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IMPACT OF ZnO NANOPARTICLES ON THE BIOCHEMICAL PARAMETERS AND ENZYME ACTIVITIES OF PLANT ABELMOSCHUS ESCULENTUS

Agricultural Science

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ABSTRACT

Environmental contamination is the major threat to living and non-living things. Due to accumulation of stringent pollution load, climate changes, ecological imbalance and infertility of soil, the application of nanotechnology on agriculture is getting significant attention on current scenario. The nanotechnology has an innovative, promising and emerging field which improves the biological systems exposed to excess of nanoparticles. The aim of present research focused on the impact of ZnO nanoparticles on the biochemical parameters and enzyme activities of Abelmoschus esculentus. The ZnO nanoparticles treatment of experimental plants significantly enhanced biochemical parameters such as nitrogen, protein, chlorophyll-a and chlorophyll-b and catalase activities meanwhile decreased activities of nitrate reductase with increasing concentrations than control.

KEYWORDS

Nanotechnology, ZnO nanoparticles, biochemical parameters, enzyme activities.

INTRODUCTION

Nanoparticles are extraordinary structural and physicochemical characteristics with a dimension from 1nm to 100nm. Applications of the nanoparticles are in medicine, biology and engineering by their physical, chemical and biomedical properties (Nel *et al.,* 2006). Nanomaterials are grouped into metal oxides, clays, carbonaceous and semiconductor materials. Zinc Oxide nanoparticles are one of the most common metal oxides used for the several applications (Klaine *et al.,* 2008) and inappropriate handling is also caused environmental contamination (Dimkpa *et al.,* 2012).

The applications of nanomaterials are broad-spectrum such as fluorescent biological labels, drug and gene delivery, detection of proteins, tissue engineering, biodetection of pathogens, tumours destruction, probing of DNA structure, etc. (Salata, 2004). Due to miniature dimension of nanoparticles, the nanotechnology focuses to sustainable agricultural processes. The nanotechnology has many potential advantages such as reduction of agricultural inputs, enrichment of food quality and safety, enhancement of absorbing nanoscale nutrients from the soil ecosystem (Ram Prasad *et al.,* 2017). From this study, in agriculture, to reduce the amount of spread chemicals, minimize nutrient losses in fertilization and increased yield through pest and nutrient management.

The implication of the nanotechnology research in the agricultural sector is become to be necessary even key factor for the sustainable developments. In the agri-food areas pertinent applications of nanotubes, fullerenes, biosensors, controlled delivery systems, nanofiltration, etc. were observed (Ion et al., 2010; Sabir et al., 2014). This technology was proved to be as good in resources management of agricultural field, drug delivery mechanisms in plants and helps to maintain the soils fertility. The nanoparticles play a significant role in the productivity through control on nutrients (Mukhopadhyay, 2014). Plants are the essential components of all ecosystems and they closely interact with their surrounding environment containing nanoparticles. However, it is largely unknown whether any toxicity results from exposure to nanoparticles or from released ions (Parsons *et al.,* 2010). The nanoparticles can enter into plant roots via the apoplastic pathway and they are transported to shoots through the vascular system. Thus, their uptake depends on the plant anatomy and composition, shape, and size of NPs. The uptake of ZnO nanoparticles has been reported in perennial ryegrass, *Lolium perenne* (Lin and Xing 2008), copper nanoparticles in mung beans, *Phaseolus radiatus* and wheat, *Triticum aestivum* (Lee *et al.,* 2008). The aim of this study was to investigate the impact of ZnO nanoparticles on the biochemical parameters and enzymes activities of *Abelmoschus esculentus.*

MATERIALS AND METHODS

Synthesis and preparation of ZnO nanoparticles

Zinc oxide nanoparticles were synthesized by the sol–gel method as nano-powder prepared by mixing a methanol solution. 2g of zinc acetate dehydrate was dissolved in a 15ml of distilled water and 8g of sodium hydroxide was dissolved in a 10ml of distilled water. After that the NaOH solution was added into the solution of zinc acetate with a constant stirring. Then 100ml of ethanol was added the solution containing both sodium hydroxide solution and zinc acetate. After the reaction, and evaporation of solvents in oven white ZnO nano-powder was formed (Khalaf et al., 2017). The solutions for treatments were prepared from ZnO nano-powder in six different concentrations such as $50mgL^{-1}$, $100mgL^{-1}$, $150mgL^{-1}$, $200mgL^{-1}$ and $250mg\ L^{-1}$ with appropriate control (distilled water).

Experiment design

Eight seeds of *A.esculentus* were sowed in six different plastic pots and add different concentrations of Zinc oxide nanoparticles such as $50mgL^{-1}$, $100mgL^{-1}$, $150mgL^{-1}$, $200mgL^{-1}$ and $250mgL^{-1}$ with control were added into the pots regular time intervals. After completion of 15^{th} day and 30^{th} day of experiments the plants were harvested and washed thoroughly using distilled water. The plant samples (root, shoot and leaves) were oven-dried at $80^{o}C$ for 15min, ground well and used for further biochemical analysis such as total nitrogen content, protein content and chlorophyll a (Chl a) and chlorophyll b (Chl b) content were studied and nitrate reductase and catalase activities also analysed.

Biochemical parameters

Total nitrogen content

The total nitrogen content was determined by the method of Markham (1942). In a microkjeldahl flask 100mg of dried powder of plant samples was taken and added 100gm of catalyst for digestion. 3ml of sulphuric acid and 1ml of hydrogen peroxide were added into the flask for digestion. The colourless solution was collected in a volumetric flask. 5ml of digest was transferred into the distillation unit with 10ml of 40% NaOH was distilled for 15 minutes and the ammonia liberated into boric acid indicator until the pink colour turned into green colour. The solution titrated against N/100 HCl until pink colour reappeared. The total nitrogen present in the sample was calculated by the 1ml of N/100 HCl = 0.14mg of nitrogen.

Protein content

Fresh plant materials were collected, boiled in 10ml of 80% ethanol and homogenized. The samples were centrifuged at 6000 rpm for

20min. 0.5ml of samples were taken from the supernatant and added with equal amount of 1N sodium hydroxide. After 10min incubation, added 2.5ml of reagent C and 0.5ml of Folin-Ciocalteu's phenol reagent after 10min. After appearance of violet-blue colour the samples were stand for 30min the colour intensity was measured in UV spectrophotometer at 650nm (Lowry *et al.,* 1951).

Chlorophyll a and b content

1gm of leaf samples were cut finely and mixed gently using a clean pestle and mortar. 20ml of 80% acetone and 0.5gm of powder of $MgCO_3$ were added to this homogenized material. The samples were gently ground and refrigerated at 4°C for 4 hrs. The samples were centrifuged at 500rpm for 5mts. The supernatant were transferred into 100ml volumetric flask which were made up to 100ml using 80% acetone. The colour absorbance of the solution was measured by 645 and 663nm wavelength of spectrophotometer (Kamble *et al.,* 2015).

Enzyme activities

Nitrate reductase activity

The activities of nitrate reductase were determined by the method adopted by Jaworski (1971). 250mg of fresh plant samples were incubated in 4.5ml of medium which contained 100mM phosphate buffer, 3% potassium nitrate and 5% propanol. From this medium, 0.4ml was treated with 0.3ml of 3% sulphanilamide in 3N hydrochloric acid and 0.3ml of 0.02% N-1, naphthyl ethylene diamine dihydrochloride. The solutions were measured at 540nm and appropriate standard solution which prepared from $NaNO_2$.

Catalase activity

Activities of catalase were slightly modified by the method of Aebi (1984). 1.5ml of phosphate buffer, 1.2ml of H_2O_2 solution and 50µl of enzyme were used as the reaction mixture. The blank was without enzyme and optical density was measured by UV spectrophotometer at 240nm.

RESULTS AND DISCUSSION

After completion of experiments, the plants samples of the plant species were analysed the biochemical parameters such as nitrogen content, protein content, chlorophyll-a and –b and enzyme activities such as nitrate reductase and catalase. All the biochemical and enzymatical indicators exhibit significant variations.

The increased levels of nitrogen content were observed with increasing days and the highest values were recorded in 150mlL^{-1} concentrations and it was declined in higher concentrations 200mlL^{-1} and 250mlL^{-1} (Fig.1). Nitrogen produces rapid growth, improves quality of plants, enhances growth and increases the protein content due to ZnO nanoparticles act as nanofertilizers. The increased nitrogen content may be attributed to the soil and climatic conditions play an important role in uptake and utilization of nitrogen. Optimum availability of nitrogen content is important for their suitable intake by plants (Bloom, 2015).

The results were clearly showed that the increased trends of protein contents were found up to 150mlL^{-1} meanwhile it was decreased in higher concentrations as well as increasing days (Fig.2). The plant protein contents play various enzymatic, structural and functional roles such as photosynthesis, biosynthesis, storage, etc. The protein compounds such as enzyme complexes are quickly reacts to fluctuating environmental conditions and ensure plants resistance to stress (Olga *et al.,* 2016).

The significant increment of chlorophyll-a and b were observed while increasing experimental days as well as the higher chlorophyll content were recorded in Chl-a than Chl-b (Fig.3). This may be due to the Chl-a is the primary pigment while others pigments including Chl-b are accessory pigments (Srichaikul *et al*., 2011; Kamble *et al.,* 2015). The increased Chl-a and Chl-b were noticed up to 150mlL^{-1} subsequently it was declined in higher concentrations (200mlL^{-1} and 250mlL^{-1}). This is due to the influence of higher accumulations of nanoparticles, micronutrient sufficiency in soil and high penetration of sunlight can be cause the high chlorophyll content (Morikawa *et al*., 2006; Dantas *et al*., 2007).

The nitrate reductase activity showed a gradual decrease from control to higher concentrations (Fig.4). This was due to the pH and temperatures influence the nitrate reductase (Marek and Dominika, 2013). Besides presence of nitrate, the activities of enzyme depends from many factors such as water, light intensity etc. (Tripathi and Mukesh Gautam, 2007; Marek and Dominika, 2013).

The catalase activities were increase significantly with increasing experimental days as well as with increasing concentrations upto 150 ml L^{-1} and slightly it was decreased in higher concentrations (Fig.5). Increased activity of catalase enzyme due to adaptations which helps to overcome the damage to the tissue metabolism by reducing toxic level (Sudhakar *et al.,* 2001) and decreased activity might be due to ZnO nanoparticles stress. Catalase is antioxidant enzyme, which acts as an important role in detoxifying H_2O_2 into water and oxygen and protects the cell from oxidative damage through reactive oxygen species (Mhamdi *et al.,* 2010; Ravi kiran and Aruna, 2010).

CONCLUSION

In agriculture, nanotechnology has been enhances the crop production with quality farming systems. Engineered nanomaterials adopted for sustainable agriculture which leads novelty, fast growth and enormity to meet the expectations of worldwide food scarcity. The potential of nanoparticles encourages a new green revolution. Therefore, further profound research is needed to alter the fate of agriculture and association with biomacromolecules present in living organisms and ecosystem.

Fig. 1. Total nitrogen content (µ mol NO_2 h^{-1} g^{-1}leaf fresh wt.) of plant *A.esculentus* at different concentrations of ZnO nanoparticles

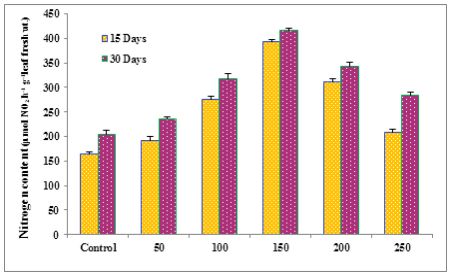


Fig. 2. Protein content (µmol H_2O_2 h^{-1} g^{-1} leaf fresh wt.) of plant *A.esculentus* at different concentrations of ZnO nanoparticles

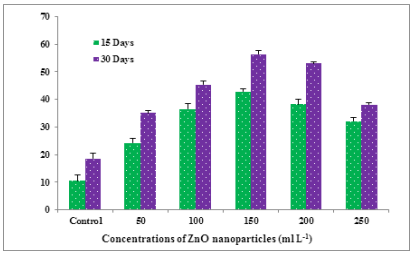


Fig. 3. Chlorophyll-a and chlorophyll-b content (mg/g fr wt x10^{-4}) of plant *A.esculentus* at different concentrations of ZnO nanoparticles

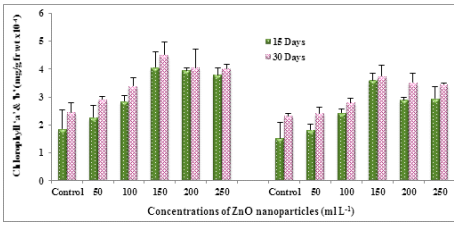


Fig. 4. Nitrogen reductase activity (µM Nitrate formed g^{-1} dry wt h^{-1}) of plant *A.esculentus* at different concentrations of ZnO nanoparticles

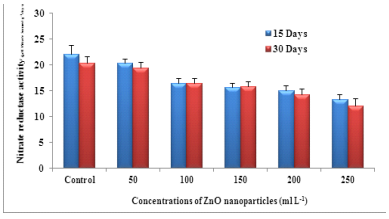
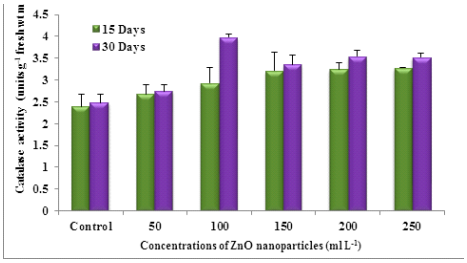


Fig. 5. Catalase activity (units g^{-1} fresh wt min^{-1}) of plant *A.esculentus* at different concentrations of ZnO nanoparticles



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