Effect of nanoparticles on growth and metabolism of some crop plants

Abstract

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Abstract

Nanotechnology includes synthesis, characterization, exploration and application of nano sized materials in the event of science and technology. Human’s pursuit for innovation, finding explanation of problems, and upgrading the industrial yield with energy efficient and cost effective materials has opened the opportunity of nanotechnology. Nanotechnology is an enormously potent technology. There are spectacular developments in the field of nanotechnology within the recent past years, with various developed methodologies to synthesize nanoparticles (NPs), with specific form and size which are counted for specific needs. Nanotechnology offers a lot of new developments in the fields of catalysis, photonics, optoelectronics, biological tagging, pharmaceutical applications, environmental pollution control, drug delivery systems, material chemistry, biobased nanotechnology, biosensors, nanodevices in molecular nanotechnology, biomedicine for diagnosis, therapeutic drug delivery and treatment development of many diseases and disorders. NPs exhibit new or improved properties which are supported with specific characteristics such as size, distribution and morphology. The critical particle size of the crystal structure and the size effect differ with the materials. NPs exhibit various effects depending on the material used to produce NPs and properties like solubility, transparency, colour, absorption or emission wavelength, conductivity, melting point and catalytic behaviour.

Synthesis of NPs can be broadly categorized into two approaches i.e. top-down and bottom up approaches. Several methods are used for synthesis of NPs such as physical, chemical, enzymatic and biological. Biogenic synthesis method to produce NPs is eco-friendly and free of chemical contaminants for biological applications where purity is concerned. This method uses various biological
entities as reducing and stabilizing agents such as extract, enzymes or proteins of a natural product. The nature of these biological entities also influences the structure, shape, size and morphology of synthesized NPs.

The rapidly-growing world population is projected to reach 9.6 billion by the year 2050 (UN, 2013), a 30% increase with reference to that in 2010s. Moreover, preferences towards meat-based diet and increasing demands for bio-energy crops are also driving an ever increasing demand for global agricultural production. In the context of sustainable agriculture, applying innovative nanotechnology in agriculture including fertilizer development is regarded as one of the promising approaches to increase significant crop production to feed the world's rapidly-growing population. Currently, positive and negative effects of NPs on plants have been reported. The researchers are engaged to study the effects of NPs on plant germination and growth with the goal to promote their use for agricultural applications.

The objective of the present study was undertaken to explicate a basic part of an exclusive plan of investigation and understanding in the field of plant NPs interaction. The effect of selected metal oxide NPs on the important commercial crop plants *Solanum lycopersicum* L. and *Brassica oleracea* var. botrytis L. on germination, physiology, biophysical and biochemical parameters and the toxic effect have been examined. Copper and zinc are important micronutrients, which are required for the plant growth and reproduction. The present work may help broaden our understanding of positive and negative aspects of these NPs in structured way, may prove promising to enhance the crop production via natural and low-cost additives. The present thesis entitled “Effect of nanoparticles on growth and metabolism of some crop plants” represents a description of the research conducted in the plant physiology laboratory in the Department of Botany, University of Allahabad, Allahabad during the years 2014-2017. The effect of green synthesized and chemically synthesized NPs were comparatively
assessed. One of the objectives of this thesis is to analyze the role of ZnO NPs in mitigation of salt stress in tomato plant. In this thesis, we have also discussed the role of CuO NPs in plant growth and development and evaluated the comparative study between two exposure media i.e., sand medium and foliar spray exposure. We conducted an experiment to evaluate the best exposure medium of NPs which can affect the plant growth and development. The comparative effects of ZnO NPs and zinc sulphate (ZnSO₄) ionic media were also recorded for germination and growth of Solanum lycopersicum in filter paper and sand culture, respectively. The climatic condition of India along with the present soil texture of Gangetic plain has been taken into account to conduct this work.

**Green synthesis of nanoparticles using flower extract of Thymus serpyllum L.**

The air dried flower extract of Thymus serpyllum has been used for the synthesis of ZnO and CuO NPs and are found to be a good reducing and surface stabilizing agents. The aqueous solutions of zinc acetate dihydrate Zn(CH₃COO)₂.H₂O and copper acetate monohydrate Cu(CH₃COO)₂.H₂O were mixed separately in double distilled water (DDW) under continuous stirring. After stirring, flower extract was added drop by drop into the above aqueous solutions. These mixtures were subjected to magnetic stirring and heated until colour change was observed. The synthesis of NPs was depicted by the colour change of the reaction mixture i.e. zinc acetate solution which is colourless and changes its colour to milky white and copper acetate solution which is blue in colour changes its colour to black on addition of flower extract. The presence of biomolecules as characterized by Fourier transforms infrared spectra analysis (FTIR) reveals that phytochemicals viz. flavonoids, polysaccharide, carboxylic acid, etc. are present on the surface of ZnO and CuO NPs. Biomolecules might act as stabilizing agent for the synthesis of NPs. The nature of synthesized NPs was analyzed by UV–vis
spectroscopy (UV-vis), X-ray diffraction (XRD), particle size analyzer (PSA), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The synthesized NPs were with an average size of 20–50 nm and mostly spherical.

**Comparative effects of green and chemically synthesized nanoparticles on growth and metabolism of *Solanum lycopersicum* L.**

Seeds of tomato were separately treated with different concentrations of green synthesized ZnO NPs and chemically synthesized ZnO NPs. The effect of these treatments on seed germination, seedling growth, pigment and sugar contents as well as on the activities of LP and antioxidant enzyme was studied. Seed germination was affected by both green and chemically synthesized ZnO NPs. Maximum negative effect in germination and seedling growth was observed at the highest concentration of chemically synthesized NPs as compared to the green synthesized NPs. Biophysical parameters of plants significantly increased under lower doses of green synthesized NPs. In the similar concentration of chemically synthesized NPs the increased shoot length, root length, fresh weight and dry weight was quite insignificant. The lowest concentration of both green and chemically synthesized NPs did not exhibit significant effect on total chlorophyll. However, the pigments decreased with the increasing concentration with maximum decrease at the highest concentration of both green and chemically synthesized NPs. At low concentration of both chemically and green synthesized NPs the level of sugar improved significantly with an increase of 14 and 23% respectively. Insignificant reduction in sugar content was recorded at the highest concentration of both chemically and green synthesized NPs. The antioxidant enzymes were activated in response to higher concentrations of both green and chemically synthesized NPs. The activities of SOD increased significantly in case
of chemically synthesized NPs. The dark blue spots by NBT staining and formation of dark brown formazan by DAB staining on leaves of seedlings exposed to different concentrations of ZnO NPs shows that superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) production is comparatively more in the leaves treated with the highest concentration of chemically synthesized NPs as compared to green synthesized NPs and control.

**Effects of copper oxide nanoparticles on Brassica oleracea var. botrytis L. by foliar spray and root exposure method**

The effect of exposure to different concentrations of CuO NPs on cauliflower growth was evaluated after 40 days of exposure. As comparison between foliar and root exposure treatments the reduction rate of root length and fresh weight biomass of plant was higher in root exposure medium than foliar exposure medium. It is possible that higher concentration of CuO NPs given by root exposure treatment could have reached toxic levels in root that reduced the root length. The increase of more than 25 folds in the Cu content was observed in roots at the highest concentration of CuO NPs by root exposure medium but increase was only 2 folds in roots treated by foliar spray method as compared to their controls. While comparing foliar and root exposure methods of CuO NPs treatments, it was found that NPs given by root exposure method have been translocated towards shoot while NPs given by foliar exposure remains accumulated in traces at shoot region. In pigment, a decrease of around 40% was observed in cauliflower at the highest concentration of CuO NPs by foliar exposure medium while it was only around 20% in root exposure medium as compared to control. Total soluble sugar decreased in the plants linearly with the increasing concentration of CuO NPs. The NR activity decreased upon exposure to higher concentrations of CuO NPs. The decrease in activity of NR may be due to low rate of absorption of NO$_3^-$ under the influence of CuO NPs. It was observed
that the application of CuO NPs by both foliar and root exposure methods showed a significant rise in EL, H$_2$O$_2$ and MDA content as compared to their respective control. SOD activities also followed the same trend. The CAT activities were more stimulated by application through foliar spray method.

**Effects of zinc oxide nanoparticles in mitigation of salt stress in *Solanum lycopersicum* L.**

In this study, seeds of tomato plants were grown in plastic pots filled with sterilized sand and exposed to Control (C), 125 mM NaCl (S$_1$), 250 mM NaCl (S$_2$), 50 mg L$^{-1}$ ZnO NPs +125 mM NaCl (N$_1$S$_1$), 50 mg L$^{-1}$ ZnO NPs +250 mM NaCl (N$_1$S$_2$), 100 mg L$^{-1}$ ZnO NPs +125 mM NaCl (N$_2$S$_1$) and 100 mg L$^{-1}$ ZnO NPs +250 mM NaCl (N$_2$S$_2$). The treatment (S$_2$) with NaCl of concentration 250 mM caused 46, 54, 70 and 79.5% reduction in germination percentage, radicle and plumule length and seedling vigour index (SVI) respectively. Application of ZnO NPs increased growth and diminished effects of salt stress. Combined ZnO NPs and NaCl treatment (N$_2$S$_2$) caused increase of 60, 64 161 and 219% in germination percentage, radicle and plumule length and SVI respectively as compared with NaCl treated plants. Salinized plants showed a reduction in plant growth parameters i.e. root length, shoot length, fresh weight, and dry weight. There was reduction in photosynthetic pigments viz. total chlorophyll and carotenoids, sugar content and NR activity in the plants exposed to high salt concentration. On the other side, salinity stress boosted the MDA, EL and H$_2$O$_2$ level as well as the activities of SOD and CAT in stressed plants over control plants. However, application of ZnO NPs mostly stimulated growth of stressed plants, which was accompanied by increase in the levels of photosynthetic pigments, sugar content and NR activity. On the contrary, priming with ZnO NPs caused a decrement in the contents of MDA, EL, H$_2$O$_2$ as well as in the activities of SOD and CAT. It is concluded from the results of this study that priming with ZnO NPs, especially N$_2$
(100 mg L\(^{-1}\) ZnO), lessened the negative impacts of NaCl on tomato plants through enhancing photosynthetic pigments, adjusting osmoregulation, and decreasing the contents of MDA and EL. Further, protection under NaCl stress was achieved via recovering the activities of antioxidant enzymes. Further efforts are required to gain a full understanding of how ZnO NPs mitigate the adverse effects of salinity stress in plants.

**Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of *Solanum lycopersicum***

In the present study, ZnO NPs were rapidly synthesized at room temperature by treating zinc acetate dihydrate with the flower extract of *Elaeagnus angustifolia* (Russian olive). The FTIR spectrum revealed the presence of phytochemicals in the flower extract mediated ZnO NPs. Seeds of tomato were separately treated with different concentrations of synthesized ZnO NPs and zinc sulphate (ZnSO\(_4\)) salt suspensions, common zinc supplement. The effect of these treatments on seed germination, seedling vigour, chlorophyll, protein and sugar contents as well as LP and the activities of antioxidant enzyme were studied. Relative water content (RWC) of leaves, a marker of crop-water status, brought out an interesting trend where in nano form of zinc solution resulted in lowering of leaf turgiscence. The highest 6.1 mM concentration reduced RWC to greater extent as compared with 1.2 mM concentration. Leaves of plants treated with 6.1 mM concentration of ZnO NPs recorded maximum reflectance while it was minimised in plants treated with 1.2 mM concentration of NPs. The effect of synthesized ZnO NPs on seedling vigor, pigment, protein and sugar content was found affirmative at lower concentrations contrary to control and ZnSO\(_4\) salt. Amount of Chl \(a\) and Chl \(b\) registered maximum value at lower 1.2 mM concentration of ZnO NPs while carotenoid content was found maximum in 1.2 mM concentration of ZnSO\(_4\) salt. The inhibitory effect at higher concentration of
NPs indicated importance in the precise application of NPs, in Zn deficient system, where plant response varies with concentration. To the best of our knowledge this is the first report on *Elaeagnus angustifolia* mediated synthesis of ZnO NPs and their effects on germination and physiological activity of tomato.

**Synthesis, characterization and application of ruthenium oxide nanoparticles on growth and metabolism of *Brassica oleracea* L.**

In the present study, we evaluated the effects of various concentrations of RuO$_2$ NPs on germination, growth and metabolism of *Brassica oleracea* var. botrytis (cauliflower) seedlings in hydroponic culture. NPs synthesized by laser ablation method are free from chemical contamination. Characterization of RuO$_2$ NPs was carried out by UV-vis spectroscopy and field emission SEM. High colloidal stability of laser synthesized RuO$_2$ NPs has facilitated their dispersity and hence longer time availability in the plant growth media. RuO$_2$ NPs stimulated seed germination, radicle and plumule length, number of leaves, plants height in lower concentrations and decreased at higher concentrations. Photosynthetic pigment, protein and sugar content and NR activity were stimulated by RuO$_2$ NPs up to 10.8 μg/mL concentrations while inhibited under higher concentrations. Leaves of plants treated with the highest concentration recorded maximum value of percentage reflectance while minimum in plants treated with the lowest concentration. RuO$_2$ NPs slightly declined the LP at the lowest concentration while further increase was concentration dependent. The present study is an attempt to explore the safe level of RuO$_2$ NPs on plants with least impact. It is concluded that the lower doses of RuO$_2$ NPs significantly improved the seed germination and seedlings growth of cauliflower by improving the metabolism of the crop plant.
Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants *Solanum lycopersicum* and *Brassica oleracea* var. botrytis

Study on the ecological consequence of metal oxide NMs has improved rapidly 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 CuO NPs by using *Morus alba* leaf extract as reducing agent. XRD and TEM analysis revealed the monoclinic phase and 20–40 nm size respectively. The existence of reducing and capping agents was revealed by 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. In the present study, seed germination of both crops exhibited almost similar trends but the adverse effect was more eminent in cauliflower. Bioaccumulation of Cu was also investigated by atomic absorption spectroscopy (AAS). Analysis of Cu content in roots of cauliflower and tomato plants exposed to 500 mg L$^{-1}$ CuO NPs showed the presence of 487 ± 43.93 and 665 ± 40.53 mg/Kg of Cu respectively and their control plants also contained trace amounts of Cu. The greater accumulation of Cu NPs in the tomato plants can be simply explained by its root morphology. The roots of tomato have the greater surface area as they bear numerous thin walled roots. 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 LP, EL, 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 examination of leaves of treated plant with NBT and DAB showed a concentration dependent increase in O$_2^-$ and H$_2$O$_2$
formation in leaves. The histochemical visualization highlights the spatial organization of oxidant biochemistry occurring in response to metal stress. In the present study during stress condition the accumulation of $O_2^-$ appeared to be traced in mesophyll tissue while $H_2O_2$ accumulation was found in the vascular tissue.

**Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants**

Over the past decade, incorporation of NMs into agricultural practices like nanofertilizers and nanopesticides has gained a lot of attention. Progress and application of fertilizers in nanoforms are one of the effective options for considerable improvement of the agricultural yield worldwide. ZnO NPs are considered as a bio safe material for biological species. Earlier studies have shown the potential of ZnO NPs in stimulation of seed germination and plant growth as well as disease suppression and plant protection by its antimicrobial activity. However, both positive and negative effects of ZnO NPs on plant growth and metabolism at various developmental periods have been documented. Uptake, translocation and accumulation of ZnO NPs by plants depend upon the features of NPs as well as the anatomy of the host plant. This review summarizes the applications of ZnO NPs as nanofertilizer in crop production. It is an attempt to examine and record the possible mechanism of antimicrobial activity of ZnO NPs. Biological synthesis of ZnO NPs and their uptake, translocation and biotransformation in plants via various routes have also been examined.

The present study deals with the green synthesis of NPs and their role in growth and metabolism on crop plants. Green synthesized ZnO NPs proved beneficial in low concentration for tomato as compared to chemically synthesized ZnO NPs. Affirmative response was noticed with ZnO NPs at lower concentration.
in comparison to ZnSO$_4$ salt and control while NPs at higher concentration were found detrimental. The use of exogenous application of ZnO NPs under salt stress condition has been found to be quite effective to alleviate salt induced damages in tomato seedlings. While comparing foliar and root exposure method of CuO NPs, it was found that NPs given by root exposure method had been translocated towards shoot while NPs given by foliar exposure remains accumulated in traces at shoot region. The accumulation of CuO NPs in tomato and cauliflower plant species was dose dependent and the results show that NPs are more actively accumulated by tomato plants than cauliflower possibly due to the difference in root morphology. These findings call for further exploration and analysis of the mechanism of nanoparticle transport within the plant. The exposure of plants to NPs and the effects of such an exposure on plant systems could open a new path of research in the field of nano-agronomy. Concerns about the toxicity of NPs need to be addressed, and special care should be taken in the application of NPs to various products.