RESEARCH ARTICLE OPEN ACCESS

Comparative Assessment of the Adverse Effect of Silver Nanoparticles to *Vigna Radiata* and *Brassica Campestris* Crop Plants.

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ABSTRACT
Inspite of very wide application of different types of nanoparticles in different commercial fields including pharmaceutical and food industries, the toxic effects of these nanoparticles on living systems have not been clearly established. Increased applications of nanoparticles by human beings lead to accumulation of more and more nanoparticles in the environment which ultimately affect the ecosystem. The current study focused on phytotoxicity of silver nanoparticles to *V.radiata* and *B.campestris* crop plants. Effect on seedling growth by nanoparticles is comparatively more than ions solution during treatment period. The test plants exposed to nanoparticle shows that the average particle size was about 25.3 nm which was determined by X-Ray Diffractions spectrophotometer. In addition, result from Fourier Transform Infrared spectrometer reported no change in chemical composition on the basis of vibrations of functional group of molecules in treated root samples. However, Scanning Electron Microscope images revealed depositions of isolated small and spherical nanoparticles in root cells. The nanoparticles appeared to be either filling the epidermal crypt or adhering onto the root surface of test plants.

Key words: Fourier Transform Infrared spectrometer, Nanoparticles, Phytotoxicity, Scanning Electron Microscope and X-Ray Diffractions spectrophotometer

I. INTRODUCTION

In recent years the development and implementation of engineered nanoparticles has increased dramatically. It was projected that nanoparticle industry will reach $1 trillion in 2015 from earlier $4 billion in 2005 [1]. Increased in number of nanoparticle products and its utilization in diverse field can lead to intentionally or unintentionally release into the ecosystem [2, 3]. It was expected that the use of nanotechnology will rapidly increase but understanding the impact of these materials on biological and ecological systems is still lagging behind [4]. Researchers have little notable data regarding the impacts of engineered nanoparticle on terrestrial crops especially agricultural crops. The current use of nanoparticle from medicine to pesticides may represent a significant pathway of exposure to humans, plants and animals through food chains. The impact of five different nanoparticles of 2000 mg/L on germinations of six different plants was at first reported [5]. Plants exposed to carbon nanoparticles accumulated particles on outer root surfaces but interestingly inspite of this specific toxicity and uptake was not at all observed [6]. $C_{60}(OH)_{20}$ reported permeable to cell wall and the particles were excluded by the cell membranes lead to cell damage [7]. Adverse effect of silver nanoparticles in edible crop plants is also reported in *Phaseolus radiatus and Sorghum bicolor.* It was reported in agar test that *P. radiatus and S. bicolor* showed a concentration dependent growth inhibitions by silver nanoparticles [8]. Phytotoxicity of silver nanoparticles was also reported in *Oryza sativa.* TEM images revealed deposition of nanoparticles inside roots which damaged the cell wall and vacuoles [9]. The present study shows an overview of adverse effect of silver nanoparticles on *B. campestris* and *V.radiata.* The data will help in understand the toxic limit of silver nanoparticles used in industry for commercial purposes which are intentionally or unintentionally released in ecosystem.

II. MATERIAL AND METHODS:

2.1 Preparation of Silver nanoparticles:

Silver nanoparticles were synthesized by chemical reduction method. It was prepared by adding molar concentration of silver nitrate solution in distilled water which was reduced by molar concentration of Sodium borohydride solution. Tween-20 was added which as a stabilizer of nanoparticles [9]. Silver ion solution was also prepared without adding Sodium borohydride and Tween-20.

2.2 Exposure Assay:

B. campestris (variety: M-27) and *V.radiata* (variety: K-851) was selected for the phytotoxicity

study. The seeds were collected from Assam seed corporations, Assam, India. The seeds of crop plants were allowed to germinate in moist condition for one week. Uniform seedlings were selected to grow in Hoagland nutrient solution for another one week. The seedlings were transferred to growth chamber in controlled environment. The nanoparticle solutions alongwith Hoagland nutrient solution was stirred with a glass rod in every 12 hours. Four different concentrations (0 µg/ml, 50 µg/ml, 500 µg/ml and 1000 µg/ml) of nanoparticle and ion solution were selected for the phytotoxicity study [9].

2.3 XRD Analysis:

The treated root samples (1000 µg/mL) were studied by X-ray diffraction (Model-XPERT PRO) at Instrumentation and USIC department, Gauhati University. Samples were washed under tap water and finally rinsed with distilled water. The samples were heated in an Argon atmosphere at 400 °C for 1.5 hour. This process was done to crystallize the particles and to burn away the organic tissues.

2.4 FTIR Analysis:

Both nanoparticles (1000 µg/mL) treated and untreated roots were analysis for FTIR spectra. The sample were at prepared by KBr pellets method operated in FTIR spectrophotometer (Model-Brucker, Vector 22) to investigate the functional groups and to investigate the possible binding site with nanoparticles.

2.5 SEM observations:

Analysis of treated (both 1000 µg/mL nanoparticle and ion) and untreated solution was done at Sophisticated Analytical Instrument Facility, NEHU, Shillong. The root samples were prepared by standard procedure method operated in SEM (Model-JEOL JSM 6360) instrument at NEHU.

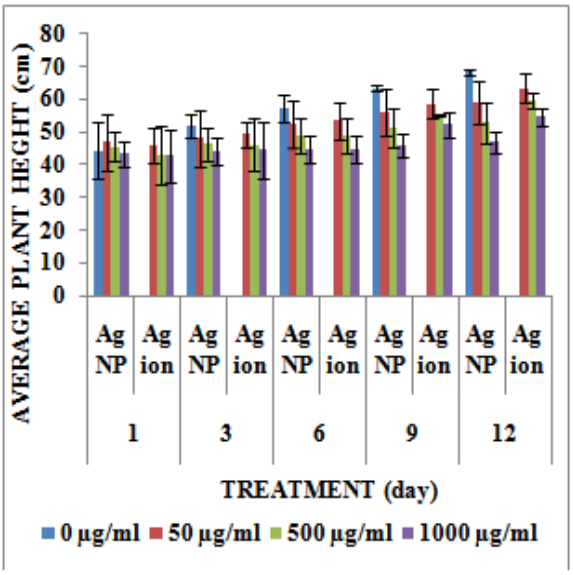
2.6 Statistical analysis:

In every experiment, each treatment was conducted with three replicates. The results were presented as mean ± SD (standard deviations). The statistical analysis of experimental data utilized the Student's *t*-test. Statistical significance was accepted when the probability of result assuming the null hypothesis (p) is less than 0.05. All the statistical calculations were done by SPSS 16 Versions software.

III. EXPERIMENTAL FINDINGS:

3.1 Phytotoxicity effect on the growth of Seedlings:

The seedling growth in test plants remains unaffected initially during treatment period. *V.radiata* did not showed any significant inhibition on growth at 500 µg/mL (p=0.997) and 1000 µg/mL (p=0.998) of Ag nanoparticle solution compared to control. It was also found that beyond 50 µg/mL Ag^+ ion solutions, seedling growths in *V.radiata* and *B.campestris* remains unaffected till day 1. The seedling growth in all test plant was not affected by the type of treatment and exposure time till 3rd day. No significant retardation in *V.radiata* and *B.campestris* seedling growth was reported at 50 µg/mL, 500 µg/mL and 1000 µg/mL of both Ag nanoparticle and ion solution when compared to control plant till 6th day. The seedling growth in *B.campestris* didnot showed any significant effect when exposed to Ag nanoparticle and ion solutions. No significant retardation was observed at 50 µg/mL concentration of Ag nanoparticle and ion solution on seedling growth of test plants. 1000 µg/mL (p=0.012) of Ag nanoparticle solution showed significant inhibition in *V.radiata* seedling growth compare to control. Treatment with Ag ion solution of 500 µg/mL (p=0.040) and 1000 µg/mL (p=0.007) resulted in significant inhibition on seedling growth in *V.radiata* when compare to control after 9th day. 50 µg/mL of nanoparticle and ion solution didnot showed any significant inhibition on seedling growth in *V.radiata* and *B.campestris*. 500 µg/mL of Ag nanoparticle solution showed significant inhibition in *V.radiata* (p=0.042) when compare with control seedling growth. The seedling growth in *V.radiata* was significantly inhibited at 1000 µg/mL of Ag nanoparticle and ion solution. While *B.campestris* seedlings showed significant inhibition at only 1000 µg/mL of Ag nanoparticle solution compared to control at the end of treatment period (12th day).



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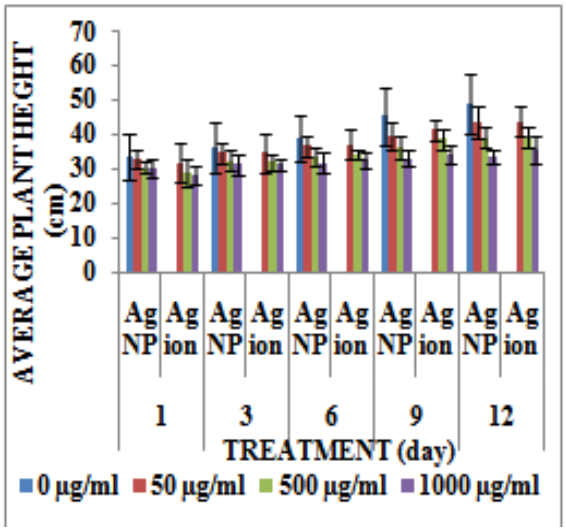

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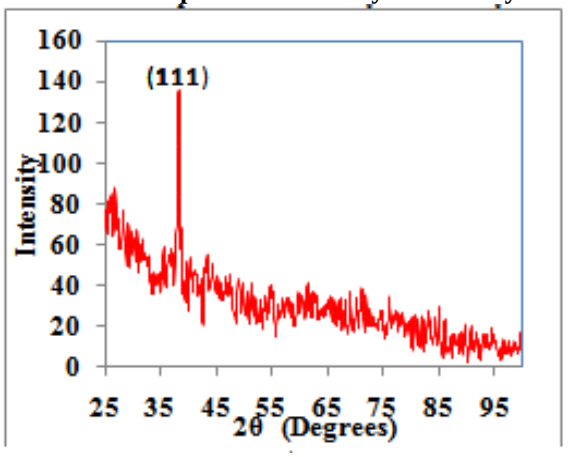
Fig 1: Effect of Ag nanoparticle and ions on seedling growth of (A) *V.radiata* and (B) *B.campestris* in Hoagland nutrient solution during 12 days of treatment.

3.2 Detection of particle in root by XRD analysis:



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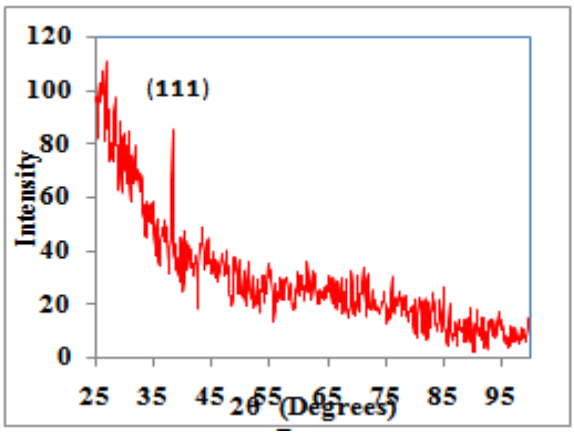

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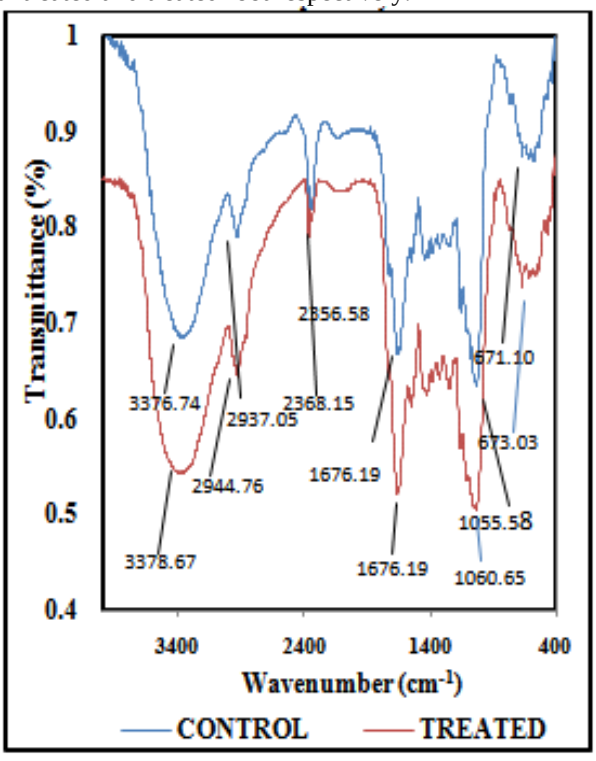
Fig 2: XRD diffraction pattern of (A) *V.radiata* and (B) *B.campestris* roots treated in 1000 µg/mL of Ag nanoparticles solution after 12 days of treatment.

The results of XRD analysis on selected test plant roots during treatment period is given in Fig 2. Roots of all test plants exposed to 1000 µg/mL nanoparticle solution resulted in similar XRD pattern except its intensity.

The XRD analysis showed a single peak of Braggs reflection that may index on the basis of the Face Cubic Centre structure (111) of silver in all test plants. From the Fig, it has been assumed that, 25.3 nm size particle must have entered into the root cell of *V.radiata* and *B.campestris* during the treatment period. However for the size confirmations, roots of selected species were observed under SEM.

3.3 FTIR analysis of treated and untreated root sample:

Fig 3 (A) shows *V.radiata* assigned to –OH stretching band which appears at 3376.74 cm^{-1} and 3378.67 cm^{-1} in untreated and treated root respectively. While –COOH show stretching anti-symmetric band at 2937.05 cm^{-1} in untreated and 2944.76 cm^{-1} in treated root cell for acid dimer. The over tone band of –COOH at 2356.58 cm^{-1} and 2368.15 cm^{-1} was for acid group in untreated and treated root respectively. The stretching –C=C band at 1676.19 cm^{-1} found in both untreated and treated root. The stretching anti-symmetric couple band was observed at 1055.58 cm^{-1} and 1060.65 cm^{-1} in untreated and treated root respectively.



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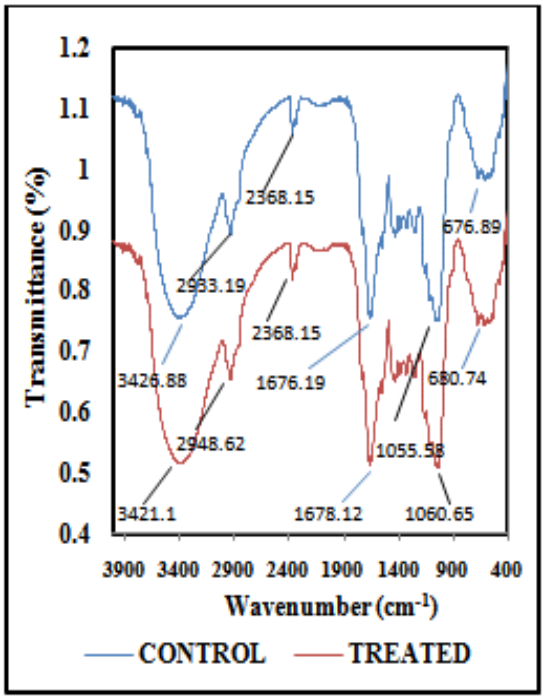

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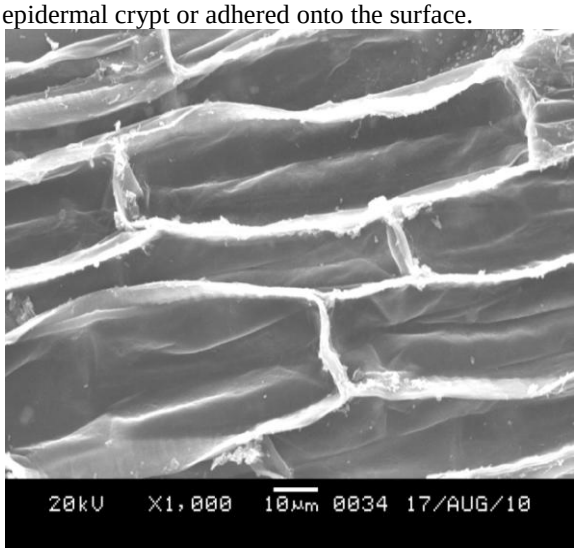
Fig 3: FTIR absorptions spectra of (A) *V. radiata* and (B) *B.campestris* roots in Hoagland nutrient medium after 12 days of treatment.

Aromatic ring was assigned for untreated and treated root at 671.10 cm^{-1} and 673.03 cm^{-1} wavenumber respectively. Fig 3 (B) represents broad – OH stretching band at 3426.88 cm^{-1} and 3421.10 cm^{-1} in untreated and treated root of *B.campestris*. Stretch anti-symmetric band at 2933.19 cm^{-1} and 2948.62 cm^{-1} of – COOH group in both untreated and treated sample respectively was for acid dimer. Moreover the over tone band at 2368.15 cm^{-1} was for –COOH group in both untreated and treated root. The stretch – C=C band appeared at 1676.19 cm^{-1} and 1678.12 cm^{-1} in untreated and treated sample respectively. – C-O to – C-C showing peak at 1055.58 cm^{-1} and 1060.65 cm^{-1} in untreated and treated sample respectively was for primary amine structure. Aromatic ring was observed at 676.89 cm^{-1} and 680.74 cm^{-1} by untreated and treated root.

3.4 Observation of root cell using Scanning Electron Microscope:

Fig 4.1 shows SEM image of *V.radiata* roots under different treatments (control, 1000 µg/ml of Ag^+ and 1000 µg/ml Ag nanoparticle treatment). Root surface in control and Ag^+ ion treatments were free from particle adherence (Fig 4.1A and Fig 4.2 B). However, adsorption of Ag particle and their aggregation on the root surface was evident (Fig 4.1C). It was clearly observed that penetration of Ag nanoparticle has taken place through root surface when exposed to Ag nanoparticle solution. The enlarged portion (Fig 4.1D) of Fig 4.1C, reveals that the particles observed were either filled in the epidermal crypt or adhered onto the surface.



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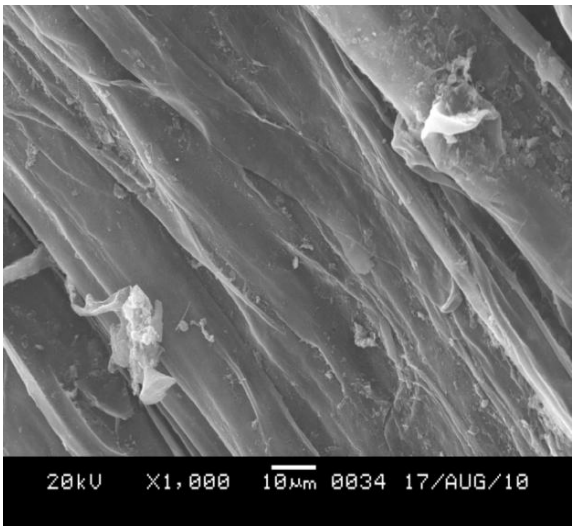

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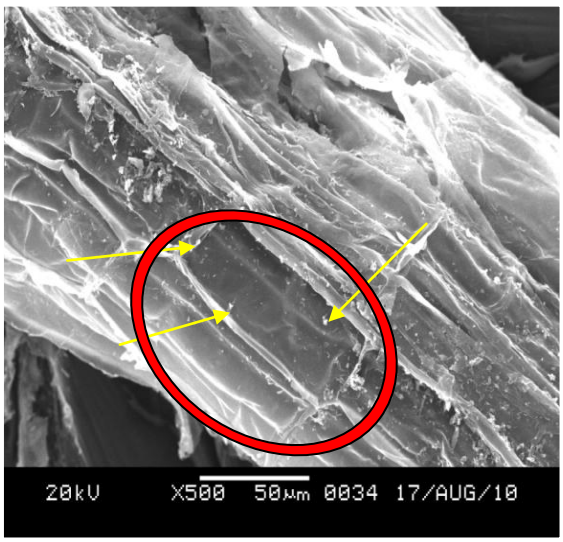

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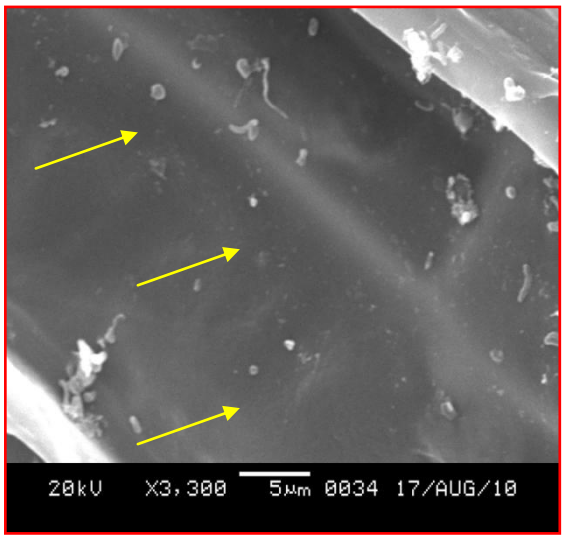

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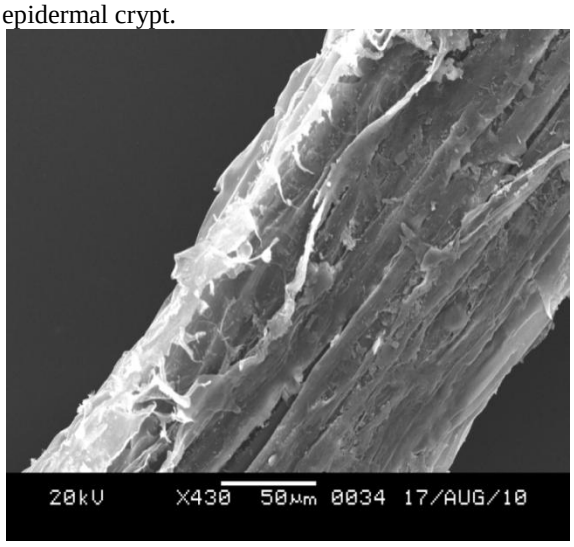
Fig 4.1 : Scanning Electron Microscope images of *V.radiata* root under treatment of *(*A) Control, (B) 1000 µg/ml Ag^+, (C) 1000 µg/ml Ag nanoparticles (D) Magnified portion of Image C.

SEM image of *B.campestris* roots under control treatment is shown in Fig 4.2A. Here we observed that, the root surface was free of particle adherence. Treatment with 1000 µg/ml of Ag^+ ion solution also showed absence of any particle adhered onto the root surface (Fig 4.2 B). But deposition of particle on the root surface was clearly observed in Fig 4.2 C. The enlarged image of Fig 4.2 C, clearly established that the penetration of Ag nanoparticle has taken place through root surface. The individual nanoparticles are clearly shown in Fig 4.2 D, which were either adhered onto the surface or filled in the epidermal crypt.



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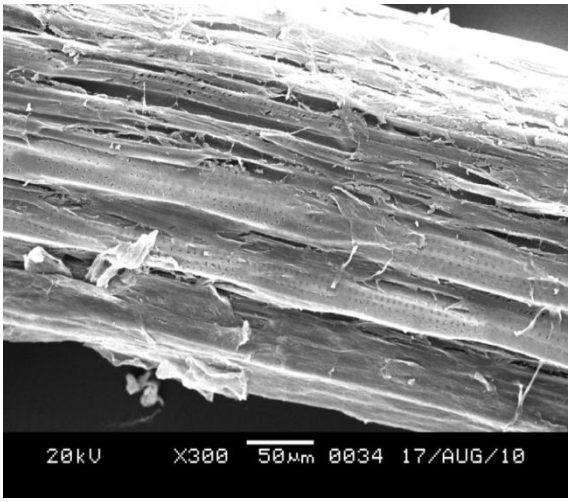

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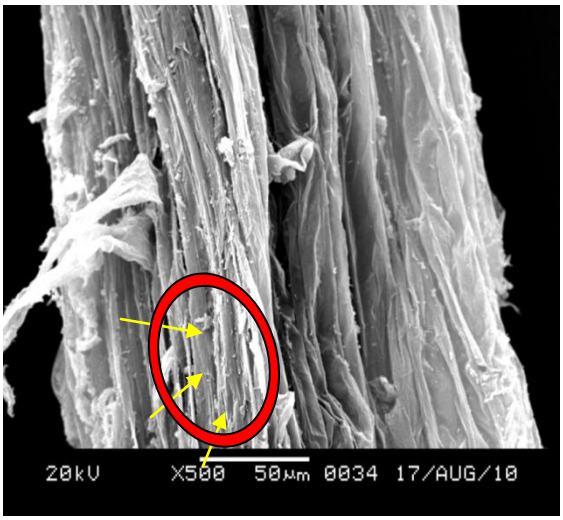

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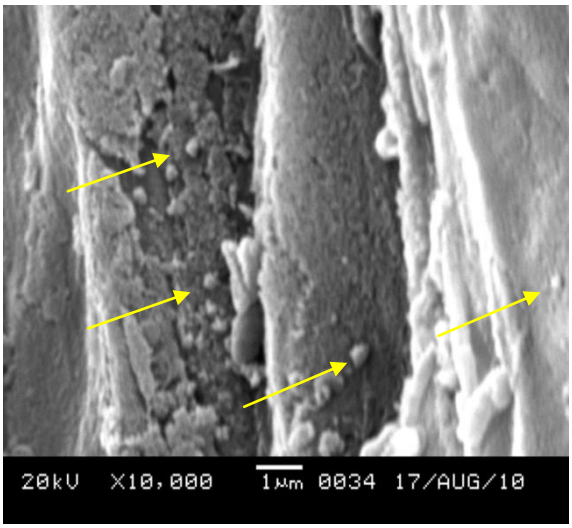

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Fig 4.2 : Scanning Electron Microscope images of *B.campestris* root under treatment of *(*A) Control, (B) 1000 µg/ml Ag^+, (C) 1000 µg/ml Ag nanoparticles (D) Magnified portion of Image C.

IV. DISCUSSIONS:

4.1 Effect of silver nanoparticles and ions on early plant growth:

The test plants exhibited increases in seedling growth with time, but at different rate depending on the species. However, there was no significant reduction in seedling growth at 50 µg/mL, 500 µg/mL and 1000 µg/mL of Ag nanoparticle and ion solution in test plants after 1st and 3rd day of exposure. The toxicity of Ag nanoparticle and Ag^+ ion solution in *V.radiata* and *B.campestris* seedlings were evident and increased with increase in concentration of both Ag nanoparticle and Ag^+ concentration. Similar result was also observed in ryegrass seedlings which did not showed any significant inhibition with ZnO and Zn^{2+} solution when exposed to lower concentration of 50 µg/mL [10]. Significant retardation in seedling growth after 12th day was observed in *V. radiata* when exposed to 500 µg/mL Ag nanoparticle solution. Interestingly, 1000 µg/mL Ag nanoparticle solution showed significant inhibition of seedling growth of all test plants at the end of treatment period (12 days). However 1000 µg/mL Ag^+ ion solution showed significant inhibition of seedling growth in *V. radiata* compared to control after 12 days of exposure. Studies found that 1000 mg/L of copper nanoparticle when exposed to *P. radiatus* and *T. aestivum* shows adverse effects on seedling growth [11]. Both *V.radiata* and *B.campestris* seedling (dicot plant) also showed adverse effect on seedling growth at 1000 µg/mL Ag nanoparticle solution after treatment period. Ag nanoparticle of 1nm to 50 nm size (as observed under SEM) which inhibited seedling growth in test plants supports our result. Similar result was also obtained in Ryegrass seedling when exposed to different concentrations (50 µg/mL, 500 µg/mL and 1000 µg/mL) of ZnO nanoparticles and Zn^{2+} ions during the treatment periods. However the toxic symptoms were more prevalent in Zn^{2+} than ZnO nanoparticles. The seedling almost withered to death with 1000 µg/mL Zn^{2+} solution [10].

4.2 Detection of silver particle on treated root by XRD:

It was found that Ag particle which penetrated inside the root cell was of 25.3 nm size (using Debye Scherrer equations). *V.radiata* and *B.campestris* root cells showed presence of a single peak at 2θ=38.13º (Fig 2). The crystalline nature of Ag nanoparticles was confirmed by Bragg's reflections which may index on the basis of face centre cubic (111). Using Debye Scherrer equations, the average particle size was found to be 9 nm and shows single Bragg's reflections i.e 111 which results in crystalline nature [12]. The single peak but of different intensity and the crystalline nature of Ag nanoparticles detected by XRD analysis completely support our result.

4.3 FTIR observation of treated and untreated root:

FTIR-Spectrometer, in the range of 400 – 4000 cm^{-1}, was applied to elucidate the molecular structure of the studied samples (Fig 3). Change in chemical composition can be detected on the basis of vibrations of functional group of molecules by nanoparticle solutions. In our finding, no such changes were reported in the functional group of both treated (by Ag nanoparticle solution) and untreated root cells. Absorption peaks at 3376.74 cm^{-1} and 3378.67 cm^{-1} wavenumbers of *V.radiata* was assigned for –OH functional group in both untreated and treated root respectively. 2937.05 cm^{-1} and 2356.58 cm^{-1} wavenumber in untreated root cell and 2944.76 cm^{-1} and 2368.15 cm^{-1} wavenumbers in treated root cell of *V.radiata* was assigned for –COOH functional group. 1676.19 cm^{-1}, 1060.65 cm^{-1} and 673.03 cm^{-1} wavenumber of *V.radiata* treated root cell was assigned for –C=C band, – C-O to – C-C and Aromatic ring functional group respectively. 3426.88 cm^{-1} and 3421.10 cm^{-1} wavenumbers in untreated and treated root cell of *B.campestris* respectively represents broad –OH stretching band. Absorbance peak at 2948.62 cm^{-1} and 2368.15 cm^{-1} wavenumbers was assigned for – COOH group in treated root cell of *B.campestris.* At 1678.12 cm^{-1}, 1060.65 cm^{-1} and 680.74 cm^{-1} wavenumber of treated root cell of *B.campestris* was assigned for – C=C , – C-O to – C-C and Aromatic ring functional group respectively. It was reported that the FTIR spectra of water hyacinth showed change in absorbance on shoot compared to root. This change may be due to the dominant cellulosic structure in the shoot [13]. It supports our result as change in absorbance was also observed in treated root cell when compared to untreated root cell. However change in absorbance and wavenumber did not showed any change in functional group in both treated and untreated root cell.

4.4 Detection of nanoparticles on root by Scanning Electron Microscope:

The Ag nanoparticles in treated root cells of our experiment were found to be remains isolated and adhered to root surfaces. The particles were observed to be adhered onto the surface of *V.radiata* and *B.campestris* root cells (Fig 4.1 and Fig 4.2). It was reported that ryegrass root surface were free from particle adherence when exposed to control and Zn^{2+} treatments. However ZnO particles were found to be undergoing adsorptions and it aggregated on root surfaces. ZnO particles were reported to be either filled in the epidermal crypt or adhered onto the surfaces [10]. Similar result was obtained when we

exposed *V.radiata* and *B.campestris* root surface with 1000 µg/mL of nanoparticle and ion solution alongwith control treatment for 12 days. The root surfaces of selected test plants were free from particle under control and Ag^+ ion treatment.

V. CONCLUSION:

The present study is a scientific analysis of potential negative effects that silver nanoparticles may have on selected common crops, viz, *V.radiata* and *B.campestris* especially on their early phase of growth. Nanotechnological research has increased strongly and attracted many scientists towards it, but its consequences are poorly known. Recently negative impacts of different nanoparticles on the environment have been studied and its toxic potentials in the environment have been ascertained. The fact that plant at seedling stage can accumulate silver nanoparticles in the cells and tissues has been demonstrated by this study. Once silver nanoparticles enter inside the cells, it may cause damage to the vacuoles and cell walls integrity and probably affect other cell organelles too. Retardation of growth during seedling stage was due to considerable absorption of silver nanoparticles by the root cells. Lower dose of silver nanoparticles concentration appears to be potentially toxic to the three crops plants tested. Comparatively a higher amount of silver ions is required than nanoparticles for adverse effect on the same plants. The researcher should mainly focus on interaction between nanoparticles and environmental matrices (water, sediments and soils) and its ecotoxicity studies.

VI. ACKNOWLEDGMENT:

The Author is thankful to Sophisticated Analytical Instrument Facility, NEHU, Sophisticated Analytical Instrument Facility (SAIF) under University Science Instrumentation Centre (USIC), Gauhati University, Guwahati and The Institute of Advanced Study in Science and Technology, Guwahati for providing instrumental help during the research work.

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