



Original Research Article

Effect of zinc oxide nanoparticles on cytology and seed germination in onion

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A B S T R A C T

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In protection of environment, nanotechnology is finding applications in photocatalysis, a process in which light promotes a reaction between compound such as pesticide residues and nanomaterial without the latter being consumed. Such a process would be useful in decomposition of water for agriculture and human safe. In food safety, photocatalysis could find uses in cleansing the surface of fresh fruits and vegetables with toxic agrochemical residues and in destroying bacteria on such produce. Zinc oxide (ZnO) nanoparticles (NPs) have a potential application as a bacteriostatic agent and can be used to control the spread and infection of variety of pathogens. In present investigation, different concentration (0.0, 10,20,30 and 40 $\mu\text{g ml}^{-1}$) of ZnO Nps were prepared in distilled water and used for the treatment in onion seeds to study the effect on cell division, seed germination and early seedling growth. Decreased Mitotic Index (MI) and increase in chromosomal abnormalities were observed in higher treatments of zinc oxide nanoparticles. Seed germination increased in lower concentrations, however showed decrease in values at higher concentrations. Germination indices showed increased values in lower concentrations; however these decreased significantly at higher concentrations.

Introduction

Nanoparticles are microscopic particles with at least one dimension less than 1000 nm. For this, these particles are very attractive materials to handle in biological system. Nanoparticles are found to be very suitable in sensing and detection of biological structures and systems (Singh *et al.*, 2008). Metal nanoparticles appear in different shapes such as nano-powder or nano-cluster or nano-crystal and different sizes ranging from 2 nm to 1000 nm.

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures (Kongara *et al.*, 2007). Man engineered nanomaterials have received a particular attention because of their positive impact in improving many sectors of economy, including consumer products, pharmaceuticals, cosmetics, transportation, energy and agriculture, etc. These are

being increasingly produced for a wide range of applications within industry (Novack and Bucheli 2007; Roco 2003b).

Scientific community is debating about the risks and benefits of the many manufactured nanomaterials into the environment (USEPA 2007) and in order to evaluate their potential adverse effects on the ecosystems and on human health the scientists are working with increasing attention to this topic. The literature on the ecotoxicity of nanoparticles and nanomaterials as well as the chemistry of both manufactured and natural NPs are given in some reports (Handy *et al.*, 2008a, b; Yu-Nam and Lead 2008).

Since, these particles are used in many consumer products and it is projected that these NPs will enter into various ecosystems, where their actions are not known. Therefore, organisms which will interact with NPs are expected to have beneficial or adverse effects. The interactions between microorganisms and metals have been well documented and ability of microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation (Zahra *et al.*, 2010).

The biocidal properties of the NPs have significant practical relevance. Antibacterial and antifungal properties of metal NPs can be tapped to control bacterial and fungal organisms responsible for crop losses. However, it must be very clear that these NPs should not have any adverse effect in plant systems. Hence, in present investigation it was planned to study the influence of ZnO NPs on mitotic cell division, seed germination and seedling growth in onion.

Materials and Methods

Zinc oxide (ZnO) NPs about \cong 20 nm sizes were obtained from the researchers in the field of nanomaterial synthesis. Seeds of Local onion variety were procured from NRC (National Research centre for onion and garlic) Rajgurunagar.

Influence of nanoparticles on mitotic cell division and mitotic cytotoxicity

Root tips (1-2 cm) were treated with ZnO, washed and fixed in 1:3 acetic acid-ethanol mixtures for 24 hours and preserved in 70% alcohol. Root tips were hydrolyzed in 1.0 N Hydrochloric acid and squashed in a drop of 2% Acetocarmine. Total 1000 cells were screened for mitotic abnormalities.

Effect of nanoparticles on seed germination

The graded concentrations (00, 10, 20, 30, and 40 $\mu\text{g ml}^{-1}$) of ZnO nanoparticles were added aseptically to sterilized petriplates lined with Whatman no. 1 filter paper. Surface sterilized seeds of onion were germinated (20 seed per plate) in each concentration of nanoparticles. Similar experiment without nanoparticles was conducted as control. After 10 days of treatment, seedlings were harvested and shoot and roots of seedling were separated. Seedling growth in terms of root length, shoot length, fresh weight and dry weight were recorded and results were compared to see effect of nanoparticles on seed germination and early seedling growth.

Germination Indices

Parameters like PI(Promptness index), GSI(Germination stress tolerance index), PHSI(Plant height stress tolerance index),

RLSI(Root length stress tolerance index), DMSI(Dry matter stress tolerance index) are calculated by using following formulae.

(a) Promptness index

$$(PI) = \frac{n}{d} \times 2(1.0) + nd \times 4(0.75) + nd^2 \times 6(0.5) + nd^3 \times 8(0.25)$$

Where n is the no. of seed germinated at day "d"

(b) Germination stress tolerance index

$$(GSI) = \frac{PI \text{ of stressed seeds}}{PI \text{ of control seeds}} \times 100$$

(c) Plant height stress tolerance index

$$(PHSI) = \frac{\text{Plant height of stressed plant}}{\text{Plant height of control plant}} \times 100$$

(d) Root length stress tolerance index

$$(RLSI) = \frac{\text{Root length of stressed plant}}{\text{Root length of control plant}} \times 100$$

(e) Dry matter stress tolerance index

$$(DMSI) = \frac{\text{Dry matter of stressed plant}}{\text{Dry matter of control plant}} \times 100$$

Results and Discussion

The influence of ZnO NPs on mitotic division expressed as MI is given in Tabel.1. Seeds treated with ZnO NPs of higher concentrations (30 and 40 µg/ml⁻¹) showed significant reduction in the mitotic index. This decrease in mitotic index was found to be statistically highly significant both in 30 and 40 µn/ ml⁻¹ concentration ZnO NPs .The MI was remarkably

reduced in roots treated with 40 µg/ ml⁻¹ ZnO NPs with a minimum value of 34.86%.

Table.1 shows the percentage of abnormal cells for different treatments with ZnO NPs The treated roots, compared with control, showed significant increase in the percentage of total abnormal cells with the increase of ZnO NPs concentrations. The highest percentage of abnormal cells, recorded in roots treated with 40 µg/L, was 17.16% compared with that of 1.86% for control set. ZnO NPs induced different types of mitotic abnormal cells in the roots of onion. These abnormal cells mainly include stickiness, laggards, chromosome bridges and ring chromosomes as well as irregular prophases anaphases, c-metaphases and cells with micronuclei as pointed up in Fig. 2.

Stickiness of chromosomes at metaphase and anaphase were very significant. It clearly reveals the polymerisation effect of ZnO NPs on nucleic acid of the chromosome. Patil and Bhat (1992) suggested that stickiness is a type of physical adhesion involving mainly the proteinacious matrix of chromatin material. Sticky bridges at anaphase recorded in higher doses could be attributed to chromosomal stickiness (EI-Khodary *et al.*, 1990), and chromosomal breakage and reunion (Haliem 1990). Induction of chromosome bridges and breaks may be lead to loss of genetic material (Salam *et al.*, 1993).

The clastogenic aberration like ring chromosome was also recorded at higher concentration of ZnO NPs, formation of ring chromosome can be ascribed to the chromosomal breakage and broken sticky chromosome ends formed due to effect of ZnO NPs. The precocious movement of

the chromosome might have been caused by the early terminalisation, stickiness of chromosomes and/or because of the movement of chromosome ahead of the rest during anaphase (Permjit and Grover, 1987). Very high frequencies of c-metaphase, disturbed anaphase and un-oriented chromosome at anaphase indicate partial inhibition of mitotic apparatus due to oxidative stress exerted by higher concentration of ZnO NPs.

Binucleate cells that were recorded frequently at higher concentration of ZnO NPs might have formed due to the inhibition of cell wall development at telophase. The micronuclei, observed at higher concentration might have originated from lagging chromosomes at ana-telophase or from a chromosome fragments (Badar and Ibrahim, 1987). Formation of micronuclei is a true mutagenic aspect, which may lead to a loss of genetic material and micronuclei have been regarded as an indication of the mutagenicity of their inducers (Raun *et al.*, 1992). Kumari *et al.* (2011) reported that ZnO NPs exert cytotoxic and genotoxic effects, including lipid peroxidation, decreasing of the mitotic index and increasing of the micronuclei and chromosomal aberration indices on root cells of *Allium cepa*. Therefore we can conclude that higher concentration of ZnO NPs can be mitoinhibitory but concentration could not be genotoxic or cytotoxic.

Results pertaining to seed germination and early seedling growth clearly indicate that ZnO NPs at lower concentration promoted seed germination and seedling growth, but at higher concentration reduced seed germination and seedling growth. Highest concentration (40 $\mu\text{g ml}^{-1}$) of ZnO NPs

showed significantly low germination percentages i.e. 78.28% and maximum percent seed germination was observed in 20 $\mu\text{g ml}^{-1}$ concentration of ZnO NPs i.e. 96.52, whereas untreated seeds showed 94.28 % seed germination (Table. 2). Lower concentrations of ZnO NPs (10 and 20 $\mu\text{g ml}^{-1}$) showed significant enhancement in shoot and root lengths, however higher concentrations (30 and 40 $\mu\text{g ml}^{-1}$) of ZnO NPs showed decreased root length, shoot length and total seedlings height. There was no major difference in root shoot ratio in all treatments however an increasing trend was seen from lower to higher concentrations. Similar trend as in seedling height was seen in case of fresh and dry weight of the seedlings after ZnO NPs treatments (Table. 2).

Data with respect to the promptness index (PI) clearly show that PI increased up to 20 $\mu\text{g ml}^{-1}$ concentration of ZnO and decreased significantly both in 30 and 40 $\mu\text{g ml}^{-1}$ concentration of ZnO NPs. Germination stress tolerance index (GSI) indicates speed of seed germination in control and treated seedlings. Higher GSI (101.90) was exhibited by 20 $\mu\text{g ml}^{-1}$ concentration of ZnO NPs and lower GSI (78.05) was observed in 40 $\mu\text{g ml}^{-1}$ ZnO NPs treatment. Plant height stress tolerance index (PHSI) values showed increase in 10 and 20 $\mu\text{g ml}^{-1}$ ZnO NPs treatment than control, conversely sudden and significant decrease in PHSI values i.e. 87.15 and 74.64 were noted in 30 and 40 $\mu\text{g ml}^{-1}$ concentrations of ZnO NPs respectively. Root length stress tolerance index (RLSI) value showed increase i.e. 106.31 in 10 $\mu\text{g ml}^{-1}$ ZnO NPs treatment over control and significant decrease in RLSI values (78.88) was observed in 40 $\mu\text{g ml}^{-1}$ concentrations of ZnO NPs.

Table.1 Effects of ZnO NPs on mitotic cell division and mitotic abnormalities in *Allium cepa*

Concentration of ZnO NPs ($\mu\text{g ml}^{-1}$)	No. of observed cell	No. of dividing cell	No. of abnormal cell	% MI	% Chromosomal abnormality
00.00	1000	470	8.72	47.04	1.86
10.00	1000	510	11.82	51.22	2.32
20.00	1000	520	23.28	52.14	4.48
30 .00	1000	400	42.72	40.64	10.68
40.00	1000	340	58.36	34.86	17.16
CD 5%		5.26	2.82	2.68	2.86

Table.2 Effect of ZnO NPs on seed germination of *Allium cepa*

Concentration of ZnO NPs ($\mu\text{g ml}^{-1}$)	Germination %	Shoot Length (cm)	Root length (cm)	Seedlings Height (cm)	Root-Shoot Ratio	Fresh Wt. (mg)	Dry Wt. (mg)
00.00	94.28	7.63	4.12	11.75	0.54	429.12	63.38
10.00	95.84	7.76	4.38	12.14	0.56	476.28	70.44
20.00	96.52	7.58	4.36	11.94	0.58	468.92	68.38
30 .00	86.62	6.46	3.78	10.24	0.59	398.08	59.12
40 .00	78.28	5.52	3.25	8.77	0.59	356.26	52.42
CD 5%	1.26	1.12	0.26	0.66	0.08	12.18	2.92

Table.3 Effect of ZnO on PI, GSI, PHSI, RLSI, and DMSI of *Allium cepa*

Concentration of ZnO NPs ($\mu\text{g ml}^{-1}$)	PI	GSI	PHSI	RLSI	DMSI
00.00	86.75	100.00	100.00	100.00	100.00
10.00	88.25	101.74	103.32	106.31	111.14
20.00	88.40	101.90	101.62	105.83	107.89
30 .00	81.52	93.96	87.15	91.75	93.28
40 .00	78.05	89.98	74.64	78.88	82.71
CD 5%	1.08	1.26	1.34	1.64	2.88

Fig.1 Effect of ZnO on seedling growth in Onion.



Figure.2 Effect of ZnO on mitotic cell division in Onion

A: C-metaphase, B: Ring chromosomes, C and D: Anaphase bridges, E: Metaphase showing precocious movement of chromosome, F and G: Telophase bridges and H: Binucleate cell with micronuclei

Dry matter stress tolerance index (DMSI) value showed the similar trend (Table. 3). Lee *et al.*, (2008) studied the effect of copper nanoparticles on bean (*Phaseolus radiatus*) and wheat (*T. aestivum*) plants. They observed decrease in growth parameters in the seedlings due to copper nanoparticles. However Zheng *et al.*, (2005) observed significant enhancement in the growth of spinach in lower concentrations of nano TiO₂ as compared to higher concentrations. Lin and Xing (2007) evaluated phytotoxicity of five types of metallic nanoparticles in six higher plant species and indicated that seed germination was not affected except for the inhibition of nano ZnO in *Lolium multiflorum* and *Zea mays*. They indicated that inhibition of root growth varied significantly among nanoparticles and plants, and it is partially correlated to nanoparticle concentration.

The result shows that ZnO at lower concentration enhances cell division, mitotic index with minimum cell divisional abnormalities and also increased seed germination, promptness index, and seedling growth that indicate the lower concentration is not harmful to the cell division and early seedlings growth.

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