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Morphological and biochemical analysis of impact of iron oxide nanoparticles on *Cicer arietinum and glycine max*

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Abstract
Nanotechnology, a new emerging and fascinating field of science, which also permits advanced research in many areas, and nanotechnological discoveries, could open up novel applications in the field of biotechnology and agriculture. Nanomaterial provide great opportunity in the field of agriculture because of their unique physiochemical properties. Nanotechnology in agriculture is currently focused on targeted agriculture, which involves the use of NPs with unique characteristics to promote crop and livestock productivity. It is possible to improve food quality, reduce global food production, plant protection, plant and animal disease detection, plant growth monitoring, and reduce waste by "sustainable expansion".The interaction of nanoparticles with plant result in several physiological, morphological, and genotoxic changes, and their understanding is important for the effective use of nanotechnology in agriculture. Researchers suggest that both positive and negative response of nanoparticles on plant growth and development depending upon the properties of nanomaterials, mode of application as well as plant species. e.g., been shown that a mixture of nanoscale SiO_2 and TiO_2 can increase nitrate reductase activity in soybean (*Glycine max*), enhance its ability to absorb and utilize water and fertilizer, stimulate its antioxidant system, and apparently hasten its germination and growth. Studies on the uptake, translocation and biotransformation, and risks of application of nanomaterials on agriculturally important crops are recent research focus for understanding the physiological, biochemical, and molecular mechanism of plant in relation to nanoparticles.

Keywords: metal oxide nanoparticles, nanomaterial, antioxidants, plant growth, seed germination, biotransformation.

1. Introduction

Chickpea and soybean belong to the legume family and they are annual crops. They are used worldwide for their dry seeds. Chickpeas and Soybean are rich in protein, carbohydrates, dietary fiber, and dietary minerals. Due to the increase in population, nutritional and quality food is demand and need for modern society and more production of food too but we have not enough land for farming. For solving this problem we need to switch on nanotechnology.

Nanotechnology, a new emerging and fascinating field of science, which also permits advanced research in many areas, and Nano technological discoveries, could open up novel applications in the field of biotechnology and agriculture. In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers. Nanoparticle, and ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10^{-9} meter). Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics and manufactured nanoparticles. This Nanoscale (1-100 nm in at least one dimension) materials have gained unprecedented attention because of their vast array of applications in agriculture, the environment, and health [1, 2, 3].

Nanotechnology in agriculture is currently focused on targeted agriculture, which involves the use of NPs with unique characteristics to promote crop and livestock productivity [4, 5]. In nanotechnology, it is possible to improve food quality, reduce global food production, plant protection, plant and animal disease detection, plant growth monitoring, and reduce waste by "sustainable expansion [6, 7, 8, 9]. Applications of nanotechnology in agriculture include fertilizers for plant growth and yield, sensors for soil quality monitoring, and pesticides for pest and disease management. Nanoparticles occur widely in nature and are objects of study in many sciences such as chemistry, physics, geology, and biology. Being at the transition between bulk materials and atomic or molecular structures, they often exhibit phenomena that are not observed at either scale. The production of nanoparticles with specific properties is an important branch of nanotechnology. In general, the small size of nanoparticles leads to a lower concentration of point defects compared to their bulk counterparts [10].

2. Materials and Method

2.1. Synthesis of Metal Oxide Nanoparticles

We made iron oxide nanoparticles by coprecipitation method. Iron nanoparticles were synthesized in size 30 to 60 nm by coprecipitation method where sodium hydroxide (NaOH) was used as a reducing agent [11]. For the preparation of iron oxide nanoparticles, A mixture of 0.6M ferric chloride ($FeCl_3$) and 0.3M ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) were dissolved in 100 mL of deionized water and let them stirred and mixed at 25°C for 1 h under the nitrogen atmosphere. In a separate flask, a 2 M solution of NaOH was prepared in deionized water. Later, 100 mL sodium hydroxide solution (2M) will be added into the solution containing ratio ferric to ferrous 2 to 1 dropwise using the burette. NaOH was added drop by drop into the beaker at the rate of one drop per second to reduce the solution until the pH of 10±0.5 was obtained. A dark black precipitate confirms the formation of the iron-oxide nanoparticle. The precipitate was removed from the supernatant by centrifugation at 15, 000 rpm for a min and repeatedly washed with absolute ethanol. The 15 Freshly prepared nanoparticles were surface characterized for shape and size using a scanning electron microscope (SEM). Iron oxide nanoparticles were <30nm in size and weight was 51 mg/ml from synthesized solution.

2.2. Seeds treatment

Seeds of *Glycine max* and *Cicer arietinum* were soaked in six solutions, each solution had varying concentrations of iron oxide nanoparticles which were control or 0 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. Seeds were soaked in these solutions for 24 hours. After being removed from the soaked, the seeds of *Glycine max* and *Cicer arietinum* were ready for sowing

2.3. Estimation of Chlorophyll

The estimation of chlorophyll done by the dimethyl sulphoxide method (DMSO) extraction procedure. Leaves were collected randomly from the soybean and chickpea plants. Chickpea and soybean leaves were taken into separate petri dishes and were chopped into fine pieces. 0.05g samples from these chopped leaves were added in replicated tubes each containing 10 ml dimethyl sulphoxide (DMSO). The tubes were incubated at 60℃ for 3 hours in an oven by providing gentle shake twice. After it, supernatants were examined under the spectrophotometer, the absorbance of the extract and read at 645 and 663 nm in a spectrophotometer against DMSO blank. Chlorophyll a, chlorophyll b, and total chlorophyll were calculated by following formulas which were given by [12].

- Chlorophyll a (mg/g fresh weight) = (12.7×A663-2.63×A645) X V/1000 x V
- Chlorophyll b (mg/g fresh weight) = (22.9×A645-4.48×A663) X V/1000 x V
- Total Chlorophyll (mg/g fresh weight) = (20.2×A645+8.02×A663) X V/1000 x V

3. Result and Discussion

3.1. Morphological Characters Study under the Effect of Iron Oxide Nanoparticles *(In-Vitro*)

Seed germination percentage of Glycine max and Cicer arietinum were calculated by the formula seed germination =1000 x germination seeds / total number of seeds and seedling vigour index (SVI) was calculated by this formula, SVI = germination (%) x [root length (cm) +shoot length (cm)].

To investigate the promontory or inhibitory effects of iron oxide nanoparticles on chickpea and soybean plant growth, treated and surface sterilized seeds were inoculated on Murashige and Skoog (MS) agar supplemented with same concentration of iron oxide nanoparticle used for the treatment.

The application of different concentrations (0 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm) of iron oxide nanoparticles on Glycine max and Cicer arietinum seeds when they grow in In-vitro condition. The germination rate in *Cicer arietinum* increased by 28.5 % (400 ppm iron oxide) in Fe nanoparticles compared to control (0 ppm) while this germination rate in soybean increased by 50 % at 200 ppm solution compared to control. Seedling vigour index (SVI) in Cicer arietinum increased by 94.7 % at 400 ppm response to control whereas SVI in soybean increased by 90.7% at 200 ppm compared to control sample. The result indicated that iron oxide nanoparticles are beneficial in both crops but nanoparticles should be delivered in low dose, otherwise it's possess a metal toxicity.

Iron oxide nanoparticles gave better results compared to copper oxide nanoparticles when soybean seeds were treated by them. Copper oxide nanoparticles give 76.66% highest germination and 1530.71 highest seed vigour index in soybean at 200 ppm solution while iron oxide nanoparticles give 90% highest germination and 1877.4 SVI in soybean at 200 ppm [13].

The following table shows the comparison between iron oxide and copper oxide nanoparticles effects on germination and seed vigour index (SVI) in soybean.

3.2. Morphological Character Study and Yield under the Effects of Iron Oxide Nanoparticles (Field Condition)

The overnight soaked seeds of chickpea and soybean with varying concentrations of iron oxide nanoparticles were sown in the field.

All measures were taken and at maturity. Results showed a significant increase in the germination and in height of plants. These results were as promising as the previous result of researchers implicating a thought that nanoparticles when delivered in low doses can be beneficial in growth and productivity.

At high doses of nanoparticles possess a threat of metal toxicity. The results were in compliment with the work conducted previously the application of Nano-iron oxide significantly affects both chickpeas and soybean and causes increasing growth and photosynthesis. Nano-iron oxide compared to other treatments such as organic materials and iron citrate facilitated the photosynthate and iron transferring to the leaves of both crop chickpeas and soybeans.

The use of iron fertilizer increased the mean of total iron concentration and total absorption of iron in *Glycine max & cicer arietinum.* Average concentrations of iron and phosphorus on the grain did not affect, but the ratio of phosphorus to iron increased [14].

Iron at concentration of 2.5 mg kg-1 in soil increased dry matter weight of soybean, but higher levels of iron decreased soybean growth [15].

3.3. Estimation of Chlorophyll Content

The DMSO method estimated the chlorophyll a, chlorophyll b, and total chlorophyll content showed the significant change in both chickpea and soybean under the effect of administered iron oxide nanoparticles. Chlorophyll a, chlorophyll b and total chlorophyll in chickpeas increase by 20.43%, 30.76 % and 23.4% respectively at 400 ppm concentration of iron oxide nanoparticle in response to control whereas chlorophyll a, chlorophyll b and total chlorophyll increased to 21.7%, 26.4% and 23.7% respectively in soybean at 200 ppm concentration of iron oxide nanoparticle in response to control. At high concentration (i.e Beyond 400ppm and 200ppm of iron oxide nanoparticle), metal oxide nanoparticles possess a toxicological threat. In plants, oxidative stress and signs of senescence include loss of chlorophyll and protein and decline in membrane permeability, all of which lead to a progressive reduction in photosynthetic capacity.

4. Tables and Figures

Table1: effects of iron oxide nanoparticles on germination and seed vigour under varying concentration.

Nano- Particles	Cicer arietinum				Glycine max			
	Germination %	Root length (cm)	Shoot length (cm)	Seed vigour index (SVI)	Germination %	Root length (cm)	Shoot Length (cm)	Seed vigour index (SVI)
Control	70	14.1	13.3	1918	60	1.3	15.1	984
200 ppm	80	16.4	16.8	2656	90	2.6	18.26	1877.4
400 ppm	90	19.8	21.7	3735	70	1.5	16.1	1232
600 ppm	70	17.1	18.4	2485	50	0.92	9.8	536
800 ppm	50	15.3	16.5	1590	40	0.78	7.2	319.2
1000 ppm	40	13.1	15.1	1132	30	0.60	5.1	171

Table 2: Effect of Iron oxide and copper oxide nanoparticles on germination and SVI in soybean under varying concentration.

Nano- particles	Soybean seeds treated by Iron oxide nanoparticles				Soybean seeds treated by Copper oxide nanoparticles			
	Germination %	Root length (cm)	Shoot length (cm)	Seed Vigour Index (SVI)	Germination %	Root length (cm)	Shoot length (cm)	Seed Vigour Index (SVI)
Control	60	1.3	15.1	984	66.66	1.2	14.1	1019.89
200 ppm	90	2.6	18.26	1877.4	76.66	2.3	17.66	1530.71
400 ppm	70	1.5	16.1	1232	63.33	1.06	13.26	906.88
600 ppm	50	0.92	9.8	536	56.66	0.85	9.5	586.43
800 ppm	40	0.78	7.2	319.2	43.33	0.71	6.7	321.07
1000 ppm	30	0.60	5.1	171	23.33	0.53	4.4	115.01

Table 3: Effects of iron oxide nanoparticles on chickpea and soybean plants height under varying concentration.

Treatments	Cicer arietinum plant height in cm	Glycine max plants height in cm
Control	46.5	50
200 ppm	53.2	61.5
400 ppm	60.5	54
600 ppm	52.8	48.1
800 ppm	43.1	44.7
1000 ppm	40.6	41.8

Table 4: Effect of iron oxide nanoparticles on chlorophyll content in chickpea and soybean under varying concentration.

	Chickpeas Chlorophyll content (mg/g fresh weight)			Soybean chlorophyll content (mg/g fresh weight)		
Treatments	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Total Chlorophyll
Control	1.86	0.78	2.64	2.16	1.51	3.67
200 ppm	1.97	0.95	2.92	2.63	1.91	4.54
400 ppm	2.24	1.02	3.26	2.41	1.58	3.99
600 ppm	1.91	0.83	2.74	1.95	1.20	3.15
800 ppm	1.78	0.71	2.49	1.82	0.98	2.80
1000 ppm	1.61	0.53	2.14	1.65	0.82	2.49

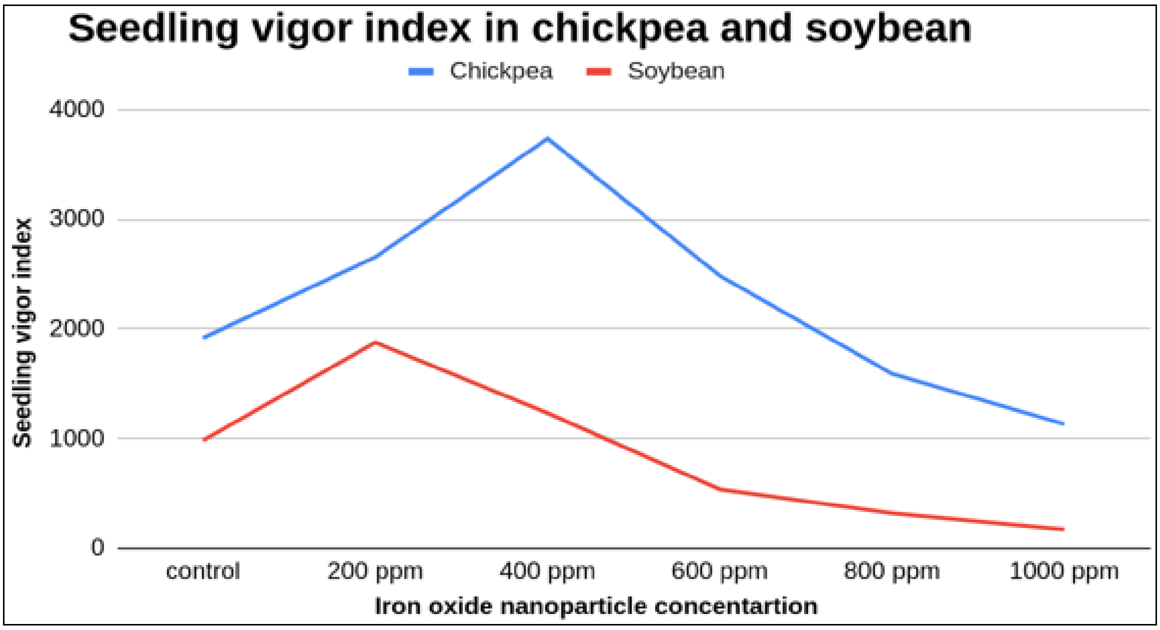

Fig1: Seedling vigor index in chickpea and soybean under varying concentration of iron -oxide nanoparticles.

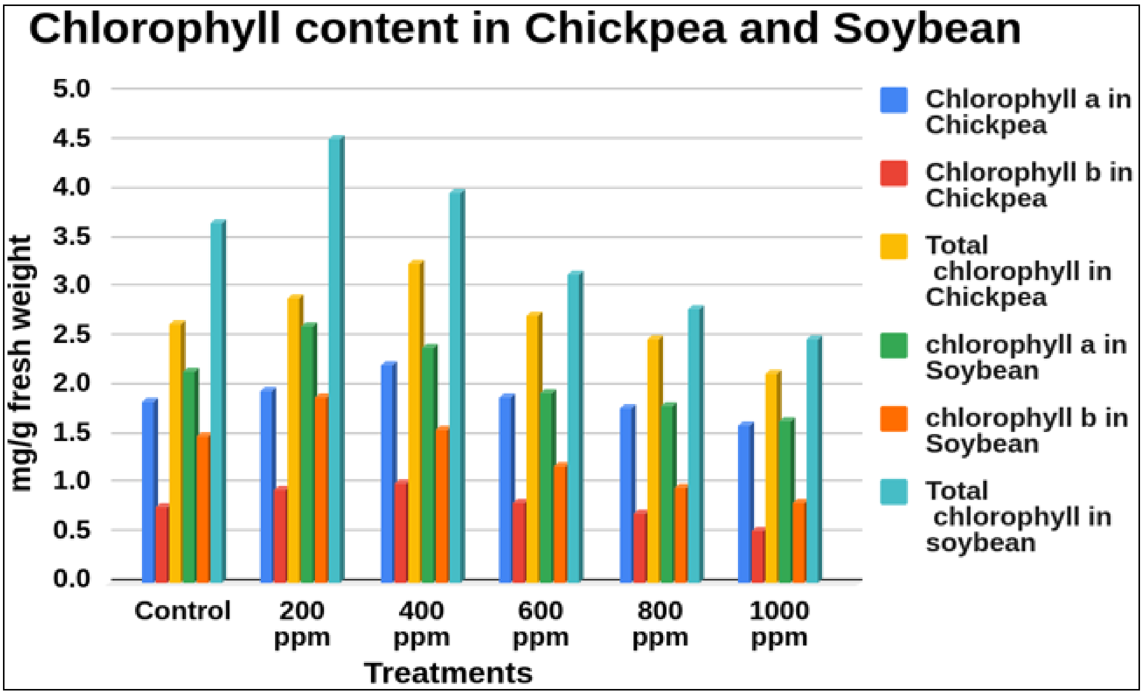

Fig 2: Chlorophyll content in chickpea and soybean under varying concentration iron-oxide nanoparticles.

5. Conclusion

Iron oxide nanoparticles proved that these are effective in both chickpea and soybean, these nanoparticles helps in increasing growth, development, and productivity in plants. *Cicer arietinum* and *Glycine max* are a great source of protein. Iron-oxide nanoparticles were more effective in elevating the protein and oil content. Iron oxide nanoparticles increased the seedling vigor index (SVI) by 94.7%, 90.7% respectively in Chickpea and soybean, highest increase in SVI was recorded at 200 ppm and 400 ppm compared to control. Iron-oxide nanoparticle at a dose of 200ppm and 400ppm in soybean and chickpea respectively, gives a more promising effect in terms of germination, root length, shoot length elongation, and SVI as compared to control.

Administration of iron oxide nanoparticles resulted in an increase by 23% in chickpea and 50% in soybean germination respectively at 400 ppm and 200 ppm concentration in comparison to control. The iron oxide nanoparticles increased total chlorophyll by 23.4% in chickpea at 400 ppm whereas this increased 23.7% in soybean at 200 ppm response to control. The antioxidant properties of Cicer arietinum and soybean are enhanced when administered with iron oxide nanoparticles. So it can be concluded that iron oxide nanoparticles are effective nanoparticles and a relatively low dose can be promising in increasing the quality and quantity of the chickpeas and soybean but high dose of iron oxide nanoparticles possess metal toxic effect.

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