

# **Plant-mediated biosynthesis of nanoparticles by leaves extract of *Cannabis* species and a study of its effect on the growth and physiology of *Vigna radiata*.**

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## **ABSTRACT**

The synthesis of these nanoparticles using readily available biological materials have immense applications in various field and there has been rapid development in the use of organisms in this area due to the ease of formation of nanoparticles and their growing success. This study focuses on the synthesis of Iron oxide nanoparticles by the use of *Cannabis* leaves extract in an ecofriendly and sustainable manner. The synthesized iron oxide nanoparticles were then characterized through UV- Visible spectrophotometer and FTIR. Graded concentrations of the synthesized FeO NPs (5, 25, 45 and 65%) were made and a study of its effect on various growth parameters such as germination percentage, root and shoot length, number of leaves, plant height, fresh and dry weight, chlorophyll and protein content on a valuable economic pulse, *Vigna radiata* was done. The results show that the iron oxide nanoparticles had significant positive effects on the germination and growth of *Vigna radiata* plants at lower concentrations, however, it had slightly inhibitory effects at higher concentrations. Therefore, this study reveals its significance in finding out the potentiality of green synthesized iron oxide nanoparticles in boosting the overall growth of *Vigna radiata* plants at a suitable concentration.

Keywords: Green synthesis, iron oxide nanoparticles, *Cannabis sativa*, *Vigna radiata*, growth parameters.

**INTRODUCTION**

Nanotechnology has broad applications in almost all the accessible science and technological fields. Nanotechnology and its role alongside nanoparticles is ever increasing in several fields of science, research, development and including day to day life. Nanoparticles are ultrafine particles which have particle size ranging between 1-100 nanometers in size. They have unique physicochemical properties and they also play a role in serving as 'magic bullets' which contain chemicals, herbicides or genes that aim at the plant parts to release their content [7,13, 10, 5]. Among the nanoparticles which has been mostly investigated and applied, iron oxide nanoparticles also come under the category [2]. Iron oxide can be found in variety of forms because it is a transition metal. Iron oxide nanoparticles possess physical and chemical properties basing upon which these nanoparticles bear enormous applications in many fields like chemistry, physics and material science. Iron oxide nanoparticles can be prepared using many different methods [3].

The process of generating nanoparticles through biological methods using non-toxic and eco-friendly ways has drawn more attention and different methods for the biosynthesis of nanoparticles using required metal salts has also been developed. It is advantageous because of its limited environmental impacts in contrast to other physicochemical methods, as well as its ability to produce nanoparticles, which are free of contamination, in large amount just in short span of time. Moreover, the biological routes can give products of a better and more desired shape and size as compared to physical and chemical routes of manufacture [6].

Synthesis of nanoparticles by using the different plant parts is entirely novel and effective, and the need for increased pressure, additional energy, higher temperature and harmful chemicals is not at all necessary. Some of the researchers have also described about the synthesis of nanoparticles by using eco-friendly materials like leaf extract, bacteria and fungus which offers

numerous advantages and compatibility for pharmaceutical and other biomedical applications [11]. The whole process takes place in a single step reaction rapidly and efficiently, administered at room temperature and pressure. The organic components contained in the plant extracts helps in the capping of the nanoparticles therefore, no additional emulsifier or capping agents are required for the synthesis. Moreover, plant extracts can also reduce the metal ions, the bio-reduction of nanoparticles by mixtures of biomolecules present in plant extracts is environmentally benign, yet chemically complex. The overall synthesis procedure is facile, consistent, easily scaled up, sustainable, economical and environmentally benign [8, 4, 6].

Nanoparticles have already found its use in various applications along with in vitro analysis although its implementation as a medicinal purpose is generally on an experimental basis. Drugs obligated to nanoparticles assert to be more significant over the regular mode of medications. Several nanoparticles have wide ranging antimicrobial activity contrary to animal and human pathogens, larvicidal against mosquitoes and plasmodial pathogens causing diseases such as malaria and filariasis. There have also been reports about the implementation of nanoparticles in food packaging for antimicrobial purposes. Application of nanoparticles in crop protection and agriculture also play an emerging and significant role [6]. In the field of agriculture, nanoparticles have been particularly aimed to improve the efficiency and sustainability of agricultural practices by minimizing the input and generating less waste, in contrast to conventional products and approaches, and also for improving the fertilization process, increasing the productivity through enhancement of nutrients and reducing the needs of plant protection products [1,12].

The present study is focused on the bio-synthesis iron oxide nanoparticles by leaf extract of *Cannabis* and to study the effect of green synthesized Iron oxide nanoparticles on the germination and early seedling growth of *Vigna radiata*, which is commonly known as mungbean or green gram, as well as the outcome of foliar sprays of Iron oxide nanoparticles on physiology as well as other growth attributes of *Vigna radiata* plants.

## **MATERIALS AND METHODS**

### **Collection of the plant sample**

Fresh and healthy leaves of *Cannabis sativa* were collected from the locality in Satnampura, Phagwara, Punjab.

### **Preparation of the aqueous extract from *Cannabis sativa***

25g of the *Cannabis sativa* leaves were taken and washed with tap water, further rinsed carefully with distilled water. It was then cut into fine pieces. In an Erlenmeyer flask, the sample was taken and aqueous extract was made by boiling the leaf pieces in 100ml distilled water at 80°C for about 15 minutes. The extract was cooled down to room temperature and filtered through filter paper (Whatman). This extract had been collected in a clean dried beaker and stored at 4°C for the synthesis of the required nanoparticles.

### **Preparation of Iron Oxide Nanoparticles**

An Erlenmeyer flask of 250 ml was taken, 20ml of the extract of *Cannabis* leaves was obtained and added drop wise in 100 ml of 0.01M FeCl<sub>3</sub>.H<sub>2</sub>O solution at room temperature. Within 2-3 minutes the color changed to greenish black indicating that the Iron oxide nanoparticles have been formed. Purification of the solution of iron oxide nanoparticles were performed by centrifugation at 12,000 rpm for 15 min, after which the pellet was re-dispersed in deionized water. The Iron oxide nanoparticles were dried at 80°C in a hot air oven and kept in an air tight flask to be used further

### **Characterization of the synthesized Iron oxide Nanoparticles**

Absorbance spectrum was analyzed by using Shimadzu UV- 1800 spectrophotometer within the range of 800-200 nm. 0.1 ml of the sample was obtained in a test tube and diluted with 3ml distilled water. The solution was then filled in a quartz cuvette with path length of 10mm.

Distilled water served as the baseline. FTIR analysis was done through FTIR-8400S, Shimadzu, Japan with 4cm-1 resolution. The transmission mode of the analysis was done within the range of 400-4000 cm-1 for identifying the functional groups in the leaves extract which resulted in the synthesis of the required nanoparticles.

### **Preparation of different concentrations of Iron oxide nanoparticles**

Stock solution of 200ml of iron oxide nanoparticles was prepared. For bioassays tests, different concentrations of 5%, 25%, 45% and 65% iron oxide nanoparticles were obtained from the stock and diluted with distilled water to make the final volume of 100ml. The solutions were kept in air tight containers and stored at 4°C for further use.

### **Collection of *Vigna radiata* seeds**

*Vigna radiata* seeds were purchased from the local market in Hargobind Nagar, Phagwara, Punjab. Then the surface sterilization of *Vigna* seeds had been done using 5% sodium hypochlorite, which was kept for not more than 10 minutes. Then these were rinsed several times with distilled water.

### **Seed Germination Assay**

The experiment was performed in petri plates under laboratory conditions. In this, 15 petri dishes were sterilized and lined with Whatman no. 1 filter paper. *Vigna* seeds were placed in the petri plates and moistened with different concentrations of FeO NP nanoparticles i.e. 5%, 25%, 45% and 65%. Distilled water was given to seeds kept in control, at a controlled temperature of  $25 \pm 1$ . The treatments were done in three replications. For germination test, 20 seeds were taken and the final germination percentage was calculated after 7 days to see effect of nanoparticles on seed germination. Seeds with radical length of 2mm or more were considered to be germinated.

Germination % = no. of seeds germinated/total no. of seeds taken x 100

For measurement of the length of the root and shoot, *Vigna radiata* seeds were soaked in distilled water and kept overnight. 5 seeds were taken and then, the measurements were taken after interval of 3, 6, 9, 12 and 15 days and it was expressed in cm.

### **Foliar spray bioassay**

15 plastic pots were taken and filled with sandy loam soil. 4-6 healthy seeds of *Vigna radiata* were sown in each pot. All the plants that were sown in the definite soil medium had been watered frequently to overcome stress due to water scarcity. After 20 days from sowing, the plants were treated with concentrations of nanoparticles i.e. 5%, 25%, 45% and 65% every 24 hours by foliar spray method which was continued 20 days. Distilled was sprayed to plants in control. At the time of harvest, plants were uprooted gently from the pot and certain growth parameters were determined such as the height of the plant, number of leaves, including fresh weight (FW) and dry weight (DW) of the plant.

Chlorophyll and protein content were also determined in the leaves of *Vigna radiata*. The estimation of the chlorophyll content was done using Arnon (1949) method, through which 80% acetone was used for extraction because of its solubility. The optical density of the samples was recorded using Spectrophotometer at 645nm and 663nm. Estimation of the total protein content was done through Lowry's method in which the absorbance of the sample was evaluated at 660nm. A calibration curve was estimated for BSA standards for the determination of the protein content in the leaves of *Vigna radiata*.

### **RESULTS AND DISCUSSIONS**

The green synthesis of iron oxide nanoparticles had been achieved by adding the leaves extract of *Cannabis sativa* drop wise into FeCl<sub>3</sub> solution. When the leaves extract was added to the yellow colored solution of FeCl<sub>3</sub>, the colour gradually changed to greenish black within 2 minutes. This is the first indication of the formation of the iron oxide nanoparticles.

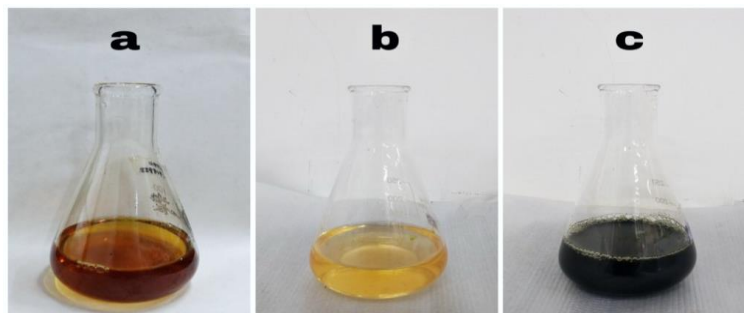


Figure 1: Green synthesis process a) *Cannabis* leaves extract b) 0.01M FeCl<sub>3</sub> solution c) synthesized iron oxide nanoparticles

### Characterization of the green synthesized Iron oxide nanoparticles

The UV-Visible spectrum of the synthesized iron oxide nanoparticles showed absorption peak at 265nm. This result showed some uniformity to the UV-Visible results of the green synthesized iron nanoparticles performed by Pattanayak and Nayak in 2012, in which they found the absorption peaks at 216nm and 268 nm confirming the formation of the required nanoparticles.

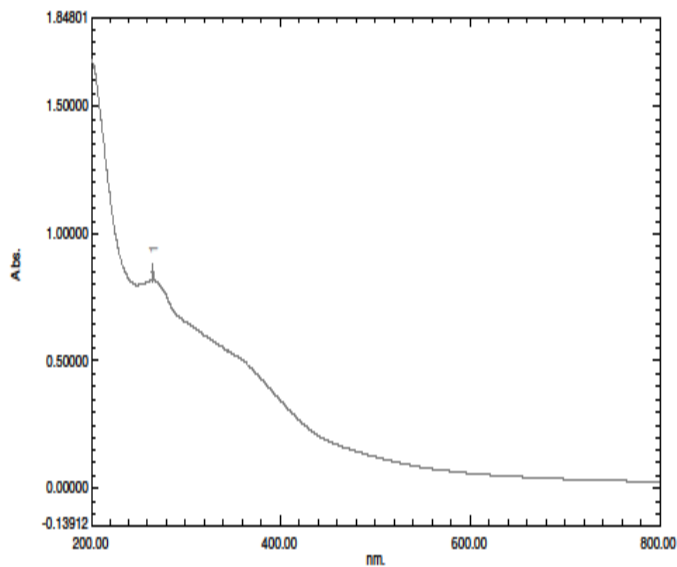


Figure 2: UV-Visible spectrum of the synthesized Iron oxide nanoparticles

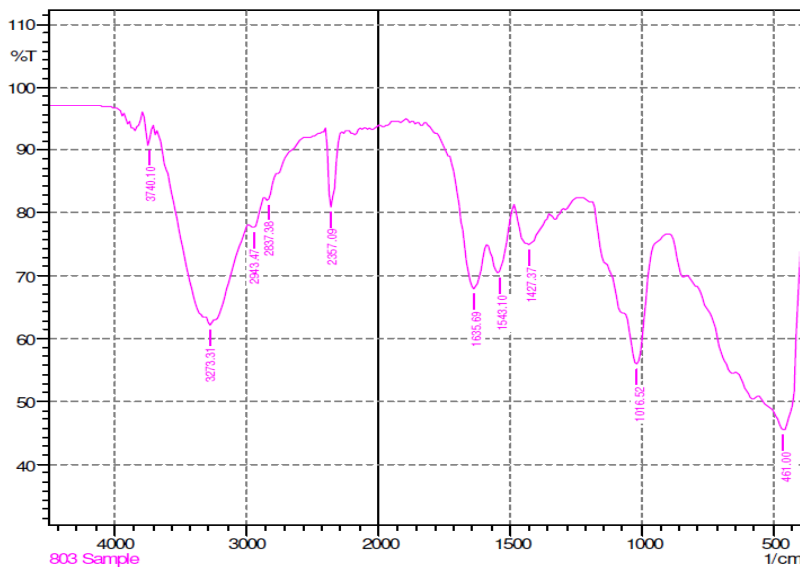


Figure 3: FTIR spectra of the synthesized Iron oxide nanoparticles

FTIR spectroscopy has been done in order to detect the biomolecules that are present in the nanoparticles synthesized using the leaves extract of *Cannabis sativa*. The FTIR analysis of the iron oxide nanoparticles showed a wide stretched peak at  $3273\text{ cm}^{-1}$  attributing to O-H group of alcohols. The absorption peak at  $2943\text{ cm}^{-1}$  attributes to the C-H stretch of alkanes. The peak at  $1635\text{ cm}^{-1}$  indicates the stretching of C=C due to alkenes and the peak at  $1427\text{ cm}^{-1}$  attributes to the O-H group of carboxylic acids. The presence of these functional groups confirms that the leaves extract of *Cannabis sativa* is involved in the reduction, capping and in the synthesis of the iron oxide nanoparticles.

### **Germination and Seedling growth**

The germination of the seeds contributes a suitable basis for the growth, development and productivity of plants. In the germination test, the results showed that iron oxide nanoparticles showed significant results at lower concentration than at higher concentrations. The treatments with different concentrations of iron oxide nanoparticles gave different effects to the growth of plants. Mean of three tests showed 100% germination at both 5% and 25%, thereby revealing increased germination of mung bean seeds by treatment with iron oxide nanoparticles as compared to those seeds treated with control. The minimum germination percentage of the seeds was 85.6% at 45% concentration. The interaction of iron oxide nanoparticles on the early seedling growth also showed excellent results on mung bean seeds treated with FeO nanoparticles at lower concentrations. Both the root and shoot length was found to be maximum at 5% FeO nanoparticles concentration i.e. 10.45cm for root and 1.7cm for shoot, while the minimum was observed at 65% i.e. 6.43cm for shoot and 1cm for root. The root length of seeds in control were measured 9.91cm, thereby revealing that the seeds treated with FeO nanoparticles at lower concentrations were superior compared to treatment with higher concentrations and control as well. Thus, through the seed experiment, the results revealed that the iron oxide nanoparticles enhanced the growth at lower concentrations. However, treatments with higher concentrations reduced the growth of the mung bean seeds. Similar observations were also recorded by Shankamma *et. al* [9].



**Foliar spray bioassay**

In case of growth attributes studied in pot experiment, it was observed that treatment with the green synthesized Iron oxide nanoparticles gave a positive effect at lower concentrations and it also proved superior than control treatments. Maximum number of leaves recorded was 8.33 at 5%, while the minimum number of leaves was 6.66 at 65% and the plants in control had a mean of 8 leaves. The maximum plant height measured was 20.56cm at 5%, while the minimum height measured was 16.06cm at 65% concentration and in control plants it was 18.733cm.

Highest value for fresh weight was 1.66g at 25% while the lowest value recorded for fresh weight was 0.833g at 65%. In control plants the fresh weight was 1.6g. As for dry weight the highest value was 0.24g at 25% followed by 0.23g at 25%. The lowest value for dry weight was 0.14g at 65%, and control plants weighed 0.21g. The experiment revealed decrease in the various parameters studied like the number of leaves, height of the plants, including fresh and dry weight with increase in concentration of Iron oxide nanoparticles.



Figure 4: *Vigna radiata* plants after 20 days of treatment. a)5% b)25% c)45% d)65% e) Control



Figure 5: *Vigna radiata* plants uprooted from pots after 20 days of treatment to study the effect of varying concentrations of FeO NPs on growth of the plant.

**Table 1:** Effect of different concentrations of green synthesized FeO NPs on the germination of *Vigna radiata* seeds within 7 days

<b>Concentrations</b>	<b>Germination Percentage Mean <math>\pm</math> Standard Deviation</b>
Control	92.22 $\pm$ 1.922
5%	100 $\pm$ 0
25%	100 $\pm$ 0
45%	85.6 $\pm$ 3.259
65%	86.2 $\pm$ 3.637

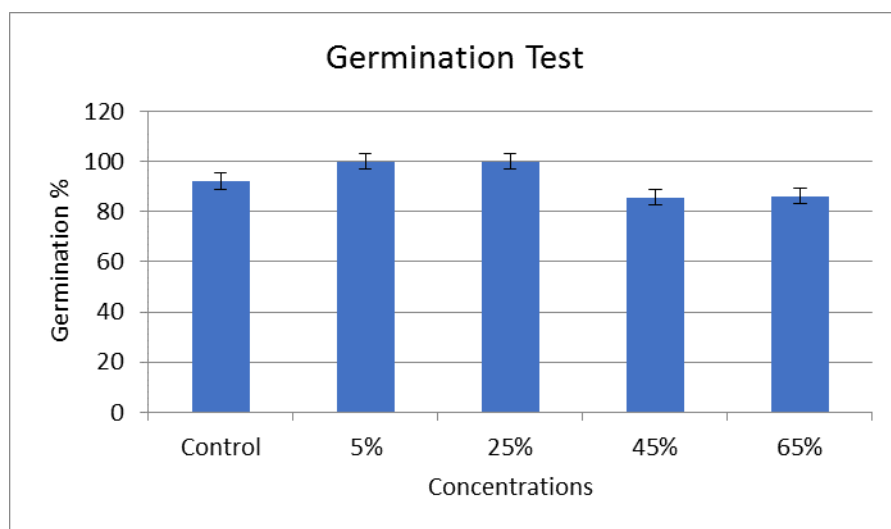


Figure 6: Effect of different concentrations of green synthesized FeO NPs on the germination of *Vigna radiata* seeds within 7 days.

**Table 2:** Results showing the root length of *Vigna radiata* seeds treated with varying concentrations of FeO NPs within 15 days.

	Mean $\pm$ Standard Deviation of Root length (cm)

Concentrations	Day 3	Day 6	Day 9	Day 12	Day 15
Control	1.186 ± 0.443772	2.9 ± 0.243311	5.3 ± 0.34641	7.516 ± 0.470354	9.91 ± 0.185203
5%	1.446 ± 0.481802	4.333 ± 0.522407	5.833 ± 0.650718	8.043 ± 0.427824	10.46 ± 0.667108
25%	1.606 ± 0.491664	3.683 ± 0.493997	5.656 ± 0.560387	7.72 ± 0.378201	9.596 ± 0.623084
45%	1.44 ± 0.242487	2.893 ± 0.695509	4.87 ± 0.121244	6.403 ± 0.363737	7.47 ± 0.491223
65%	0.986 ± 0.014633	2.186 ± 0.408085	3.843 ± 0.73542	5.406 ± 0.442869	6.43 ± 0.365103

**Table 3:** Results showing the shoot length of *Vigna radiata* seeds treated with varying concentrations of FeO NPs within 15 days.

Concentrations	Mean ± Standard Deviation of Shoot length (cm)				
	Day 3	Day 6	Day 9	Day 12	Day 15
Control	0	0.166 ± 0.152753	0.66 ± 0.23094	1.133 ± 0.251661	1.573 ± 0.185023
5%	0	0.266 ± 0.057735	0.8 ± 0.1	1.236 ± 0.063509	1.7 ± 0.096437
25%	0	0.133 ± 0.23094	0.56 ± 0.288675	1.103 ± 0.20502	1.47 ± 0.213776
45%	0	0	0.3 ± 0.1	0.866 ± 0.152753	1.233 ± 0.152753

65%	0	0	$0.333 \pm 0.057735$	$0.66 \pm 0.057735$	$1 \pm 0.173205$

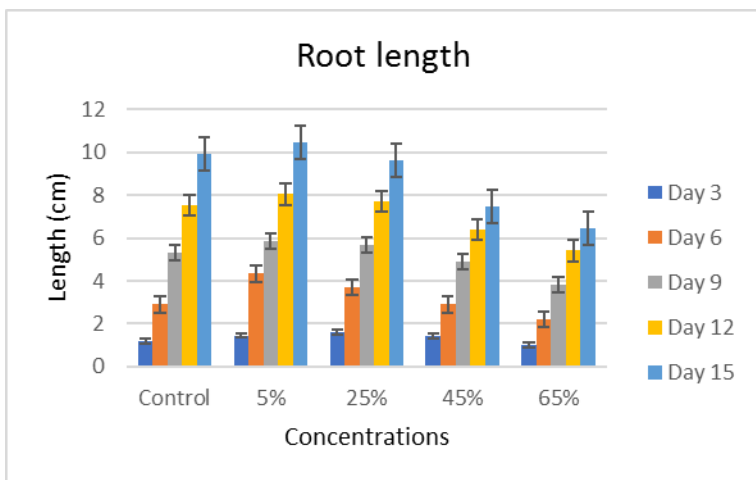


Figure 7: Root length of seeds treated with varying concentrations of FeO NPs within 15 days.

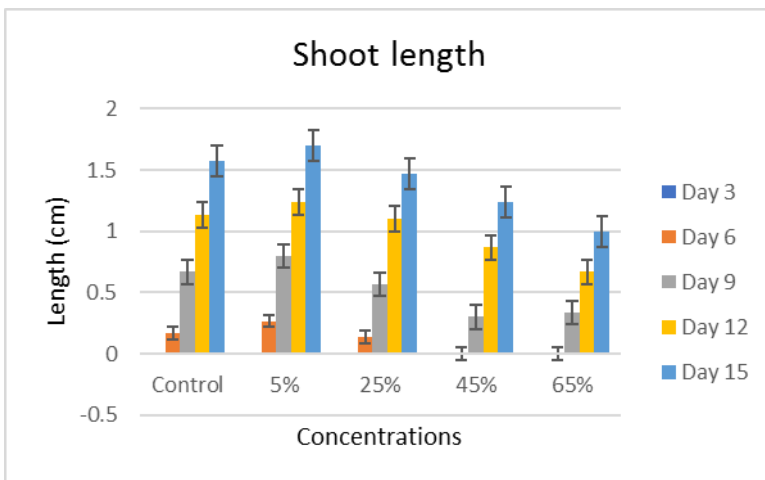


Figure 8: Shoot length of seeds treated with varying concentrations of FeO NPs within 15 days.

**Table 4:** Effect of foliar sprays on the growth of *Vigna radiata* plants sprayed with varying concentrations of FeO NPs for 20 days

Concentrations	Mean ± Standard Deviation			
	Number of leaves	Plant height (cm)	Fresh weight (g)	Dry weight (g)
Control	$8 \pm 1$	$18.733 \pm 0.378594$	$1.6 \pm 0.43589$	$0.21 \pm 0.026458$
5%	$8.333 \pm 0.57735$	$20.566 \pm 1.115049$	$1.533 \pm 0.378594$	$0.233 \pm 0.025166$
25%	$7.667 \pm 1.527525$	$18.233 \pm 0.404145$	$1.667 \pm 0.321455$	$0.24 \pm 0.043589$
45%	$7 \pm 1$	$16.866 \pm 0.51316$	$1.033 \pm 0.208167$	$0.173 \pm 0.015275$

65%	$6.667 \pm 1.154701$	$16.066 \pm 0.971253$	$0.833 \pm 0.11547$	$0.14 \pm 0.017321$
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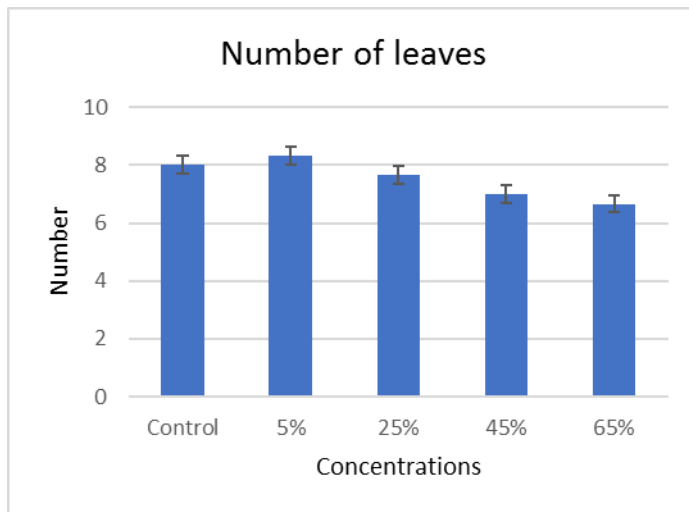


Figure 9: Count of the number of leaves in response to treatment with different concentrations of FeO NPs on *Vigna radiata*.

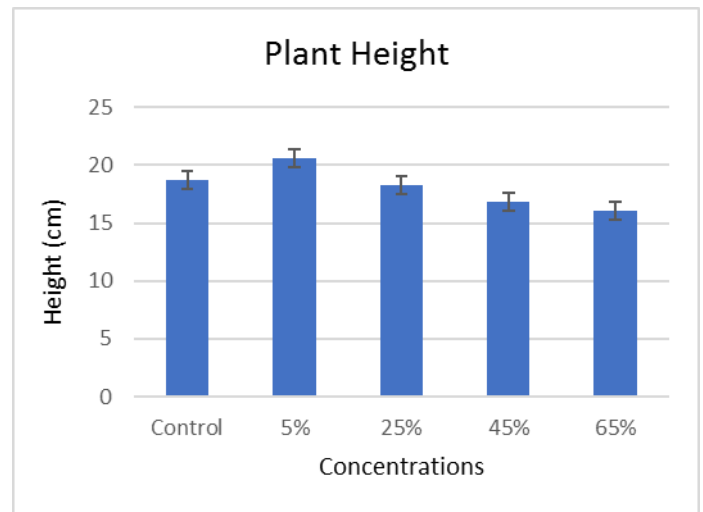


Figure 10: Result showing the height of *Vigna radiata* plants sprayed with varying concentrations of FeO NPs for 20 days.

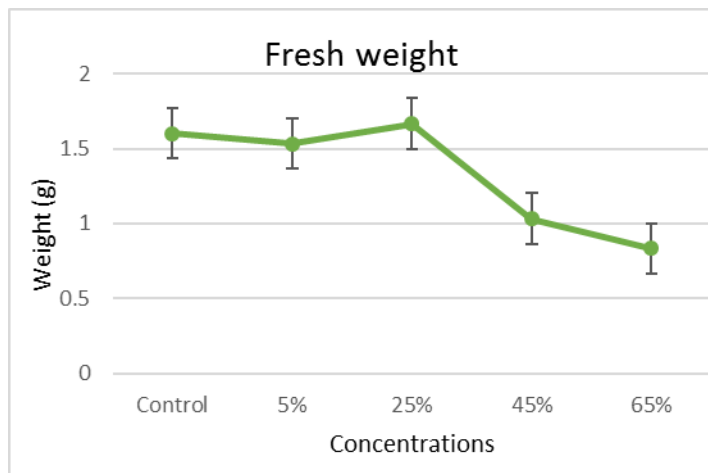


Figure 11: Fresh weight of *Vigna radiata* plants in response to treatment with different concentrations of FeO NPs

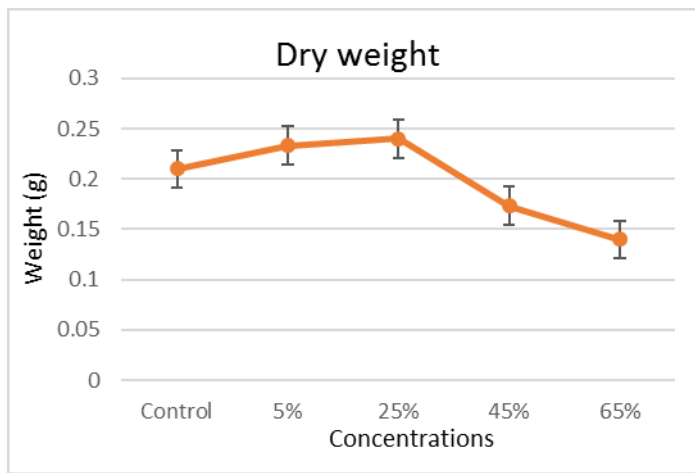


Figure 12: Dry weight of *Vigna radiata* plants in response to treatment with different concentrations of FeO NPs

**Chlorophyll content in the leaves of *Vigna radiata***

The chlorophyll content had been evaluated in fresh mung bean leaves extracted using 80% acetone. The result of the total chlorophyll content varied from 0.08453333 mg/g FW to 0.0628 mg/g FW. The chlorophyll content was highest in control plants although the leaves treated with 5% FeO NPs had just a slight decrease in the chlorophyll content (0.0837 mg/g FW). The lowest value was observed in the leaves treated with 65%. Therefore, it was seen that with the increase in the concentration of iron oxide nanoparticles, there was decrease in the total chlorophyll content in the leaves of *Vigna radiate* as was observed by [2].

**Protein content in the leaves of *Vigna radiata***

The result of the protein content present in the leaves of *Vigna radiata* are shown in table 7. The values varied from 1.221 to 0.654 mg/g. The protein concentration was found to be highest in control followed by lower concentrations of the iron oxide nanoparticles. 5% concentrations of FeO NPs had a protein concentration of 1.2006 mg/g, while at 65% the value was 0.654mg/g which was the minimum value. Figure 21 demonstrates about the declination of the protein content at different concentrations of iron oxide nanoparticles. The protein concentration at 5%

was slightly lesser than that of control. However, comparing to control, the treatments given at higher concentrations led to decrease in the protein content drastically. Thus, basing on the protein content in *Vigna radiata* leaves, it was observed that the iron oxide nanoparticles had significant effects at lower concentrations than at higher concentrations, since the protein content was found to be higher at lower concentrations of iron oxide nanoparticles and vice versa.

**Table 5:** Results showing the protein content in the leaves of *Vigna radiata* treated with varying concentrations of FeO NPs.

Concentrations	Mean ± Standard Deviation of chlorophyll content in leaves.		
	Chlorophyll a mg/g FW	Chlorophyll b mg/g FW	Total chlorophyll content Mg/g FW
Control	0.0251 ± 0.01193035	0.0376 ± 0.002357965	0.0845 ± 0.003494758
5%	0.0245 ± 0.000960902	0.1406 ± 0.176122751	0.0837 ± 0.003459769
25%	0.0220 ± 0.001320353	0.0346 ± 0.00406325	0.0735 ± 0.003251666
45%	0.0234 ± 0.000916515	0.0249 ± 0.001361372	0.065 ± 0.008861151
65%	0.0262 ± 0.005943344	0.0225 ± 0.003080584	0.0628 ± 0.001078579

**Table 6:** Results showing the protein content in the leaves of *Vigna radiata* treated with varying concentrations of FeO NPs.

Concentrations	Total protein content (mg/g) Mean ± Standard Deviation
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Control	$1.221 \pm 0.009643651$
5%	$1.2006 \pm 0.008020806$
25%	$0.954 \pm 0.013453624$
45%	$0.701 \pm 0.0090185$
65%	$0.654 \pm 0.00321455$

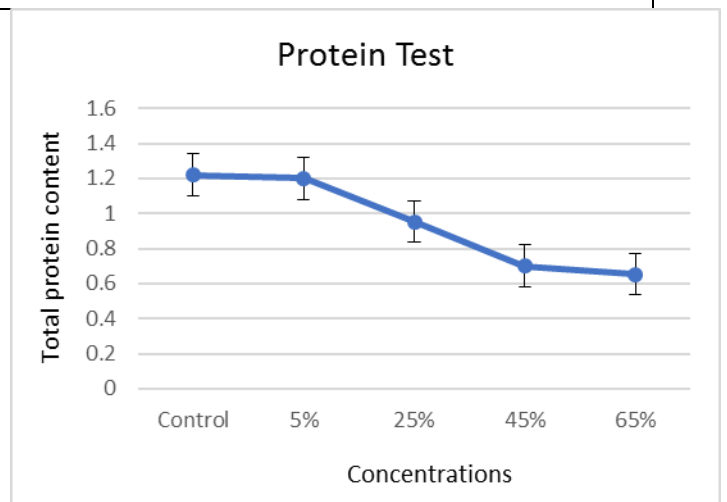
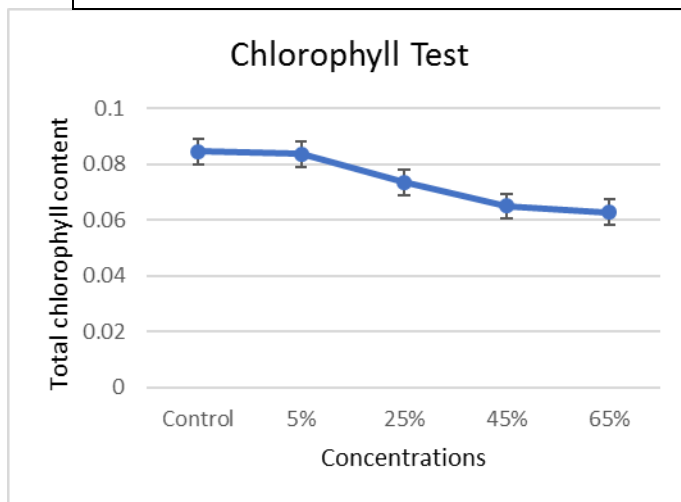


Figure 13: Total chlorophyll content in leaves of *Vigna radiata* in response to treatment with different concentrations of FeO NPs.

Figure 14: Total protein content in leaves of *Vigna radiata* in response to treatment with different concentrations of FeO NPs.

**CONCLUSION**

Enhanced germination of the seeds including the early growth of the plants is a requisite for attaining healthy and high yield of crops. The stimulatory effects seen at the early growth stages may be continued up to the mature stages of the plants as well, and even the plant productivity can also be improved by treatment with iron oxide nanoparticles in low concentration. The iron oxide nanoparticles, at an appropriate exposure, could be implemented in agricultural sectors.



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