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Phytotoxicity effect of Silver nanoparticles on *Oryza sativa.*

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Abstract: Phytotoxicity on *Oryza sativa* was studied by directly exposing it to silver nanoparticles solutions. Seedlings were allowed to grow at room temperature in Hoagland's nutrient solutions prior to treatment. Particles were prepared in distilled water by Chemical reductions method. U.V-Vis spectra analysis confirms its formations at 400 nm wavelength. 12 days of phytotoxicity periods in 1000µg/ml concentrations of silver nanoparticles leads to adverse effects on plant species. Analysis of particles was initially determined by XRD spectrophotometer and was found to be 25 nm in size. FTIR spectrophotometer results shows that there is change in intensity and shifts in positions of peak in treated roots as compared to control sample. Slight variations in peaks and its intensity of *Oryza sativa* roots confirm involvement of functional group such as carboxyl, Amine, hydroxyl etc in nanoparticle binding. TEM images revealed that various particle sizes deposited inside the root cells. Interestingly, it was found that during penetrations of particles inside the roots cell, they damaged the cell wall as well as vacuoles to enter. It may be due to the penetrations of large particles entering through small pores of cell walls. This study directly tells the relevant of effects of silver nanoparticles inside cellular mechanisms during phytotoxicity periods.

Key words: Nanoparticles, Phytotoxicity, XRD (X-Ray Diffractions) spectrophotometer,TEM (Transmission Electron Microscope), FTIR (Fourier Transform Infra Red) spectrophotometer.

Introductions:

Engineered nanoparticles have three different unique characteristics - Size, structure and properties. These nanoparticles were widely used by mankind in different applications. Silver particles widely used in antibacterial activity, cosmetics industry etc. Nanoparticles released into environmental by many different ways which may can be intentional or accidental resulting in toxic effects to organisms. Silver nanoparticle of 40 nm sizes causes toxic effects in *Chlamydomonas reinhardtii* algae [1, 2]. Effects on *Cucurbita pepo* growth was also inhibited by the same nanoparticles [3]. Studies of effect of silver nanoparticles on various organisms were very limited. Toxicity to various organisms depends on nature of particles, sizes, concentrations, exposure time. Research of uptake, toxicity and effects of nanoparticles in higher plant needs quick attentions [4]. However there were some studied regarding toxic effects of nanoparticles in plants. *Lemna minor* L. was studied for phytotoxicity effects with low concentrations of nanoparticles having different particle sizes (20 nm and 100 nm). Effect was more as exposure time increases resulting significant inhibitions [5]. Effects of silver nanoparticles results reductions in biomass and transpirations rate than bulk silver. The toxicity effect on *Cucurbita pepo* was more prevalent in nanoparticles than bulk silver solutions (4.4 to 10 times more) [6]. Phytotoxicity on the development of *Arabidopsis thaliana* by four different metal oxide nanoparticles namely, aluminum oxide (nAl2O3), silicon dioxide (nSiO2), magnetite (nFe3O4) and zinc oxide (nZnO). Toxic effects by ZnO were more in seed germinations, root elongations and number of leaves than other particles [7]. In our present studies, we have synthesized silver nanoparticles by chemical reductions method. Effect

on *Oryza sativa* seedling by nanoparticles during Phytotoxicity periods was observed by TEM, FTIR and XRD analysis. XRD and TEM explain depositions of nanoparticles inside cells. Breakage in cell wall and vacuole was observed under TEM explains intracellular depositions and its toxicity in plant cells.

2. Materials and Methods

2.1 *Synthesis of Silver nanoparticle solutions*:
All chemicals used in the experiments were analytical grade and was purchased from Merck Ltd. Nanoparticles were synthesized by chemical reductions method. Preparations of nanoparticles solutions were done by slight modifications from the standard method [8, 9] where Silver nitrate was reduced by Sodium borohydride in distilled water. Tween-20 was added to prevent aggregations of nanoparticles.

2.2 *Seedling growth:*
Oryza sativa, Variety-Jaya was selected as model crop plants for our phytotoxicity studies Seeds were collected from Assam seed corporations, Assam, India. The seeds were allowed to germinate in moist conditions for 2 weeks in dark conditions. Healthy and uniform seedlings were selected and allowed to grow in Hoagland's nutrient solutions for 1 week. Finally the seedlings were exposed to different concentrations of nanoparticles solutions at different time intervals (Phytotoxicity period).

2.3 *XRD analysis:*
X-ray diffractions (Model-XPERT PRO) were used for analysis. Nanoparticles treated roots were washed in double distilled water for 3-4 times and dried at 80°C for 72 hours. A gram of finely powdered sample is thoroughly mixed in mortar and pestle to get a fine homogenous mixture. The samples were prepared and put uniformly on a glass slide.

2.5 *TEM observation:*
Effect of nanoparticles on *Oryza sativa* roots at the end of Phytotoxicity period was studied by TEM analysis. The samples were prepared by standard protocol used at RSIC, Shillong (Model- JEOL JSM 100 CX). Samples was fixed in 1% Glutaraldehde than washed in 0.1 M buffer, 1% Osmium tetraoxide was used for post-fixations and again washed with 0.1 M buffer. The samples were dehydrated in acetone, infiltrated and embedded in epoxy resin.

2.6 *FTIR measurements*:
FTIR spectra for both Control and particle treated roots were obtained by KBr pellets methods operated on FTIR spectrophotometer (Model- Brucker, Vector 22) to investigate the functional groups present in the root of *Oryza sativa* and to look into possible binding sites with nanoparticles.

3. Results

3.1 *Formations of Nanoparticles*
Reducing agent Sodium borohydride reduces Silver ions into nanoparticles, assuming total conversions. Tween-20 has the property to prevent aggregations of particles and hence used as surfactant. Our preliminary results shows that 0.1% of Tween-20 donot show any significant effect on seed germinations. 1000 µl of 10^{-3} M of Silver nitrate aqueous solutions was reduced by 500 µl of 6.6 X 10^{-3} M Sodium borohydride aqueous solutions in distilled water which was heated upto the boiling temperature followed by vigorous shaking [10]. Appearance of yellow colour and spectral peaks at 400 nm shows formations of Nanoparticles. TEM images of root cells revealed its spherical shape and average size of 20 nm in diameter.

3.2 *Growth conditions during Phytotoxicity periods*:
Silver nanoparticles of three different concentrations 50µg/ml, 500µg/ml and 1000µg/ml were selected for studies. However we will mainly focus on the effects of higher concentrations throughout the study. Initially surface sterilizations of seeds were done with Sodium hypochlorite solutions. Seeds were allowed to germinate in moist conditions for 1 week. Healthy and uniform seedlings were allowed to grow at Hoagland's nutrient solutions for 1 week before beginning of Phytotoxicity periods. The toxicity periods of particles exposed to seedlings were continuing till 12 Days alongwith control. Seedling was allowed to grow in nutrient solutions with additions of different concentrations of particles at 25 ± 5ºC. To prevent aggregations it was stirred with glass rod after every 12 hours.

3.3 *XRD analysis*
XRD analysis of Treated roots (1000 µg/ml) at the end of Phytotoxicity periods was selected for study. Roots were uniformly crushed in mortar and pestle after drying at 80ºC for 72 hours. A fine homogenous powder was put on a glass slide. XRD patterns revealed that the particle is Face Centre Cubic lattice structure (111). The Average particle size can be calculated by using Scherrer's relation: $D = K\lambda / \beta \cos\theta$. After calculating, we found that the Average particle sizes are around 25 nm.

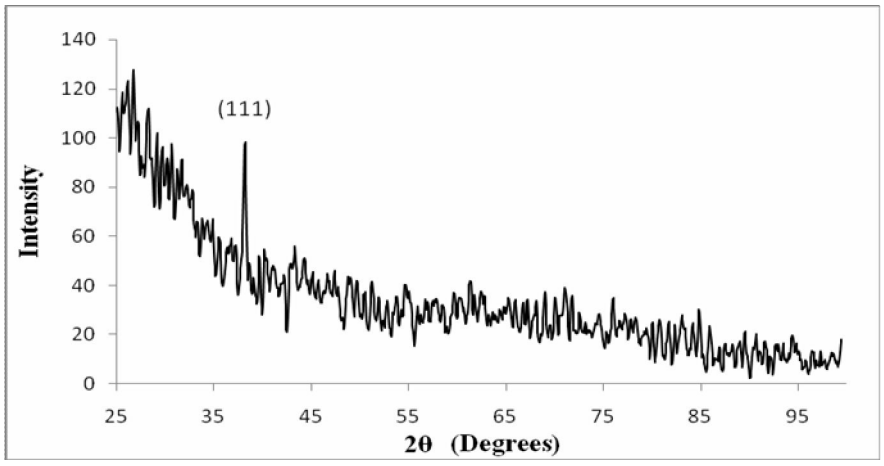


Figure 1: XRD patterns of Silver nanoparticles on *Oryza sativa* roots at the end of phytotoxicity periods.

3.4 *FTIR study:*

FTIR analysis was carried out to find out the possible biomolecules interactions with nanoparticles in case of both treated (1000µg/ml) and untreated root samples. The FTIR spectra of both untreated and treated roots with silver nanoparticles obtain absorptions peaks located in the regions 400-3900 cm^{-1}. The absorptions peak at around 3370.95 cm^{-1} and 3365.51 cm^{-1} can be assigned as absorptions peak of – OH or – NH_2 in both Control and treated roots. Wavelength of 2950.55 cm^{-1} ,2962.22 cm^{-1} ,2368.17 cm^{-1} and 2348.08 cm^{-1} show peaks assigned to – COOH group in both types of treatment. Moreover wavelength of 1626.19 cm^{-1} and 1636.87 cm^{-1} represent –NH_2 group having primary structure of protein while absorptions peak of 1066.58 cm^{-1} and 1078.45 cm^{-1} wavelength represents – C-O or – C-C functional group. 686.89 cm^{-1} and 672.13 cm^{-1} wavelength of both treatments represented Aromatic ring.

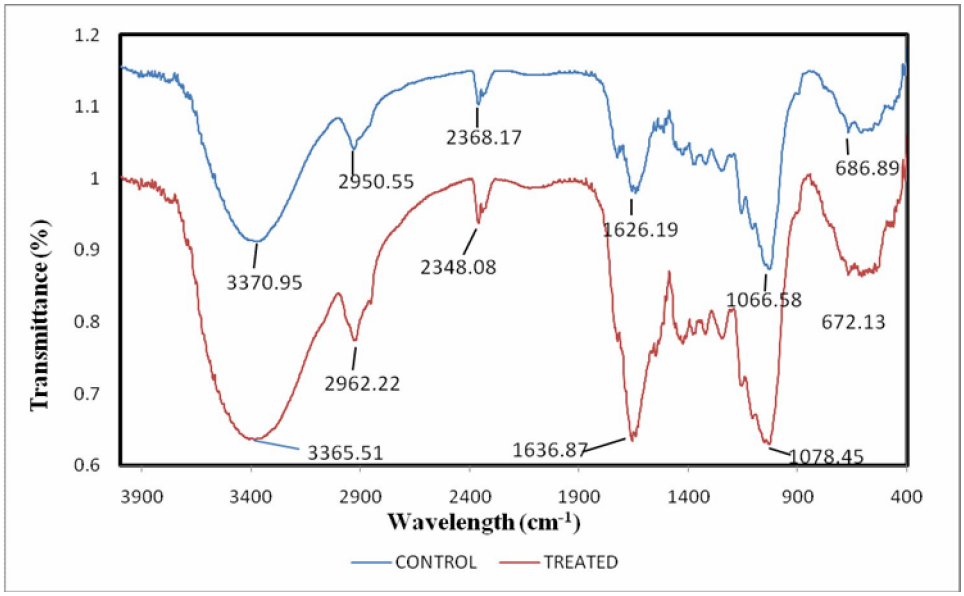
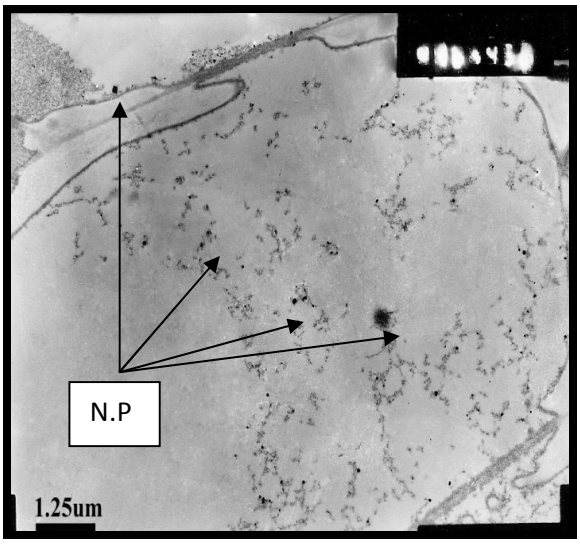


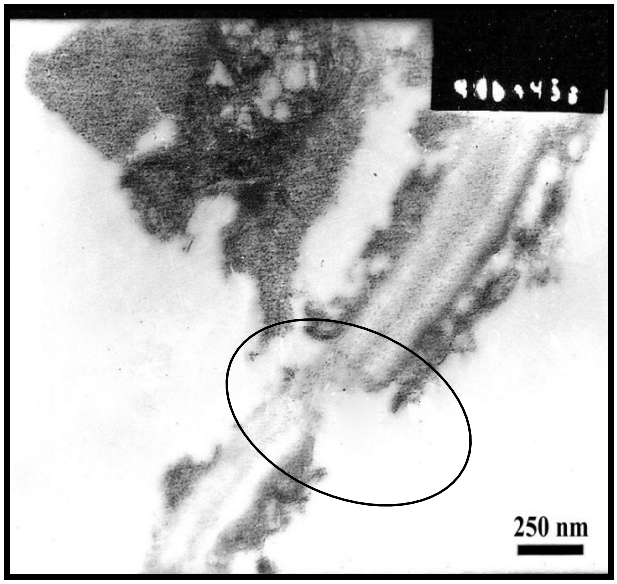
Figure 2: FTIR spectra of *Oryza sativa* - Untreated and Treated roots with Silver nanoparticles.

3.5 *TEM micrographs:*

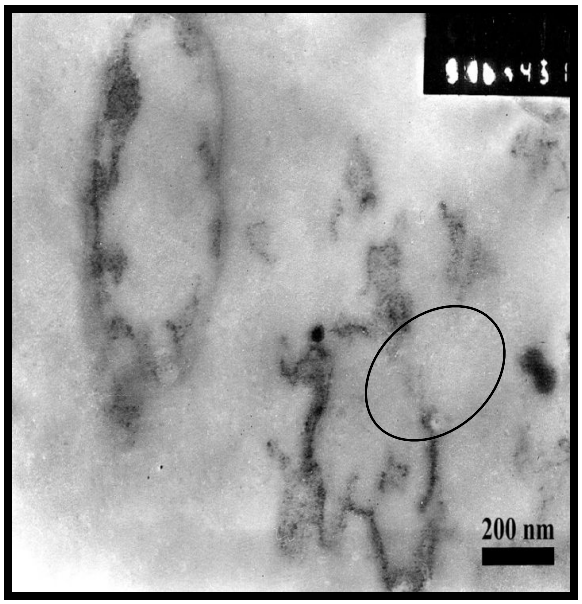
TEM analysis reported depositions of silver nanoparticles inside the root cells. Uptake of nanoparticles (1000µg/ml) was observed under TEM at the end of Phytotoxicity periods. Figure 3 (A) clearly shows Cross sectional view of *Oryza sativa* root cells indicates depositions of particles (dark dots) inside the cells. Nanoparticles of different sizes were found inside the cell however average size of 25 nm were predominantly recorded. Interestingly, these small particles had caused adverse effect on damage to external and internal portions of cells. Figure 3 (B) shows breakage in cell wall of the roots. There may be penetrations of particles by breaking the cell walls. If we observed the figure 3 (C), damaged in vacuoles have been also reported. The entry of particles is clearly seen by breaking the vacuoles. Accumulations of nanoparticles and its toxic effects on test plant species is totally depend upon the concentrations and exposure time.



A



B



C

Figure 3: TEM images of Oryza sativa roots at the end of Phytotoxicity period by Silver nanoparticles (1000µg/ml) - (A) Whole cell, (B) Cell breakage in the cell wall and (C) Damage in the vacuoles inside the cell.

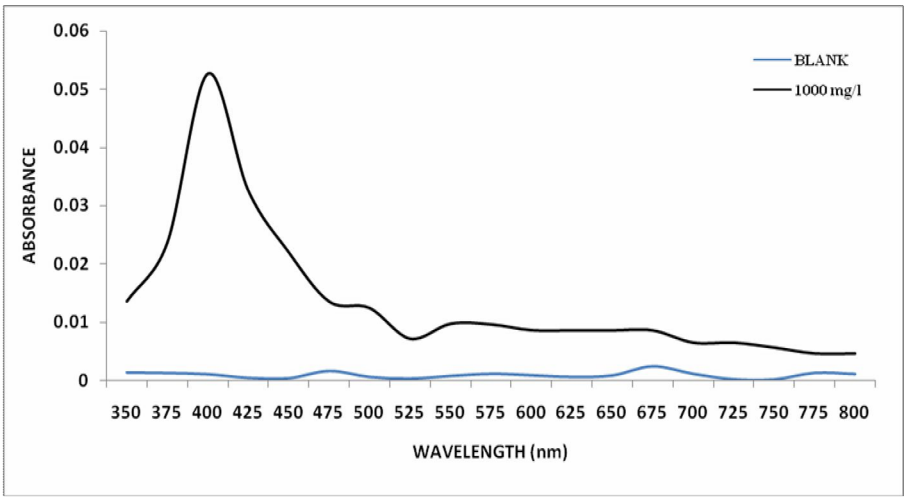


Figure 4: UV-Visible absorptions spectrum of silver nanoparticles immediately after preparations.

Discussions:

Phytotoxicity studies of nanoparticles in this report are mainly restricted to highest concentrations (1000µg/ml). Synthesis of silver nanoparticles was done by chemical reductions methods. The preparations of nanoparticles were done in distilled water. Formations of particles were confirmed by U.V-Vis absorptions spectra at 400 nm (Figure 4). The absorptions spectra are due to Plasmon excitations of particles [11]. Distributions and particle sizes were mainly depending upon spectral analysis [10]. Engineered nanoparticles were always exposed to ecosystem as they were easily released into the surrounding environment. Small particle size nanoparticles may become more toxic to plants. The phytotoxicity of *Oryza sativa* was lasted for 12 days. XRD analysis of treated roots at the end of the toxicity period shows Bragg's reflections (111) may index on the basis of Face Centre Cubic structure of silver. This reveal out the depositions of particles inside the cells. The diffractions pattern of nanoparticles deposited in roots are shown in Figure 1.

The XRD patterns clearly shows particles are crystalline in nature. The size of particles penetrates can be easily calculated from the graph (2θ=38.13). After calculating, the average particles found to be 25nm in size. However, there are limitations in these techniques that the particles may not be uniformly distributed in the sample.

Various functional groups have been identified such as Carboxyl, hydroxyl, amine, carbonyl etc. The FTIR spectra shows absorptions spectra in both cases (treated and untreated) were in the regions of 400 cm^{-1} - 3900 cm^{-1}. In case of Control, absorptions peak at 3370.95 cm^{-1} wavelength characteristics of symmetrical stretch vibrations of – OH and – NH_2 functional groups. – COOH group characteristics were observed at 2950.55 cm^{-1} and 2368.17 cm^{-1} wavelength describing Antisymmetric stretch due to acid dimers. Wavelength of 1626.19 cm^{-1} shows presence of –NH_2 group which describes a bend in absorptions peak due to primary structure of protein. The distinct peak observed at 1066.58 cm^{-1} explains Antisymmetical stretch of – C-O or – C-C functional groups. The bend absorptions peak at 686.89 cm^{-1} wavelength describes presence of Aromatic ring. However changes in intensity and shift in positions of the peaks in case of treated sample was observed. The shifting of peaks suggests involvements of Carboxyl, hydroxyl, amine, carbonyl etc functional groups in binding of Silver nanoparticle to the root cells. The adsorptions of nanoparticles on *Oryza sativa* roots is may be due to the mechanisms of complexations with functional groups, physical adsorptions, chemical reactions with surface sites, ion exchange and surface precipitations [12,13]. Uptake and translocations of carbon nanoparticles was also reported in *Oryza sativa* [14]. They found that fullerene C70 can easily taken by roots and transport to shoots. However if C70 particles is entered through leaves and it moves to downwards with the help of phloem [14]. One important hypothesis was established regarding transportations of smaller particles inside the

cells. Cell walls thickness of about 5 to 20 nm functions as natural sieves which transports small particles passes through large pores to enter in the protoplasm. Eventually, same authors had also admitted that new and large pores were created for passaging of larger particles [1, 2]. Breakage of cell wall and vacuoles in root cells of test species described the toxicity of particles. Depositions of silver nanoparticles inside the root cell by smaller particles have also been reported in our study (Figure 3A). Different particle sizes inside was observed under TEM. Presence of variable size and compositions was also reported in intracellular portions of various plants species [14]. One of the pathways was also reported where particle size of 20 nm silver nanoparticles may be transported inside the cells through plasmadesmata [15]. Particles must be entered through cell wall and plasma membrane of root cells. Xylem is one of the main passages of uptake and transportations to shoot and leaves of plant. Pores size of cell wall was in the range of 3-8 nm, smaller than engineered nanoparticles[16]. Damaged in intracellular level may be due to surface area or size of particles interacts with plant roots. However more study is required to know the uptake mechanisms and toxicity of particles in intracellular damages.

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References

1. Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.J., Quigg, A., Santschi, P.H., Sigg, L. Environmental behaviour and ecotoxicology of engineered nanoparticles to algae, plant and fungi , 2008a 17, 372-386.
2. Navarro, E., Piccipetra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L., Behra, R.,. Toxicity of silver nanoparticles to Chlamydomonas reinhardtii, Environmental Science and Technology, 2008b, 42, 8959-8964.
3. Stampoulis, D., Sinha, S.K., White, J.C. Assay-dependent phytotoxicity of nanoparticles to plants. Environmental Science and Technology, 2009, 43, 9473-9479.
4. Characterising the Potential Risks Posed by Engineered Nanoparticles. 2007, Department for Environment and Rural Affairs, London, UK.
5. Eva, J. G., Lesley, C. B and Jamie, R. L, Phytotoxicity of silver nanoparticles to Lemna minor L, Environmental Pollution, 2011, Article in press.
6. Craig, M and Jason, C. W., Toxicity of Silver and Copper to Cucurbita pepo: Differential Effects of Nano and Bulk-Size Particles, 2011, Environ Toxicol , 26: n/a. doi: 10.1002/tox.20667
7. Chang, W. L., Shaily, M., Katherine, Z., Dong, Li., Yu-Chang, T., Janet, B and Pedro, J.J. A., Developmental phytotoxicity of metal oxide nanoparticles to Arabidopsis thaliana, Environ. Toxicol. Chem. 2010; 29:669–675
8. Li, X., Zhang, J., Xu, W., Jia, H., Wang, X., Yang, B., Zhao, B., Li, B and Ozaki, Y. Mercaptoacetic Acid-Capped Silver Nanoparticles Colloid: Formation, Morphology, and SERS Activity. Langmuir, 2003, 19, 4285-4290.
9. Gogoi, S.K., Gopinath, P., Paul, A., Ramesh, A., Ghosh, S.S and Chattopadhyay, A., Green Fluorescent protein-Expressing Escherichia coli as a model system for investigating the Antimicrobial Activities of Silver Nanoparticles. Langmuir, 2006, 22, 9322-9328.
10. Khanna, P.K., Singh, N., Kulkarni, D., Deshmukh, S., Charan, S and Adhyapak, P.V., Water based simple synthesis of redispersable silver nano-particles. Materials Letters, 2007, 61, 3366-3370.
11. Bae, C.H., Nam, S.M and Park, S.M., Formation of silver nanoparticles by laser ablation of a silver target in NaCl solution. Applied Surface Science, 2002, 197, 628e634.
12. Gupta, V.K and Rastogi, A., Biosorption of lead from aqueous solution by green algae Spirogyra species: kinetic and equilibrium studies, J. Hazard. Mater. 2008, 152 (1), 407–414.
13. Srividya, K and Mohanty, K. Biosorption of hexavalent chromium from aqueous solutions by Catla catla scale: equilibrium and kinetics studies, Chem. Eng. J. 2009, 155, 666–673.

14. Lin, S., Reppert, J., Hu, Q., Hunson, J.S., Reid, M.L and Ratnikova, T. Uptake, translocation and transmission of carbon nanomaterials in rice plants. Small, 2009, 1128–32.
15. Ma, X., Lee, J.G., Deng, Y and Kolmakov, A., Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation, Science of the Total Environment, 2010, Article in press.
16. Carpita, N.C and Gibeaut, D.M. Structural models of primary cell walls in flowering plants: consistency of molecular-structure with the physical properties of walls during nanoparticles and their short and long term dynamics inside the growth. Plant J, 1993, 3, 1-30.

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