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Infuence of chemically synthesized copper nanoparticles and cupric ions on oxalate oxidation system in germinating *Sorghum* grain

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We have earlier reported the effects of chemically synthesized copper nanoparticles (CuNPs) on oxalate oxidase (OxOx) activity, extracted from the shoot tissue of germinating grain sorghum i.e. *in vitro*. Here, we tried to study this effect *in vivo* and compare it with those of Cu^{2+}. We describe herein, characterization of CuNPs and their effects on oxalate oxidation system i.e. OxOx activity, total oxalate and H_2O_2 content *in vivo* i.e. in shoot tissues/leaves of germinating grain Sorghum (*Sorghum vulgare* L). To achieve it, grain sorghum seeds were grown up to 10 days in laboratory, irrigated with Hoagland's solution containing either CuNPs (1.0 ppm) or Cu^{2+} (1.0 ppm) after 4 days of germination. Control were irrigated with Hoagland solution only. The shoot/leaves of the seedling plants were harvested at 4, 6, 8 and 10 day of germination and analysed quantitatively for OxOx activity, soluble protein, H_2O_2 and total oxalate. The growth of the Sorghum seedling plants supplemented with CuNPs and Cu^{2+} was decreased significantly (P <0.1) at all growth stages compared to control. This inhibitory effect of CuNPs was higher than Cu^{2+}. CuNPs decreased the activity of OxOx but Cu^{2+} had no effect at day 10. Both CuNPs and Cu^{2+} decreased the specific activity of OxOx and H_2O_2 content but increased total oxalate content at day 10. The decrease in H_2O_2 content in both CuNPs and Cu^{2+} supplemented shoot tissues with concomitant increase in oxalate content confirmed the decreased activity of OxOx in CuNPs and Cu^{2+} supplemented seedling plants.

Keywords: CuNPs, Germination, Metal toxicity, OxOx activity, Puhana, *Sorghum vulgare*

Materials, when reduced in size to 1-100 nm in their dimension, exhibit drastic changes in their physicochemical, optical, magnetic, catalytic and electrical properties. Among these nanomaterials, metal nanoparticles (NPs), such as silver, gold, copper and iron NPs are used in catalysis, electronics, sensing, photonics, environmental cleaning and in agriculture[2]. Most of the metal NPs release free radicals, which cause oxidative stress. The surface of free radicals can activate the redox cycle and cause toxicity[3]. Metal NPs exert toxic effect by entering directly into the cell, causing an oxidative damage to the cell structures and molecules. Copper is one of the most widespread inorganic pollutants. This active transition metal is involved in many redox processes in plants and animals. In plants, copper ions (Cu^{2+}) are the component of regulatory proteins, which participates in electron transport in photosynthetic and respiratory chains. Cu^{2+} acts as a cofactor for number of enzymes, such as phenol oxidases, ascorbate oxidase, and superoxide dismutase[4,5]. Plants need only trace amounts of Cu^{2+} and their high concentrations are toxic for them. Free Cu^{2+} can unspecifically bind to the -SH groups of enzymes, which results into the loss of their secondary structure[6,7]. Cu^{2+} at higher concentrations damage the thylakoid membranes, disturbing the function of photosystem II and the water oxidizing complex of chloroplasts[5,8]. Toxic effects of Cu^{2+} on plant metabolism and metallo-enzymes has been reviewed recently[9]. Although the influence of genotypes, growth regulators and basal media on direct differentiation of shoot buds of leaf segments of plant has been reported[10], there is no report available on the effect of metal ion and its nanoparticles on early growth of plant, specifically on its metalloenzymes.

Oxalic acid is synthesized as a byproduct of respiratory break down of carbohydrates and proteins which is stored as soluble salts (sodium and potassium oxalate) and insoluble salts (calcium and magnesium salt) in higher plants. There are two enzymes responsible for degradation of oxalate in higher plants, oxalate decarboxylase and oxalate oxidase. The former enzyme has been detected in pea seedling while latter has been studied in a number of

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higher plants. Oxalate oxidase (Oxalate: Oxygen oxidoreductase, EC 1.2.3.4) (OxOx) is known to catalyze the aerobic oxidation of oxalate into CO_2 and H_2O_2 in plants[11]. In grain sorghum seedlings, OxOx is a metalloprotein, which requires Cu^{2+} for its activity[12]. The enzyme has also been described as germin like protein, which regulates the germination of cereals like sorghum and wheat[13,14]. A previous report from our laboratory has revealed that CuNPs increased OxOx activity, extracted from germinating grain sorghum shoot tissues, in *vitro*[1]. However, the effects of CuNPs on oxalate oxidation system in germinating grain sorghum have not been studied *in vivo*. The present study, describes the chemical synthesis of CuNPs ,their characterization and effects of CuNPs and Cu^{2+} on activity/ specific activity of OxOx, total oxalate (soluble and insoluble), and H_2O_2 *in vivo* i.e. in shoot tissues of germinating grain sorghum.

Material and Methods

Materials

4-Aminophenazone, oxalic acid (from Sigma), horseradish peroxidase, solid phenol, copper sulphate ($CuSO_4.5H_2O$), sodium hydroxide pellet, ethylene diamino-tetra-acetic acid (EDTA), sodium borohydrate ($NaBH_4$), potassium nitrate (KNO_3), calcium nitrate [$Ca(NO_3)_2$], magnesium sulfate ($MgSO_4.7H_2O$), potassium di-hydrogen phosphate (KH_2PO_4), succinic acid, sodium carbonate (Na_2CO_3), Folin and Ciocalteu's (F.C.) phenol reagent, mercuric chloride ($HgCl_2$), ethanol, acetone, sodium potassium tartarate, bovine serum albumin (BSA) (from SRL), were used. All these chemicals were of analytic reagent (AR) grade. Grain sorghum seeds (*Sorghum vulgare*, variety Puhana, commercial name) were purchased from local market. Double distilled water (DW) was used throughout the study.

Preparation of CuNPs

CuNPs were prepared by chemical reduction method[15]. Aqueous solution of NaOH (0.1M, 10 mL) was added to an aqueous solution of $CuSO_4$ (0.4M, 100 mL) and EDTA (0.1M, 10 mL). The mixture was heated to 60°C on a hot plate magnetic stirrer and aqueous solution of $NaBH_4$ (0.1M, 10 mL) was added drop wise under continuous stirring. The reddish brown coloured precipitates were obtained which indicated the formation of CuNPs. These precipitates of CuNPs were kept for half an hour to settle down. The supernatant was removed with micropipette without disturbing NPs layers. The sediment/product (CuNPs) were washed thrice with ethanol and acetone separately and finally dried in an oven at 40°C for 10 min and stored dry at room temperature (30±5°C).

Characterization of CuNPs

The characterization of CuNPs was carried out by transmission electron microscop*e* (TEM) at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi. X-ray diffraction (XRD) at National Physical Laboratory (NPL), New Delhi, Fourier transform infra-red (FTIR) spectra at Physics Department of M.D. University, Rohtak.

Collection of plant materials

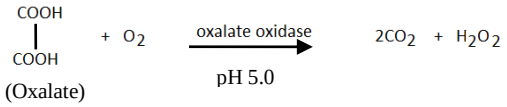
Three sets of grain sorghum seedling plants were raised from their seeds in the laboratory as described[16]. The seeds of grain sorghum were soaked in deionized water overnight, surface sterilized with 0.1% $HgCl_2$ for one min, washed with DW several times and allowed to germinate in Petri dishes lined with a double layer of moist filter paper at room temperature (33±5°C).After 4 days of germination, one set of the seedling plants i.e. control was irrigated daily with Hoagland's nutrient solution consisting of $Ca(NO_3)_2.4H_2O$ (1.18 g /L), KNO_3(0.51 g/L), $MgSO_4.7H_2O$ (0.49 g/L) and KH_2PO_4 (0.14 g/L) in DW, while second set of seedling plants i.e. Cu^{2+} supplemented seedling plants were irrigated daily with Hoagland's solution containing Cu^{2+}i.e. $CuSO_4$ (1.0 mg/L or 1.0 ppm), while the third set of seedling plants i.e. CuNPs supplemented seedling plants were irrigated daily with Hoagland's solution containing CuNPs (1.0 mg/L or 1.0 ppm) and maintained in a photoperiod of 8-10 h/day. The seedlings plants in each group were harvested at 4, 6, 8, 10 days of germination and their shoot tissues/leaves were separated with sharp scissor, measured their height and surface area washed in chilled DW, dried between folds of filter paper, weighed and stored immediately at –20°C until use.

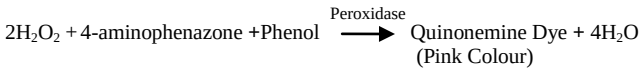
Extraction of OxOx

Crude OxOx from grain sorghum leaves was extracted in cold DW at 4°C as described[16]. Frozen leaves (1 g) were homogenized in cold DW (3 mL) in 1:3 ratio (w/v) in a chilled mortar with pestle. The homogenate was squeezed through a double layer of cheese cloth and the filtrate was centrifuged at 15000×g for 30 min in cold (4°C). The supernatant /aqueous extract was collected and used as a source of crude OxOx. It was stored at −20°C until use.

Assay of OxOx

OxOx was assayed in 15 mL test tubes, wrapped with black carbon paper as described[11] based on the following chemical reactions:





The reaction mixture containing 1.7 mL of 0.05 M sodium succinate buffer (pH 5.0), 0.1 mL of $CuSO_4$ solution (10^{-2}M) and 0.1 mL of crude enzyme (0.1 mg/mL) was pre-incubated at 37°C for 2 min. The reaction was then started by adding 0.1 mL of oxalate (10 mM). After incubation at 40°C in dark for 10 min, 1.0 mL of colour reagent was added and kept at room temperature (25±3°C) for 15 min in dark to develop the colour. In blank, the enzyme was replaced by equal quantity of reaction buffer, while in control, heat denatured enzyme (heated in boiling water for 2 min) was used. A_{520} of the reaction mixture was read in Spectronic-20 against control. H_2O_2 content generated during the reaction was extrapolated from a standard curve between H_2O_2 concentration and A_{520} prepared in 0.05M sodium succinate buffer pH 5.0. The colour reagent, consisted of 50 mg of 4-aminophenazone, 100 mg solid phenol and 1 mg horseradish peroxidase (Rz=3.0) per 100 mL of 0.4M sodium phosphate buffer, pH 7.0 and was stored in amber coloured bottle at 4°C when not in use[17] freshly every week. The soluble protein in various enzyme preparations was determined by Lowry method[18]. One unit of enzyme is defined as the amount of enzyme, which utilizes 1.0 nmole of H_2O_2 per min under the standard assay conditions. Specific activity of enzymes was calculated as a ratio of unit activity per mg of soluble protein.

Determination of total oxalate

The total oxalate (both soluble and insoluble oxalate) was quantified as described[12]. The procedure for soluble oxalatewas the same as described for theassay of OxOx, except that oxalate solution (0.1 mL)was replaced by partially purified sorghum oxalate oxidase. The insoluble oxalate was determined by boiling 50 mg of pellet,obtained during extraction of crude OxOx, in 2 mL 6N HCl.The dissolved pellet (0.1 mL) was used in the assy above assay of OxOx in place of oxalate solution was used to determine insoluble oxalate.The content of both soluble and insoluble oxalate was calculated from the standard curve of oxalate concentration *vs*. A_{520} using the partially purified OxOx. To prepare partially purified OxOx, solid ammonium sulfate was added slowly into crude OxOx (15000×g supernatent) to achieve a final saturation of 0-75%. The resulting solution was kept overnight and centifuged at 10000×g for 30 min. The pellet was collected, washed carefully and redissolved in minimum volume of chilled 0.05M sodium succinate buffer pH 5.0 and stored at 4°C until use.

Statistical analysis

The results are mean ±SE (n=3). The data were compared using student' t test. All the data are mean of three replication.

Result and Disscussion

Preparation and characterization of CuNPs

CuNPs were prepared from from $CuSO_4$ solution by chemical reduction method using $NaBH_4$. The appearance of reddish brown coloured precipitates indicated the formation of CuNPs. On drying at 40°C for 10 min, the suspension of CuNPs turned into dark brown coloured powder. TEM images of CuNPs revealed their spherical shape with the diameter in the range, 4.06 nm to 8.43 nm at 150000X magnification and 5.28 to 16.6 nm at 100000X magnification (Suppl. Fig. 1A. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*)). Earlier,we have reported the spherical structure of CuNPs with the diameter in the range 13-58 nm[1]. The difference in the diameter of CuNPs could be due to the difference in methods used for the preparation of NPs. XRD pattern of CuNPs (JCPDS card No. 01-080-1268) exihibited characteristics peak of CuNPs at 36.46º, 42.34º, 50.42º, 61.36º and 73.58º (Suppl. Fig. 1B). There was no characteristic peaks of impurities revealing the high purity of the prepared CuNPs. FTIR spectra of CuNPs was recorded in the range 500-4000 cm^{-1}, which showed its characteristic peaks at 600, 1200 and 1700 nm (Suppl. Fig. 1C).

Effect of CuNPs and Cu^{2+} supplementation on growth of germinating grain sorghum plants

The results (Table 1 and Suppl. Figs. 2 & 3 A-C) revealed that both CuNPs and Cu^{2+} each at 1 ppm significantly decreased (P <0.01) the growth of the grain sorghum seedlings at day 10, compared to control. However, CuNPs caused more reduction in growth compared to that for Cu^{2+} at all growth stages.

Earlier, the toxic effect of CuNPs on growth of seedling plants of mung bean and wheat have been reported[4]. However, the effect of CuNPs on wheat growth was concentration dependent, as it had no effect at 0.2 to 0.8 ppm but decreased the growth at 1 ppm and increased it at 10, 20, 30 ,40 and 50 ppm, when grown in soil in pots[19]. Both CuNPs at 1.0 ppm and Cu^{2+} at 0.5 ppm suppressed photosynthesis in *Elodea densa* plants, when incubated in their water solution or suspension[20] CuNPs and Cu^{2+} might have also suppressed photosynthesis in grain sorghum seedling plants leading to their decreased growth. The decrease in growth of grain sorghum seedling plants by CuNPs could be correlated with the observed decrease in oxalate oxidase activity and H_2O_2 content in CuNPs supplemented grain sorghum/seedling plants. OxOx activity is required to soften the hard tissue by catalyzing degradation of oxalate, which otherwise forms insoluble salt with Ca^{2+}. OxOx generates H_2O_2 from oxalate, which might play an important role in cellular regulation, as H_2O_2 stimulates glucose transport, glucose incorporation into glycogen, HMP shunt pathway, lipid synthesis, release of calcium from mitochondria and arachidonate from phospholipids, poly ADP ribosylation and insulin receptor tyrosine kinase and pyruvate dehydrogenase but inactivates glycolysis, lipolysis, recyclation of lysophospholipids, ATP synthesis, superoxide dismutase and protein kinase[21]. Most of the nanoparticles produce free radicals in plants which cause oxidative stress. Biological oxidative stress may cause inflammation, cell destruction, and genotoxicity. The particle surface of the free radicals can activate the redox cycle and cause particle toxicity[3]. CuNPs might exert toxic effect by penetrating directly into the cell, supposedly by causing oxidative damage to cell structures and molecules. These necessities are to be studied further.

Effect of CuNPs and Cu^{2+} on oxalate oxidase activity in grain sorghum seedling plants

Fig. 1A shows the effect of CuNPs and Cu^{2+} supplementation on OxOx activity in shoot tissue of grain sorghum seedling plants at day 4, 6, 8 and 10 after germination. Our results revealed that supplementation of CuNPs caused a significant decrease (P <0.01) in OxOx activity at day 10

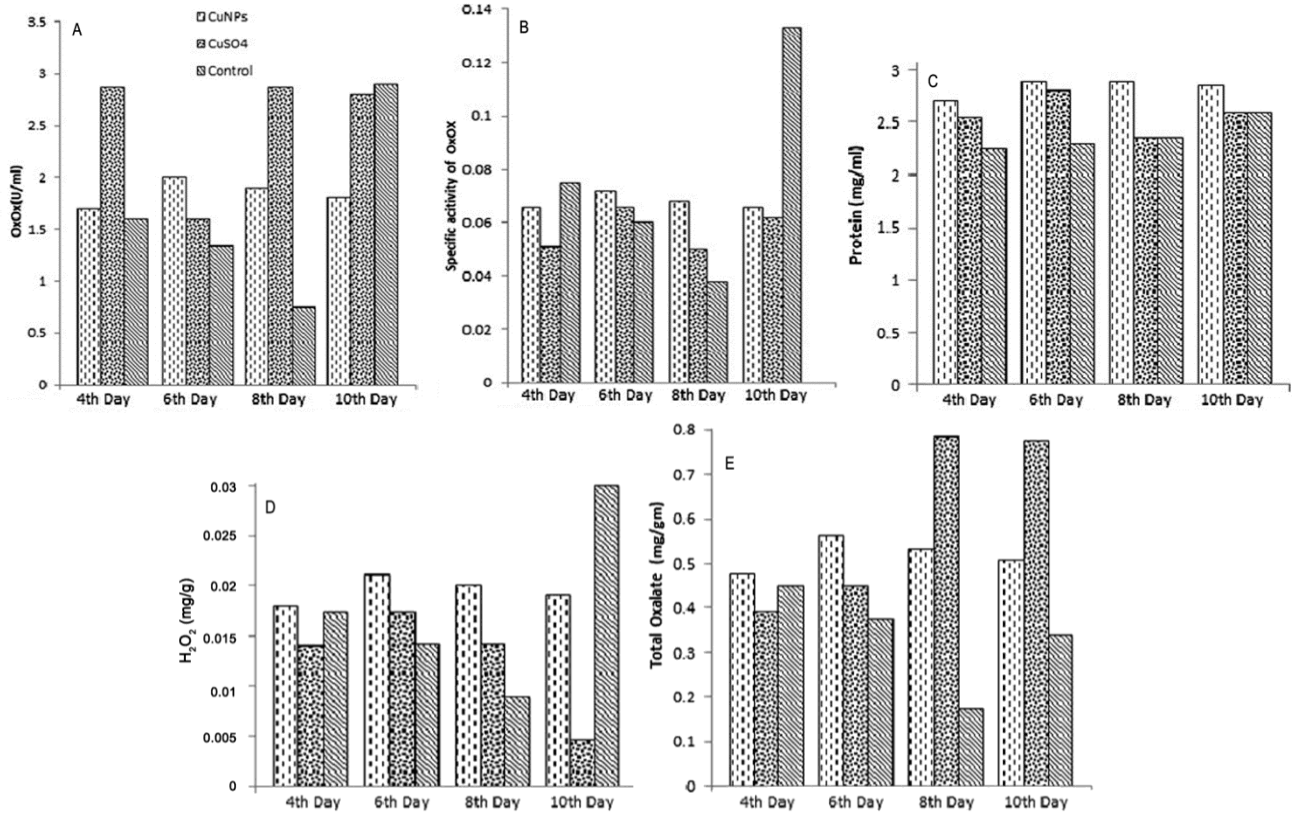


Fig. 1 — (A) Oxalate oxidase activity; (B) Specific activity of oxalate oxidase; (C) Soluble protein content; (D) H_2O_2 content; and (E) Total oxalate content in shoot tissues of grain sorghum seedling plants growning in Hoagland's solution supplemented with CuNPs and Cu^{2+}at 1.0 ppm at various growth stages. Control were grown in Hoagland's solution only.

Table1 — Effect of CuNPs and Cu^{2+} on seedling plants of grain sorghumat different growth stages

Samples	Days	Mean ±SE length of shoots(in cm)	SD and SE (in cm)	Mean length of leaves (in cm)	SD & SE (in mm)	Leaf Mean areas (in mm)	SD & SE (in mm)
	4	9.38±1.56		1.0		0.0	
CuNPs	6	10.90±1.56	3.126	2.11	2.112	0.3	0.064
	8	16.56±1.56	&	5.72	&	0.4	&
	10	13.24±1.56	1.563	4.21	1.056	0.42	0.032
	4	9.38±1.26		0.98		0.2	
Cu^{2+}	6	13.46±1.26	2.537	3.98	2.176	0.4	0.205
	8	14.92±1.26	&	5.65	&	0.7	&
	10	15.18±1.26	1.268	5.54	1.088	0.46	0.102
	4	11.67±1.16		3.07		0.4	
Control	6	14.91±1.16	2.325	5.14	3.038	0.5	0.105
	8	17.35±1.16	&	8.04	&	0.65	&
	10	14.40±1.16	1.16	6.81	1.519	0.47	0.052

[Each data is the mean of 30 samples at 4^{th} Day, 32 samples at 6^{th} Day, 24 samples at 8^{th} Day and 27 samples at 10^{th} Day]

compared to control. Nevertheless Cu^{2+} didn't affect OxOx activity at day 10. However, OxOx activity was increased in CuNPs supplemented shoot tissue at day 6 and 8, compared to control. OxOx activity was also increased in Cu^{2+} supplemented shoot tissue at day 4 and 8 but almost similar at day 6 and 10. Over all, CuNPs decreased OxOx activity, while Cu^{2+} had no effect at day 10 (Fig. 1A). These observations are in contrast to our earlier report on the effect of CuNPs, which showed 30% increase in OxOx activity in 10 day old grain sorghum seedling plants by CuNPs[14]. The decreased activity of OxOx in the present study could be due to inhibition of enzyme by aggregated form of Cu^{2+} ions i.e. CuNPs.

Specific activity of OxOx was decreased highly significantly (P <0.001) in both Cu^{2+} and CuNPs supplemented shoot tissue at day 10 compared to control. However, there was no significant change ($P \leq 0.05$) in the specific activity of OxOx in both the cases at day 4, 6 and 8 (Fig. 1B). The reason for decreased specific activity of OxOx (Fig. 1B) in CuNPs and Cu^{2+} supplementedshoot tissue could be due to increased OxOx but enhanced soluble protein content compared to control (Fig. 1C).

Effect of CuNPs and Cu^{2+} on H_2O_2 content in grain sorghum seedling plants

Fig. 1D shows H_2O_2 contentin shoot tissue of grain sorghum seedling plants supplemented with CuNPs and Cu^{2+} at day 4, 6, 8 and 10. There was significant decrease (P <0.01) in H_2O_2 content in both CuNPs and Cu^{2+} supplemented plants compared to control at day 10. The decrease was more in case of Cu^{2+} compared to CuNPs at all the growth stages. The decrease in H_2O_2 might be due to the low activity of OxOx in the presence of CuNPs and Cu^{2+} (Fig. 1D). This can also be correlated with the antioxidant system of the seedling plants, which is likely to be affected by CuNPs and Cu^{2+}. Earlier, AgNPs and CeO_2NPs influenced the antioxidant system in germinating *Arabidopsis thaliana* and rice, respectively[22-24].

Effect of CuNPs and Cu^{2+} on total oxalate content in grain sorghum seedling plants

Fig. 1E exhibits the total oxalate (soluble plus insoluble) in grain sorghum seedling plants supplemented with CuNPs and Cu^{2+} at 4, 6, 8 and 10 day. The total oxalate was increased significantly (P <0.01) in CuNPs and Cu^{2+} supplemented seedling plants compared to control at day 10. However, this increase was more in Cu^{2+} supplemented seedling plants compared to CuNPs. The increased oxalate content could be due to decreased OxOx activity in CuNPs and Cu^{2+} supplemented plants. OxOx is known to degrade oxalate to CO_2 and H_2O_2 under aerobic condition. In the present study, decrease in H_2O_2 with concomitant increase in oxalate content in CuNPs and Cu^{2+} supplemented seedling plants confirm the decreased activity OxOx. The decrease in OxOx activity was higher in the presence of CuNPs compared to that of Cu^{2+}. Since sorghum OxOx is known to be stimulated by Cu^{2+} *in vitro*, the possibility of decreased de novo synthesis of OxOX in the presence of CuNPs cannot be ruled out at this stage.

Conclusion

The results have demonstrated that supplementation of CuNPs and Cu^{2+} at 1.0 ppm in germinating grain sorghum plants decreased their growth at day 10, followed by decrease in OxOx activity and H_2O_2 content but increase in total oxalate, compared to control.

Conflict of interest

The authors declare no conflict of interest.

References

1 Chauhan N, Hooda V & Pundir CS, *In vitro* effect of metal oxide nanoparticles on barley oxalate oxidase. *J Nanopart Res*, 15 (2013) 1493.

2 Pundir CS, Introduction to Enzyme and Nanotechnology, In: *Enzyme Nanoparticles: Preparation, Characterization, Properties and Applications.* (Elsevier, Ireland), 2015, 1.

3 Lee WM, An YJ, Yoon H & Kweoon HS, Toxicity and bioavailability of copper nanoparticles to terrestrial plants mungbean (*Phaseolus radiates*) and wheat (*Triticum aestivum*): Plant agar test for water soluble nanoparticles. *Environ Toxicol Chem*, 27 (2008) 1915.

4 Chernavina IA, *Physiology and Biochemistry of Trace Elements*. (Vysshaya Shkola, Moscow), 1970.

5 Yruela I, Copper in plants. *Braz J Plant Physiol*, 17 (2005) 145.

6 Yu E, Zolotukhina & Gavrilenko EE, Copper, Cadmium, Iron, Zinc and Manganese byproteins in aquatic macrophytes. *Fiziol Rast*, 37 (1990) 651.

7 Nekrasova GF & Maleva GF, Development of anti-oxidant reactions in Elodea canadensis leaves upon short term exposure to high Ni^{2+}, Zn^{2+}, and Cu^{2+} concentrations. In: *Modern Plant Physiology: From Molecules to Ecosystems. Proceedings of International Conference*, 2007, 278.

8 Patsikka E, Kairavuo M, Sersen F, Aro E-M & Tyystjarvi E, Excess copper predisposes photosystem II to photo-inhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll, *Plant Physiol*, 129 (2002) 1359.

9 Anderson E, Peiter E & Kupper H, Trace metal metabolism in plants. *J Exptl Biol*, 69 (2018) 909.

10 Kumar KR, Singh KP, Raju DVS, Bhatia R & Panwar S, Influence of genotypes, growth regulators and basal media on direct differentiation of shoot buds from leaf segments of marigold (*Targets* spp.), *Indian J Exp Biol*, 57 (2019), 30.

11 Hodgkinson A, *Oxalic acid in Biology and Medicine*, (Academic Press, NY), 1977, 104.

12 Satyapal & Pundir CS, Purification and properties of an oxalate from leaves of grain sorghum hybrid CSH-5. *Biochem Biophys Acta*, 1161 (1993) 1.

13 Kuchhal NK, Satyapal & Pundir CS, Partial purification, some properties and possible physiological role of an oxalate oxidase from grain sorghum leaves. *Indian J Plant Physiol*, 36 (1993) 159.

14 Lane BG, Dunwell JM, Ray JA, Schmit MR & Cuming AC, Germin, a protein marker of early plant development, is an oxalate oxidase. *J Biochem*, 268 (1993) 12239.

15 Batra B, Lata S, Sharma S & Pundir CS, An acrylamide biosensor based on immobilization of hemoglobin onto multiwalled carbon nanotubes/copper nanoparticles/poly-aniline hybrid film. *Anal Biochem*, 433 (2013) 210.

16 Pundir CS & Nath R, Occurrence of an oxalate oxidase in Sorghum leaves. *Phytochemistry*, 23 (1984) 1871.

17 Potezny N, Bias R, Oloughlin PD, Edward JB, Rofe AM & Conyers RA, Urinary oxalate determination by immobilized oxalate oxidase in continuous flow system. *Clin Chem*, 29 (1983) 16.

18 Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the folin phenol reagent. *J Biol Chem*, 193 (1951) 265.

19 Hafeez A, Razzq A, Mahmood T & Jhanzab HM, Potential of copper nanoparticles to increase growth and yield of wheat. *J Nanosci Adv Technol*, 1 (2015) 1.

20 Nekrasova GF, Ushakova OS, Ermakov AE, Uimin MA & Byzov IV, Effect of copper (II) Ions and copper nanoparticles on Elodea densaplanch. *Russian J Ecol*, 42 (2011) 458.

21 Ramasarma T, H_2O_2 has a role in cellular regulation, *Indian J Biochem Biophys*, 27 (1990) 269.

22 Fenik SI, Trofimyak TB, Blyum & Ya B, Mechanism of tolerance formation in Brassica juncea plants exposed to high copper concentrations. *Uspsovrem Biol*, 115 (1995) 261.

23 Rico CM, Hong J, Morales MI, Zhao L, Barrios AC, Zhang JY, Peralta-Videa JR & Gardea-Torresday JL, Effect of cerium oxide nanoparticles on Rice: A study involving the antioxidant defense system and *in vivo* fluorescence imaging. *Environ Sci Technol*, 47 (2013) 5635.