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Research Article

CYTOTOXICITY OF NANOPARTICLE ON THE SEEDLING OF THE PLANTS BELONGING TO THE FABACEAE FAMILY

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ABSTRACT

The research was carried out to show the influence of Silver, Iron and Sulphur Nanoparticles on seed germination under *in-vitro* condition on different morphological and biochemical parameters. In the present study, Silver (AgNPs), Iron nanoparticles (Fe NPs) and Sulphur nanoparticles (SNPs) were synthesized by chemical reduction process. UV-vis spectrophotometer analysis revealed that synthesized AgNPs, FeNps and SNPs are in nano size range and the influence of synthesized NPs were studied on germination and seedling growth of *Vigna radiata* (Green Gram). Seeds of samples were surface sterilized using ethanol 90%, soaked in a sterile distilled water for 60min, then soaked in silver, Iron and Sulphur nanoparticles at 100 mg/L (100 ppm) concentrations for 15 minutes. The work was initiated from treated and non-treated seed embryos of sample. The effect of nanoparticles on the morphological and biochemical level was detected. Protein, amino acid, sugar, Tannin, alkaloid and flavonoid content were used as biochemical parameters to estimate the effects of AgNPs, FeNP and SNPs on the metabolism of seedlings. The Results indicated that nanoparticles positively influenced the growth of the plants for most of the parameters studied, such as seed germination, root growth, biochemical metabolism of plants i.e. primary and secondary metabolites.

Keywords: Cytotoxicity, Silver nanoparticles, Iron nanoparticles, Sulphur nanoparticle, UV spectrophotometer, metabolites.

1. INTRODUCTION

Nanotechnology is a field of interdisciplinary research where functional systems are engineered at nano scale. Among the different types of metallic NPs, Silver and Iron nanoparticles (AgNPs and FeNPs) have been mainly used as preliminary material due to its natural abundance, low cost production, non-toxic nature with good electrical and optical properties. For this investigation, we have synthesized silver, Iron and Sulphur nanoparticles by the chemical reduction process. Seed germination is an important process which indicates the beginning of plant development [1]. During the process of water absorption, NPs (Nanoparticles) may also be absorbed and enter seeds through coat pores. Entry of NPs into seeds has been reported previously and has been shown to be primarily dependent on NPs size and concentration [2]. Recently, many studies have showed the physiological responses of plant seedlings to nanoparticles during germination [3]. We have selected seeds of *Vigna radiata* (Green gram) as samples because it is an annual legume crop, grown primarily for it is protein and energy-rich dry seeds. It is also rich in variety of active compounds for health benefits such as primary and secondary metabolites [3]; they are of great medicinal values [4] and have many important roles in human and animal nutrition. Nanotechnology can present solution to increasing the value of agricultural products and environmental problems [5].Taking this into consideration, the present investigation was undertaken to show the influence of Silver, Iron and Sulphur Nanoparticles on seed germination under in-vitro condition and on different morphological and biochemical parameters.

2. Material and Method

2.1. Collection of samples and Chemicals

Seeds of *Vigna radiata* were collected from the local market of Patna, Bihar. All the chemicals and reagents used in this study were purchased from SRL Pvt. Limited, Mumbai.

2.2. Synthesis of silver Nanoparticles

50 ml of 0.001 M $AgNO_3$ was put on magnetic stirrer. To this solution, 5 ml of 1% sodium citrate was added drop by drop and was mixed vigorously. Colour change of the solution was observed after half an hour (Grey).Then it was cool to room temperature and stored in brown bottle [6].

2.3. Synthesis of Iron Nanoparticle

15g $FeCl_3.6H_2O$was dissolve in 150ml pure water with stirring and then 2g $NaBH_4$added to the solution and temperature was increased to 80°C.The colour of the solution change from bright to dark.

2.4. Synthesis of Sulphur Nanoparticle

5gmof solid sodium thio-sulphate and 5g of oxalic acid was dissolved in double distilled water separately and left for 40 minutes at room temperature for completion of the reaction. After equilibration the sample was sonicated in water bath a yellow precipitate was formed.

2.5. Treatment with nanoparticles

Seeds were sterilized by washing with 90% ethanol for 5min followed by thorough washing with sterile distilled water. Then the seeds were soaked in the sterile distilled water, after that seeds were soaked in copper (100mg/ml) and silver nanoparticles (100mg/ml) for 15 minutes [7]. After soaking, seeds were dried, crushed and stored in air tight container for further use.

2.6. Germination of seeds:

Treated and non-treated seeds were germinated on the cotton fitted in Petri dishes for 15 days under the yellow bulb as light source.

2.7. Measured parameters:

We have measured following parameters to see the effect of our synthesized nanoparticles on the plant *Vigna radiata*.

2.8. % of germination:

Percentage (%) of Germination was calculated using the following formula [7]: -

$$\% \text{ of Germination} = \frac{\text{No. seeds germinated}}{\text{Total seeds cultured}} \times 100$$

Seedling biomass was measured after 14 days of germination [8]. The effect on the plant height (roots and shoot length) and leaf colour are also observed.

2.9. Chlorophyll Analysis

Chlorophyll content was estimated by previously reported method [9]. The absorbance of the solution was estimated at 645nm and 663nm wavelength against the solvent (acetone) blank. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

- Total Chlorophyll = Chlorophyll a + Chlorophyll b
- Chlorophyll a = 12.7(A663) - 2.69(A645)
- Chlorophyll b = 22.9(A645) - 4.68(A663)

2.10. Quantitative estimation

The sample was screened for the presence of Protein, Sugar and Amino acid by using following standard method of estimation.

2.11. Protein estimation

Total protein content was estimated by Lowrymethods [10]. Briefly, 0.25 g of dry powdered samples was homogenized with 10 ml of cold acetate buffer (0.1 M). The homogenate was filtered. The filtrate was centrifuged at 5000rpm for 15 min. in a centrifuge, the supernatant contains soluble proteins. 1 ml from this supernatant was taken in another tube and 5ml of alkaline reagent was added to it. After adding alkaline reagent, the solution was kept undisturbed for 10 minutes. Then, after 10 minutes, 0.5 ml Folin-Ciocalteau's reagent was added. Its absorbance was read at 750 nm against the blank by using a spectrophotometer. The protein content was calculated by using a standard curve prepared from BSA (bovine serum albumin).

2.12. Total soluble sugar estimation:

Total soluble sugar was estimated by the method of Dey [11]. Briefly, 500 mg each of fresh normal plant material was homogenized with 10.0 ml of 80% ethanol. Each sample was centrifuged at 2000 rpm for 20 minutes. The supernatants were collected separately to 1.0 ml of alcoholic extract; 1.0 ml of 5% phenol was added and mixed. Then 5.0 ml of 96% sulphuric acid was added rapidly. Each tube was gently agitated during addition of sulphuric acid and then allowed to stand in water bath at 26-30°C for 20 minutes. The OD of the characteristic yellow orange colour thus developed was measured at 490 nm in a spectrophotometer after setting for 1000mg/L transmission against the blank, standard curve was prepared by using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight of tissue.

2.13. Total free amino acid estimation:

Total free amino acid was estimated by using Sircelj [12]. Briefly, 100 mg of dry powdered plant material of each sample was extracted in 10 ml of 80% ethanol and centrifuged at 8,000g for 15min. The supernatant containing the alcohol-distilled water and 1 ml of ninhydrin reagent was shaken vigorously and then heated in a boiling water bath for about 20 min and added to 5ml diluents (equal volumes of water and n-propanol). Results were expressed in terms of µg of amino acid per gram of dry tissue.

3. RESULTS AND DISCUSSION

3.1. UV- Vis Spectrophotometer

In the present study *Albizia lebbeck* leaf extract were used as reducing agent for the synthesis of silver and copper nanoparticles respectively. Silver and copper nanoparticles were successfully synthesized in *Albizia*. Further these synthesized nanoparticles were characterized by UV-Vis spectral analysis at the range of 200-800 nm using UV-Vis Spectrophotometer.

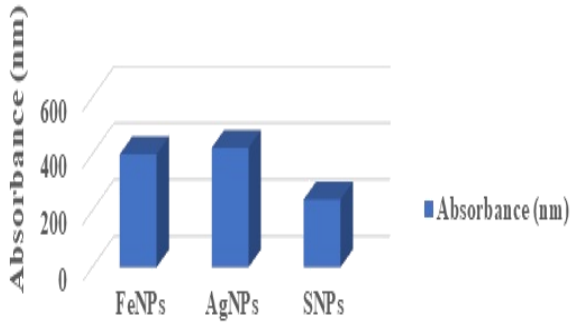

Fig. 1: UV- Vis Spectrophotometer of FeNPs, AgNPs and SNPs for *Albizia*

The colour intensity of the synthesized AgNPs and FeNPs increased with the duration of incubation period. In case of Silver nanoparticles, the colour changed from colourless to pale yellow within 10 min and then to brown in 60 min, the colour change to dark brown after 24 h and there was no significant colour change afterwards. The synthesis of Ag nanoparticles was confirmed by measuring the UV-Vis spectrum of the reaction media. As shown in Fig. 1, the absorption spectrum of the Ag nanoparticles, solution showed surface Plasmon band of 423 nm, confirmed the synthesis of Ag nanoparticles, in *Albizia*. Similar results were observed by Ahmad et al in which silver nanoparticles were synthesized using *Punica granatum* [8].In case of iron nanoparticles, colour changed from orange to brown black that confirmed the presence of Fe nanoparticles. The UV-Vis spectra of Fe nanoparticles synthesized using *Albizialebbeck* showed absorption peak maximum at 400nm as shown in Fig 1. Similar results were obtained for the synthesis of Fe nanoparticles on the *Capparis zeylanica* plant with little variation in the previous work [13].Analysis through X-Ray Diffraction and FTIR, crystalline nature of the nanoparticles was confirmed.

3.2. FTIR

Infrared Spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance.

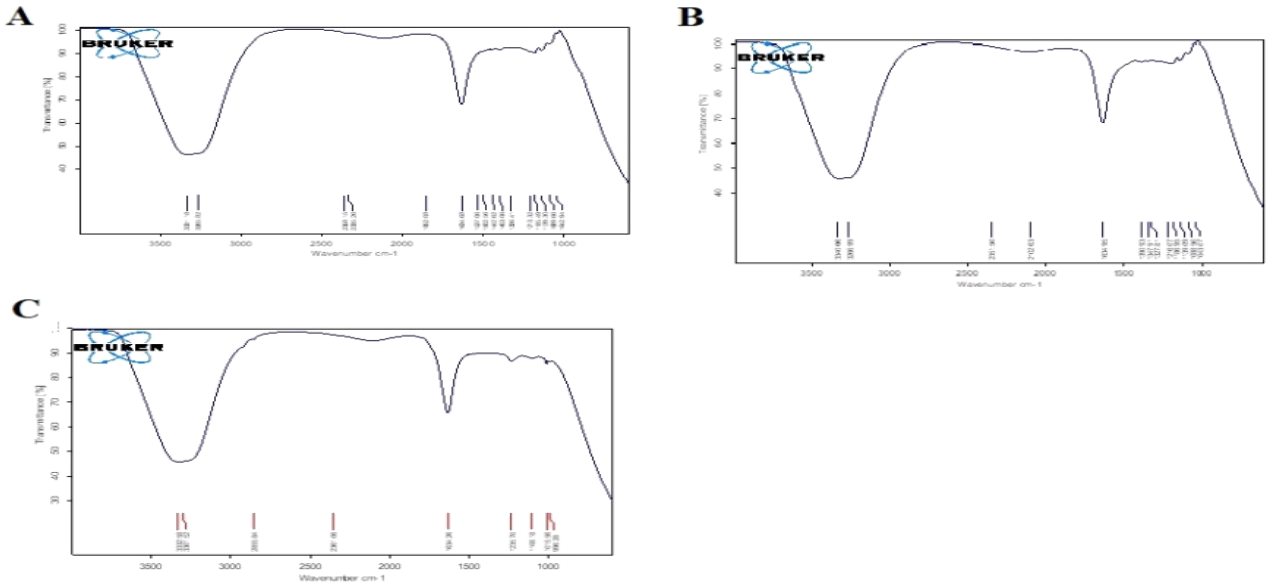

Fig. 2. Graph indicating the Iron nanoparticle (A), Silver nanoparticle (B) and sulphur nanoparticle (C) for *Albizia*

The particles were analysed under FTIR for the size conformation. It is an effective analytical technique for detecting functional groups and characterizing covalent bonding information. FTIR spectrum analysis of the functional group present in *Albiziia lebbeck* extract, the Silver and Iron nanoparticle was confirmed by change in the FTIR spectrum after the synthesis. The fig. 2 showed the peak between 3904 cm^{-1} to 2927.35cm^{-1}. The peak at 1629.40 cm^{-1} and 1693cm^{-1} shows the symmetric stretching vibration of COOH [14] and the reduction of silver ion to silver nanoparticle. For Iron the fig 3 showed the peak between 1709.30 cm-1 to 1453.09 cm^{-1} correspond to O-H stretching H-bonded alcohol and phenol, the stretching property of Hydroxyl group act as a reducing agent for synthesis of iron nanoparticle [15].

3.3. XRD

As shown in figure 3 the presence of structural peaks in XRD patterns and the average crystalline size clearly illustrate that the nanoparticles synthesized by green method were Nano crystalline in nature with average particle size 2θ correspond to ±15nm for iron [16] however in case of silver nanoparticle. The whole spectrum of 2θ values ranging from 10° to 80° with particle size 44nm, the diffraction peak was found to be broad around the base indicating that the nanoparticles are nanosized and spherical in shape. The diffraction of nanoparticle was slightly deflected probably due to fewer biomolecules of stabilizing agent or enzyme present in the extracts [17].

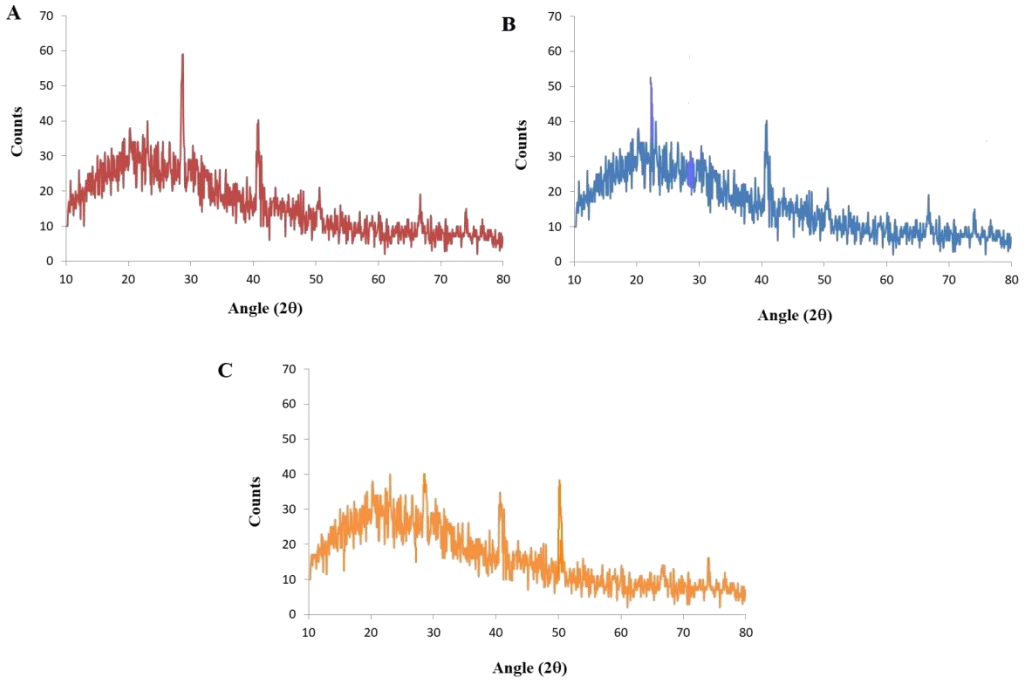

Fig. 3. XRD pattern of iron nanoparticles (A), Silver nanoparticle (B) and sulphur nanoparticle (C) by *Albizia*

3.4. Morphological Parameter

Incubated seeds were germinated on petridishes and were observed at the interval of 2 days. After that, morphological and biochemical parameters were tested. We have taken only few morphological and biochemical parameters for our investigation. Treated and non-treated seeds were germinated on cotton for 15 days. The observations were taken at an interval of two days. The final observation was taken after 15 days [7]. In case of *Vigna radiata* we have observed that % of germination, plant height, shoot and root length was more in AgNPs treated seeds followed by FeNPs treated seeds than SNPs treated seed as compared to the control but there is not so much differences obtained in the plant height, shoot and root length in FeNPs and SNPs treated samples with control (Fig.4 A, B). We can also observe the changes in

the colour of the growing leaves. Plant biomass, i.e., plant fresh weight and dry weight increases in FeNPs treated sample followed by SNPs treated than AgNPs treated sample as compared to the control (Fig. 4 C). The present study (Fig. 4 D) revealed that after 20 days of treatment of the seeds of *Vigna radiata* in NPs solution, the stress caused by SNPs is found to be greater than FeNPs and AgNPs as compare to the control. The plant height, shoot length and root length significantly increased in AgNPs treated sample but the morphological growth parameter was not found desirable in case of FeNPs and SNPs due to the stress.

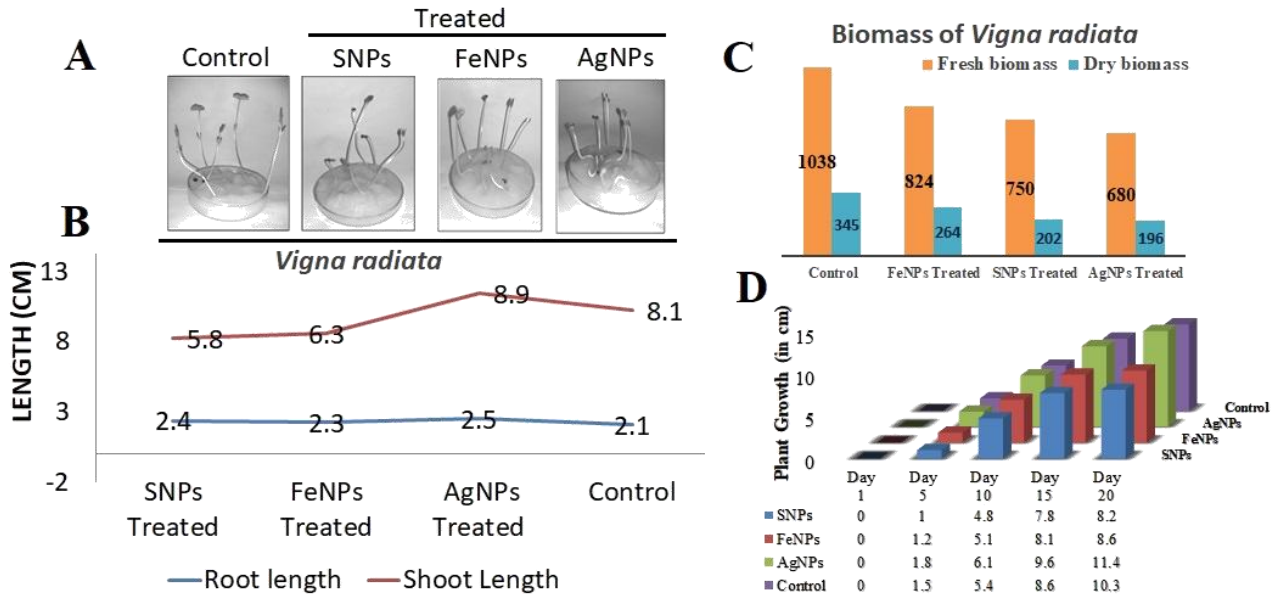

Fig.4. Effect of SNPs, AgNPs and FeNPs on plant biomass

Table 1: Effect of AgNPs, SNPs and FeNPs on morphological parameters

Physical Parameters	Non-Treated (Control)	Treated		
		FeNPs	AgNPs	SNPs
% of Germination	100%	70%	80%	50%
Seedling Vigour Growth	1080	574	824	430
Day of Emergence of First Leaf	3	6	4	7
Number of leaves	16	8	10	4
Plant Height (cm)	10.3	8.6	11.4	8.2
Shoot Length (cm)	8.1	6.3	8.9	5.8
Root Length (cm)	2.1	2.3	2.5	2.4
Plant Fresh Weight (mg)	1038	824	680	750
Plant Dry Weight (mg)	345	264	196	202
Colour of Leaves	Light Green	Dark Green	Light Green	Dark Green

According to (table1) In AgNPs treated plants, the leaves colour is lighter than the FeNPs and SNPs treated plant samples. It is incidental that AgNPs have positive effects as compared to FeNPs and SNPs on plant height, root length and shoot length. Fresh and Dry plant biomass of FeNPs (824gm, 264 gm) is seen to be more than SNPs (750gm, 202 gm) and AgNPs show decreased in biomass (680gm, 196gm) as compare to control (1023gm, 345 gm), decrease in root length and biomass indicate intensification in toxicity [18]. NPs accumulation seizes the growth of the plant hence SNPs show more stress as compare to FeNPs and AgNPs treated seeds. The plant height, shoot length and root length significantly increased in AgNPs treated sample. (11.4 cm, 9cm, 2.5 cm, respectively) as compared to control (10.3cm, 8.1 cm, 2.1 cm), but there is no significant differences

obtained in FeNPs treated sample (8.6 cm, 6.3 cm, 2,1 cm) as compared to SNPs treated sample(8.2cm, 5.8 cm, 2.4 cm). From the table 1, we can also see the changes in the colour of the growing leaves, i.e., green coloured leaves in control, dark green coloured leaves in AgNPs treated plants.

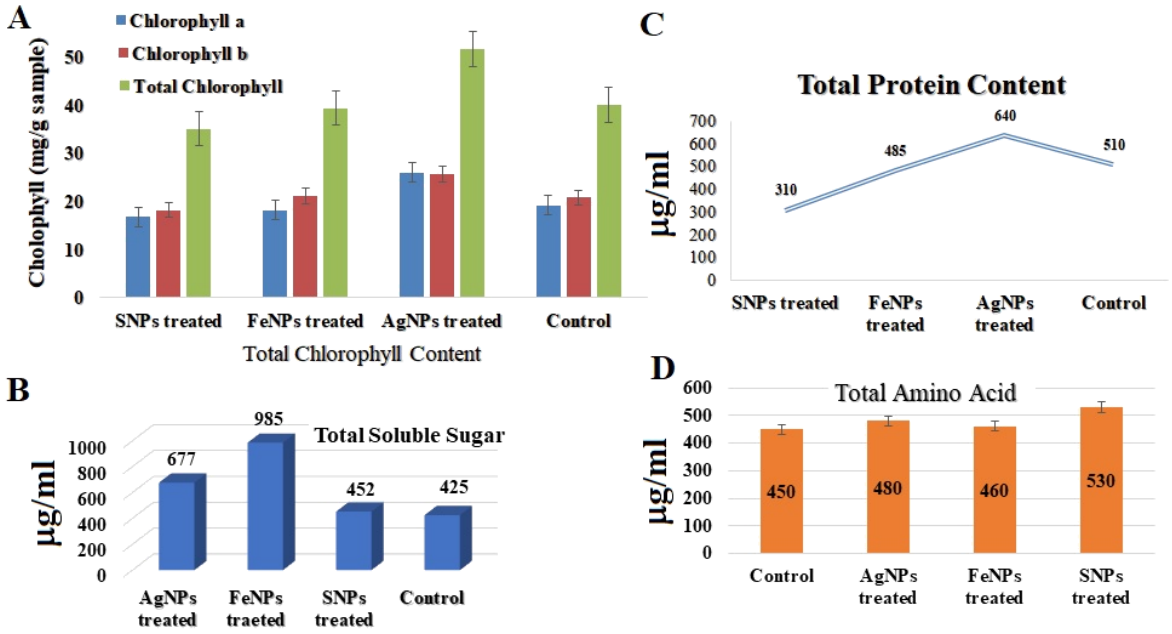

Fig. 5: Effect of SNPs, AgNPs and FeNPs on biochemical parameters A) Chlorophyll content B) Total Soluble Sugar, C) Total Protein Content, D) Total Amino acids

3.5. Biochemical parameter

We all know that chlorophyll is the most important element in the photosynthesis. The leaves obtained after the germination, were employed to determine their contents.

3.6. Total Chlorophyll Content

It is evident from the above figure (Fig.5 A) that in case of *Vigna radiata*, the Chlorophyll a, chlorophyll b and total chlorophyll contents are found to be present higher in AgNPs treated sample followed by FeNPs treated sample in comparison to the control. Chlorophyll a, chlorophyll b and total chlorophyll content was found to be greater in the AgNPs treated plant samples (51.78mg/g) of *Vigna radiata*, while there were no significant differences obtained in the plant treated with FeNPs (39.43) and control (40.14mg/g). SNPs treated sample (35.13mg/g) show decreased chlorophyll content. Because all NPs treatment samples resulted in the change in chlorophylls contents, we suspected that the rate of photosynthesis may be affected in both the NPs treated plant samples. This was in conformity with the earlier findings where they reported increase in the concentration of both NPs, the chlorophyll content decreases with 2-3 mg/g sample [19, 20].

3.7. Total soluble sugar (TSS) content

It is evident from the results in Fig. 5B, that the absorbance of SNPs is found to be least i.e. 0.452 in comparison to AgNPs that has a absorbance of 0.677 and FeNPs has shown maximum absorbance i.e 0.985 respectively. Among all treatments, highest TSS content was found in the FeNPs treated samples followed by AgNPs treated samples of *vigna radiata* which was found to be(0.985mg/ml,0,677mg/ml), in comparison to control (0.425mg/ml), while in case of SNPs treated seeds it was found to be (0.452mg/ml). The result is Similar to the reports of enhanced carbohydrate content with AgNPs and FeNPs treatment in wheat [21]; we could also find significant improvement of TSS in comparison to the control.

3.8. Protein Content

From results in Fig. 5C we have observed that higher protein content was found to be present in AgNPs treated sample followed by FeNPs treated sample in comparison to the control, protein was very low in SNPs

treated sample in comparison to the control. we have observed that higher protein content was present in seed extract of *Vigna radiata* treated with AgNPs (0.64mg/ml) While there were no significant difference in protein content in the seed extract treated with FeNPs (0.485mg/ml) in comparison to the control (0.510mg/ml), SNPs treated seeds show decreased content of protein (0.310mg/ml).

3.9.Total free amino acid content

Our result reveals that stress caused by Ag, Fe and S nanoparticles hampered free amino acid content of the *Vigna radiata*. Total free amino acid content is found maximum in SNPs treated sample when compared to control. Total free amino acid content is maximum in SNPs treated seeds sample, however there is not much difference is observed in FeNPs and AgNPs treated seeds as compare to the control. The reduced absorption of NPs might be used as a catalyst for improvement in biochemical metabolism of plants. Our findings are in line with previous reports where they observed that if FeNPs concentration increases, it causes stress which in turn increases the total free amino acid content by 50-100 μg/ml and hence disturb the metabolic processes [22, 23].

The present investigation has demonstrated the effect of silver, Iron and sulphur nanoparticle on the seed germination behaviour of *Vigna radiata*. All the nanoparticles positively influenced the growth of the plants for most of the parameters studied. In this study we observed the toxicity of three different nanoparticles SNPs, FeNPs and AgNPs on *Vigna radiata* which has occupied an important place in human nutrition as rich source of protein in the diet of consumers of India and western diet. We know that metabolites are the most important biomolecules required for the basic metabolic processes. From our investigation we have noticed that the content of metabolites have been increased or decreased when the plants were treated with AgNPs, FeNPs and SNPs as compared to control, similarly the nutritional value of the samples also increase or decrease**.** NPs have enhanced the % of seed germination, shoot length and root length whereas higher concentrations inhibited the growth, or it cause death of the plant. Hence AgNPs, FeNPs and SNPs not only used as test material to reveal their non-toxicity mechanism in plants but also for determining their biocompatibility and identifying their potential agriculture applications in crop improvement and food productivity. It is interesting to note that lower doses of NPs were found to be non-toxic while favoured seed germination, seedling growth and metabolism of the test plant to some extent. Once the nanoparticles enter seeds, it has long-lasting effects on the germination, growth and biochemical profiling. Further study is required to explore the minimum and favourable concentrations of NPs which may be beneficial for plant growth and metabolism to increase the productivity as well as safe release in the environment.

Conflict of Interest: *Authors claim no conflict of interest.*

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