

SYNTHESIS OF SILVER NANOPARTICLES AND ITS ADVERSE EFFECT ON SEED GERMINATIONS IN *ORYZA SATIVA*, *VIGNA RADIATA* AND *BRASSICA CAMPESTRIS*.

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ABSTRACT:

The adverse effect due to toxicity of silver nanoparticles was evaluated using seed germination test with *Oryza sativa*, *Vigna radiata* and *Brassica campestris* exposed to different concentrations of silver nanoparticles ($\mu\text{g/ml}$). Silver nanoparticles were synthesized by chemical reductions method where AgNO_3 is reduced by NaBH_4 in aqueous medium at room temperature. Tween-20 was added as surfactant to prevent aggregations of nanoparticles. Formations of Silver nanoparticles were investigated by UV-Visible (UV-Vis) spectroscopy, Transmission Electron Microscope (TEM) and X-Ray Diffractions observations. The U.V-Vis analysis of nanoparticles shows absorptions peak at 400 nm. XRD analysis revealed the size whereas TEM helps to know its shape as well as size of nanoparticles. Inhibitory effect due to Tween-20 at different concentrations was studied on test seeds. Silver nanoparticles show adverse effect on seed germinations, root and shoot growth on these three plant species when they were soaked and incubated at different concentrations of nanoparticles. Inhibitions of root growth occurred at 1000 $\mu\text{g/ml}$, 1200 $\mu\text{g/ml}$ and 1600 $\mu\text{g/ml}$ for *Oryza sativa*, *Brassica campestris* and *Vigna radiata* respectively. Comparatively inhibitions of roots by ions was found to be 4500 $\mu\text{g/ml}$, 6000 $\mu\text{g/ml}$ and 3000 $\mu\text{g/ml}$ *Oryza sativa*, *Brassica campestris* and *Vigna radiata* respectively. Effect of Tween-20 was also observed. It was found that retardations of roots length in *Oryza sativa* and *Brassica campestris* occurs at 1% while *Vigna radiata* shows at 0.5% of Tween-20. A preliminary study was also performed in order to find out the possible toxic effects by different molar concentrations of Silver nitrate solutions and Sodium borohydride solutions on the seeds of three plant species since they have been used as raw materials for nanoparticle synthesis.

Key words: Nanoparticles ;Root growth ; Seed germination; Shoot growth and Tween-20.

[I] INTRODUCTIONS

Nanobiotechnology is a new emerging field of research. These particles are measured in the scale of nanometer size which is one-billionth of a meter. Nanoparticles exhibit a number of properties than the bulk materials. These small size nanoparticles can modify the physiochemical properties of the materials, which can lead to adverse biological effect on living cells [1]. Silver nanoparticles have received much attention worldwide due to attractive physical and chemical properties. Silver nanoparticles can be synthesized using various methods: chemical, electrochemical [2], γ -radiations [3], photochemical [4], laser ablations [5] etc. Silver nanoparticles can be synthesized through reduction of silver nitrate by sodium borohydride along with stabilizing agents in aqueous solutions. Stabilizing agents are necessary for protecting the growth of

nanoparticles by aggregations [6]. A lot of studies has been reported on positive and negative effects of nanoparticles on higher plants. Nano- SiO_2 and nano- TiO_2 show increase in nitrate reductases in *Glycine max*, it also enhances the ability to absorb and utilize water, it stimulates antioxidant systems and hastens its germination and growth [7]. Due to its variable shape and size, it is difficult to predict the positive or negative effect and its mode of action in the environment and within living systems [8]. Alumina nanoparticles show inhibitions to root elongations of corn, cucumber, soybean, cabbage and carrot [9]. ZnO nanoparticles also inhibit root elongations of ryegrass, radish and rape [10]. In this study silver nanoparticles were prepared by chemical reductions method in the presence of Tween-20 as stabilizing agent. Its formation was confirmed by U.V-Vis spectrophotometer and

TEM. The effect of silver nanoparticles and silver ions (both in $\mu\text{g/ml}$ concentrations), sodium borohydride and silver nitrate (both in Molar concentrations) and Tween-20 (v/v concentrations) was studied in seed germination and seedling growth on *O.sativa*, *V.radiata* and *B.campestris* species.

[II] MATERIALS AND METHODS

2.1 Chemicals

Silver nitrate (AgNO_3 , 99.5%, Merck), Sodium borohydride (NaBH_4 , 95%, Merck) and Tween-20 from Merck were used as received without further purifications. Double distilled water was used in all the experiments.

2.2 Seeds

Seeds of three plants species namely *Oryza sativa* (Variety: Jaya), *Vigna radiata* (Variety: K-851) and *Brassica campestris* (Variety: M-27) were collected from Assam seed corporations, INDIA. These selective plants are rich in carbohydrates (*Oryza sativa*), proteins (*Vigna radiata*) and fats (*Brassica campestris*). The seeds were small in size and used extensively in our diets. Seeds were kept in a dry place in dark conditions before used.

2.3 Seeds germinations

Surface sterilizations of seeds were done by immersing them into 10% sodium hypochlorite solutions for 10 minute. Then they were soaked in distilled water, silver nanoparticle suspensions, silver ions solutions, sodium borohydride solutions, silver nitrate solutions and Tween-20 suspensions for 2 hours after being rinsed with distilled water for three times [11]. The germinations process was done by putting a piece of filter paper into petridish, and 5 ml of test medium was added into it. 10 Sterilized seeds were transferred into each Petri plate and placed them into an incubator. In every experiment control seeds were also taken for comparison with the treated ones. The germination process was haltered, percentage of seed germinations, seedling root and shoot length in different treatments was measured.

2.4 Preparations of solutions

2.4.1 Preparations of Silver nanoparticle and Silver ions solutions:

The silver nanoparticles were prepared by chemical reductions method in which AgNO_3 aqueous solutions was reduced by NaBH_4 aqueous solutions with slight modifications from the standard method of preparations [12,13]. 1000 μl of 10^{-3} M AgNO_3 aqueous solutions was added in 100 ml of distilled water along with 0.1% of Tween-20 which presence act as stabilizers. Then it will be heated to boiling and add 500 μl of 6.6×10^{-3} M of NaBH_4 aqueous solutions [14]. During these process solutions was mixed vigorously. Solutions were heated until colour changed to pale yellow. Than it was removed from the heating element and keep it to cool at room temperature. The solutions should be kept at amber color bottle to keep away from sunlight. Silver ions were also prepared like above but in absence of sodium borohydride and Tween-20 to know its effects in seed germinations and seedling growth. Silver ions don't show any color changes but it should be protected and kept at amber color bottles.

2.4.2 Preparations of different concentrations of Tween-20, Sodium borohydride and Silver nitrate solutions:

Sodium borohydride and Silver nitrate are the important raw materials during preparations of silver nanoparticles by chemical reductions method. Their effect at different molar concentrations (10^{-1} - 10^{-5} M) was observed in the three plant species. Effect of Tween-20 which is used as surfactant has also been taken for studies since it is used to prevent aggregations of nanoparticles.

2.5 Characterizations of nanoparticles

The characterizations of nanoparticles was achieved by different techniques.

U.V-Vis spectrophotometer (Hitachi, Model no.3210) was used to get the absorptions peak to confirm the synthesis of silver nanoparticles. All spectra of different concentrations of nanoparticles prepared in distilled water were analyzed.

X-ray diffractions (Model-XPRT PRO) were used for analysis. The silver nanoparticles were prepared by pipetting a small amount of well-mixed particle solution. The solutions were

prepared uniformly thin films on a glass slide. The silver nanoparticles particles were found to be amorphous and had to be crystallized before using XRD.

However the size and shape of particles were obtained under Transmission Electron Microscope (Model-JEOL JSM 100 CX). It was observed when a drop of particle solutions was dried on a copper grid.

2.6 Statistical analysis

In every experiment, each treatment was conducted with three replicates. The results were presented as mean \pm SD (standard deviations). The statistical analysis of experimental values was compared with the control ones. Statistical significance was done by ANOVA test. It was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05.

[III] Results and Discussions

Nanobiotechnology has received great significance in every aspect of life. Different nanoparticles have shown different toxic mechanisms in test materials. Zinc nanoparticles have shown its effect on mice resulting in vomiting, diarrhoea and even death. Similarly Aluminum and Alumina nanoparticles show significant effect in growth of plants. In this report we have found significant inhibitions in rate of seed germination, root and shoot growth by silver nanoparticles when compared with silver ions. Preliminary examinations also shows individual toxic effects of Silver nitrate, Sodium borohydride and Tween-20 at particular concentrations.

Nanoparticles was prepared by reduction of silver nitrate solutions by sodium borohydride solutions in presence of Tween-20. The U.V-Vis absorptions spectra has proved quite sensitive to the formations of silver colloidal because silver nanoparticles exhibit an intense absorptions peak due to the surface Plasmon excitations. UV-Vis spectra of different concentrations of nanoparticles (50 μ g/ml, 500 μ g/ml and 1000 μ g/ml) shows absorbance peak at around 400 nm. This indicates total conversions of silver ions to silver nanoparticles.

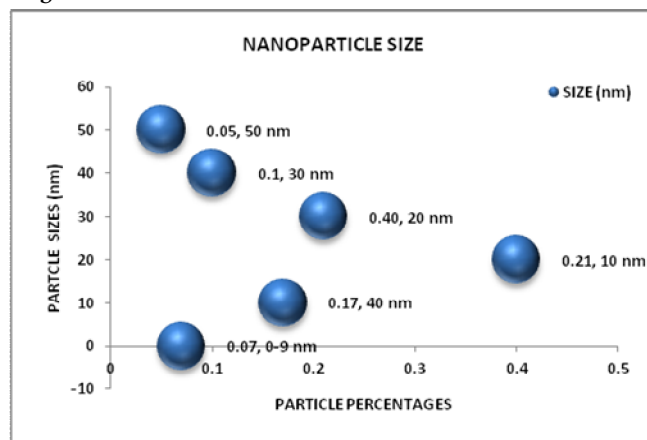
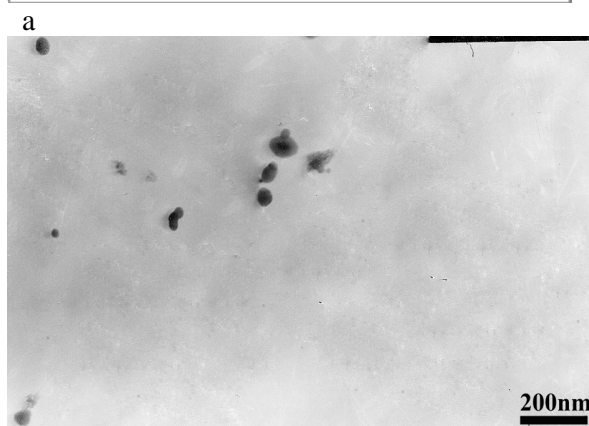
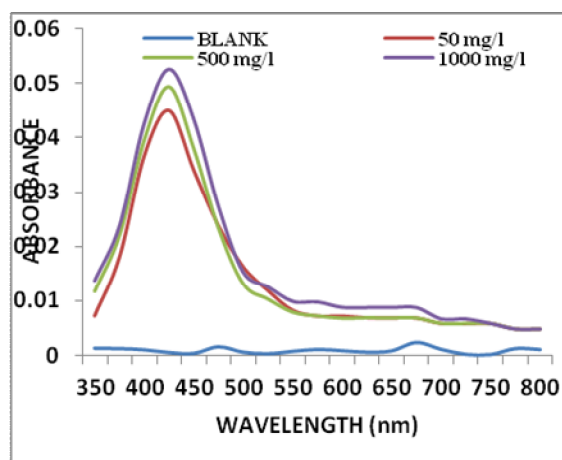


Figure 1: (a) UV-Visible absorptions spectrum (at 400 nm) of different concentrations of silver nanoparticles, (b) TEM images of Silver nanoparticles formed during preparations by chemical reductions methods and (c) Size distributions of silver nanoparticles as observed under TEM.

XRD pattern of silver nanoparticles shows the peak at 38.13 degrees. By using Debye-scherrer formula, average size of silver nanoparticles was calculated to be around 25 nm in size. However TEM studied confirmed about the shape and size of silver nanoparticles. It was

found that the average size of silver nanoparticle was around 20 nm and spherical in shape. Similar single peak was also observed by Das et al, 2009 when silver ions were reduced by ethanol. XRD measurements in centrifuged bacterial medium indicate presence of silver nanoparticles by showing a single peak [14].

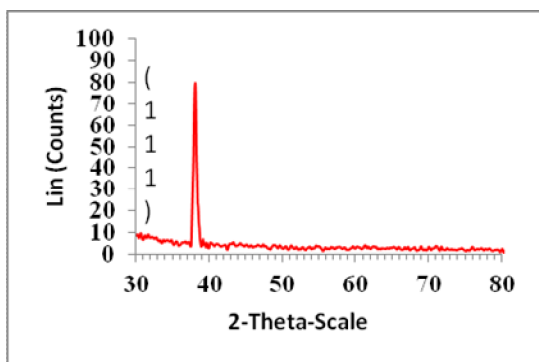
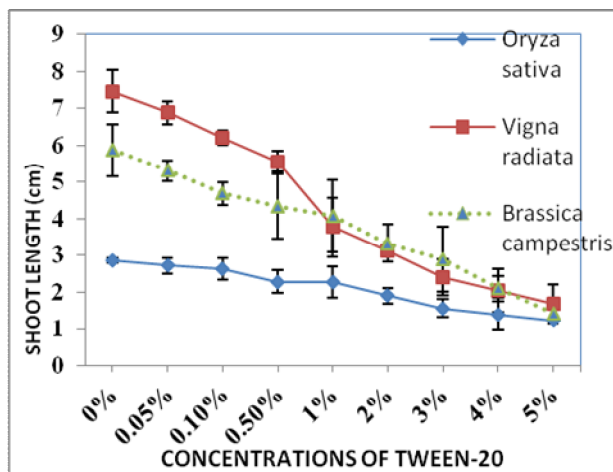
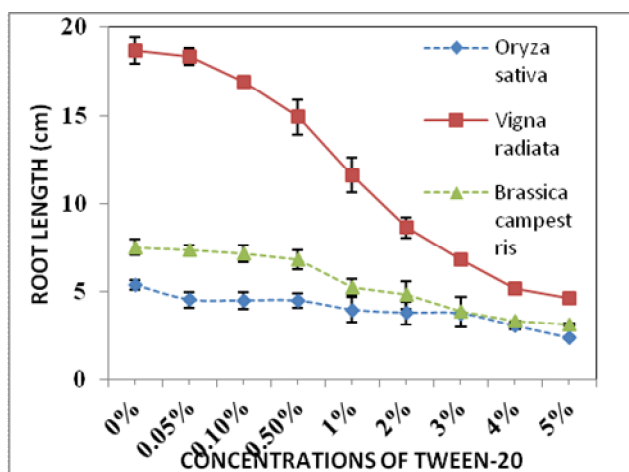


Figure 2: XRD spectrum of silver nanoparticles.

Tween-20 is used as a surfactant in the preparations of silver nanoparticles. It prevents aggregations of nanoparticles. In our study we have study different percentage of Tween-20 prepared in distilled water to see its effects on seed germinations, root and shoot length. Seeds of different plants species were first soaked for two hours and then incubated at $30^{\circ} \pm 2^{\circ}\text{C}$ for 7 days. The influence of different percent of Tween -20 was observed on germinations. In this work, 1 mm was used as the minimum length to be called as roots.

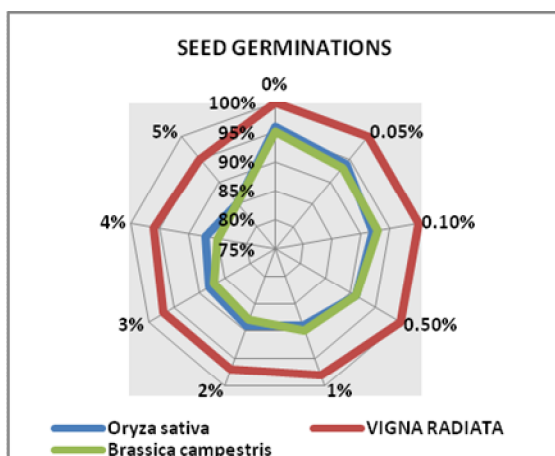


b



c

Figure 3: Effect of Tween-20 on seed germinations (a), root growth (b) and shoot growth (c) in different plant species.



a

* The mean difference is significant at the 0.05 level

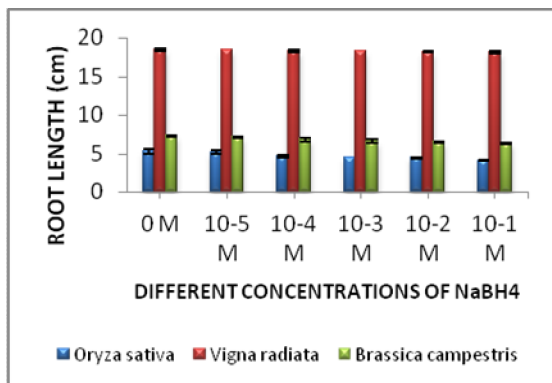
	<i>Oryza sativa</i>		<i>Vigna radiata</i>		<i>Brassica campestris</i>	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	-	-	-	-	-	-
0.05%	0.348	0.999	0.998	0.858	1.000	0.970
0.1%	0.294	0.987	0.787	0.087	0.994	0.396
0.5%	0.274	0.301	0.000*	0.003*	0.758	0.137
1%	0.018*	0.282	0.000*	0.000*	0.001*	0.056
2%	0.006*	0.016*	0.000*	0.000*	0.000*	0.003*
3%	0.005*	0.001*	0.000*	0.000*	0.000*	0.001*
4%	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
5%	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

Table 1: Effect of Tween-20 on growth of seeds (*P* value compared to control)

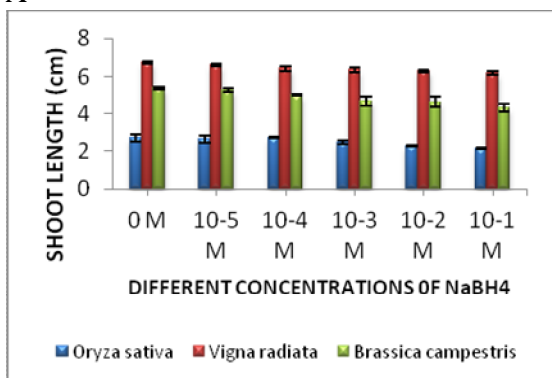
As per our findings we have taken 0.1% of Tween-20 as a surfactant for preventing aggregation of nanoparticles. At these particular concentration Tween-20 does not show any negative effect on root and shoot length. The inhibitions of root growth was found at 0.5% Tween-20 *Vigna radiata* ($p=0.000$) when compared with control. However in case of *Oryza sativa* ($p=0.018$) and *Brassica campestris* ($p=0.001$) the inhibitions of root occurred at 1% Tween-20. Similarly shoot growth was also inhibit at 2% of Tween-20 both in *Oryza sativa* ($p=0.016$) and *Brassica campestris* ($p=0.003$) whereas *Vigna radiata* ($p=0.003$) was significantly inhibited at 0.5%. Tween-20 effects on different parts of plants have been reported depending upon concentrations. Inhibitions and stimulations effects on growth of pea stem sections were also reported by different types of surfactants [15]. This surfactant has tremendous impact on biological systems rather than surface tensions and preventions from aggregations of particles. Tween-20, Tween-80 and Tergitol NPX were studied on cucumber seedlings at different concentrations. It was reported that increasing the concentrations above 0.01 % resulted in significant in further repressions of elongations which was due to chemical activity [16]. In our cases there was no significant effect of Tween-20 was observed at 0.05 % and 0.1 % concentrations in all the three species. Our result supports that increase in concentrations

effects seed germinations, root and shoot elongations of *Oryza sativa*, *Vigna radiata* and *Brassica campestris*.

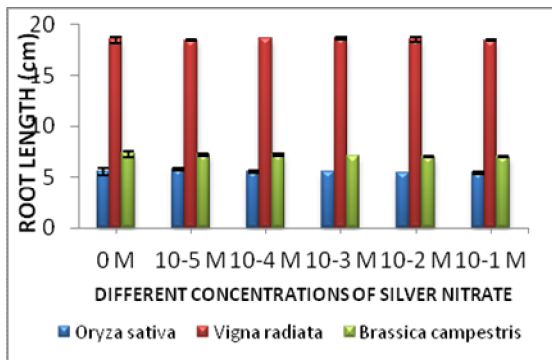
Different molar concentrations of sodium borohydride and silver nitrate were used to find out the toxic limits of respective against root and shoot growth. These chemicals were used as raw materials during the preparation of silver nanoparticles. Seeds were soaked and incubated as same as above at different molar concentrations. Both AgNO_3 and NaBH_4 at 10^{-3} M concentrations donot show any significant differences in root growth when compared with the control one (Table 2 and Table 3). However in case of shoot growth of *Vigna radiata* (in case of NaBH_4) it shows significant differences with control one (Table 3). Individual toxicity of silver nitrate was found to be more prevalent in seed germinations of *Vigna radiata* [17]. This may be due to the presence of Cobalt ions which overcome the effects of silver ions on germinations and growth. Similar results were also obtained in case of barley seed germinations where silver ions inhibited seed germinations where cobalt was effect less and cobalt ion overcome the silver ion inhibitions for germinations [18]. To maintain homogeneity in treatments, we have used 10^{-3} M concentrations of AgNO_3 and NaBH_4 to prepare silver nanoparticle solutions.



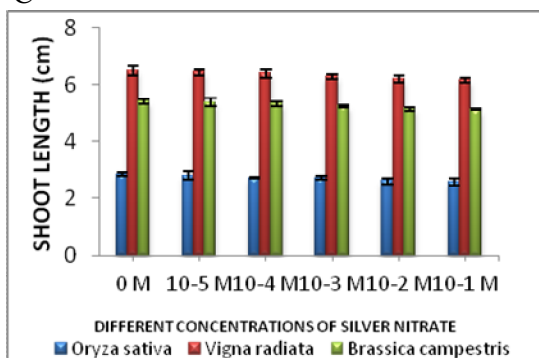
A



B



C



D

Figure 4: Effect of Sodium borohydride (A, B) and Silver nitrate (C, D) on root and shoot growth on 7 Days of treatment.

	<i>Oryza sativa</i>		<i>Vigna radiata</i>		<i>Brassica campestris</i>	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	-	-	-	-	-	-
10 ⁻⁵ M	1.000	0.988	1.000	0.969	0.999	0.977
10 ⁻⁴ M	0.989	1.000	0.760	0.186	0.664	0.570
10 ⁻³ M	0.171	0.657	0.877	0.032	0.444	0.465
10 ⁻² M	0.028*	0.075*	0.236	0.001*	0.033*	0.327*
10 ⁻¹ M	0.007*	0.014*	0.048*	0.000*	0.001*	0.057*

* The mean difference is significant at the 0.05 level

Table 2: Effect of Sodium borohydride on growth of seeds (P value compared to control)

	<i>Oryza sativa</i>		<i>Vigna radiata</i>		<i>Brassica campestris</i>	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	-	-	-	-	-	-
10 ⁻⁵ M	0.984	0.741	0.960	0.993	0.807	0.989
10 ⁻⁴ M	0.576	0.607	0.137	0.181	0.251	0.727
10 ⁻³ M	0.101	0.180	0.073	0.076	0.053	0.084
10 ⁻² M	0.005*	0.023*	0.001*	0.001*	0.002*	0.010*
10 ⁻¹ M	0.000*	0.000*	0.000*	0.000*	0.000*	0.007*

* The mean difference is significant at the 0.05 level

Table 3: Effect of Silver nitrate on growth of seeds (P value compared to control)

Silver nitrate and Sodium borohydride were also used during the preparations of silver nanoparticles. So, it is quite necessary to examine its individual effect on root and shoot growth when compared to control silver nitrate solutions do not show reductions in root and shoot growth at all Molar concentrations. In case of sodium borohydride there is a significant reduction in root and shoot growth at 10⁻² M and 10⁻¹ M concentrations.

It was earlier reported that 2000 mg/l (among the concentrations of 20, 200, 2000 mg/l) of the nano-Al₂O₃ suspensions shows inhibitions of root growth on five different plant species [13]. The concentrations of 2000 mg/l were, initially used to see the effect of silver nanoparticles on seed germination, root and shoot growth. Germination of seeds and application of nanoparticles during germination will be carried out following the method described by Lin and Xing (2007).

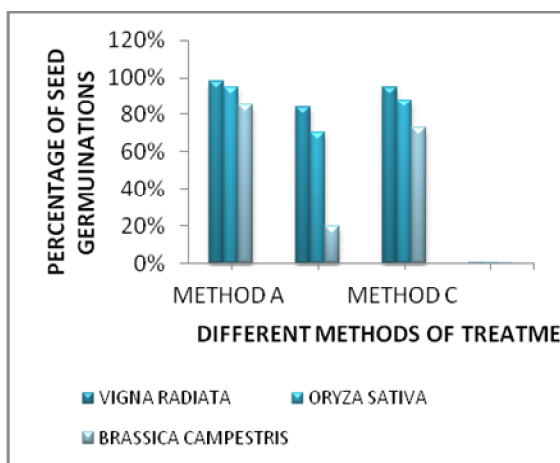
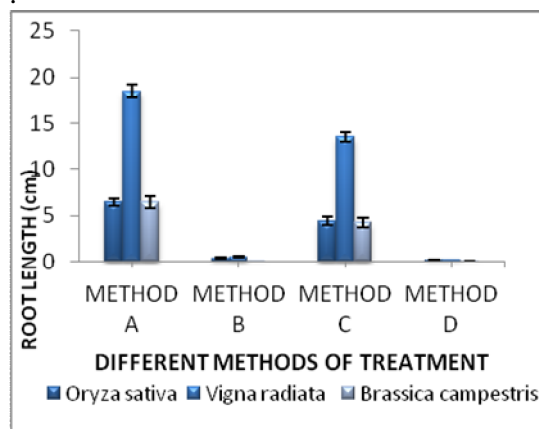


Figure 5: Percentage of seed germination of three different plant species by different methods of treatments.

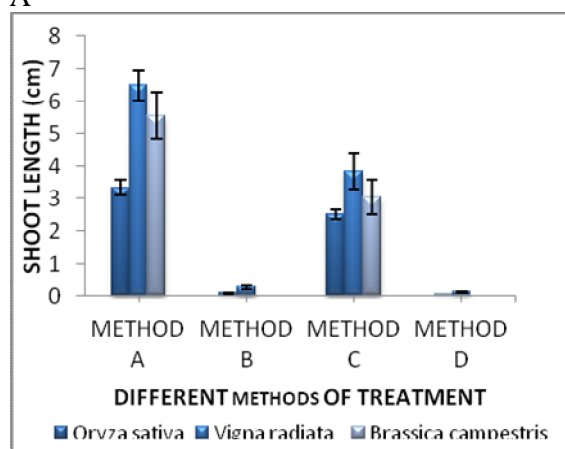
We have used different methods of treatment to observe the phytotoxicity of silver nanoparticles in plants. In the case of Method D where seeds are soaked and incubated under nanoparticles suspensions shows significant decreased in root and shoot length when compared with Method A. Method A treatment was used a control where we soaked and incubated the seeds under distilled water. However there is little significant growth in Method C but Method B shows inhibitions root and shoot length. The growth rate of different methods of treatment is shown in ascending order.

Method D < Method B < Method C < Method A

It signifies the effect of silver nanoparticles on seed germinations when they were exposed directly to the plant species. However nature has provided some extent to provide defense mechanisms against the low concentrations of silver nanoparticles suspensions



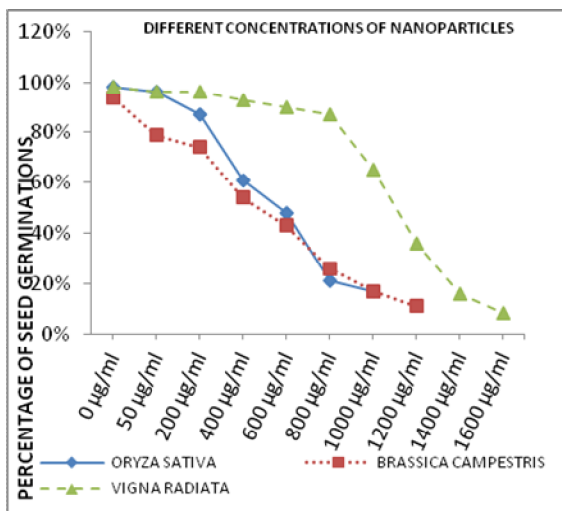
A



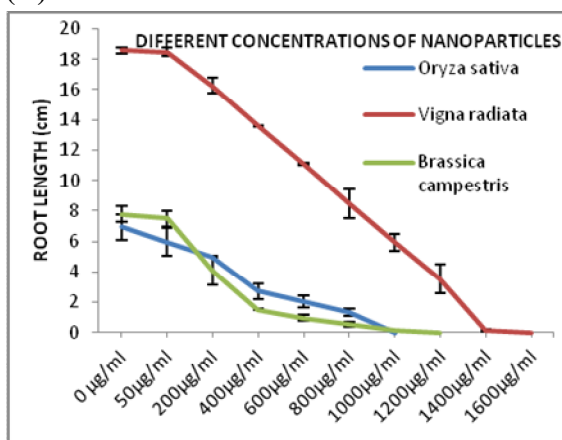
B

Figure 6 : Effect of different methods of treatment in root (A) and shoot (B) growth.

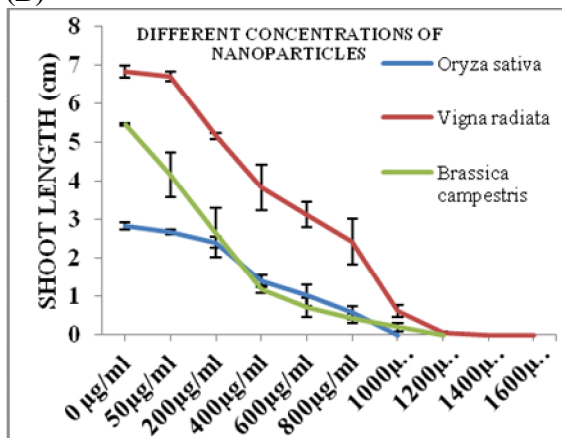
Different concentrations of silver nanoparticles show variations in seed germinations, root growth and shoot growth when they were treated with same. It was found that the higher the concentrations the more adverse effect on plant species. The effect of silver ions was also found but it is not like the effect of silver nanoparticles when compare with silver nanoparticles. Seed germinations, root length and shoot length of *Oryza sativa*, *Vigna radiata* and *Brassica campestris* treated with silver nanoparticles were given in figure below.



(A)



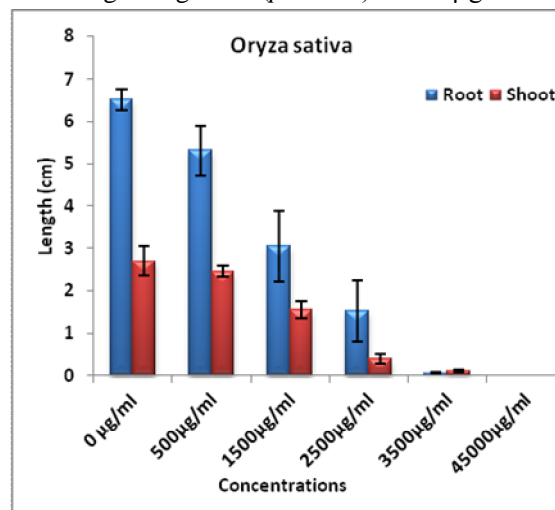
(B)



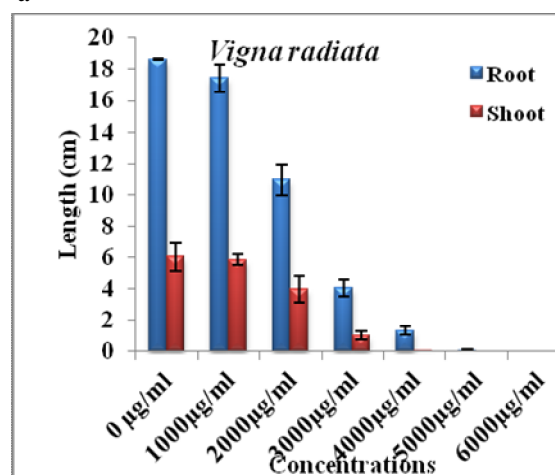
(C)

Figure 7: Effect of different concentrations of silver nanoparticle on seed germinations (A), root growth (B) and shoot growth (C) on different plant species (Day-7). The effect of different concentrations of silver nanoparticles was observed differently in selected plant species. Direct treatment with silver nanoparticles cause adverse effect on root

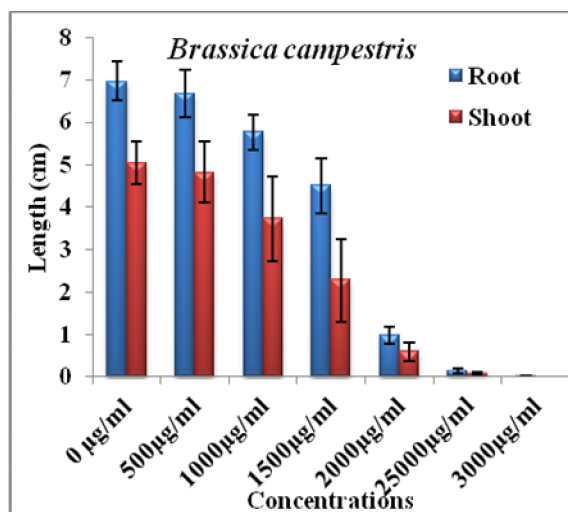
and shoot elongations of these selected plants. The most effected plant species was *Oryza sativa* and *Brassica campestris*. The terminations of root growth for *Oryza sativa* and *Brassica campestris* was 1000µg/ml and 1200µg/ml respectively by silver nanoparticles. It was found that *Oryza sativa* shows significant inhibitions of root growth ($p=0.022$) and shoot growth ($p=0.000$) from 200 µg/ml and 400 µg/ml respectively when compared with the control one. Similarly *Vigna radiata* also shows retardations of both root ($p=0.001$) and shoot ($p=0.000$) growth at 600 µg/ml and 400 µg/ml but the root growth is totally terminated at 1600 µg/ml. Interestingly *Brassica campestris* shows more resistance in shoot ($p=0.003$) growth at 50 µg/ml towards nanoparticles treatments. It starts inhibiting root growth ($p=0.000$) at 400 µg/ml.



a



b



c

Figure 8: Effect of silver ions on root (a) and shoot (b) growth in *Oryza sativa*, *Vigna radiata* and *Brassica campestris*

In case of *Oryza sativa* the root length does not show inhibitions at 500µg/ml ($p=0.114$) but when the concentrations of ions is increased to 1500µg/ml ($p=0.000$) then it shows significant decrease in root elongations when compared with control. This same inhibitions was happened to *Brassica campestris* ($p=0.034$) and *Vigna radiata* ($p=0.000$) at 1000 µg/ml and 2000 µg/ml respectively. In case of shoot growth the growth is retarded at 1500 µg/ml for *Oryza sativa* ($p=0.000$) and 2000 µg/ml *Vigna radiata* ($p=0.003$) however *Brassica campestris* ($p=0.002$) shows retardations at 1500µg/ml. Thus these reports can easily illustrate the effect of silver nanoparticles on seed germination, root and shoot growth of three plant species.

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