Effect of Hydroxyapatite nanorod on chickpea (*Cicer arietinum*) plant growth and its possible use as nano-fertilizer

Niranjan Bala[1], Anindita Dey[1], Sukhen Das[1]*, Ruma Basu[2] and Papiya Nandy[1]

1. Physics Department, Jadavpur University, Kolkata 700032, India
2. Physics department, Jogamaya Devi College, Kolkata, 700026, India

Abstract

Engineered nano particles may have a variety of effects on plant systems which is not well studied as yet. We have studied for the first time the beneficial effect of hydroxyapatite (HAP) nanorod on seed germination and growth of chickpea plant. HAP nanorods have been synthesized by sol gel technique and then characterized. Chickpea plants have been allowed to germinate and grow in sterile sand containing HAP nanorod and this resulted in enhancement of both germination rate and plant growth radically. The maximum increase was observed in the presence of 1 mg/ml Hap-nanorod where the plant growth rate was more than two times over the control. Electron microscopic study provided the evidence of accumulation of nanoparticles within the plant tissue. These nanoparticles have great potential to be used as nano-fertilizer.

Keywords: Hydroxyapatite nanorod; electron microscopy; nano-fertilizer; plant biomass production; α-amylase activity; nanoparticles accumulation

Bala, N., A. Dey, S. Das, R. Basu and **P. Nandy.** 2014. 'Effect of Hydroxyapatite nanorod on chickpea (*Cicer arietinum*) plant growth and its possible use as nano-fertilizer'. *Iranian Journal of Plant Physiology*, 4 (3), 1061-1069.

Introduction

Nano-sized particles have always existed in nature and interacted with our environments. Like all living beings of eco-system plants also get exposed to such nanoparticles (NPs) and respond accordingly (Guzman, 2006; Zhao, 2007; Ghosh, 2010). The advent of nanotechnology has provided a wealth of various engineered nanoparticles (ENPs) viz. metal oxide nanoparticles, carbon nano tubes, fullerenes, nano wires, magnetic nanoparticles etc. which exhibit novel physical, chemical, and biological characteristics (Chen, 2012; Poland, 2008; Warheit, 2008).

These new entrants in nature may have a variety of effects on plant systems. Various studies showed positive or negative effects of ENPs on experimental systems *in vitro.* Most of the NPs accelerate the possible toxicological and pathological risk to human health, especially in drug delivery system (Warheit, 2008). Poland et al. (2008) showed that multiwalled carbon nano tube (MWCNT) caused cancer in mice (Poland,

*Corresponding author
E-mail address: sdasphysics@gmail.com
Received: January, 2014
Accepted: March, 2014

2008) and also damaged DNA in human lungs (Lindberg, 2009). High concentrations of some ENPs reduced plant growth and increased the permeability of bacterial cells (Doshi, 2008; Ling and Xing, 2007; Nel, 2006; Racuciu, 2007). ENPs may also prevent photosynthesis by reducing nutrient absorption (Navarro, 2008). In our laboratory we have already observed that nano-mullite showed neutral effect whereas its metal-amended derivatives showed toxic effect on mung bean plant growth (Dey, 2011).

However, beneficial aspects of ENPs have now been explored by researchers in different fields like biosensing, drug delivery (Zanello, 2006; Panyam and Labhasetwar, 2003; Harrison and Atala, 2007), biodetection of pathogens (Chan and Nie, 1998), detection of proteins (Xue, 2012; Nam, 2012; Nietzold and Lisdat, 2012), and in cancer therapy (Rosenberg, 1985; Connor, 2005; Peng, 2008). Lu et al (2002) reported that TiO_2 and SiO_2 nanoparticles increased the synthesis of nitrate reductase in *Glycine max* which in turn facilitated growth and germination by increasing the efficiency of its water uptake machinery. Mondal et al. (2011) studied the effect of MWCNTs and oxidized MWCNTs (OMWCNTS) on mustard plant seeds and reported enhancement both in germination rate and plant growth, the reason being the increase in water uptake capability of seed membrane. Studies on animal system proved that nano calcium phosphates were very well tolerated and got absorbed in animal system (Svitlana, 2013; He, 2002). Hydroxyapatites (HAPs) are also used in traditional gene delivery system (Reischl and Zimmer, 2009; Chowdhury, 2004; Roy, 2003; Naqvi, 2012), as a vehicle for targeted drug delivery system (Liong, 2008; Shubayev, 2009; Epple, 2010) and in biomedical applications (Reischl and Zimmer, 2009; Chowdhury, 2004; Roy, 2003).

In the current study for the first time the effect of nano-HAP was investigated on chickpea plant growth where the nano-HAP has been chosen for its high biocompatibility as well as biodegradability (Zhao, 2007). Synthesis of α-amylase content was also measured in sprouted nano-HAP treated chickpea seeds.

Materials and Methods

Synthesis of crystalline nano-calcium phosphates (nano HAP)

Crystalline nano-calcium phosphates particles (nano-HAP) were synthesized in room temperature (20 °C) by standard technique (Cai, 2007). All the reagents used in the experiments were obtained from Merck (Germany).

Characterization of nano-HAP

X-ray diffraction patterns of the crystalline nano HAP were analyzed in powder diffractometer, Model D8, BRUKER AXS, using Cu K_α radiation (α = 0.15425 nm in the range of 2θ from 10 ° to 80 °. Thermogravimertic analysis of nano-HAP was done by DTG-60H, simultaneous DTA-TG apparatus, SHIMADZU from 25 °C to 1100 °C in nitrogen environment at a flow rate of 50 cc/min. High Resolution Transmission electron microscopic study (JEM – 2100 HRTEM, JEOL, Japan) was performed to examine the morphology and estimate the particle size.

The typical chemical groups of nano-HAP were analyzed by Fourier transform infrared (FTIR) spectroscopy, using a JASCO FTIR instrument-410. The range of the analysis was 4000 to 400 cm^{-1}, where the pellets were first prepared as 1% samples in Potassium Bromide (KBr) for the analysis.

Seed collection

Certified seeds of chickpea (*Cicer arietinum*) were collected from the local market of Jadavpur, Kolkata having an average germination rate greater than 85% as shown by a preliminary study.

Preparation of nanoparticle solution

Nano-HAP was suspended directly in double distilled water by sonication in an ultrasonic bath (Model No. 229, Imeco Ultrasonic, India) for one hour. After that the samples were prepared at different concentrations accordingly.

Germination study

To start with, all seeds were immersed in 10% sodium hypochlorite solution for surface sterilization (ISTA, 1976). To analyze percentage of seed germination, 25 individual seed samples were transferred to beakers containing sterilized sand, moistened with distilled water or NPs solution as the case may be. Germination data were recorded at every 24 h interval following International Rules for Seed Testing Association (ISTA, 1976). Seeds were considered to be completely germinated when the radicle attained a length of 1 mm and plumule was just unfolded. All the experiments were repeated four times with 3 replications in each case.

Seeds were treated in different media and treated seeds were designated as followings:

S0- Distilled water treated,
S1- HAP (0.5 mg/ml) treated,
S2- HAP (1.0 mg/ml) treated, and
S3- HAP (1.5 mg/ml) treated.

Methods for measurement of other parameters

Number of seed germinated per 100 seeds was treated as germination percentages and T_{50} was recorded after repeating germination process thrice with 6 replications in each case.

Shoot-root growth and dry weight (wt) accumulation of plants were recorded after ten days of treatment as accumulated dry weight per 100 grams of fresh weight. The data were analyzed by statistical method of ANOVA using EXEL-STAT.

For agricultural purposes germination index (GI) is used as an indicator of phytotoxicity in soil (Tam and Tiquia, 1994). The percentage of GI was calculated according to a standard method (Batish, 2007).

Seed vigor index (SVI) was calculated by multiplying germination % by seedlings length. Alpha amylase activity and reducing sugar content was determined from crude extract of sprouting seeds. For crude extraction, 8 germinating seeds from S0 and S2 of 2 days were washed thoroughly in double distilled water (dd H_2O) and homogenized in chilled mortar-pestle with 15 ml ice cold Na-phosphate buffer (0.1 M, pH 7.0). The homogenate was centrifuged at 10000 rpm for 15 min at 4 °C. The supernatant was collected as crude extract and was used for measuring the alpha amylase activity and reducing sugar content (Cui, 2002). Absorption of reducing sugar was measured at 520 nm in a UV-VIS spectrophotometer (Parkin Elmer Lambda 25) to calculate the amount of reducing sugar content and alpha amylase activity.

FTIR and HRTEM of control (S0) and HAP treated (S2) plant

Plants from S0 and S2 set were dried in oven (80 °C). These plants were then grounded to fine powder for FTIR (JASCO FTIR instrument-410) analysis.

For HRTEM (JEM-2100, JEOL, Japan) stem segments of approximately 3 mm length were collected from S0 and S2, respectively. The stems were fixed in 2.5% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.1) for 8 h and post fixed with OSO_4. The samples were dehydrated in an ethanol series and embedded in Spurr's epoxy resin. Ultrathin sections were obtained using an ultramicrotome and stained with uranyl acetate and basic lead citrate.

Results

Characteristics of nano HAP

All peaks of the X-ray diffraction pattern of HAP nanoparticles (Fig. I, a) were assigned by the JCPDS File no. 24-0033. From Fig. I (a), it was clear that the sample was well crystalline since the peaks were sharp in nature. The 100% peak (211) of the sample was observed at $2\Theta = 31.74°$ indicating the presence of HAP. Other types of crystalline calcium phosphate particles such as octacalcium phosphate (OCP), tricalcium phosphate (TCP), and dicalcium phosphate dihydrate (DCPD) were not detected in XRD. CTAB, used for controlling the particle size was not detected, as the residual amount had been eliminated during the process of washing.

Differential thermal and thermogravimetric (DTA-TG) (DTG-60H, Shimadzu) analysis was conducted from room temperature (~30 °C) to 1100 °C (Fig. I, b). No significant change was observed except with negligible weight loss of nano-HAP, indicating that no phase transition took place during heating.

Fourier transform infrared spectra (FTIR) of the synthesized sample showed the typical hydroxyapatite phase (Fig. I, c). The band at ~1035 cm^{-1} corresponded to the PO_4^{3-} (P=O) stretching band, the peaks at ~563 cm^{-1} and ~603 cm^{-1} were associated with PO_4^{3-} (P-O) bending mode while the band at ~ 960 cm^{-1} was due to symmetric stretching of (PO_4^{3-}) (Trommer, 2009). The band at ~ 633 cm^{-1} indicated the presence of structural -OH in HAP (Panda, 2003). The bands at ~874 cm^{-1} and ~1458 cm^{-1} indicated the interaction of HPO_4^{2-} with carbonate and the presence of CO_3^{2-}, respectively (Hu, 2011). The bending mode at ~3368 cm^{-1} and ~1638 cm^{-1} appeared due to adsorbed water in the sample whereas the broad band in the range of ~2500-3700 cm^{-1} originated due to symmetrical and asymmetrical stretching vibrations of OH group (Hu, 2011; Lin, 2007).

HRTEM micrograph (Fig. I, d) of the sample showed typical rod-shaped nano-HAP of length ranging from 40 - 80 nm and diameter 15 - 30 nm.

Our studies showed that the germination process was facilitated in presence of nano-HAP. In case of control sample (S0) the germination percentage was a maximum of 88% in 60 h. But in case of S2 the germination percentage was 100% and it took place within 54 h whereas in case of S1 100% germination was observed in 60 hr. Details of germination percentage and T50 are given in Fig. II (d) and Table 1, respectively. The value of T50 decreased in the presence of nano-Hap with a minimum for sample S2. In the presence nano-HAP the germination process was

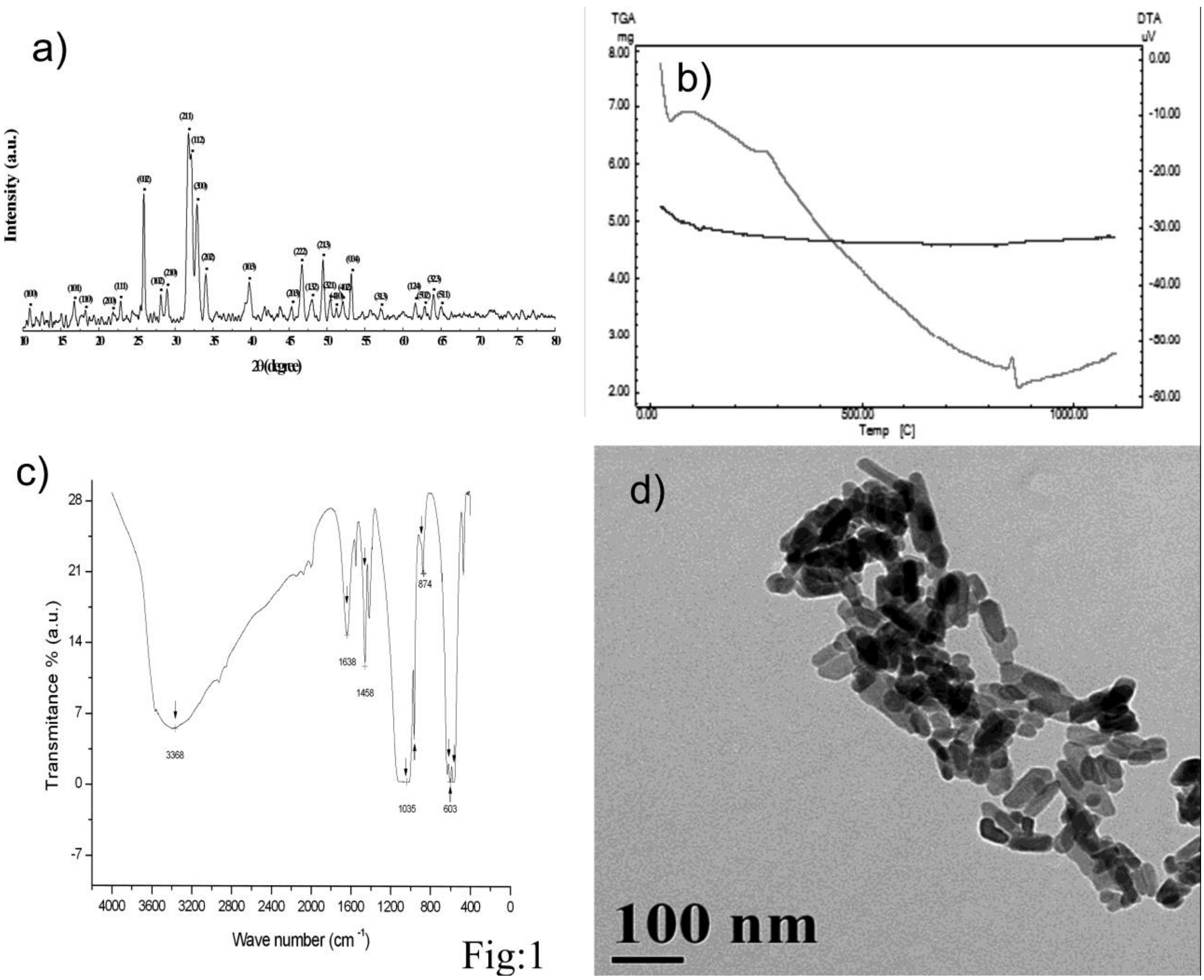

Fig. I. Characterization of nano-HAP.a) X-ray diffraction pattern, b) DTA-TG spectra of prepared sample, c) FTIR spectrum and d) High Resolution TEM micrograph of sample.

favored as reflected in the values of germination %.

Growth, GI, SVI, and dry weight accumulation

Seedling growth was maximum in case of S2. Fig. II (a, b, c) and Table 2 show the growth pattern of seedlings after 3, 6, and 12 days, maximum value being ~40 cm in case of S2. Both the GI and SVI (Table 1) were also maximum in case of S2 over S0, S1 and S3. Dry weight accumulation was also highest in S2 in comparison with the others indicating maximum growth and vigor of S2. So we observed that the values of all indexes pointed to the favored germination and plant growth in the presence of nano-HAP.

Data were statistically analyzed by one way ANOVA. Values showing the same alphabets are significant at P< 0.001 statistical level.

Reducing sugar content and α-amylase activity

We also observed increase in the values of reducing sugar content and α-amylase activity in S2 compared to S0 [Fig. III, a and b] which may be due to the increase in activity of growth hormone gibberellins (GAs) (Fincher, 1989).

FTIR and HRTEM study of control (S0) and HAP treated (S2) plant

No significant difference was found between the FTIR spectra of S0 and S2 (Fig. III, c), which indicated that major quantity of incorporated nano-HAP in the plant tissue had been utilized subsequently.

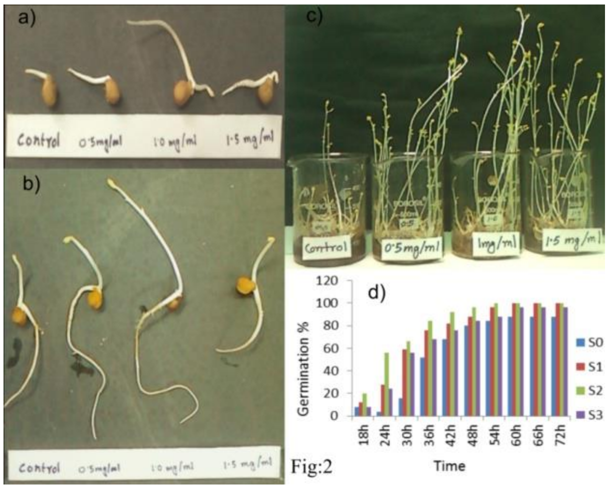

Fig. II. Growth patterns of experimented plants and germination rate. a) Seedlings at 3 day stage, b) at 6 day stage, c) at 12 day stage and d) germination percentage of seeds in accordance to time (hour)

However, HRTEM micrograph of S2 (Fig. IV, c and d) indicated the presence of trace amount of HAP, which was absent in S0 (Fig. IV, a and b). However, in S2, the rod shaped morphology of HAP was not observed.

Discussion

Researchers have reported in many articles about the adverse effect of varieties of ENPs (Nel, 2006; Dey, 2011; Holsapple, 2005; Oberdorster, 2005; Monica, 2009). However, little studies have been done so far about the beneficial effect of ENPs on plant system. Mondal et al., 2011) have shown that MWCNTs and OMWCNTs enhanced seed germination and plant growth rate of mung been plant due to increased activity of aquaporins.

Table 1
T_{50}, GI, SVI, and dry weight accumulation

Treatment	T_{50} (hr)	Germination Index (GI)	SVI	Dry weight (gm)/100gm of Fresh weight*		
				Shoot	Root	Total Seedling
S0	35	100.000	1850.64	7.886 ± 0.354	8.091 ± 0.088	15.977 ± 0.077
S1	29	165.964	3180.00	14.763 ± 0.110	13.071 ± 0.140	27.834 ± 0.329
S2	23	191.919	4118.00	15.519 ± 0.161	12.744 ± 0.323	28.263 ± 0.415
S3	30	134.007	3002.88	8.966 ± 0.031	9.974 ± 0.197	18.940 ± 0.345

* Values represented in the table were Mean value ± SD and were obtained from 10 randomly selected plants

In our present study we have shown for the first time the beneficial effect of nano-HAP on chickpea seeds. Zheng et al (Zheng, 2005) reported that vigor of spinach seedlings germinated from aged seeds increased with application of proper concentration of TiO_2 nanoparticles but the cause behind such behavior is still not clear. In our study we have considered 1 mm of radicle emergence as germination indicator. In germination, seed coat plays an important role by not only protecting the embryos but also helping in water absorption

Table 2
Plant height at 12 days

Treatment	Height (cm)
S0	21.03
S1	31.8
S2	41.18
S3	31.28

Data were statistically analyzed by one way ANOVA. Values showing same alphabets are significant at P< 0.001 statistical level.

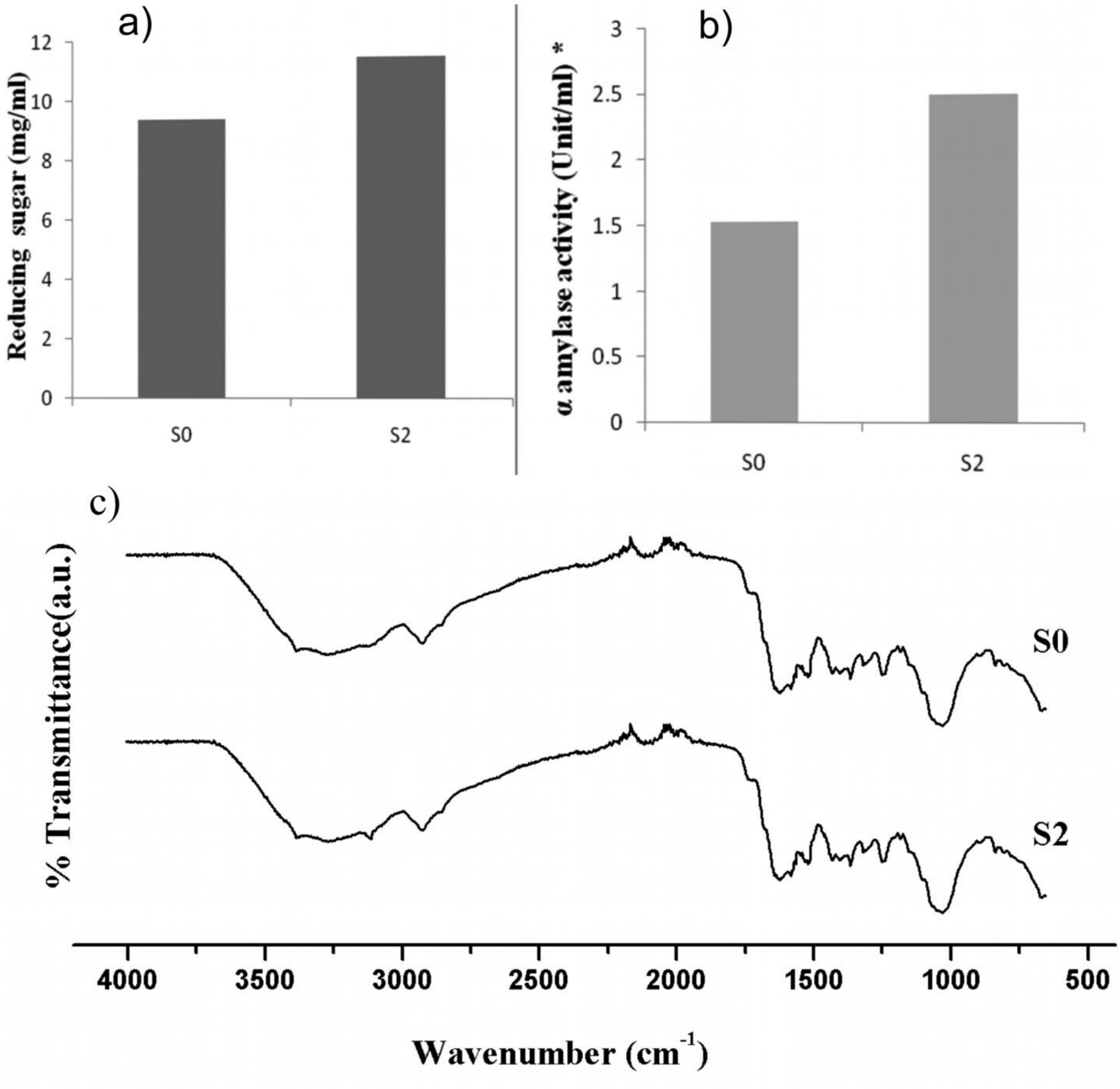

Fig. III. a) Reducing sugar content, b) α amylase activity (* One unit activity of alpha amylase is defined as 1 mg of glucose release per 10 min) and c) FTIR of S0 and S2 plant powder owing to its selective permeability. Decrease in T_{50} and increase in germination percentage by application of nano-HAP also indicated the insertion of nano-HAP through gram seed coat as the nanoparticles which cannot penetrate seed coat possibly have neutral effect on germination rate and $T_{50.}$ We observed good response of nano-HAP at quite low concentration (1 mg/ml) over the control whereas at 1.5 mg/ml concentration, growth rate decreased which implied that increase of plant growth rate was maxim`um at an optimum concentration of nano-HAP.

Dry weight of a plant generally determines its growth and vigor. Dry weight accumulation after 12 days was higher in case of all HAP treated seeds with a maximum in case of S2. Comparing FTIR results of S0 and S2 we can propose that in S2 major part of incorporated HAP had been utilized subsequently by the plant tissues for their metabolic processes, which was also supported by the HRTEM micrograph. HRTEM micrograph of S2 showed the presence of trace amount of HAP NPs in treated plants but they lost their rod shaped morphology. From this result we can also conclude that as major part of the nano-HAPs is utilized by the plants, the risk of bioaccumulation as well as chance of toxicity in higher trophic level is reduced**.**

This result is of great importance from agricultural perspective as we can apply this technique for increasing germination rate and vigor of crop plants with a very little concentration of nano-HAP.

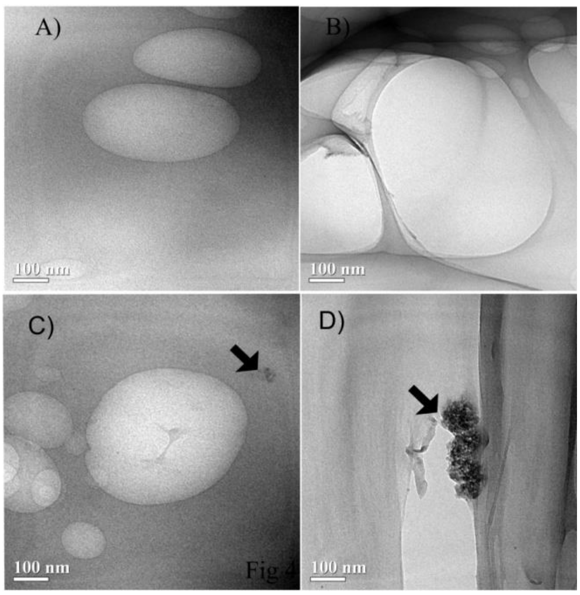

Fig. IV. HRTEM micrograph of stem. A) and B) for control (S0) plant, C) and D) for nano–HAP treated plant (S2) showing presence of nanoparticles .

Conclusion

Though majority of EPNs are phytotoxic, we have observed beneficial role of nano-HAP in seed germination and plant growth regulation in Chickpea. The possible reason for such beneficial role is the increase in activity of growth hormone gibberellins. As HAP is found to be biocompatible it may be used as fertilizer.

Acknowledgement

We would like to thank Defence Research and Development Organization (DRDO), Defence Ministry, India, for their financial support.

References

Batish DR, K. Lavanya H. P. Singh and **R. K. Kohli .** 2007. 'Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea'. *Plant. Growth. Regul*. 51:119–128.

Cai Y, Y. Liu, W. Yan, Q. Hu. J. Tao , M. Zhang, Z. Shi and **R. Tang.** 2007. 'Role of hydroxyapatite nanoparticle size in bone cell proliferation'. *J. Mater. Chem.* 17: 3780-3787.

Chan WCW and **SM Nie.** 1998**.'** Quantum dot bioconjugates for ultrasensitive nonisotopic detection'. *Science*, 281(5385):2016-2018

Chen X., H. Lv , M. Ye , S. Wang , E. Ni ,F. Zeng , C. Cao ,F. Luo and **J. Yan .**2012. 'Novel superparamagnetic iron oxide nanoparticles for tumor embolization application: preparation, characterization and double targeting'. *Int. J Pharm*. 426(1-2):248–255.

Chowdhury EH, M. Kunou , M. Nagaoka, AK Kundu, T. Hoshiba and **T. Akaike.**2004. 'Mammalian integrin-targeted gene delivery: A common approach for advanced viral and non-viral vectors'. *Gene. Ther. Mol. Biol*. 9:431-444.

Connor E.E., J. Mwamuka,A. Gole, C.J. Murphy and **M.D. Wyatt**. 2005. 'Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity'. *Small*. 1: 325 -7.

Cui K.H.,S. B. Peng, Y.Z. ZXing, C. G. Xu,S. B. Yu and **Q. Zhang**.2002. 'Molecular dissection of seedling-vigor and associated physiological traits in rice'.*Theorappl Genet*. 105:745-753.

Dey A.,R. Basu, S. Das and **P. Nandy** .2011. 'Study on the phytotoxicity of nano mullite and metal-amended nano mullite on mung bean plant'. *J. Ecotoxicol. Environ. Monit*. 13:1709-1715.

Doshi R., W. Braida, C. Christodoulatos, M. Wazne and **G. O'Connor** .2008. 'Nano-aluminum: transport through sand columns and environmental effects on plants and soil communities'. *Environ. Res*. 106:296-303.

Epple M., K. Ganesan, R. Heumann, J. Klesing, A. Kovtun, S. Neumannb and **V. Sokolova** .2010. 'Application of calcium phosphate nanoparticles in biomedicine'. *J. Mater. Chem*.20:18-23.

Fincher G.B. 1989.' Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains'. *Annu Rev Plant Physiol Plant Mol Biol*.40:305–345.

Ghosh M. , M. Bandyopadhyay and **A. Mukherjee** 2010. 'Genotoxicity of titanium dioxide nanoparticles at two trophic levels: Plant and human lymphocytes'. *Chemosphere*. 81: 1253–1262.

Guzman KAD, M.P.Finnegan and **J. F. Banfield** .2006. 'Influence of surface potential on aggregation and transport of titania nanoparticles'. *Environ. Sci. Technol*. 40:7688-7693.

Harrison BS and **A. Atala.** 2007.'Carbon nanotube applications for tissue engineering' *Biomaterials*.28: 344-353.

He Q, A.R. Mitchell, S. L. Johnson, C.W. Bartak T. Morcol and **S. J. Bell** .2002. 'Calcium phosphate nanoparticle adjuvant'. *Clin. Diagn. Lab. Immun*.9(5):1021-1024.

Holsapple M., W. Farland , T. Landry,N. Monteiro-Riviere, J. Carter, N. Walker and **K. Thomas** . 2005. 'Research strategies for safety evaluation of nanomaterials, part II: toxicological and safety evaluation of nanomaterials, current challenges and data needs'. *Toxicol. Sci*. 88:12-17.

Hu X., H. Shen, K. Shuai, E. Zhang,Y. Bai, Y.Cheng, X. Xiong, S. Wang, J. Fang and **S. Wei .** 2011. 'Surface bioactivity modification of titanium by CO_2 plasma treatment and induction of hydroxyapatite: In vitro and in vivo studies'. *Appl. Surf. Sci*.257:1813–1823.

ISTA. 1976. 'International Seed Testing Association'. *Seed. Sci. Technol*.4:51-177.

Lin K., J. Chang, R. Cheng and **M. Ruan**. 2007. 'Hydrothermal microemulsion synthesis of stoichiometric single crystal hydroxyapatite nanorods with mono-dispersion and narrow-size distribution'. *Mater Lett*. 61(8–9):1683–1687.

Lindberg H. K., G. C. Falck, S. Suhonen ,M. Vippola , E. , J. Catalán,K. Savolainen and **H. Norppa.** 2009. 'Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro'. *Toxicology Letters*, 186(3):166-73.

Ling D.H. and **B. S. Xing**. 2007. 'Phytotoxicity of nanoparticles: inhibition of seed germination and root elongation'. *Environ. Pollut*. 150:243-250.

Liong M., J. Lu, M. Kovochich , T. Xia , S.G. Ruehm, A.E. Nel, F. Tamanoi and **J.I.Zink** . 2008. 'Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery'.*ACS Nano*. 2:889-96.

Lu C.M.,C. Y. Zhang, J. Q. Wen, G.R.Wu and **M. X. Tao** .2002. 'Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism'. *Soyabean science*.21:168-172.

Mondal A., R. Basu, S. Das and **P. Nandy**. 2011. 'Beneficial role of carbon nano tubes on mustard plant growth-an agricultural prospect'. *J. Nanopart. Res*. 13(10):4519-4528.

Monica R.C. and **R. Cremonini**. 2009. 'Nanoparticles and higher plants'. *Caryologia*. 62: 161-165.

Nam E.J., E.J.Kim, A. W. Wark, S. Rho,H. Kim and **H. J. Lee.** 2012. 'Highly sensitive electrochemical detection of proteins using aptamer-coated gold nanoparticles and surface enzyme reactions'. Analyst, 137(9):2011-2016.

Naqvi S.A., N. Maitra, M. Z. Abdin, MD. Akmal, I. Arora and **MD. Samim**. 2012. 'Calcium phosphatenanoparticle mediated genetic transformation in plants'. *J. Mater. Chem* .22:3500-3507.

Navarro E., A. Baun, R. Behra, N. B. Hartmann ,J. Filser ,A. J. Miao ,A. Quigg ,P.H. Santschi and **L. Sigg .** 2008. 'Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants and fungi'. *Ecotoxicology*, 17(5):372-386.

Nel A., T. Xia T,L. Mädler and **N. Li.** 2006. 'Toxic potential of materials at the nanolevel'. *Science,* 311:622-627.

Nietzold C. and **F. Lisdat** . 2012. 'Fast protein detection using absorption properties of gold nanoparticles'. *Analyst*, 137:2821.

Oberdorster G., A. Maynard and **K. Donaldson** . 2005. 'Principles for characterizing the potential human health effects from exposure to nonmaterial: elements of a screening strategy' .*Particle Fibre Toxicol*.6, 2:8.

Panda R.N., M. F. Hsieh, R. J. Chung and **T. S. Chin** . 2003. 'FTIR, XRD, SEM and solid state NMR investigations of carbonate-containing hydroxyapatite nano-particles synthesized by hydroxide-gel technique'. *J. Phys. Chem. Solids*, 64:193–199.

Panyam J. and **V. Labhasetwar**. 2003. 'Biodegradable nanoparticles for drug and gene delivery to cells and tissue'. *ADV. DRUG DELIVER REV*. 55: 329-347.

Peng X.H., X. Qian, H. Mao , A. Y. Wang , Z. Chen, S. Nie and **D. M. Shin** . 2008. 'Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy'. *Int. J. Nanomedicine*, 3:311-321.

Poland C.A. ,R. Duffin ,I. Kinloch , A. Maynard, W.A.H. Wallace , A. Seaton ,V. Stone , S. Brown , W. MacNee and **K.Donaldson** . 2008. 'Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study'. *Nature Nanotechnology*, 3:423-428.

Racuciu M. and **D. E. Creanga**. 2007. 'TMA-OH coated magnetic nanoparticles internalized in vegetal tissue'. R*omanian Journal of Physics*.52:395-402.

Reischl D. and **A. Zimmer**. 2009. ' Drug delivery of siRNA therapeutics: potentials and limits of nanosystems'. *Nanomedicine*, 5:8-20.

Rosenberg S.J., S. A. Loening, C. E. Hawtrey, A. S. Narayana and **D. A. Culp** . 1985. 'Radical prostatectomy with adjuvant radioactive gold for prostatic cancer: a preliminary report'. *J Urol*.133:225-227.

Roy I., S. Mitra, A. Maitra and **S. Mozumdar** . 2003.'Calcium phosphate nanoparticles as novel non-viral vectors for targeted gene delivery.' *International Int. J. Pharm*. 250: 25-33.

Shubayev V. , T. R. Pisanic and **S. Jin**. 2009. 'Magnetic nanoparticles for theragnostics'. *Adv. Drug. Deliv.Rev*. 61(6):467-77.

Chernousova , S. , J. Klesing , N. Soklakova and **M. Epple**. 2013. 'A genetically active nano-calcium phosphate paste for bone substitution, encoding the formation of BMP-7 and VEGF-A. *RSC Adv***.** 3:11155-11161.

Tam, NFY and **S. M. Tiquia**. 1994. 'Assessing toxicity of spent litter using germination technique'. *Resour. Conserv.Recycl*. 11:261-274.

Trommer R. M., L. A. Santos and **C. P. Bergmann**.2009.Nanostructured hydroxyapat ite powders produced by a flame-based technique. *Mater. Sci. Eng. C.* 29(6):1770–1775.

Warheit D.B. 2008. 'Nanoparticles: health impacts? *'Mater Today* .7:32-35.

Xue Q, L. Wang and **W. Jiang**. 2012. 'A versatile platform for highly sensitive detection of protein: DNA enriching magnetic nanoparticles based rolling circle amplification immunoassay'. *Chem. Commun*. 48:3930-3932.

Zanello L.P., B. Zhao, H. Hu and **R. C. Haddon**. 2006. 'Bone Cell Proliferation on Carbon Nanotubes'. *Nano letter*, 6:562-567.

Zhao Y.,Y. Zhang , F. Ning , D. Guo and **Z. Xu .** 2007. 'Synthesis and cellular biocompatibility of two kinds of HAP with different nanocrystal morphology'. *J Biomed. Mater Res. B. Appl. Biomater*. 83(1):121-6.

Zheng L., F. S. Hong,S. P. Lu and **C. Liu** . 2005. 'Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach'. *Biol. Trace Elem Res.* 104: 83-91.