Range Mgmt. & Agroforestry 38 (2) : 208-214, 2017
ISSN 0971-2070

Influence of copper oxide and zinc oxide nano-particles on growth of fodder cowpea and soil microbiological properties

R. Srinivasan*, A. Maity, K. K. Singh, P. K. Ghosh, Sunil Kumar, M. K. Srivastava, A. Radhakrishna, Rahul Srivastava and Bandana Kumari
ICAR-Indian Grassland and Fodder Research Institute, Jhansi-284003, India
*Corresponding author e-mail: srinivasmic@gmail.com
Received: 7th June, 2017 Accepted: 4th December, 2017

Abstract
A study was conducted in net house to record the effects of seed treatment with varying levels of nano copper oxide (nCuO; 25, 50, 100 and 200 ppm) and nano zinc oxide (nZnO; 250, 500 and 750 ppm) on the growth of cowpea and bioaccumulation of minerals in plants under pot culture condition. It was found that 25 ppm of nCuO enhanced germination percentage, while seedling length and biomass were maximum at 50 ppm compared to control and RDF $CuSO_4$ (@20 kg/ha) treatment. Concentration of nCuO at 100 ppm recorded maximum shoot length (77.3 cm), however, 50 ppm of nCuO had significantly higher root length (20.4 cm) and nodulation in cowpea. It was found that the availability of Cu in plant system was enhanced with increase in concentration of nCuO up to 100 ppm (17.8 µg/g). Highest availability of Zn (45.4 µg/g) in plant dry matter was recorded at 25 ppm of nCuO. The influence of nCuO particles on the soil microbiological properties was also studied, and it was found that there was no significant difference between control and nCuO treatments up to 100 ppm in terms of both dehydrogenase activity and total bacterial count (TBC). However, decrease in dehydrogenase activity and TBC was observed at 200 ppm. Soil microbial biomass carbon (SMBC) was maximum at 50 ppm of nCuO. In nZnO treatments (up to 750 ppm), all microbiological parameters studied were higher when compared to control.

Keywords: Bioaccumulation, Cowpea, Germination, Growth, Microbiological properties, Nanoparticles

Introduction

The use of nanoparticles (NPs) has recently received a lot of attention by the agricultural researchers (Pourkhaloee *et al.*, 2011; Haghighi *et al.*, 2012), especially by those investigating seed characteristics, although their exact mechanisms of actions are not well understood. Nanomaterials, because of their tiny size, show unique characteristics and have greater surface area than bulk materials, and due to this larger surface area, their solubility and surface reactivity tend to be higher (Ruffini and Cremonini, 2009). Application of nanotechnology to agricultural crop is the emerging areas of research for sustainable intensification of crop production. Nanotechnology applications reduce the rate of fertilizer nutrients loss through leaching and increase their availability to plants which ultimately leads to reduced water and soil pollution (Cui *et al.*, 2006). Naderi and Danesh-Shahraki (2011) reported that nano-fertilizers enhanced nutrient use efficiency, reduced soil pollution, and reduced fertilizer application. The rates of NPs uptake, intracellular localization as well as biomineralization in different plants have been reported (Mailander and Landfester, 2009; Verma and Stellacci, 2010), but it is difficult to generalize the nature of NPs for their improved efficiency or productivity in plants.

In the recent past, ZnO NPs have been studied as a nutrient to increase the efficiency of plant fertilization, but the larger surface area of nanoparticles did not ensure improved solubility or even higher availability of Zn^{2+} for plants (Lombi *et al.*, 2012). Seeds of several plants (*Zea mays*, *Glycine max*, *Cajanus cajan* and *Abelmoschus esculentum*) were coated with ZnO NPs for their use as a Zn supplement. The germination test were then carried out with coated and uncoated seeds, which indicated a better germination percentage (93-100%) due to the nZnO coating when compared to uncoated seeds (80%). However, a pot culture experiment conducted with coated seeds revealed that the crop growth with nZnO coated seeds was similar to that observed with soluble Zn treatment applied as zinc sulfate heptahydrate (Adhikari *et al.*, 2016). ZnO NPs, synthesized by soil fungi in a concentration 10 mg/L was also found to enhance the mobilization of native phosphorus in the mung bean (*Vigna radiata*) rhizosphere. Indeed, the presence of ZnO NPs in the rhizosphere affected root nodulation, delayed the onset of nitrogen fixation and caused early senescence of nodules (Huang *et al.*, 2014). However, there is paucity of information on effect of NPs on growth attributes of fodder crops. Hence, this study was conducted to determine the effect of nCuO and nZnO on seed germination and plant growth attributes of fodder cowpea. Since copper and zinc are the two trace elements, whose deficiency is wide spread in soils and animal feed resources of Central India in general and Bundelkhand region in particular.

Materials and Methods

***Nano-particles and seed treatment*:** The experiment was laid out in completely randomized design with three replications. Nanoparticles used in this experiment were procured from Sisco Research Laboratories Pvt. Ltd Mumbai, India. Seed treatments comprised of different concentrations of nCuO (25, 50, 100 and 200 ppm), nZnO (250, 500 and 750 ppm) and their macro salts as recommended dose of fertilizer (RDF: $CuSO_4$ and $ZnSO_4$ @ 20 kg/ha) and a control without nano or its macro salts. The size (APS) and purity of nCuO were 40 nm and 99% and the corresponding values for nZnO were 30 nm and 99.9%, respectively. Seed treatment with NPs and macro salts was carried out by soaking in distilled water with different concentrations under shaking for 6 hours and followed by air drying and sowing was carried out in the net house. Plants were harvested at 60 and 115 days after sowing and plant growth attributes such as shoot length, root length, fresh weight *etc*. was observed. Copper and zinc content in plant dry matter were estimated following standard method using Atomic Absorption Spectrometry.

***Germination percentage*:** One hundred seed per replicate was placed in moist filter paper in 11 cm diameter petri dish in three replications. Germination count was recorded every 2 days for 8 days after sowing (DAS). The final count of germination was recorded on the 8th day according to International Seed Testing Association (ISTA, 1995) rules and number of normal seedlings was expressed as percent germination. Germination Index (GI) was calculated according to the following equation-

Germination Index (GI) = % germination in each treatment/ % germination in control

***Seedling vigour index*:** Shoot and root lengths were recorded for the calculation of vigour index (VI) in each treatment and the VI was calculated as per the formula given under (ISTA, 1996; Maisuria and Patel, 2009).

Seedling vigour index = (average root length + average shoot length) x % seed germination

***Soil microbiological properties*:** Total bacterial population was enumerated from the rhizospheric soil through serial dilution and pour plate technique. Other properties like dehydrogenase activity (Tabatabai, 1982) and soil microbial biomass carbon (SMBC; Jenkinson and Powlson, 1976) were recorded.

Statistical analyses: Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to compare the differences between mean values for each attribute using statistical software WASP 2.0**.**

Results and Discussion

***Germination traits of cowpea seeds*:** A significant positive influence on germination, seedling length and weight was observed for all nCuO treated cowpea seeds compared to those of unexposed control germination (Table 1). Among nCuO treatments, 25 ppm concentration recorded highest germination percentage (100) and maximum seedling length (16.2 cm) which was superior (P<0.05) to control (97.5% and 14.8 cm) and RDF (97 % and 10.3 cm). Maximum seedling fresh weight was

Table 1. Effect of nCuO treatments on germination traits of cowpea seeds

Treatments	Germination (%)	Seedling length (cm)	Seedling fresh weight (g)	Vigour Index	Germination Index
Control	97.5^{bc}	14.8^{b}	1.89^{d}	1443^{b}	1.00^{bc}
RDF ($CuSO_4$)	97^{c}	10.3^{d}	1.32^{e}	999^{d}	0.99^{c}
25 ppm	100^{a}	16.2^{a}	2.19^{c}	1620^{a}	1.03^{a}
50 ppm	99.5^{ab}	13.1^{c}	4.43^{a}	1303^{c}	1.02^{ab}
100 ppm	97^{c}	14.8^{b}	2.85^{b}	1435^{b}	0.99^{c}
200 ppm	97^{c}	15.3^{ab}	2.62^{b}	1484^{b}	0.99^{c}
SEm	0.69	0.34	0.08	36.94	0.01
CD (P=0.05)	2.14	1.05	0.26	115.1	0.02

Means bearing different superscripts in a column differ significantly (P<0.05)

recorded at 50 ppm of nCuO (4.43 g) compared to control (1.89 g) and $CuSO_4$ (1.32 g). However, 25 ppm of nCuO recorded maximum seedling vigour index (1620) and germination index (1.03) than all other treatments. Farhat *et al.* (2015) reported observable increase in germination percentage of wheat (*Triticum aestivum*) on soaking in nano-particles (silver and iron) suspension for two hours but it was reduced under the influence of copper NPs. However, shoot growth was significantly increased on soaking in NPs suspension and incubation in distilled water in case of copper NPs.

Similarly, positive influence on germination, seedling length, seedling fresh weight, vigour index and germination index was observed for all nZnO treated seeds compared to those of unexposed control (Table 2). Among nZnO treatments, 750 ppm of nZnO recorded highest germination percentage (97.2) and maximum seedling length (17.6 cm) which was significantly ($P<0.05$) superior to control (94.2% and 13.8 cm) and RDF (95.2% and 14.6 cm). It also recorded the highest seedling fresh weight (3.49 g), vigour index (1710.1) and germination index (1.03) when compared to RDF and control. Our findings were in agreement with earlier studies, where zinc oxide nanoparticles were reported to enhance plant growth and their development. Application of Zn naoparticles at lower concentration resulted in improved seed germination in wheat (Ramesh *et al.*, 2014) and maize (Suriyaprabha *et al.*, 2012). Peanut seeds treated with 1000 ppm nanoscale ZnO had maximum germination (100%) and seedling vigour index (1701), but increased concentration (2000 ppm) led to decrease in seedling vigour index (Prasad *et al.*, 2012). ZnO NPs induced a significant improvement in *Cyamopsis tetragonoloba* plant biomass, shoot and root growth, root area, chlorophyll and protein synthesis (Marschner, 1993). Mahajan *et al.* (2011) reported that ZnO NPs promoted the root and shoot length, root and shoot biomass in *Vigna radiata* and *Cicer arietinum*.

***Growth attributes of fodder cowpea*: ** Nano CuO treatments recorded significant differences in most of the plant growth parameters. 100 and 200 ppm of concentrations recorded significantly higher shoot length (77.3 and 76.7 cm respectively) than RDF (63.8 cm) and control (56.0 cm) treatments (Table 3). Maximum fresh weight, root length and number of root nodules per plant were recorded at 50 ppm of nCuO which was significantly superior to control and RDF. Maximum seed yield was recorded in 25 ppm concentration. Though the observations on shoot fresh weight, number of pods and weight of pods were found non-significant, most of the nCuO treatments recorded enhanced values than control. Hafeez *et al.* (2015) reported that Cu-NPs (10, 20, 30, 40

Table 2. Effect of nZnO treatments on germination traits of cowpea seeds

Treatments	Germination (%)	Seedling length (cm)	Seedling fresh weight (g)	Vigour Index	Germination Index
Control	94.2^d	13.8^d	2.02^d	1299^e	1.00^d
RDF ($ZnSO_4$)	95.2c	14.6^c	2.29^c	1389^d	1.01^{bc}
250 ppm	94.5^{cd}	16.7^b	2.32^c	1575^c	1.00^{cd}
500 ppm	96.2^b	17.2^{ab}	3.12^b	1650^b	1.02^{ab}
750 ppm	97.2^a	17.6^a	3.49^a	1710^a	1.03^a
SEm	0.315	0.166	0.064	14.17	0.004
CD (P=0.05)	0.99	0.52	0.20	44.7	0.013

Means bearing different superscripts in a column differ significantly ($P<0.05$)

Table 3. Effect of nCuO on growth attributes of fodder cowpea

Treatments	Shoot length (cm)	Fresh weight (g/plant)	Root length (cm)	No of root nodules per plant	No. of pods	Seed yield (g/pot)
Control	56.0^c	63.7^d	12.4^c	14.1^d	2.61	14.5^c
RDF ($CuSO_4$)	63.8^b	80.8^{b}c	16.0^b	20.2^{bc}	4.44	20.9^b
25 ppm	72.2^a	89.4^{ab}	17.5^b	19.4^c	4.06	26.7^a
50 ppm	74.0^a	93.5^a	20.4^a	26.4^a	4.33	20.2^b
100 ppm	77.3^a	72.9^{cd}	15.2^b	22.8^b	3.11	16.5^c
200 ppm	76.7^a	64.2^d	11.5^c	15.9^d	3.83	15.3^c
SEm	2.4	3.4	0.8	0.9	0.84	1.0
CD (P=0.05)	7.7	10.8	2.7	2.8	NS	3.3

Means bearing different superscripts in a column differ significantly ($P<0.05$)

and 50 ppm) applied to soil in pots significantly increased growth and yield of wheat as compared to control and they also recorded that 30 ppm of Cu-NPs had significantly higher chlorophyll content, leaf area, number of spikes/pot, number of grains/spike, 100 grain weight and grain yield.

Nano ZnO treatments also resulted significant increase in most of the growth attributes. 500 ppm of concentration recorded significantly higher shoot (133.9 cm) and root (17.6 cm) length and fresh weight (203.4 g) than RDF and control treatments (Table 4). All NP treatments also recorded enhanced nodulation over control. Maximum number of pods per plant and seed yield/pot was recorded by 750 ppm of concentration. Prasad *et al.* (2012) also reported that plant height was significantly increased with 400 and 1000 ppm nanoscale ZnO compared to control and the respective bulk $ZnSO_4$ concentration. Seeds treated with 1000 ppm concentration of nanoscale ZnO recorded highest plant growth (15.4 cm) due to extended inter-nodal length.

Zinc nanoparticles resulted in greater plant biomass, root and shoot growth, chlorophyll content in *Cyamopsis tetragonoloba* (Raliya and Tarafdar, 2013). Mahajan *et al.* (2011) observed better root and shoot length and shoot biomass when applied Zn nanoparticles in *Vigna radiata* and *Cicer arietinum*. Bao-shan *et al.* (2004) reported improved agronomic parameters due to SiO_2 nanoparticles in *Latix olgensis* including height, root collar sphere, root length and number of lateral roots. Application of silicon nano-tubes improved nutrient uptake in maize plants when applied at lower doses (Suriyaprabha *et al.,* 2012). Prasad *et al.* (2012) reported that 30.5% and 38.8% higher pod yield was recorded in groundnut with application of nanoscale ZnO at 2 g/15L + NPK compared to NPK alone and 29.5% and 26.3% higher pod yield compared to chelated zinc at 30 g/15 L + NPK.

***Accumulation of copper and zinc in fodder cowpea*:** Nano CuO treatments recorded enhanced micronutrients (Cu and Zn) accumulation in dry matter as compared to control and at par with its bulk form. 100 ppm of concentration recorded maximum copper (17.8 µg/g) which was at par with RDF (17.6 µg/g). However, 25 ppm recorded maximum zinc content (45.5µg/g) which was significantly superior to all treatments (Fig 1). Enhancement in accumulation of micronutrients was also reported earlier by few workers. Carbon nanofibers (CNFs) mediated controlled release of the Cu NPs led to effective translocation of the Cu-CNFs from the root to the shoot of the plants (Ashfaq *et al*., 2017). Copper accumulation increased with increasing exposure times (days) and a significantly higher accumulation was observed in roots than shoots and leaves. Dimkpa et al. (2015) observed increase in shoot accumulations of Cu (3.8 fold) and Na (1 fold), whereas decrease in Fe (0.4 fold), Mn (0.2 fold), Zn (0.5 fold) and Ca (0.5 fold) in *Phaseolus vulgaris* with CuO NP (500 ppm) exposure. Peng et al. (2016) reported that the bioavailability of CuO NPs in rice soil was reduced by 69.84% along with plant growth, but it was significantly increased by 165% after drying-wetting cycles. CuO NPs were found to be translocated from soil to plant especially to the chaff and promoted the Cu accumulation in the aleurone layer of rice. The reason for higher accumulation of zinc over copper could be attributed to the availability of micronutrients in soil and also to the intrinsic properties of the plants.

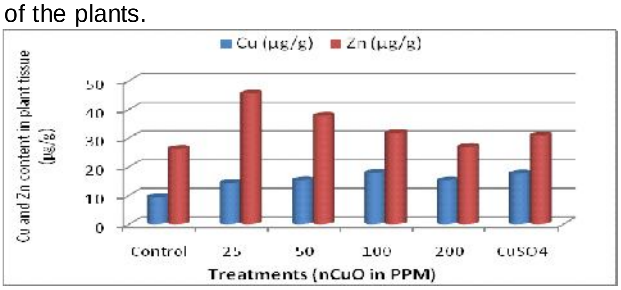

Fig 1. Effect of nCuO on plant accumulation of micronutrients in fodder cowpea

Table 4. Effect of nZnO on growth attributes of fodder cowpea

Treatments	Shoot length (cm)	Plant weight (g/plant)	Root length (cm)	No of root nodules per plant	No. of pods	Seed yield (g/pot)
Control	92.7[b]	132.7[c]	13.2[c]	11.3[b]	11.0[d]	15.1[d]
RDF ($ZnSO_4$)	103.3[ab]	191.0[ab]	15.7[b]	14.3[b]	14.7[c]	19.1[c]
250 ppm	101.3[b]	154.4[b]	17.0[ab]	19.7[a]	17.3[b]	21.5[abc]
500 ppm	133.9[a]	203.4[a]	17.6[a]	18.7[a]	17.3[b]	21.8[ab]
750 ppm	126.6[a]	188.8[ab]	16.7[ab]	19.0[a]	21.0[a]	23.9[a]
SEm	5.3	5.3	0.56	1.03	0.54	0.8
CD (P= 0.05)	16.7	16.8	1.8	3.3	1.7	2.5

Means bearing different superscripts in a column differ significantly (P<0.05)

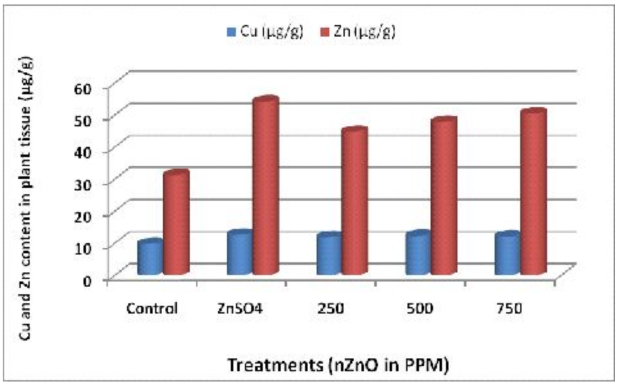

Fig 2. Effect of nZnO on plant accumulation of micronutrients in cowpea

Nano ZnO treatments also recorded enhanced copper and zinc accumulation in dry matter synergistically as compared to control and Cu was at par with its macro salt. 750 ppm of concentrationt recorded maximum zinc (50.4 µg/g) which was next to its RDF macroform (54.2 µg/g). All nZnO treatments recorded enhanced copper availability in dry matter compared to control (Fig 2). Rameshraddy *et al*. (2017) reported that ragi plants supplemented with ZnO nano particle, Zn gluconate and $ZnSO_4$ had improved leaf and seed Zn content in all the Zn treatments compared to control plants. Under well watered condition highest seed and leaf Zn content of 13.9 and 8.94 mg/100g was found in ZnO nano treatment in seed priming and foliar treatment combinations. ZnO nano treated plants showed an increase in leaf Zn content by 39.71% and seed Zn content by 62.63% over $ZnSO_4$. The result indicated the better transport and accumulation of Zn in seed and leaf with ZnO nano treatment compared to $ZnSO_4$ treatment.

***Soil microbiological properties*:** There was increase in TBC upto 100 ppm of nCuO over control and it was maximum at 50 ppm (Table 5). Maximum dehydrogenase activity was recorded at RDF $CuSO_4$. There was also increase in SMBC content in nCuO treatment upto 100 ppm and the highest SMBC was recorded at 50 ppm of nCuO, while reduction in dehydrogenase, TBC and SMBC was observed at 200 ppm. Maity *et al*. (2016) reported that soil total bacterial count was higher with the application of CuO (9.45 log CFU/g of soil) and ZnO (9.46 log CFU/g of soil) at their lower doses than higher doses in a field experiment. The concentrations of CuO and ZnO NPs were inversely proportionate with the population of bacteria. Kumar *et al*. (2011) reported that when Cu NPs were homogenously incubated under laboratory conditions only marginal changes in the microbial community were observed.

Similarly, there was increase in dehydrogenase activity, TBC and SMBC levels in nZnO treatments over control. The maximum dehydrogenase activity and TBC was

Table 5. Influence of nCuO on soil microbiological properties

Treatments	Dehydrogenase activity (TPF ppm)	Total bacterial count (log10 value)	Soil microbial biomass carbon (mg C/kg soil)
Control	30.0[b]	7.57[c]	310.3[d]
RDF ($CuSO_4$)	32.5[a]	7.22[c]	161.3[f]
25 ppm	30.4[b]	8.04[b]	427.3[c]
50 ppm	31.2[ab]	8.50[a]	566.7[a]
100 ppm	30.0[b]	8.43[a]	453.3[b]
200 ppm	27.9[c]	7.39[c]	274.7[e]
SEm	0.6	0.11	4.3
CD (P=0.05)	1.8	0.37	13.8

Means bearing different superscripts in a column differ significantly (P<0.05)

Table 6. Influence of nZnO on soil microbiological properties

Treatments	Dehydrogenase activity (TPF ppm)	Total bacterial count (log10 value)	Soil microbial biomass carbon (mg C/kg soil)
Control	24.0[b]	7.79[c]	261.0[e]
$ZnSO_4$	28.6[a]	8.16[b]	392.0[b]
250 ppm	29.8[a]	8.58[a]	331.0[d]
500 ppm	28.4[a]	8.33[b]	560.6[a]
750 ppm	28.1[a]	8.29[b]	372.3[c]
SEm	0.8	0.07	6.0
CD (P=0.05)	2.5	0.22	19.2

Means bearing different superscripts in a column differ significantly (P<0.05)

recorded at 250 ppm and maximum SMBC was recorded at 500 ppm. The dehydrogenase activity observed was significantly higher in all nZnO treatments over control and it was at par with $ZnSO_4$ (Table 6). TBC was also at par with $ZnSO_4$ except 250 ppm which was significantly higher. There was a reduction in SMBC at 750 ppm nZnO treatment but it was significantly higher when compared to control.

Shah *et al.* (2014) studied the effect of metal nanoparticles in biosolids on soil microbial community and reported that ZnO and zerovalent Cu nanoparticles were not toxic to soil bacterial community, while Ag nanoparticles and TiO_2 in biosolids changed the bacterial richness and composition in wavering pattern as a function of time. Zinc nanoparticles resulted improvement in microbial population in *Cyamopsis tetragonoloba* rhizosphere, acid phophatase, alkaline phophatase and phytase activities (Raliya and Tarafdar, 2013). Positive responses of soil bacteria were also reported earlier. When FeO NPs were applied to the soil, it had positive effects on soil microbial metabolic activity (at 1 and 10 mg/kg soil) and soil nitrification potential (at 0.1 and 1 mg/kg soil; He *et al*., 2016). These positive effects on specific microbial metabolic activity and specific nitrification potential indicated that metal or metal oxide nanoparticles could even change the C and N cycles of the agricultural soil through influencing soil microbial metabolism. A stronger effect on soil enzymatic activities was observed with nZnO than $nTiO_2$, $nCeO_2$ and nFe_3O_4 in saline-alkali soil, which was considered more susceptible to metal oxide nanoparticles than black soil (He *et al*., 2016). Therefore, the type of soil was a key component dictating the effect of metal oxide nanoparticles on the bacterial community composition and size.

Conclusion

This study indicated the influence of nanoparticles in enhancing the germination and seedling vigour, growth and micronutrient accumulation in fodder cowpea. Highest germination percentage, seedling length, seedling vigour index, germination index and maximum zinc content were recorded at 25 ppm of nCuO, while maximum fresh weight, root length and number of root nodules per plant were recorded at 50 ppm nCuO. Among nZnO treatments, highest germination percentage and seedling length, seedling fresh weight, vigour index, germination index and maximum zinc content were recorded in 750 ppm and higher shoot and root length and fresh weight were recorded in 500 ppm. Soil microbioogical properties were also influenced positively up to certain level by nanoparticle treatments. Hence, nanoparticles can be used in enhancing fodder cowpea production under optimal concentration. However, the findings need to be verified under field conditions.

Acknowledgement

The support and facilities provided by the Director, ICAR-IGFRI, Jhansi to undertake this study are duly acknowledged.

References

Adhikari, T., D. Sarkar, H. Mashayekhi and B.S. Xing. 2016. Growth and enzymatic activity of maize (*Zea mays* L.) plant: solution culture test for copper dioxide nano particles. *Journal Plant Nutrition* 39: 102-18.

Ashfaq, M., N. Verma and S. Khan. 2017. Carbon nanofibers as a micronutrient carrier in plants: efficient translocation and controlled release of Cu nanoparticles. *Environmental Science: Nano* 4: 138-148.

Bao-shan, L., D. Shao-qi, L. Chun-hui, F. Li-jun, Q. Shuchun, and Y. Min. 2004. Effect of TMS (nanostructured silicon dioxide) on growth of changbai larch seedlings. *Journal of Forest Research* 15:138-140.

Cui, H., C. Sun, Q. Liu, J. Jiang and W. Gu. 2006. *Applications of Nanotechnology in Agrochemical Formulation, Perspectives, Challenges and Strategies*. Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing. China.

Dimkpa, C.O., J.E. McLean, D.W. Britt and A.J. Anderson. 2015. Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology* 24: 119-129.

Farhat, Y., R. Abdul, M.N. Iqbal and M.Z. Hafiz. 2015. Effect of silver, copper and iron nanoparticles on wheat germination. *International Journal of Biosciences* 6: 112-117.

Hafeez, A., A. Razzaq, T. Mahmood and H.M. Jhanzab. 2015. Potential of copper nanoparticles to increase growth and yield of wheat. *Journal of Nanoscience with Advanced Technology* 1: 6-11.

Haghighi M., Z. Afifipour and M. Mozafarian. 2012. The effect of N–Si on tomato seed germination under salinity levels. *International Journal of Environmental Sciences* 6: 87-90.

He, S., Y. Feng, J. Ni, Y. Sun, L. Xue, Y. Feng, Y. Yu, X. Lin and L. Yang. 2016. Different responses of soil microbial metabolic activity to silver and iron oxide nanoparticles. *Chemosphere* 147: 195-202.

Huang, Y.C., R. Fan, M.A. Grusak, J.D. Sherrier and C.P. Huang. 2014. Effects of nano ZnO on the agronomically relevant *Rhizobium* legume symbiosis. *Science of the Total Environment* 497: 78-90.

ISTA (International Seed Testing Association) 1995. International rules for seed testing. *Seed Science and Technology* 13: 322-326.

ISTA (International Seed Testing Association). 1996. International rules for seed testing. *Seed Science and Technology* 21: 1-288.

Jenkinson, D.S. and D.S Powlson. 1976. Residual effects of soil fumigation on soil respiration and mineralization. *Soil Biology and Biochemistry* 2: 99-108.

Kumar, N., V. Shah and V.K. Walker. 2011. Perturbation of an arctic soil microbial community by metal nanoparticles. *Journal of Hazardous Materials* 190: 816-822.

Lombi, E., B. Nowack, A. Baun, and S. P. McGrath. 2012. Evidence for effects of manufactured nanomaterials on crops is inconclusive. *Proceedings of the National Academy of Sciences of the United States of America* 109: 49.

Mahajan, P., S.K. Dhoke and A.S. Khanna. 2011. Effects of nano zinc oxide particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. *Journal of Nanotechnology* 1-7. http://dx.doi.org/10.1155/2011/696535.

Mailander, V. and K. Landfester. 2009. Interaction of nanoparticles with cells. *Biomacromolecules* 10: 2379-2400.

Maisuria, K.M. and S.T. Patel. 2009. Seed germinability, root and shoot length and vigour index of soybean as influenced by rhizosphere fungi. *Karnataka Journal of Agricultural Sciences* 22: 1120-1122.

Maity, A., N. Natarajan, D. Vijay, R. Srinivasan, M. Pastor and D. R. Malaviya. 2016. Influence of metal nanoparticles (NPs) on germination and yield of oat (*Avena sativa*) and berseem (*Trifolium alexandrinum*). *Proceedings of National Academy of Sciences, India, Sect. B Biological Sciences*. DOI 10.1007/s40011-016-0796-x.

Marschner, H. 1993. Zinc uptake from soil. In: A.D. Robson (ed). *Zinc in Soils and Plants*, Kluwer Academic Publishers, Dordrecht. pp. 59-79.

Naderi, M. and A. Danesh-Shahraki. 2011. The application of nanotechnology in the formulation optimization of chemical fertilizers. *Journal of Nanotechnology* 106: 20-22.

Peng, C., C. Xu, Q. Liu, L. Sun, Y. Luo and J. Shi. 2017. Fate and transformation of CuO nanoparticles in the soil–rice system during the life cycle of rice plants. *Environmental Science and Technology* 51: 4907-4917.

Pourkhaloee, A., M. Haghighi, M.J. Saharkhiz, H. Jouzi and M.M. Doroodmand. 2011. Investigation on the effects of carbon nanotubes (CNTs) on seed germination and seedling growth of salvia (*Salvia microsiphon*), pepper (*Capsicum annum*) and tall fescue (*Festuca arundinacea*). *Journal Seed Technology* 33: 155-160.

Prasad, T.N.V.K.V., P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munuswamy, K.R. Reddy, T.S. Sreeprasad, P.R. Sajanlal and T. Pradeep. 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *Journal of Plant Nutrition* 35: 905-927.

Raliya, R. and J.C. Tarafdar. 2013. ZnO nanoparticle biosynthesis and its effect on phosphorous mobilizing enzyme secretion and gum contents in cluster bean (*Cyamopsis tetragonoloba* L.). *Agricultural Research* 2: 48-57.

Ramesh, M., K. Palanisamy, K. Babu and N.K. Sharma. 2014. Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum* Linn. *Journal of Global Biosciences* 3: 415-422.

Rameshraddy, G.J. S. Pavithra, K.N. Mahesh, Geetha and A.G. Shankar. 2017. Seed priming and foliar spray with nano zinc improves stress adaptability and seed zinc content without compromising seed yield in ragi (finger millet). *International Journal of Pure and Applied Biosciences* 5: 251-258.

Ruffini, C.M. and R. Cremonini. 2009. Nanoparticles and higher plants. *Caryologia* 62: 161-165.

Shah, V., J. Jones, J. Dickman and S. Greenman. 2014. Response of soil bacterial community to metal nanoparticles in biosolids. *Journal of Hazardous Materials* 274: 399-403.

Suriyaprabha, R., G. Karunakaran, R. Yuvakkumar, V. Rajendran, and N. Kannan. 2012. Silica nanoparticles for increased silica availability in maize (*Zea mays* L) seeds under hydroponic conditions. *Current Nanoscience* 8: 902-908.

Tabatabai, M.A. 1982. Soil enzymes. In: A.L. Page, R.H. Miller and D.R. Keeney (eds.). *Methods of Soil Analysis*, Part 2. American Society of Agronomy and Soil Science Society of America, Madison.

Verma, A. and F. Stellacci. 2010. Effect of surface properties on nanoparticle-cell interactions. *Small* 6: 12-21.