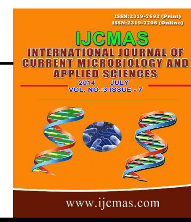

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 749-760**
http://www.ijcmas.com

Original Research Article

Effect of Titanium Dioxide Nanoparticles on Hydrolytic and Antioxidant Enzymes during Seed Germination in Onion

S.L.Laware* and Shilpa Raskar

P.G. Department of Botany, Fergusson College, Pune- 411004, University of Pune (MS), India
**Corresponding author*

Keywords

TiO_2 nanoparticles, amylase, protease, SOD, CAT, POD

A B S T R A C T

Surface sterilized onion seeds were germinated (25 seed per plate) with graded concentrations (00, 10, 20, 30, 40 and 50 μg mL^{-1}) of TiO_2 nanoparticles (NPs) in petriplates lined with Whatman no. 1 filter paper. Similar experiment without nanoparticles was conducted as control. TiO_2 NPs at lower concentration enhanced seed germination and seedlings growth in onion and however the germination and growth showed inhibition at higher concentrations. The TiO_2 NPs also induced significant changes in activities of hydrolytic and antioxidant enzymes. Activities of amylase and protease were enhanced in lower concentration, but showed decrease at higher concentrations. The activity SOD showed concentration dependent increase; however CAT and POD were found to be enhanced in the presences of 10-30 μgml^{-1} NPs but showed decreased activities in 40 and 50 μgml^{-1} NPs concentrations.

Introduction

Increasing production and use of nano-sized materials have raised concerns about their possible impacts on environmental and human health (Hood, 2004). These nanomaterials (NMs) are being produced, sold and commercially used for various purposes and even in some food products. As NMs are being used on large scale they might have entered in ecosystems. Such NMs on entering in ecosystem might have affected or effected seed germination process, plant growth and metabolism. Hence, plants should be tested to establish their response to nanomaterial stress and possible role of hydrolytic and antioxidant enzymes to NMs during seed germination and early seedling growth.

Seeds enfold sufficient quantity of food reserves which support the seed germination and early seedling growth (Zeeman et al., 2004). Germinating seeds generally exhibit high amylase and protease activities. This is because these enzymes are synthesized during seed germination to mobilize stored food for the survival of the young plant until it is capable of making its food by photosynthesis (Schramm and Loyter, 1966). Seed germination is regarded as a series of steps which normally occur prior to the emergence of the radicle from the seed coat (Mayerand and Shain, 1974). The water intake rate and seed reserve utilization are important physiological and biochemical processes associated with seed germination.

Germinating seeds, on imbibitions, consume more oxygen for activation or hydration of mitochondrial enzymes, involved in the Krebs cycle and electron transport chain (Salisbury and Ross, 1991). Furthermore, enzymes, like lipases, proteinases, phosphatise and hydrolases (Bewley and Black, 1985; Coccuni and Negrini, 1991; Washio and Ishikawa, 1992; Bernier and Ballance, 1993; Bernhardt et al., 1993) are either activated or synthesized to avail simpler substances for embryo growth. These simpler substances produced from stored food by the enzymatic actions are carried from the endosperm or cotyledons to the embryonic axis and are utilized in the synthesis of new required material (Davies and Slack, 1981; Mayer and Poljakoff Mayber, 1989). Conversion of starch to glucose is carried out by amylases (Schelgel, 2003); whereas proteases convert proteins into amino acids. The enzyme most frequently credited with the initial attack on starch granules is α-amylase, which initiates starch mobilization in germinating seeds (Trethewey and Smith, 2000; Fincher, 1989).

Reactive oxygen species generation (ROS) and oxidative stress are proposed to explain the toxicity of nanoparticles (Nel et al., 2006). Plants are generally protected against this oxidative stresses by a wide range of radical scavenging systems such as antioxidative enzymes like superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), as well as non-enzymatic compounds like carotenoids (Cameron and Reid, 2001; Zimmermann and Zentgraf, 2005).

Plants have special mechanisms to remove or inactivate reactive oxygen species (ROS) such as H_2O_2, OH^-, and O_2^- radicals that are by products of naturally occurring reactions. However, excess ROS can result in protein breakdown, lipid peroxidation in membranes and DNA injury (Choudhury and Panda, 2004). Previous studies have shown that heavy metals increases the activity of the antioxidant enzymes like catalase (CAT) and ascorbate peroxidase (APX) in plants (Lopez et al., 2007).

It has been proven that oxidative stress represents a common mechanism for cell damage induced by NPs (Pulskamp et al., 2007), and the mechanism has been validated in many NPs' toxicity studies (Yang et al., 2009). Upon entering the cell, particles may induce intracellular oxidative stress by disturbing the balance between oxidant and anti-oxidant processes. Excessive oxidative stress may also modify proteins, lipids, and nucleic acids, which further stimulates the anti-oxidant defence system or even leads to cell death. Meanwhile, with increased ROS production, NPs can cause DNA damage and increase gene expression of the death receptor (Yang et al., 2009). In addition, increased ROS induced by NPs in lysosomes can cause DNA point mutations or induce single or double strand breaks (Singh et al., 2009). Raskar and Laware (2014) observed various chromosomal aberrations in onion root tip cells treated with ZnO NPs. They ascribed these to DNA lesions and chromosome breakage. Another major oxidative stress response is intracellular Ca_2^+ release, which leads to mitochondrial perturbation and cell death (Xia et al., 2008). However, there is lack of information on the effects of NPs in onion with respect to hydrolytic and antioxidant enzymes during seed germination and early seedling growth.

Materials and Methods

Titanium dioxide (TiO_2) P25 powder of particle size ≅ 21 nm and purity > 99.5% was obtained from the researchers in the

field of nanomaterial synthesis. These were made of 80% anatase and 20% rutile. Seed of local onion variety was procured from NRC (National Research centre for onion and garlic), Rajgurunagar (MS).

The graded concentrations (00, 10, 20, 30, 40 and 50 μg mL^{-1}) of TiO_2 nanoparticles prepared in distilled water were added aseptically to sterilized petriplates lined with Whatman no. 1 filter paper. Surface sterilized seed of onion were germinated (25 seed per plate) in each concentration of nanoparticles. Similar experiment without nanoparticles was conducted as control. Seed germination and seedling growth in terms of root length and shoot length was determined after 10 days of treatment. The crude enzyme extract was prepared from 7 day old seeding.

Extraction and assay of enzyme: Treated and control seedlings were collected and cut into small pieces. Accurately 1 gm of sample was homogenized and extracted with 3ml of 0.1M phosphate buffer (pH 7.0) in pre chilled mortar and pestle. The extract was centrifuged at 4^0C in cooling centrifuge at 15000 xg for 10 minutes and supernatant was used as sources of enzymes.

Amylase (α-amylase EC. 3.2.1.1): The Amylase activity was calculated by using Jayaraman, (1981) method. Treated and control seedlings were collected and cut into small pieces. Accurately 1.0 gm of seedling sample was homogenized in pre chilled mortar with pestle, in 5 ml of 0.1 M sodium acetate buffer (pH4.8) supplemented with (10 mM NaCl). The extract was centrifuged at 4^0C in cooling centrifuge at 15000xg for 10 minutes and supernatant was used as sources of crude enzyme. Enzyme reaction mixture contained 2.0 ml of 0.1M sodium acetate buffer (pH 4.7), 0.5ml of 1% starch and 0.5ml enzyme extract in total 3 ml volume. The reaction was initiated by adding 0.5 ml of enzyme extract and reaction mixture was incubated for 10 minutes at 37 ^{O}C temperatures in water bath. After 10 minutes of incubation 2ml of Dinitrocalysilic acid (DNS) was added and reactions were heated in water bath at 100 ^{o}C for 10 minutes. Same procedure was followed for control but enzyme reaction was terminated at zero minutes by adding DNS reagent. After heating reaction mixture was diluted with distilled water to 10 ml and OD was taken at 510 nm. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 1 min at 37 ^{o}C. The enzyme activity was expressed as units g^{-1} fresh tissue.

Protease: The Protease activity was calculated by using Issac and Gokhale (1982) method. Accurately 1.0 gm of seedling sample was homogenized in pre chilled mortar with pestle, in 5ml of 0.1 M phosphate buffer (pH 7.4). The extract was centrifuged at 4^oC in cooling centrifuge at 15000xg for 10 minutes and supernatant was used as sources of crude enzyme. Enzyme reaction mixture contained 2.0 ml of 0.1M (pH 7.5) phosphate buffer, 0.5ml casein and 0.5ml plant extract in total 3 ml of volume. The reaction was initiated by adding 0.5 ml of enzyme extract and incubated for 10 minutes at 40 ^{o}C temperatures in water bath. The enzyme activity was stopped by adding 3ml of 5% H_2SO_4 solution. The proteins precipitated in reaction mixture after 60 minutes of resting were separated by centrifugation at 10,000 xg for 10 minutes. Exactly 2ml of supernatant was mixed 3ml of 2% Na_2CO_3 and 1ml of folin phenol reagent. The blue colour developed was read at 660nm. Similar procedure was followed for control but enzyme reaction was terminated at zero minutes by adding 3ml of 5% H_2SO_4 solution. The protease activity was measured by estimating the release of

tyrosine calculated from the standard curve prepared with tyrosine. One unit of protease activity was defined as the amount required for liberating 1 mg of tyrosine in 1 min at 40 ^{o}C. The protease activity was expressed as units g^{-1} fresh tissue.

Catalase (CAT, EC. 1.11.1.6): The CAT activity was calculated by using Maxwell and Bateman (1967) method. The reaction mixture contained 2.9 ml of 0.06M phosphate buffer, 10 mM H_2O_2 and 0.1 ml enzyme extract in final volume of 3 ml. The absorbance of the reaction concoction was measured at 240 nm compared to the reaction mixture without an enzyme extract. The reaction was initiated by adding 0.5 ml of enzyme extract. The decrease in absorbance was measured at 240 nm by using UV–visible spectrophotometer (Shimadzu-1700). The residual H_2O_2 concentration was calculated using extinction coefficient 0.036 μmole^{-1}ml^{-1}. One unit of CAT activity (U g^{-1} FW) was defined as the amount of CAT, which decomposed 1μmol/L hydrogen peroxide in 1 min. The enzyme activity was expressed as units g^{-1} fresh weight.

Peroxidase: (POD, EC. 1.11.1.7) The POD activity was assayed by Vidyasekharan and Durairaj (1973) method. The assay mixture of 3 ml contained 1.7ml of 0.1M phosphate buffer (pH 7.0), 1ml freshly prepare 10 mM Guaiacol, 0.1 ml enzyme extract and 0.1 of 12.3 mM H_2O_2. Initial optical density was read at 436 nm and then increase in optical density was noted at the interval of 30 seconds on UV- visible spectrophotometer (Shimadzu-1700). By using the extinction coefficient of guaiacol dehydrogenase product at 436 nm (i.e.6.36μmole^{-1}ml^{-}), the enzyme activity was calculated as units g^{-1} fresh Weight.

Superoxide Dismutase (SOD, EC1.15.1.1)**:** Superoxide Dismutase (SOD), a metal containing enzyme plays a vital role in scavenging superoxide radical. Superoxide dismutase activity was calculated by using Madamanchi et al (1994). Crude enzyme extract 100μl was mixed with a 3ml reaction cocktail: 50Mm potassium phosphate buffer (pH7.8), 13mM methionine, 2μM riboflavin, 0.1mm Ethylene diamine tetraacetic acid (EDTA) and 75μM nitroblue tetrazolium (NBT). Final volume of reaction mixture was made equal by adding distilled water. A blank was set without enzyme and NBT to calibrate the spectrophotometer and another control was set with NBT but without enzyme as reference control. The reaction tubes were exposed to 400W bulbs (4x100 W bulbs) for 15 minutes and immediately absorbance was taken at 560nm. The percent inhibition was calculated. The 50% inhibition of the reaction between riboflavin and NBT in the presences of mithionine is taken as 1 unit of SOD activity and the enzyme activity is expressed as units g^{-1} fresh tissue.

Results and Discussion

Effect of different concentrations of TiO_2 NPs on activity of hydrolytic enzymes like amylase and protease as well as antioxidant enzymes like SOD, CAT and POD was evaluated during seed germination in *Allium cepa* and results are depicted in Table 2 and 3.

Data pertaining to effect of TiO_2 NPs treatment on amylase activity indicate that amylase activity was increased up to 40 μg ml^{-1} treatments and then decreased over control at 50 μgml^{-1} treatment. Maximum increase in amylase (30.65%) was recorded at 20 μg ml^{-1} of TiO_2 NPs treatment. Data with respect to effect of TiO_2 NP treatments on protease activity indicate that protease activity was increased up to 40 μg ml^{-1}

treatments and then decreased significantly over control at 50 µgml^{-1} treatment. Maximum increase in protease (11.68%) was recorded at 40 µg ml^{-1} of TiO_2 NPs treatment.

The activities of antioxidant enzymes like, SOD, CAT and POD analysed from onion seedlings after TiO_2 treatment clearly indicate that activity of SOD increased with increasing NPs concentration. Activity of CAT increased up to 30 µgml^{-1} concentrations of TiO_2 NPs and then decreased significantly in seedlings treated with 40 and 50 µgml^{-1} TiO_2 NPs. Highest increase i.e. 30.88% over control was observed in seedlings treated with 20 µg ml^{-1} of TiO_2 NPs and then decreased in higher concentrations. Significant increase in POD activity was observed up to 30 µg ml^{-1} concentrations of TiO_2 NPs and maximum increase i.e. 10.74% over control was recorded in 30 µg ml^{-1} treatments of TiO_2 NPs. At higher concentration POD activity showed significant decrease over control.

The activities of key hydrolytic enzymes like amylase and protease in seedlings of *Allium cepa* that were grown in the presence of TiO_2 NPs were analyzed in present investigation. The results on enzyme activity revealed that NPs at their lower concentrations promoted enzyme activities, however at higher concentrations inhibited activities of hydrolytic enzymes.

The increased activity of α-amylase during seed germination is probably due to the *de novo* synthesis (Filner and Varner, 1967) of this enzyme. According to Wang et al (1988) amylase activity increases gradually during initial days of germination and convert starch to soluble sugars needed for growth of embryo axis. According to Tully and Beevers (1978) proteins are hydrolysed to free amino acids, which support protein synthesis in endosperm and embryo. Marambe et al (1992) noted a high significant correlation between the α-amylase activities; seed water uptake and subsequent percent seed germination as well as linear correlation of amylase activity with the starch degradation and increase in sugar content of the treated sorghum seeds.

It was observed that in germinating beans, the proteolytic activity increases during initial 7 days of germination and this increase was partially dependent on the embryonic axis (Gepstin and Han, 1980). The combined action of various proteolytic enzymes thus results in total degradation of storage proteins (Ikuko and Hiroshi, l980) to free amino acids needed for protein synthesis in embryonic axis. According to Baron (1979) solutes produced in seeds as a result of hydrolytic enzyme activities during the initial phases of germination promote the water movement into the seeds that contribute to the seed osmotic potential. On the other hand increased amino acid content in hypocotyls and expanding leaves significantly contributes to the water uptake by tissues (Morgan, 1984).

Findings of Navarro et al (2008) indicated that NPs can slowly penetrate into seeds and affect their metabolism *in vivo.* Khodakovskaya et al (2009) demonstrated that multiwalled carbon nanotubes (MWCNTs) can penetrate through the coats of tomato seeds after several days of co-incubation. It was further stated that once nano sized holes are created in the seed coat, oxygen transfer and water uptake might occur and drive the metabolic process for plant growth. However, NPs in large quantity are often observed in the agglomerate form and their interactions with seeds are weak so uptake of water/ oxygen is limited and such conditions can decrease seed germination.

In the present study it was observed that TiO_2 NPs treatment at the concentrations i.e. 10 to 40 µg ml^{-1} in onion seed showed enhanced activities of amylase and protease and then there was decrease in activities of these enzymes at higher concentration of NPs. The increase in seed germination and better establishment of seedlings due to lower concentrations of TiO_2 NPs might be due to accelerated water and oxygen uptake. Sufficient quantity of water and oxygen in imbibed onion seed might have accelerated the amylase and protease activities and produced soluble sugars and amino acids required for early seed germination and seedling growth. However on other hand higher concentrations of NPs might have resulted in agglomeration of particles which might have induced moisture stress and weakened uptake of water and oxygen and hence decreased seed germination (Raskar and Laware, 2013).

Antioxidant enzymes are known for ROS scavenging and are more active in the presence of biotic or abiotic stresses; hence, in order to determine the probable mechanism of toxicity of TiO_2 NPs, activities of antioxidant enzymes were assessed in 7 day old onion seedlings. From the results on enzyme activities it is clear that the production of ROS due to NPs is responsible for inducing the antioxidant enzymes.

The activity of key antioxidant enzyme i.e. SOD in *Allium cepa* seeds treated with NPs and seedling grown in the presence of NPs showed increase with increase in concentration of NPs. Superoxide dismutase are responsible to catalyze the dismutation of superoxide (O_2^{-})e. Hence, SOD enzymes are considered as important antioxidant defence in nearly all cells exposed to oxygen. In a study on wheat seedlings treated with biogenic silver NPs a significant increase in SOD activity was reported by Himakshi et al (2013). Similarly, Wang et al (2011) observed decrease in salt stress due to application of silicon NPs in alfalfa plant and they attributed this decreased salt stress to elevated activities of SOD, POD and CAT. In other study on soybean seed germination, it was found that the seeds treated with a mixture of Nano SiO_2 and Nano TiO_2 exhibited more germination and higher activities of nitrate reductase, superoxide dismutase, catalase and peroxidase (Lu et al., 2002). Authors concluded that SiO_2 and TiO_2 NPs would be better for seed germination and early seedling growth in soybean.

A report on SiO2 NPs toxicity in *Arabidopsis thaliana* clearly indicated that SiO_2 NPs are less toxic than ZnO and Fe_3O_4 NPs (Lee et al., 2010). Likewise Lin et al (2009) subjected *Arabidopsis* cells to multiwalled carbon nanotubes (MWCNTs) and reported significant decrease in the superoxide dismutase activity (SOD) in *Arabidopsis* cells exposed to MWCNTs as compared to control. However, Tan et al (2009) observed significant time dependent induction in enzymes in rice cells treated with MWCNTs at the concentration of 20 mgl^{-1}. Mohamed et al (2014) studied effect of nanoparticles on biological contamination of *in vitro* cultures of banana and reported that NPs cause an oxidation-reduction reaction and produced superoxide ion radical and hydroxide. According to them these reactive oxygen species (ROS) can be effective antimicrobial agents. Similar results were reported by Helaly and Hanan El-Hoseiny (2011) on stressed *in vitro* cultures of sweet orange. They observed higher superoxide dismutase (SOD), peroxidase (POX), ascorbic peroxidase (APX), Catalase (CAT) and glutathione reductase (GR) activities.

In another study on rice, Tang and Cao (2003) confirmed the increased yield of rice due to nano SiO_2 treatments; according to them such increase in yield could be due to increased strength and resistance to disease. Toxicity of nanostructures such as flower like ZnO capped with starch, spherical uncapped ZnO and spherical CdS on aquatic plant *Hydrilla verticillata* was examined by Mishra et al (2013). According to them spherical CdS nanoparticles were more toxic than the corresponding ZnO nanoparticles since there was a decrease in chlorophyll content and increase in catalase activity.

The results with respect to shoot and radicle (root) growth depicted in table-1 clearly indicate that root growth was significantly affected due to TiO_2 NPs at higher treatments as compared to shoots. It might be due to the fact that after emergence; growing radicles came in direct contact with NPs and subjected to more ROS stress exerted by NPs. This can be inferred by scrutinizing increased values of SOD and decrease values of CAT and POD in onion. It has been identified that the increase in SOD activity and generation of more H_2O_2 are the indicators of damage to the root growth (Dong et al., 2002).

It is well documented that antioxidant enzymes like catalases and peroxidases are known to scavenge H_2O_2 by breaking it down into water and oxygen. The excessive levels of H_2O_2 are reported to be reduced through the activities of catalase, POD and APX. Results on activities of CAT and POD enzymes responsible for elimination of H_2O_2 are given in table-3.

It is clear from data the increase in CAT activity due to NPs treatment at lower concentrations was more as compared POD activity. Higher activity of CAT in onion seedlings treated with 10-30 μgml^{-1} can be attributed to generation of more H_2O_2 and the higher activity of SOD in respective treatments (Fridovich, 1983; Bowler et al., 1992). Himakshi et al (2013) studied the effect of biogenic silver NPs in wheat seedling and noted significant enhancement in activities of CAT and POD. They ascribed these increased activities of catalase and peroxidase to marked increase in H_2O_2 through enhanced activity of SOD with biogenic silver NPs.

Comparatively lower increments in the activity of POD in onion seedlings treated with TiO_2 NPs at lower concentrations can be accredited to the remarkable activity of CAT as a key enzyme for eliminating H_2O_2, thereby regulating the activity of Peroxidase. Riahi-Madvar et al (2012) treated wheat seeds with nano scale alumina and studied the morphological characters of seedling along with antioxidant enzymes. They made similar observations with respect to SOD and reported that excessive levels of H_2O_2 might be reduced through the activities of catalase and APX.

Based on results of TiO_2 NPs effect on onion seed germination and early seedling growth it could be stated that NPs might have helped the water absorption by the seeds (Zheng et al., 2005), increased abilities of seed for absorbing and utilizing water efficiently, activated and promoted hydrolytic enzymes seed antioxidant system (Lu et al., 2002).

The NPs might have reduced ROS stress in treated onion seedling by reducing H_2O_2, superoxide radicals, and lipid peroxidation products (malonyldialdehyde content) and increasing activities of superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase enzymes as observed in spinach (Lei et al., 2008).

Table.1 Effect of TiO_2 on seed germination and early seedling growth in *Allium cepa*

TiO_2NPs (μg ml^{-1})	% Seed germination	PIOC/ PDOC	Radicle Length (cm)	PIOC/ PDOC	shoot length (cm)	PIOC/ PDOC	Radicle: shoot Ratio
Control	94.68	0.00	4.52	0.00	7.62	0.00	0.59
10 μg ml^{-1}	95.25	0.60	4.58	1.33	7.84	2.89	0.58
20 μg ml^{-1}	96.32	1.73	4.62	2.21	8.12	6.56	0.57
30 μg ml^{-1}	97.68	3.17	4.56	0.88	8.38	9.97	0.54
40 μg ml^{-1}	98.12	3.63	4.48	-0.88	8.46	11.02	0.53
50 μg ml^{-1}	92.02	-2.81	4.18	-7.52	8.16	7.09	0.51
CD 5%	1.72		0.16		0.22		0.26

CD= critical difference; PIOC= percent increase over control; PDOC= percent decrease over control

Table2 Effect of TiO_2 on hydrolytic enzymes during seed germination in *Allium cepa*

TiO_2NPs (μg ml^{-1})	**Amylase** (U g^{-1}FW)	PIOC/PDOC	**Protease** (U g^{-1}FW)	PIOC/PDOC
Control	42.28	0.00	42.81	0.00
10 μg ml^{-1}	48.56	14.85	42.96	0.35
20 μg ml^{-1}	52.45	24.05	45.24	5.68
30 μg ml^{-1}	55.24	30.65	46.09	7.66
40 μg ml^{-1}	46.28	9.46	47.81	11.68
50 μg ml^{-1}	41.36	-2.18	41.56	-2.92
CD 5%	0.12		1.42	

CD= critical difference; PIOC= percent increase over control; PDOC= percent decrease over control

Table.3 Effect of TiO_2 on antioxidant enzymes during seed germination in *Allium cepa*

TiO_2NPs (μg ml^{-1})	**SOD** (U g^{-1}FW)	PIOC/ PDOC	**Catalase** (U g^{-1}FW)	PIOC/ PDOC	**Peroxidase** (U g^{-1}FW)	PIOC/ PDOC
Control	17.64	0.00	32.64	0.00	21.98	0.00
10 μg ml^{-1}	21.22	20.29	38.52	18.01	24.06	9.46
20 μg ml^{-1}	22.74	28.91	42.72	30.88	24.34	10.74
30 μg ml^{-1}	23.04	30.61	40.16	23.04	23.98	9.10
40 μg ml^{-1}	24.38	38.21	31.98	-2.02	20.12	-8.46
50 μg ml^{-1}	25.26	43.20	31.14	-4.60	19.26	-12.37
CD 5%	2.52		2.14		2.58	

CD= critical difference; PIOC= percent increase; PDOC= percent decrease

However, at higher doses of TiO_2 NPs onion seed germination was reduced significantly and even showed reduced activity of amylase and proteases, this might be due to agglomeration of TiO_2 NPs and their weak interactions with seed or production maximum H_2O_2 and inhibition of CAT and POD at 40 and 50 μml^{-1} of TiO_2 NPs. PODs have a role in very important physiological processes like control of growth by lignification, cross-linking of pectins and structural proteins in the cell wall, and catabolism of auxins (Gaspar et al., 1991). The reduced radicle growth in onion seedlings at higher concentrations might be due to lower activity of POD and limited lignifications and cross-linking of pectins and structural proteins in the cell wall as well as inadequate cell elongation.

TiO_2 NPs at lower concentration enhanced seed germination and seedlings growth in onion and inhibited germination growth at higher concentrations. The TiO_2 NPs also induced significant changes in hydrolytic and antioxidant enzyme activities. Amylase and protease activities showed enhanced values in lower concentrations, but showed decrease at higher concentration. The activity SOD activity showed concentration dependent increase; however CAT and POD were found to be enhanced in the presences of 10-30 μgml^{-1} NPs but showed decreased activities in 40 and 50 μgml^{-1} NPs concentrations.

Acknowledgement

Authors are thankful to The Principal Fergusson College Pune (MS) for providing necessary facilities and also thankful to University of Pune (MS) for financial assistance.

References

Baron, W.M.M. 1979: Organization in Plants. Edward Arnold Limited, London., pp. 176-178.

Bernhardt, D., A. Trutwig and A. Barkhold, 1993. Synthesis of DNA and the development of amylase and phosphatase activities in cotyledons and germinating seeds of *Vaccaria pyramidata*. J. Exp.Bot. 44,695–699.

Bernier, A.M. and Balance, G.M. 1993. Induction and secretion of alpha-amylase (1–3), (1–4)–beta–glucanase and (1–3)–beta–glucanase activities in gibberellic acid and CaCl2 treated half seeds and aleurones of wheat. Cereal Chem. 70, 127–132.

Bewley, J.D. and Black, M. 1994. Seed*s:* Physiology of Development and Germination. Plenum Pres, NewYork, pp. 445.

Bhati -Kushwaha H., Kaur, A. And Malik, C.P. 2013. The synthesis and role of biogenic nanoparticles in overcoming chilling stress. Indian Journal of Plant Sciences. 2 (4): 54-62.

Bowler, C. M., Van Montague and D. Inze. 1992. 'Superoxide dismutase and stress tolerance'. Annlual Review of Plant Molecular Biology. 43, 83-116.

Cameron, A.C., Reid, M.S. (2001). 1-MCP blocks ethylene-induced petal abscission of *Pelargonium peltatum* but the effect is transient.Postharvest Biol Technol. 22, 169–177.

Choudhury, S. and S.K. Panda, 2004. Induction of oxidative stress and ultrastructural changes in moss Taxithelium nepalense (Schwaegr.) both under lead (Pb) and arsenic (As) phytotoxicity, Curr. Sci. 87, 342–348.

Cocucci, M. and Negrini, N. 1991. Calcium calmodulin in germination of *Phacetia tanacetifolia* seeds: Effects of light, temperature, fusicoccin and calcium calmodulin antagonist. Physiol. Plantarum. 82,143–149.

Davies, H.V. and Slack, P.T. 1981. The control of food mobilization in seeds of dicotyledonous plants. New Phytologist. 88, 41–51.

Dong, B., W. L. Sang, X. Jiang, J. M. Zhou, F. X. Kong, W. Hu and L. S. Wang. 2002. 'Effects of aluminum on physiological metabolism and antioxidant system of wheat (Triticum aestivum L) '. Chemosphere. 47, 87-92.

Filner, P. and Varner J. E. 1967. A test for de novo synthesis of enzymes by density labeling with H2 18O of barley α-amylase induced by gibberrellic acid. Proc. Nat. Acad. Sci. USA. 58, 1520- 1526.

Fincher, G.B. 1989. Molecular and cell biology associated with endosperm mobilization in germinating cereal grains. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40, 305-346.

Fridovich, I. 1983. 'Superoxide radical: an endogenous toxicant'. Annual Review of Pharmacology and Toxicology. 23, 239-257.

Gaspar, Th., Penel, C., Hagege, D., and H. Greppin.1991. Peroxidases in plant growth, differentiation, and developmental processes, In: *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases* (Eds. J. Lobarzewski, H. Greppin, C. Penel and Th.Gaspar), 249-280 Universite de Geneva Press, Geneva.

Gepstin, S. and Han, I. 1980. Evidence for the involvement of cytokinin in the regulation of proteolytic activity in cotyledons of germinating beans. Plant and Cell Physiol. 21(1):57-63.

Ghanati, F., A. Morita and H. Yokota. 2005. 'Effects of aluminum on the growth of tea plant and activation of antioxidant syste'. Plant and Soil. 276, 133-141.

Helaly, M.N., El-Hoseiny, H. 2011. Combined effects between genotypes and salinity on sweet orange during the developments stages of its micropropagation. Res J Bot. 6(2): 38-67.

Hood, E. 2004. Nanotechnology, Looking as we leap, Environ. Health Perspect. 112 ,740–749

Ikuko, H. and Hiroshi, M. 1980. Pumpkin (*Cucurbita sps*.) seed globuline.VI. Proteoiytic: activities appearing in germinating cotelydons. Plant and Cell Physiol. 2 l (2): 233-245.

Issac and Gokhale 1982. Autolysis: Journal of Mycological Society. 78, 389-394.

Jayaraman, J. 1981. Laboratory Manual in Biochemistry, Wiley Eastern Ltd. New Delhi, India. 122-123.

Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F. and Biris, A.S. 2009 Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano 3:3221–3227.

Lee, C.W. , S. Mahendra, K. Zodrow, D. Li, Y. Tsai, J. Braam and P. J. J. Alvarez. 2010. Developmental phytotoxicity of metal oxide nanoparticles to Arabodopsis thaliana. Environ. Toxicol. Chem. 29, 669-675

Lei, Z., Mingyu, S., Xiao, W., Chao, L., Chunxiang, Q., Liang, C., Hao, H., Xiao-qing, L., Fashui, H., 2008. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biological Trace Element Research. 121, 69–79.

Lin, C., Y. Fugetsu, F. Watari, 2009. Studies on toxicity of multi-walled carbon nanotubes on Arabidopsis T87 suspension cells, J. Hazard. Mater. 170, 578–583.

Lopez, M.L., J.R. Peralta-Videa, H. Castillo Michel, A. Martinez-Martinez, M. Duarte-Gardea, J.L. Gardea-Torresdey, 2007. Lead toxicity in alfalfa plants exposed to phytohormones and EDTA monitored by peroxidase catalase and amylase activities, Environ. Toxicol. Chem. 26. 2717–2723.

Lu, C.M., Zhang, C.Y., Wen, J.Q., Wa, G.R., Tao, M.X. 2002. Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism. Soybean Sci. 21, 168-172

Madamanchi, N. R., Donahue J.L., Cramer, C.L., Alscher, R.G., and Pedersen, K. 1994. Differencial response of Cu.Zn superoxide dismutase in two pea cultivars during a short term exposure to sulpher dioxide. Plant Mol. Bio. 26, 95-103

Marambe, B., Tadao, A. and Kenji, K. 1992. Alpha-amylase and protease activities and water relations in germinating sorghum (Sorghum bicolor Moench.) seeds as affected by animal-waste composts. Soil Sci. Plant Nutr. 38 (1):123-131.

Maxwell, D.P. and Bateman D. F. 1967.Change in the activities of some oxidases in extracts of Rhizoctonia infected bean hypocotyles in relation to lesion maturation. hytopathol. 57, 132-135.

Mayer, A. M. and Poljakoff-Mayber, A. 1975. The Germination of Seeds. 2nd Edition. Pergamon Press, London.

Mayerand, A.M. and Shain, Y. (1974) Control of seed germination. Ann. Rev. Plant Physiol. 25, 167-193.

Mishra, P., Shukla, V. K., Yadav, R. S. and Pandey, A. C. 2013. Toxicity Concerns of Semiconducting Nanostructures on Aquatic Plant Hydrilla verticillata. Journal of stress physiology and biochemistry. 9(2): 287-298.

Mohamed Naser Helaly, Mohamed Ahmed El-Metwally, Hanan El-Hoseiny, Samar Abdelaziz Omar, Nabil Ibrahim El-Sheery5 2014. Effect of nanoparticles on biological contamination of *in vitro* cultures and organogenic regeneration of banana AJCS . 8(4):612-624.

Morgan, J.M. 1984: Osmoregulation and water stress in higher plants. Annu. Rev. Plant Phvsiol. 35, 299-319

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A., Quigg, A., Santschi, P.H., Sigg, L., 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 17, 372-386.

Nel, A., Xio, T., Mädler, L., Li, N. 2006. Toxic potential of materials at the nano level. Science. 311, 622-627

Pulskamp, K.; Diabaté, S.; Krug, H.F. 2007. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol. Lett. 168, 58–74.

Raskar, S and Laware,S.L. 2013. Effect of titanium dioxide nano particles on seed germination and germination indices in onion. Plant Sciences Feed, 3 (9): 103-107.

Raskar, S.V. and S. L. Laware. 2014. Effect of zinc oxide nanoparticles on cytology and seed germination in onion. Int.J.Curr.Microbiol.App.Sci. 3(2): 467-473.

Riahi-Madvar, A., F. Rezaee, and V. Jalili. 2012. Effects of alumina nanoparticles on morphological properties and antioxidant system of *Triticum aestivum*'. Iranian Journal of Plant Physiology, 3 (1): 595. 603

Salisbury, F.B. and Ross, C.W. 1991. Plant Physiology. 4th Edition. Wadsworth Publishing Company, Belmont, California, USA.

Schelgel, H.G. 2003. Examining carbohydrate contents of Asian cassava. 5th edn, Cambridge, Gen. Biol. pp. 180-185.

Schramm, M. and Loyter, A., 1966. Purification of α-Amylase by Precipitation of Amylase-Glycogen Complexes. Meth. Enzymol. 9, 533-537.

Singh, N.; Manshian, B.; Jenkins, G.J.S.; Griffiths, S.M.; Williams, P.M.; Maffeis, T.G.G.; Wright, C.J.; Doak,

S.H. 2009. NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials. Biomaterials. 30, 3891–3914.

Tan, X., C. Lin, B. Fugetsu, 2009. Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells, Carbon. 45, 3479–3487.

Tang, S.Y. and Cao, Y.P. 2003. Effects of spraying different forms of silicon (Si) on growth as stress resistance of rice plants .Sci Fertil. 2,16-22

Trethewey, R.N. and Smith, A.M. 2000. Starch mobilization in leaves. In: Advances in photosynthesis (Eds.): R.C. Leegood, T.D. Sharkey and S. Von-Caemmerer. Photosynthesis: physiology and metabolism. Dordrecht, the Netherlands, Kluwer Academic Publishers. 9, 205-231.

Tully, R. E. and Beevers, H. 1978. Protease and peptidases of castor bean endosperm. Enzyme characterisation and changes during germination. Plant Physiol. 62, 726-750.

Vidyasekharan, P. and Durairaj, P. 1973. Shot hole syndrome in mango. Indian Phytopath..26**,** 49-55.

Wang, X., Wei, Z. Liu, D. and Zhao, G. 2011. Effects of NaCl and silicon on activities of antioxidative enzymes in roots, shoots and leaves of alfalfa. African Journal of Biotechnology. 10, 545-549.

Wang, X.D., Sun, C., Gao, S.X., Wang, L.S., Han, K. 2001. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucmis sativus*. Chemosphere. 44, 1711-1721

Wang, Y., Wang, D. M. and Liang, H. G.1988. Eifect on plumular axis on the amylase activity in cotyledons of germinating pea seeds. Acta. Phytophysiol. Sinica. 14(3): 244-249.

Washio, K. and Ishikawa, K. 1992. Structure and expression during the germination of rice seeds of the gene for a carboxypeptidase. Plant Molecular Biol. 19,631–640.

Xia, T., Kovochich, M., Liong, M., Mädler, L., Gilbert, B., Shi, H., Yeh, J.I., Zink, J.I., Nel, A.E. 2008. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano. 2, 2121–2134.

Yang, H., Liu, C., Yang, D.F., Zhang, H.S., Xi, Z. 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: The role of particle size, shape and composition. J. Appl. Toxicol. 29, 69–78.

Zeeman, S.C., D. Thorneycroft, N. Schupp, A. Chapple, M. Weck, H. Dunstan, P. Haldimann,N. Bechcold, A.M. Smith and S.M. Smith. 2004. Plastidial α-glucan phosphorylase is not required for in starch degradation in *Arabidopsis* leaves but has a role in the tolerance of abiotic stress. Plant Physiol. 135, 849-858.

Zheng, L., Hong, F., Lu, S., and Liu, C. 2005. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach. Biol Trace Elem Res.105, 83-91.

Zimmermann, P., Zentgraf, U. 2005. The correlation between oxidative stress and leaf senescence during plant development. Cell Mol Biol Lett. 10, 515–534.