UNIVERSITY OF DELHI

INNOVATION PROJECTS 2015-16

FINAL REPORT

1. **PROJECT CODE:** MH-310

2. **PROJECT TITLE**: Sugarcane to Nanoparticles: Green Nanotechnology the future(MH-310)

3. **NAME OF COLLEGE/INSTITUTION:**Miranda House

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Certificate of Originality

This is to certify that the research work carried out and the final report submitted by the Project Investigators and the students of Innovation Project having Project code MH-310 and title Sugarcane to Nanoparticles: Green Nanotechnology the future (MH-310) of Miranda House is original. Any plagiarism/academic dishonesty reported at any stage will be our responsibility.

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Utilization Certificate

Innovation Project 2015-16

Project Code MH-310

Project Title: **Sugarcane to Nanoparticles: Green Nanotechnology the Future**

Audited Financial Statement under Innovation Project scheme

College: Miranda House

Project Investigators: Dr. Saloni Bahri, Dr. Smirit Sharma Bhatia & Dr. Kalawati Saini

Grant sanctioned Rs. 5, 00, 000/- (Five lakhs only)					
Grant released 3,50,000/- + 60, 000/- = 4,10,000/- (Four Lakhs Ten Thousand Only)					
S. No.	Budget Head	Amount Sanctioned	Released Amount	Total Amount Utilized	Amount Remaining
1.	Equipment/Consumable	2,25,000/-	2,23,000/-	2,22,968/-	32/-
2.	Travel	55,000/-	27,500/-	27,500/-	Nil
3.	Stipend	1,20,000/-	1,20,000/-	1,20,000/-	Nil
4.	Honorarium	25, 000/-	2,000/-	2,000/-	Nil
5.	Stationery	20,000/-	10,000/-	10,000/-	Nil
6.	Contingency	55,000/-	27,500/-	27,500/-	Nil
Total Amount Utilized		**Rs. 4, 09, 968/-** (Four Lakh Nine Thousand Nine Hundred Sixty Eight Only)			
Amount Remaining		**Rs. 32/-** (Thirty Two Only)			

Certified that out of **Rs. 4, 10,000/-** (Four Lakhs Ten Thousand Only) Sanctioned to Innovation Project Code **MH-310 Rs. 4, 09, 968/-** has been utilized During the period of the project. The remaining amount Rs. **Rs. 32/-** (Thirty Two Only) is being returned back to the University.

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04-11-2016

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Signature of Principal

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Final Report

1. **Project Title: Sugarcane to Nanoparticles: Green Nanotechnology the future**

2. **Project Code:** MH-310

3. **Abstract:**

Our project aims at the study of the effect of copper nanoparticles on the growth of two economically important plants namely: *Sorghum bicolor*(L) Moench (Poaceae) and *Vignaradiata*(L.) R.Wilczek(mung bean, Fabaceae) under *in vitro* conditions.The copper nanoparticles wereprepared using different reducing agents (hydrazine, sucrose, fructose and sugarcane juice). The project questions the idea of the use of nanoparticles and in what quantities that may enhance the growth of the plant as they play an important role in plant metabolism and its incorporation may boost growth in various ways, which in future may prove to be helpful.

Our observations encompass the measurement of the length of the shoot and the root, number of leaves,root laterals andnature and colour of the leaves at 10, 20, 50 and 100 ppm nanoparticle concentration added to MS(Murashige and Skoog's) nutrient medium. MS basal(without nanoparticles) was the control medium. The results were compiled and the average was calculated on the basis of the visible effects at an interval of 3 days. The experiment was terminated after 10 days. *In vitro* raised plants were transferred to pots and stem sections were also cut to observe any change in the cell shape and contents anatomically.

Copper and Copper oxides Nanoparticles were synthesized.Characterizations of synthesized nanoparticles was done by UV-Visible Spectroscopy and PXRD. Effect of these synthesized Nanoparticles on growth of plants was then studied. Synthesis of copper nanoparticles in a one-phase, in water system was investigated. The Copper nanoparticles (NPs) were synthesized by chemical reduction and green method. The Copper sulphate (10mM $CuSO_4.5H_2O$) was used as a source of copper metal ions (Cu^{+2}) in each set-up. Subsequently, the metal cations were reduced using Tri-sodium Citrate (TSC) in the presence of 1 ml of Hydrazine at 373K. The green synthesis of Copper NPs was done using Fructose, Ascorbic acid (Lemon juice) and sugarcane juice. The Copper oxides(Cu_2O,CuO) were obtained with copper NPs due to the high tendency of copper to oxidize in air. The crystal structure of the particles was found to be FCC with help of Powder X-Ray Diffraction (PXRD). The expected Plasmon resonance peaks for Cu 510-531 nm, Cu2O 418-492 nm and CuO 700-790 nm was found from UV/Vis spectroscopy analysis.

4. **Introduction**: Nanoparticles of varying sizes and shapes can be synthesized by a variety of chemical and physical methods [1]. But according to many studies, these production methods are expensive, labor-intensive, and are harmfulto human health and environment[2]. This has led to the need to explore alternatives which aresafe and cost-effective [3]. One alternative is plant extract that can convert metal ions into metal nanoparticles. One of the important question raised is why not whole plant, why plant extracts? This is because the heterogeneity of the size

and morphology of nanoparticles produced in whole plants may hinder their use in applications where specific, finely tuned sizes and shapes are required. Also, extraction, isolation and purification of nanoparticles from plant material is a difficult and problematic procedure, with a low yield. Thus, *in vitro*approaches are better as they provide control over the size and shape of the nanoparticles. This is possible as purification is easy and medium's pH and reaction temperature can be changed externally. There have been various studies using extracts from a variety of different plant such as copper, gold, silver, platinum, iron, and many others [4, 5]. Green synthesis is advantageous in terms of cost because it greatly reduces the cost of reducing agent. When using chemical synthesis, the prime cost of nanoparticles is mainly determined by the cost of the metal salts and reducing agents. In the case of "green" synthesis, the bulk of the costs will be determined only by the cost of the metal salts, as extracts from plant wastes can serve as reducing agents.

5. Research problem/hypothesis/objectives

- Synthesis of Copper and Copper oxides Nanoparticles
- Characterizations by UV-Visible Spectroscopy and PXRD.
- Effect of these synthesized Nanoparticles on growth of plants under *in vitro* conditions.

6. Methodology Techniques/Sampling /Tools/Materials

(i) Requirements:

Glassware: Conical flask, beakers, measuring cylinders, test tubes, culture tubes, volumetric flask, cuvette, pipettes, stirrer, droppers, funnel.

Plant Material:*Vigna radiata* and *Sorghum bicolor* seeds, sugarcane juice

Chemicals:$CuSO_4$solution, hydrazine, sodium tricitrate, fructose, NaOH solution, ascorbic acid(lemon juice), Major solution Elements (NH_4NO_3, KNO_3, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, KH_2PO_4), Minor Elements (H_3BO3, $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, KI, $Na_2MoO4.2H_2O$), Iron ($FeSO_4.7H2O$, $Na_2EDTA.2H_2O$),Vitamins(Inositol, NicotinicAcid, Pyridoxine HCl, Thiamine HCl,Glycine), agar, Mercuric Chloride($HgCl_2$), sucrose.Tri-sodium citrate of A.R. grade, fructose,NaOH and Ascorbic acid were used to synthesize the copper NPs. The fresh juice of Lemon (Ascorbic acid) and Sugarcane were used for green synthesis of copper NPs.All the solutions were prepared in double de-ionized water.

Instruments/Machines: Autoclave, Microwave, laminar air flow cabinet, pHMeter.

Miscellaneous: Test tube stand, cotton plugs, aluminium foil, filter paper, butter paper, syringes, Millipore filters, basic geometry.

(ii) Composition of MS medium:

MAJOR ELEMENTS	Amount in g(dissolve in 500 ml)
Ammonium nitrate	1.650
Potassium nitrate	1.900
Calcium chloride dehydrate	0.440
Magnesium sulphate heptahydrate	0.370
Potassium hydrogen phosphate	0.170
MINOR ELEMENTS	
Boric acid	0.062
Manganese sulphate tetrahydrate	0.223
Zinc sulphate heptahydrate	0.086
Potassium iodide	0.008
Sodium molybdatedihydrate	0.002
IRON	
Ferrous sulphate heptahydrate	0.278
Disodium EDTA	0.373
VITAMINS	
Inosital	1.000
Nicotinic acid	0.005
Pyridoxine HCl	0.005
Thiamine HCl	0.001
Glycine	0.200

For preparing IL of solution, add 500ml MAJOR + 50 mL MINOR + 50 mL IRON + 50 mL VITAMINS = 650 mL and DISTILLEDWATER = 350 mL

(iii) Medium Preparation:

- MS (Murashige and Skoog) full strength medium was prepared by mixing requisite amount of macronutrients, micronutrients, vitamins and iron according to the table given above.
- Before that stock solutions of the different components were made in distilled water and stored in refrigerator. For *Sorghum bicolor* and *Vigna radiata* semi-solid MS medium was used.
- For making 1 Lof MS medium, weighed 30g of sucrose and transferred it into a conical flask.
- To it, added 8g agar (0.8%).Add 300ml of distilled water and heated it for2 minutes in the microwave to melt the agar.

- Then, added the components of the MS medium i.e. 500ml major stocksolution, 50ml minor stock solution, 50ml vitamins stock solution and50ml iron stock solution.
- Adjusted the pH of the medium to 5.8 using 0.1N HCl and 0.1N NaOH.
- Made the final volume upto 1L.
- MS basal medium (without nanoparticles) was autoclaved at 15 pounds percubic cm at 121^0Cfor 15 minutes.
- Nanoparticles were added to the autoclaved MS basal medium throughMillipore filter assembly in laminar flow cabinet.
- Poured the solution of nanoparticles of different concentration (10ppm,20ppm, 50ppm and 100ppm) in the culture tubes and kept overnight for solidification.

(iii) Sterilization of seeds:

- The seeds of *Vigna radiata* and *Sorghum bicolor* were washed properly under running tap water for about 10 minutes and then washed 3-4 times carefully with detergent.The washed seeds were transferred into a clean beaker.
- The seeds were then treated with 0.8% Mercuric Chloride ($HgCl_2$) solution. The solution was prepared with autoclaved distilled water and final washing was also given with sterilized distilled water.

(iv) Inoculation:

- Soon after washing the seeds, the inoculation was done in a Laminar flow chamber.Before transferring the seeds into the culture tubes containing the medium, the working table inside the chamber had to be cleaned properly with Rectified Spirit (The working hands must be clean to prevent any contamination of the culture tubes during inoculation).
- The Spirit lamp was lit.The mouth of the culture tube was heated for a few seconds and unplugged.Asterilized pair of forceps was used to transfer two seeds in each culture tube, ensuring that the forceps remained contaminant free throughout the process.The rim of the tube was heated over the Spirit lamp and the mouth of the tube was covered back with a cotton plug.This process was repeated carefully for 24 tubes containing the medium.
- Atotal of 5 racks (having 24 culture tubes each) containing medium MS- Basal, 10ppm, 20ppm, 50ppm and 100ppm respectively were inoculated following the same procedure.The samples were then kept for observation over a period of 10 days.

(v) Transfer of *in vitro* raised plants to soil: The plants of*Sorghum bicolor* and *Vigna radiata* were transferred to the earthen pots containing vermiculite.

(vi) Preparation of Copper nanoparticles (Chemical Reduction):

Tri-sodium citrate (TSC) capped copper nanostructures (Cu and Cu_2O NPs) were synthesized via chemical reduction method that can be divided into three sets. The concentration of copper sulphate was kept 10mM in each case and each experiment carried out at 373 K temperature .The concentration of TSC was 25.5 mM in case of Set-1 and the concentration of Ascorbic acid 200 mM in case of set-2 and the concentration of Fructose 100 mM , 200 mM Ascorbic acid in presence of 1g NaOH in case of Set-3. The volume of solutions was kept 100 mL in each set-up. The Copper NPs using a hot plate equipped with a constant magnetic stirring. The solution turns brownish in color after ca. 30 minutes indicating the formation of Cu/Cu_2O NPs in the electrolyte solution.

(vii) Preparation of Copper nanoparticles (Green Synthesis):

The fresh juice of sugarcane 2ml and juice of Lemon 5 ml was used instead of TSC, Fructose and Ascorbic acid as reducing as well as capping agent. The rest of all the parameters are kept same as used in chemical reduction method.The synthesis of Copper NPs with sugarcane was done in presence of 1g NaOH and also without NaOH.

(viii) Material Characterization:

The UV-visible absorption measurements were recorded with UV-vis spectrophotometer at a resolution of 1 nm. X-ray diffraction (XRD) measurements of the dried powder have been done by filling it in to the groove of quartz glass sample holder by using a Bruker D8 Advance diffractometer with Ni-filtered CuKα radiation. The data was collected in the 2θ range of 0–100^o with a step size of 0.02 and a step time of 1*s*.

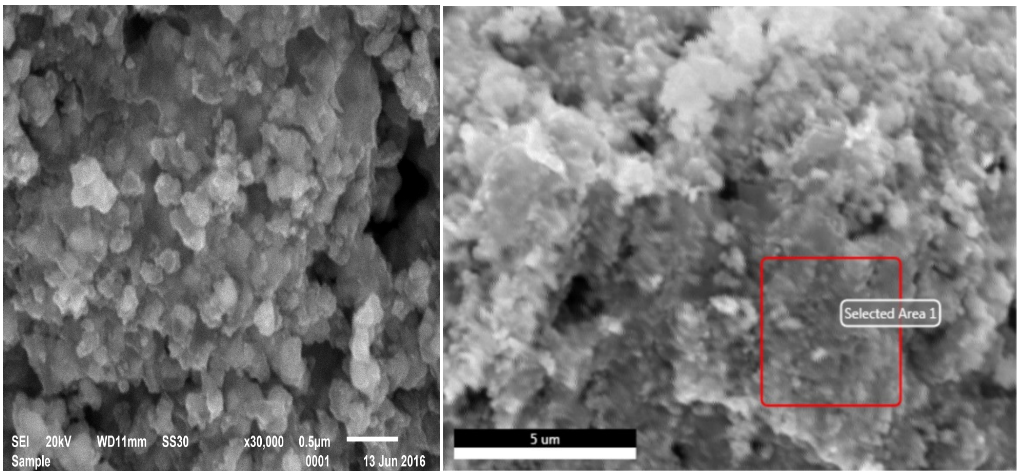

Figure 1: SEM images of Copper/ Copper Oxide nanoparticles synthesized with10mM$CuSO_4.5H_2O$+ 2.25mM TCS+ 1mLHydrazine

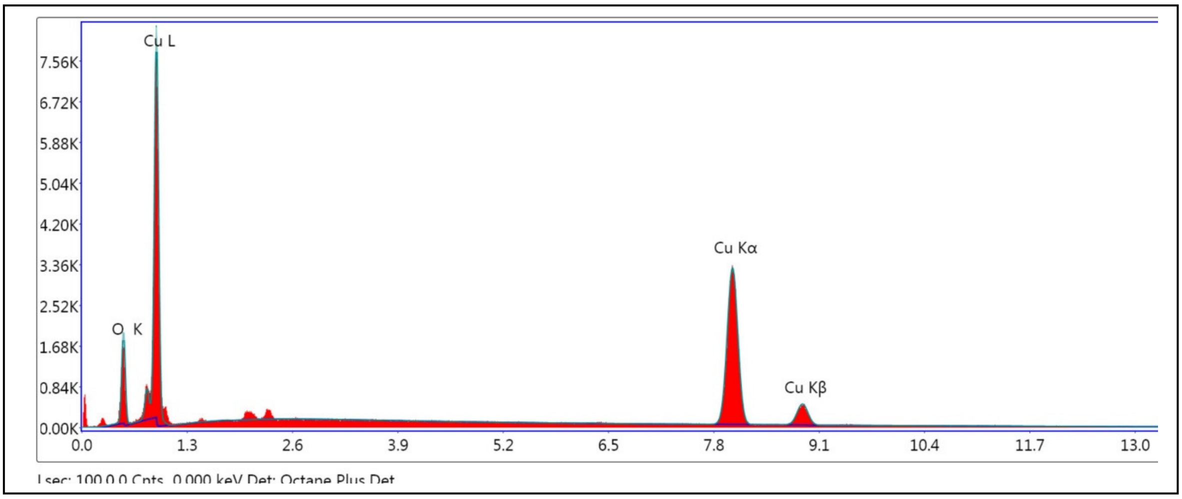

Figure 2: EDS of Copper/ Copper Oxide nanoparticles synthesized with 10mM$CuSO_4.5H_2O$+ 2.25mM TCS+ 1mLHydrazine

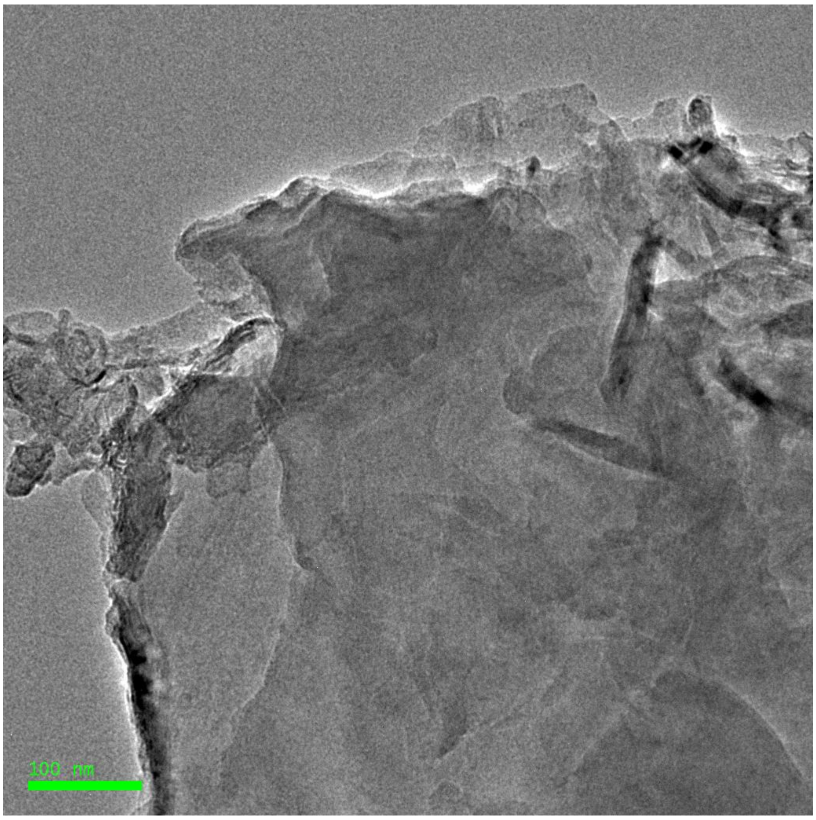

Figure 3: TEM image of Copper/ Copper Oxide nanoparticles synthesized with 10mM$CuSO_4.5H_2O$+ 2.25mM TCS+ 1mLHydrazine

The surface morphology was studied by recording the transmission electron microscopy (TEM) images of these samples by using Tecnai G^2 F 20 TWIN TMP Series of the microscope, Model FEG 200KV. SEM with EDAX analyses were also done using an energy-dispersive X-ray spectroscope (JEOL JSM 6610). It is clear from figure 1 that Cu and Cu_2O nanoparticles are agglomerated in nature. The energy dispersive spectroscopy (EDS) results of selected area of SEM image (Figure 1). The EDS analysis shows the percentage ratio as well as elemental composition of synthesized nanomaterials. In this sample Cu and Cu_2O are presented in 1:3 ratio. The figure 3 shows TEM image of copper/copper oxide nanoparticles. The TEM image shows that nanoparticles are obtained in rod shape.

7. Result and Discussion

Preparation of Nanoparticles:

At different Concentration different capping agent and with Sugarcane juice at 373 K: In this synthesis, Cu and Cu_2O NPs are formed following nucleation of the NPs by chemical reduction reaction followed by growth process. When Copper salt dissolves in deionozed water first get ionizes into +2 oxidation state (as per the following reaction) generating Cu^{2+} species which in presence of citrate capping ligands reduces back into Cu^o oxidation state (i.e. nucleation). Depending upon the concentration of Copper Salt, the number of nuclei formed may vary and thus leads to the different shapes of NPs. The Cu^o nuclei then undergo growth process to generate NPs where the growth process is controlled by diffusion of growth species which in turn depends upon the citrate. Ascorbic and Fructose additive concentration. Overall, the nucleation and growth kinetics is controlled by varying only two of the parameters related to the synthesis that are concentration of additives and temperature.

$$Cu \rightarrow Cu^{2+} + 2e^- \quad [1]$$

The as prepared sol was used for UV-vis absorption measurements. Figures 1-6 show UV–vis absorption spectra recorded on as prepared sol in aqueous solution with 25.5 mM tri-sodium citrate,100mM Fructose and 200mM Ascorbic acid and also in presence of 1g NaOH. The characteristic absorption bands with 25.5mM TSCpositioned at ca. 401 nm (surface-plasmon) has been observed for Cu_2O NPs than value of absorption bands reported at 330nm in the literature. The characteristic absorption peak has been observed at 423nm for Copper NPs Cu_2O in presence of both 100mM Fructose and 200mM Ascorbic acid. But the value for surface–plasmon has been obtained at 418nm for Cu_2O and 790 nm for CuO NPs only with fresh sugarcane juice as reported in the literature at 750nm. The broadness of the absorption band and surface–plasmon resonance probably arises from the wide size distribution of copper NPs.The characteristic value for surface–plasmon has been obtained at 510nm and 531 nm for Cu as reported at 519nm in the literatue and 473nm and 492nm Cu_2O NPs with (fresh sugarcane juice 2mL + 1g NaOH+ 25.5mM TSC) these values correlate as reported in the literature .

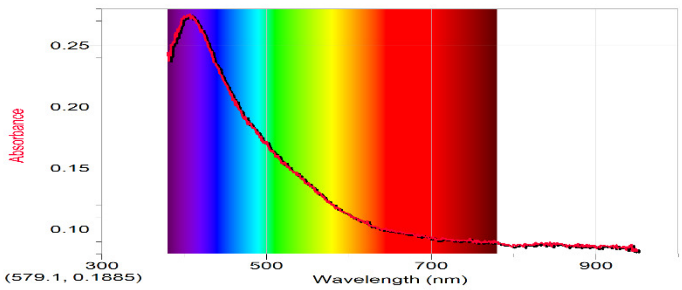

Figure 4: UV-Visible Spectra Copper NPs with 10mM $CuSO_4.5H_2O$+ 25.5mM TSC+ 1mL Hydrazine

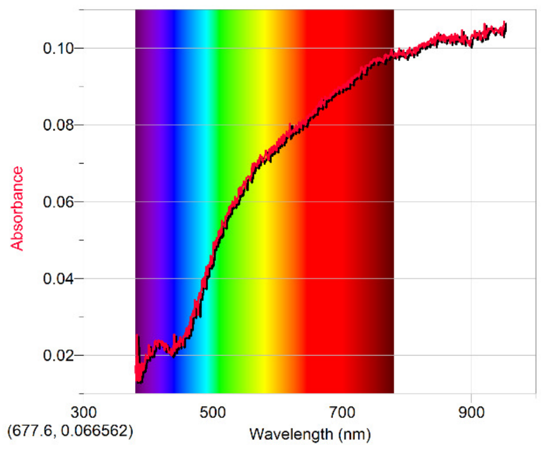

Figure 5. UV-Visible Spectra Copper NPs with 10mM CuSO4.5H_2O+ 200mM Ascorbic Acid + 1g NaOH

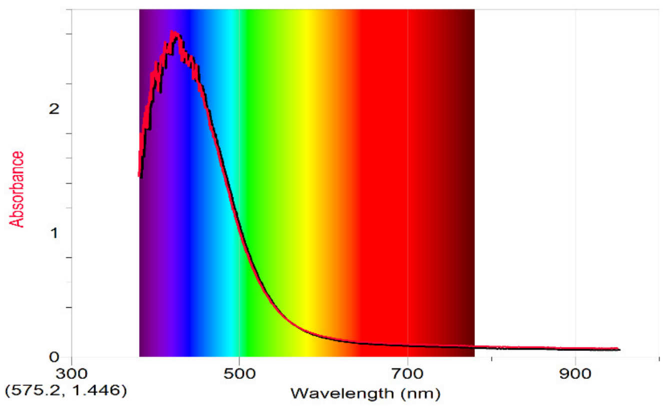

Figure 6. UV-Visible Spectra Copper NPs with 10mM CuSO4.5H_2O+ 100mM Fructose 200mM Ascorbic Acid + 1g NaOH

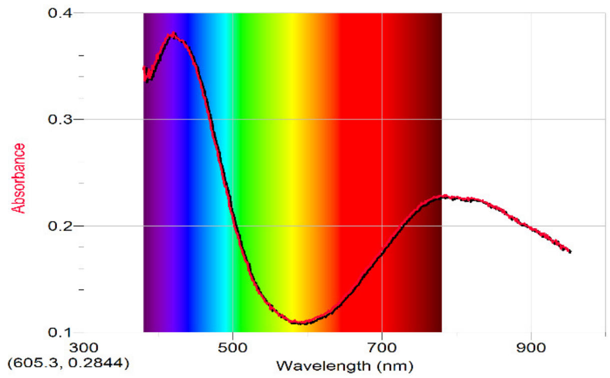

Figure 7. UV-Visible Spectra Copper NPs with 10mM $CuSO_4$.5H_2O+ 2mLSugarcane Juice.

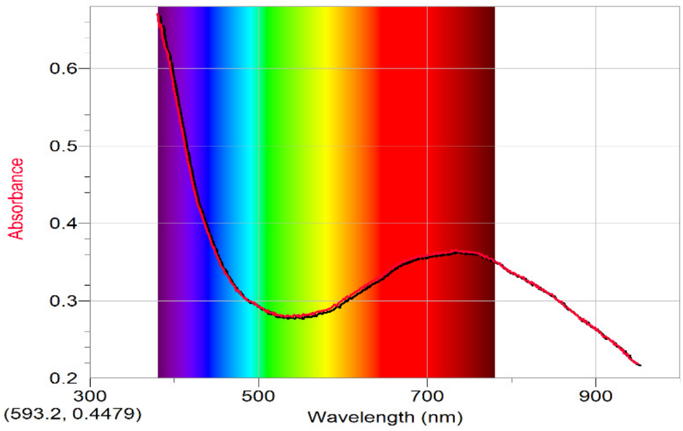

Figure8. UV-Visible Spectra Copper NPs with 10mM $CuSO_4.5H_2O$+ 2mLSugarcane Juice+ 1g NaOH

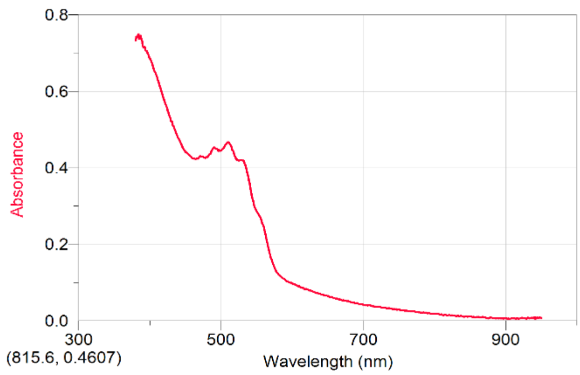

Figure 9. UV-Visible Spectra Copper NPs with 10mM $CuSO_4.5H_2O$+ 25.5mM TSC+ 2mLSugarcane Juice+ 1g NaOH

The Powder X-ray diffraction (PXRD) is shown in Figure 7. The 2θ peaks at 43.29°,50.43°,74.12°,89.92°and 95.13° belong to the fcc metallic Cu (JCPDS, PDF, File No. 04-0836). The other peaks in this diffraction pattern at 2θ values 29.55° and 36.41°, belong to Cu_2O (JCPDS, PDF, File No. 05-0667) for the the particles . indicating that Cu_2O also coexists together with copper particles. The coexisting Cu_2O is considered to be due to some oxidation in air environment. No impurity diffraction peaks have been detected confirming the high purity of the product obtained by this method. The observation of diffraction peaks intensity for all the Cu nanoparticles indicates that these are crystalline in nature.

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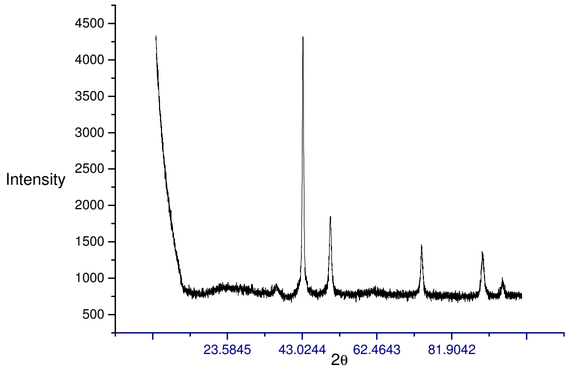

Figure 10. Powder X-Ray Diffraction Pattern of Copper NPs with 10mM $CuSO_4.5H_2O$+ 25.5mM TSC+ 1mL Hydrazine

<u>Observations for *in vitro* raised plants at different levels of copper nanoparticles:</u>

Table 1: Effect of copper nanoparticles prepared using hydrazine as the reducing agent on *in vitro* raised seedlings of *Vigna radiata*.

Medium	Seedgermin ation (%)	Average root length (cm)	Average shoot length (cm)	Average number of laterals
MS	64.5	5.58	9.23	~13
MS +10ppm	68.75	4.76	7	~11
MS + 20ppm	79.16	5.82	8.58	~12
MS + 50ppm	60.41	2.86	8.6	1
MS + 100ppm	68.75	1.33	4.20	~5

Table 2: Effect of copper nanoparticles prepared using hydrazine as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor*.

Medium	Seed germination (%)	Average root length (cm)	Average shoot length (cm)
MS	83.33	5.59	4.27
MS + 10ppm	89.58	3.95	2.96
MS + 20ppm	85.41	6.99	14.32
MS + 50ppm	68.75	3.61	3.70
MS + 100ppm	89.58	1	3.2

Table 3: Effect of copper nanoparticles prepared using hydrazine as the reducing agent on *in vitro* raised seedlings of *Vigna radiata*.

Medium	Seed germination (%)	Average root length (cm)	Average shoot length (cm)	Average number of root laterals
MS	65.5	6.00	10.33	~11
MS + 10ppm	69.75	3.70	~6	~12
MS + 20ppm	80.16	6.89	~9	~12
MS + 50ppm	64.18	2.60	6.14	~5
MS + 100ppm	60.68	1.90	3.10	~6

Table 4: Effect of copper nanoparticles prepared using hydrazine as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor.*

Medium	Seed germination (%)	Average root length (cm)	Average shoot length (cm)
MS	81.25	4.6	3.6
MS + 10ppm	93.75	5.8	5.0
MS + 20ppm	87.5	5.8	3.8
MS + 50ppm	87.5	4.1	2.8
MS + 100ppm	87.5	1.8	2.3

Table 5: Effect of copper nanoparticles prepared using fructose as the reducing agent on *in vitro* raised seedlings of *Vigna radiata*.

Medium	Seed germination (%)	Average root length (cm)	Average shoot length (cm)	Average number of root laterals
MS	75.1	4.6	2	-
MS + 10 ppm	90.20	5.2	1.8	-
MS + 20 ppm	79.1	4.8	1.5	-
MS + 50 ppm	77.1	4.5	2.3	-
MS + 100 ppm	82	4.6	2.2	-

Table 6: Effect of copper nanoparticles prepared using fructose as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor*.

Medium	Seed germination (%)	Average root length (cm)	Average shoot length (cm)
MS	79.1	4.4	2.1
MS + 10 ppm	87.5	5.0	2.1
MS + 20 ppm	77.1	4.9	1.6
MS + 50 ppm	79.1	4.9	2.0
MS + 100 ppm	80	4.8	2.0

Table 7: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor*.

Medium	Average length of root(cm)	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS	3.27	2.35	1	Open	Green
MS +10 ppm	4.65	3.05	2	Open	Green
MS + 20 ppm	3.83	2.10	2	Open	Green
MS + 50 ppm	3.76	2.40	2	Open	Green
MS + 100 ppm	1.16	1.90	1	Open	Green

Table 8: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor*.

Medium	Average length of root(cm)	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS Basal	7.05	8.01	2	Open	Green
MS +10 ppm	7.06	9.29	2	Open	Green
MS +20 ppm	8.60	10.30	2	Open	Green
MS + 50 ppm	7.94	10.64	2	Open	Green
MS +100 ppm	6.79	9.35	2	Open	Green

Table 9: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Vigna radiata.*

Medium	Average length of root(cm)	Average no. of laterals	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS Basal	4.92	13.9	9.10	2	Open	Green
MS +10 ppm	5.05	15.46	8.6	2	Open	Green
MS + 20 ppm	6.85	17.08	10.58	2	open	Green
MS + 50 ppm	6.65	20.35	11.33	2	open	Green
Ms + 100 ppm	6.82	17.87	11.53	2	open	Green

Table 10: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Vigna radiata.*

Medium	Average length of root(cm)	Average no. of laterals	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS Basal	6.00	12.13	8.19	2	Open	Green
MS + 10 ppm	7.80	12.93	10.42	2	Open	Green
MS + 20 ppm	7.52	14.79	9.54	2	Open	Green
MS + 50 ppm	6.70	13.97	9.93	2	Open	Green
MS + 100 ppm	3.78	12.97	8.14	2	Open	Green

Table 11: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Vigna radiata.*

Medium	Average length of root(cm)	Average no. of laterals	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS Basal	8.02	17.68	10.64	2	Open	Green
MS + 10 ppm	8.62	14.61	10.37	2	Open	Green
MS + 20 ppm	8.63	17.44	11.28	2	Open	Green
MS + 50 ppm	9.46	15.46	12.42	2	Open	Green
MS + 100 ppm	2.53	11.26	11.39	2	Open	Green

Table 12: Effect of copper nanoparticles prepared using sugarcane juice as the reducing agent on *in vitro* raised seedlings of *Sorghum bicolor.*

Medium	Average length of root(cm)	Average length of shoot (cm)	Average no. of leaves	Nature of leaves	Colour of leaves
MS Basal	~2	3.9	2	Open	Green
Ms + 10 ppm	~2.5	2.2	2	Open	Green
MS + 20 ppm	~4.3	3.87	2	Open	Green
MS + 50 ppm	~5.6	2.73	2	Open	Green
MS + 100 ppm	~4.0	1.97	2	Open	Green

The observations recorded show that nanoparticles do have an effect on the growth of plants. Even the method used to prepare nanoparticles affects the growth of the plants. The results show that higher concentrations of copper oxide nanoparticles decreased root and shoot length and an increased response was observed at low levels of nanoparticles added to the MS medium (Tables 1 to 12 and Figures 11 to 17).

8. **Innovations shown by the project:**
Use of green method and waste products like bagasse to make NPs is a very innovative approach to preparation of Nanoparticles.Thegreen synthesis is a novel technique which is simple and environment friendly than the previously used conventional chemical reduction methods. Thus green method is an ideal one because it is simple process, low energy consumption, high output, easy control and **no environmental pollution**. It was observed that NP synthesis by different methods affect the overall growth of seedlings raised under *in vitro* conditions.

9. **Conclusion and Future direction:**

Till now we have fabricated Copper NPs using chemical reduction and green method. The chemical reduction method is used more frequently for the preparation of stable and colloidal dispersion of Copper NPs in aqueous as well as in organic solvents. The green synthesis is a novel technique which is simple and environment friendly than the previously used conventional chemical reduction methods. Thus green method is an ideal one because it is simple process, low energy consumption, high output, easy control and no environmental pollution.It was observed that NP synthesis by different methods affect the overall growth of seedlings raised under in vitro conditions.Effect of low concentration of NPs promoted growth in terms of shoot and root morphologies whereas high concentration of NPs inhibited the growth of seedlings.Nanoparticles have so many applications for example they are used in microelectronics and as fillers, opacifiers, catalysts, semiconductors, cosmetics and drug carriers. It is feared that they can generate potentially dangerous and undesirable consequences. Their harmful effects are generally unknown.This has led to extensive research in the area of nanotoxicology. The long term benefits of our project include mapping out these harmful effects. This project does not throw light on these problems immediately but sets a perspective for further extensive research. In our opinion food security is a major global challenge. We propose that in the future nanocomposites can possibly be used as 'designer fertilizers' for growth promotion of crops.We propose to develop Cu NPs by green method at an industrial scale from baggase which is a waste product.It will be doubly useful as waste would be utilized to produce Cu NPs by green method so both steps are environment friendly. Our students are very enthusiastic about marketing the protocol for preparation of Cu NPs by green method at an industrial scale from baggase. If we are able to finalise the protocol for the said method, the same can be the idea for a major start-up.

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2.Gratzel M. 2001. Photoelectrochemical cells. Nature. 414:338–344.

3. Okuda M., Kobayashi Y, Suzuki K, Sonoda K, Kondoh T, Wagawa A, Kondo Yoshimura H. 2005. Self-organized inorganic nanoparticle arrays on protein lattices. Nano Lett 5:991–993.

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5. Murray CB., Sun S., Doyle H, Betley T. 2014. Monodisperse 3D transitionmetal (Co, Ni, Fe) nanoparticles . MRS Bull 26:985–991 Banerjee et al.Bioresources and Bioprocessing. 1:3 , 9 -10.

11. Publication/s from the work. (Attach copies)

- Choudhary, N., Malik, P., Saini, K., Bhatia S.S., Bahri, S.,Ingole, P.P., Pandey, S., Agarwal, R., Baruah, Sekhri, S., Sharma, K., Rahul, S. 2016. '**Synthesis of copper nanoparticles (Cu,Cu_2O,CuO) and characterization by UV-Visible spectroscopy and power X-Ray diffraction'** in the proceedings of National Conference in Chemistry-2016(NCC-2016) organized by Department of Chemistry, Shyamlal College, University of Delhi, at India International Centre,07-08 April 2016.

12. Conference Presentation/s (attach copies)

- Choudhary, N., Malik, P., Saini, K., Bhatia S.S., Bahri, S.,Ingole, P.P., Pandey, S., Agarwal, R., Baruah, Sekhri, S., Sharma, K., Rahul, S. 2016. **Synthesis of copper nanoparticles (Cu,Cu2O,CuO) and characterization by UV-Visible**

spectroscopy and power X-Ray diffraction. Oral presentation at the National Conference in Chemistry-2016(NCC-2016) organized by Department of Chemistry, Shyamlal College, University of Delhi, at India International Centre,07-08 April 2016.

- Bahri, S., Saini, K., Bhatia, S.S., Sekhri, S., Sharma, K., Ruhal, S., Choudhary, N., Malik, P., Baruah, S., Agarwal, R., Pandey, S., Suhail, S. & Suri, S. 2016. Poster presentation on Agronanotechnology: **An approach to increase plant productivity**. Biotikos 2016, National Symposium on Nanobiotechnology, organised by TERI University Biotechnology Society, p. 19-20, 31 March to 1 April 2016.

- Bhatia, S.S., Saini, K., Bahri, S., Pandey, S., Choudhary, N., Malik, P., Agarwal, R., Baruah, S., Sekhri, S., Sharma, K., Ruhal, S., Suhail, S., & Suri, S. 2016. Poster presentation on **Synthesis of copper nanoparticles and characterizations by using different techniques.** In: Abstracts of Indo-Portugese Workshop on Emerging Trends of Nanotechnology in Chemistry and Biology (INCB 2016), organised by Department of Chemistry, Hansraj College and Deshbandhu College, University of Delhi, Delhi, India in association with University da Madeira, Portugal, 12-13 February 2016. J. Mat. NanoSci., 3(1), S1-S22, S19. ISSN 2394-0867.

- Bahri, S., Bhatia, S.S., Saini, K., Sharma, K., Choudhary, N., Malik, P., Agarwal, R., Pandey, S., Ruhal, S., Suhail, S., Sekhri, S., Suri, S. &Baruah, S. 2015.**Effect of copper nanoparticles on seedling growth of *Vigna radiata* L. (Wilczek).** RSC Workshop on Chemistry for Tomorrow's World organized by Green Chemistry Network Centre, Delhi University, ManavRachna University, Faridabad, Shiv Nadar University, Greater Noida held at New Delhi from December 2-3, 2015, pp. 43-44.

13. Pictures related to the project.

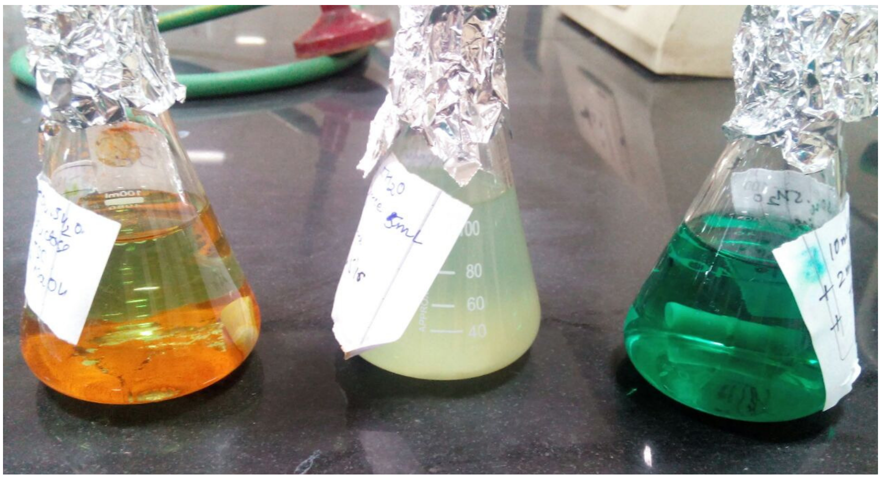

Figure 11: Nanoparticles of copper oxide

Figure 12: Cultures of *Vigna radiata* kept in the culture room.

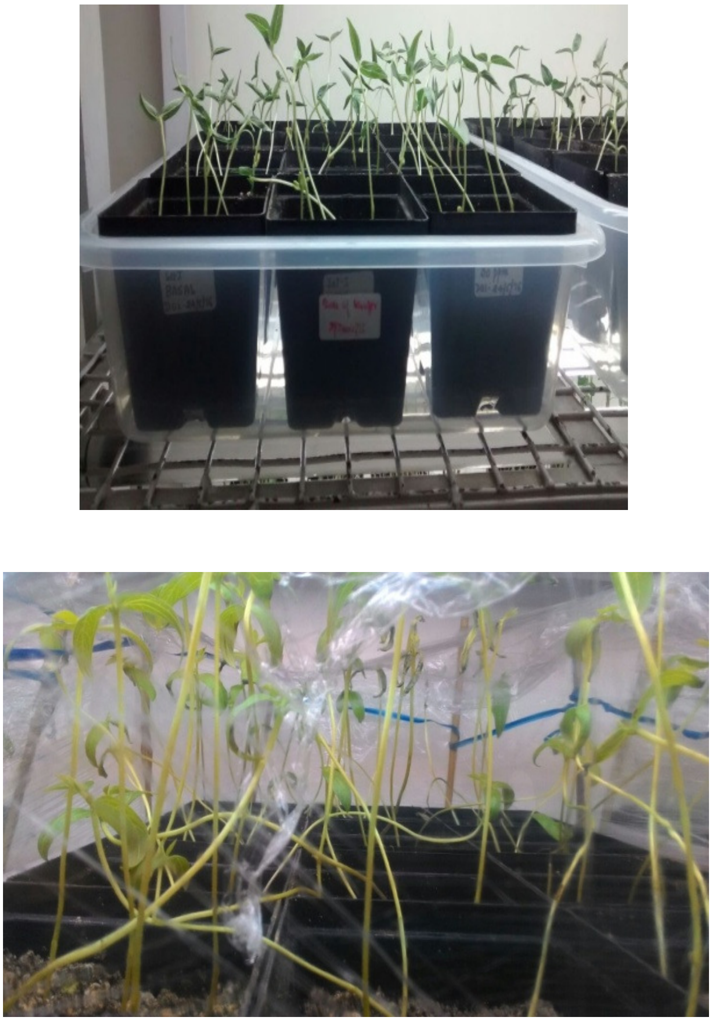

Figure 13: i*n vitro* raised seedlings of *Vigna radiata* transferred to pots.

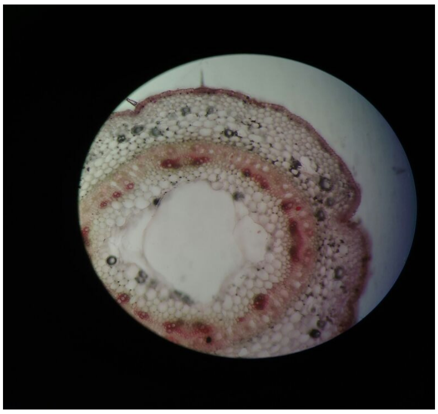

Figure 14: Transverse section of stem of *Vigna radiata* plant (MS + 100 ppm + copper oxide nanoparticles).

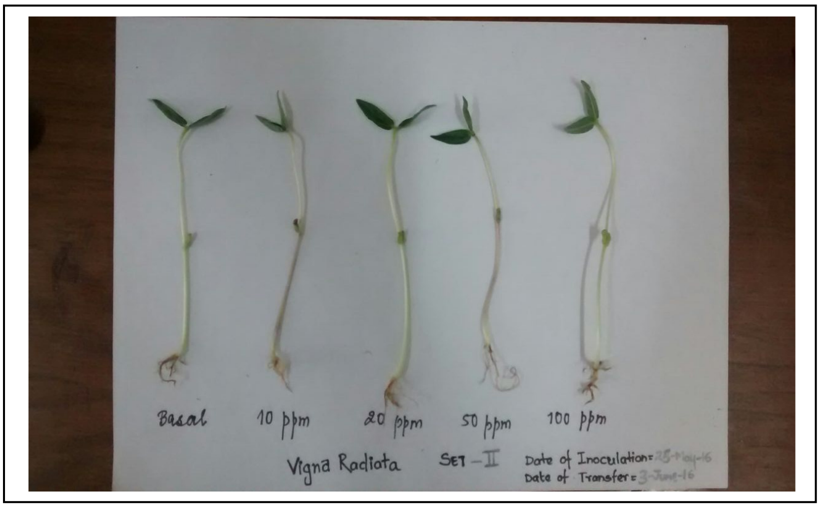

Figure 15: *In vitro* plants of *Vigna radiata* raised on Ms + copper oxide nanoparticles using fructose as reducing agent after 10 days of culture.

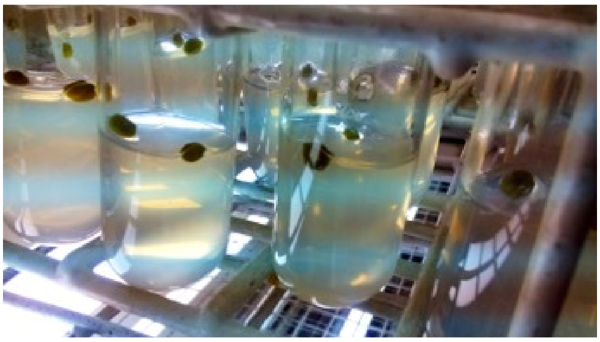

Figure 16: Seeds of *Sorghum bicolor* inoculated on MS basal and MS + copper oxide nanoparticles.

Figure 17: *In vitro* raised plants of *Sorghum bicolor* on MS basal and MS + copper oxide nanoparticles at different concentrations.

14. Annexure/Any other information

- Workshop on "Green Nanotechnology for Beginners" on 28 January, 2016 was organized in which students learnt:

a) Literature Survey

b) Formulating the problem

c) Setting up of the experiment

d) Work in a collaborative environment

e) Recording of results in a systematic manner

f) Writing of research paper

g) Organization of Workshop

h) Poster & Oral presentations in various conferences

i) *In vitro* studies

j) Medium preparation and inoculation

k) Recording observations and analysis of data

l) Photography of cultures

m) Use of filter sterilization technique