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Effect of iron oxide nanoparticles on morphology and physiology of mung bean *(Vigna radiata)*

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Abstract
The aim of present work was to study the effect of iron oxide nanoparticles on the morphology and physiology of *Vigna radiata*. Seeds of this plant were raised at different concentrations of iron oxide nanoparticles (10, 50, 100, 500, 1000 mg/l). It was revealed that presence of iron oxide nanoparticles affected the growth of *Vigna radiata* at different concentrations. Root and shoot length was found increased as the nanoparticle concentration increased from 10 mg/l-1000 mg/l so we can say that iron nanoparticles had stimulatory effect on growth of plants. Total chlorophyll and carbohydrate content was increased at 15th day in comparison to 7th day. But content of soluble protein was highly correlated with age of plant i.e. content of soluble protein decreases with increasing of age. So, content of soluble protein was higher at 7th day in comparison to 15th day.

Keywords: nanoparticle, carbohydrate, concentration, yield, bovine serum albumin

Introduction

Nanotechnology and nanomaterial's have attracted much attention because of their unique novel properties like large surface area and high reaction activities. They have been used as cosmetic additives, as highly reactive catalyst, in drug delivery system, in cell imaging and in cancer therapy. They are also used in fertilisers and pesticides. The nanoparticle are used to increase the supply of nutrients to shorts and roots of plants. Thus, they are exposed to and absorbed by plants. Their uptake by the plants affects the plant physiology in many ways. They may to be toxic to plant cell and cell organelles or they may be beneficial for the growth of plants (Monica and Cremonini, 2009). Positive and negative effects were studied by different researchers. These effects were found dose and duration of exposure and even species dependent. So, due to increase demands of nanotechnology, their potential harmful effects in agriculture as well as in other industries needs to clarify. So, the present study was done to study the effects of iron oxide nanoparticles one of the important plant *Vigna radiata* (Green Gram). The plants were raised hydroponically in hoagland solution.

Fig 1: The plantlet of *Vigna radiata* hydroponically

Materials and Methods

Chemicals

Iron (III) chloride hexahydrate, Iron (11) chloride tetrahydrate, Ammonium hydroxide, TCA (Trichloroacetate), TBA (Thiobarbituric acid), Sodium carbonate, Copper sulphate, Sodium potassium tartarate, Folin- Coicalteau reagent, Acetone, Sodium phosphate buffer, Phenol etc.

Instruments and equipments

Beakers, wire gauzes, air pumps, slides, brush, forceps, blades, droppers, glassrods, spatula, pipettes, micropipettes, cuvetts, test tubes, watch glasses, mortar and pestle, whatman filter paper, Buchner funnel, centrifuge, spectrophotometer, weighing balance, magnetic stirrer, pH meter. Micro Image Projection system was used for capturing the images of plant sections.

Preparation of nanoparticles

Iron oxide nanoparticles (Fe30, NPs) were prepared by as per the procedure of Maity and Aggarwal (2007). Required amounts of $FeCl_36H_2O$ (0.32gm) and $FeCl_24H_2O$(0.16 gm) were dissolved in 40 ml of deionized water. The solution heated at 80°C for 1 hr while being stirred. Then 5.0 ml of NH4OH (30% w/v) are added rapidly to it. The resulting suspension is vigorously stirred for another 1 hour and then cooled to room temperature. The precipitated particles was washed 5 times with hot water as well as cold water and separated by magnetic decantation and then dried at 70 °C for 1 hr. Dry powder was used for further experimentation.

Experimental design

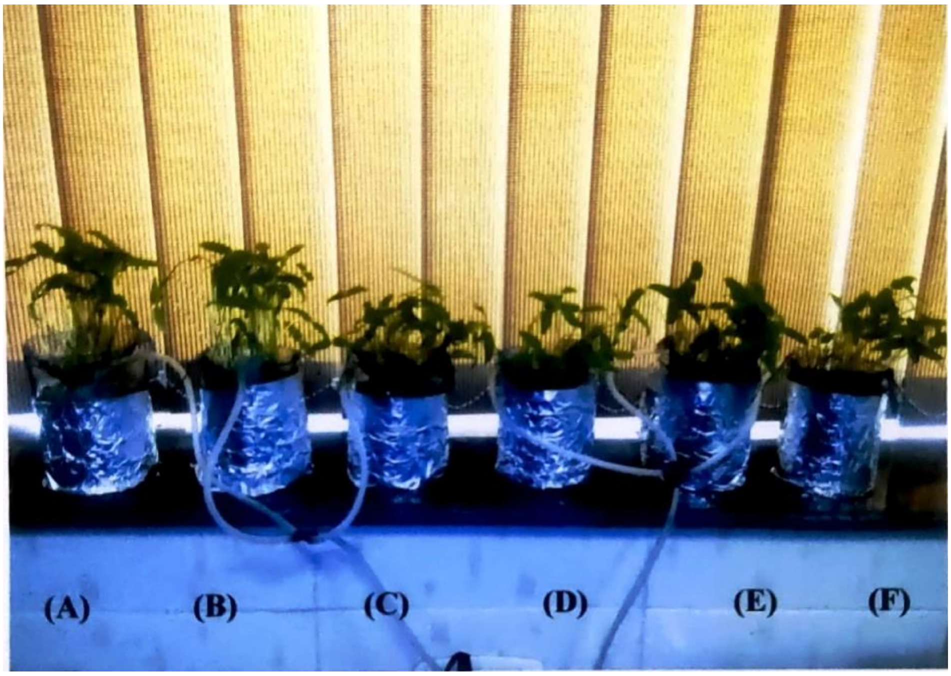

Fig 2: Experimental set up of *Vigna radiata* grown under different concentrations of iron oxide NPs, showing the actual conditions under which the plants were grown. Control is on left side (A) and the other concentrations are onward10 (B), 50 (C), 100(D), 500(E) and 1000(F) mg/l.

In order to study the effect of different concentrations of Iron oxide NPS on *Vigna radiata* growth, total protein, total carbohydrates, chlorophyll estimation and a completely randomized design was employed as shown as Fig.2. Seeds were divided in six groups on basis of treatment.

First group (A) was control and seeds were raised in absence of nanoparticles. Rest of all the groups (B, C, D, E, F) were raised under the influence of iron oxide NPS at a final concentrations (10, 50, 100, 500, 1000 mg/l) respectively. Mung bean seeds were surface sterilized using 0.1% mercuric chloride and washed three or four times with distilled water to remove any remnant of mercuric chloride as it are poisonous. Seeds from all six groups were soaked separately for 6 hours in distilled water having their respective concentrations of Iron NPs. The treated seeds were transformed in petridishes lined with moist filter paper. After 24 hours, the seeds showing the emergence of radical out of seed coat were recorded as being germinated. Percent seed germination were used to determine the effect of metal oxide nanoparticles on seed viability. Then, the plants were raised hydroponically in Hoagland's solution having the above mention concentrations of iron NPs. Proper aeration was given by using air pumps in the solutions having suspended nanoparticles. The root and shoot length were measured after every two days up to 15 days by using ruler. Chlorophyll estimation, total phenol, total protein, total carbohydrate were done on 7^{th} and 15^{th} day of treatment.

Results and Discussion

Results of root and shoot length measurement -

The mung plants were raised hydroponically in Hogland solution having 10,50,100, 500,1000 mg/l concentrations of iron nanoparticles. Proper aeration was given by using air pumps in the solutions having suspended nanoparticles. Root and shoot length were measured on alternate day upto 14th day by using ruler with the help of box. Dhoke *et al*. (2013) [5] studied the effect of iron oxide nanoparticles on *Vigna radiata* seedling and good growth was observed. Same significant effects were observed in Ocimum basilicum L. growth characterstics (branches, leaves number) by Souad A. Elfeky *et al*. (2013).Root and shoot length were found increased as the concentration of nanoparticles increased from 10 to 1000 mg/l as shown as in Table 2. So we can say that iron had stimulatory effect probably on growth of plants.

Table 1: Results were presented as mean ± S.D. for n = 10 of *Vigna radiata* from 2nd day - 14th day.

Sr. No.	Day	Concentration mg/l	Mean ± S.D. of root (cm)	Mean ± S.D. of shoot (cm)
1	2nd	Control	4.02 ± 1.1516	3.19 ± 0.6806
		10	2.92 ± 0.5770	3.56 ± 0.9958
		50	2.7 ± 0.9201	4.36 ± 0.8592
		100	3.5 ± 0.8498	4.6 ± 0.6799
		500	4.41 ± 0.5621	4.68 ± 0.8715
		1000	5.17 ± 1.2202	5.8 ± 0.9238
2	4th	Control	6.13 ± 1.6166	6.11 ± 0.9871
		10	7.58 ± 2.3785	7.23 ± 1.4048
		50	9.31 ± 1.7272	8.47 ± 1.1431
		100	9.68 ± 1.7536	9.72 ± 1.5010
		500	10.47 ± 1.9408	10.61 ± 1.3691
		1000	11.97 ± 2.3763	12.38 ± 1.6619
3	6th	Control	7.61 ± 1.7854	8.68 ± 0.7955
		10	9.51 ± 1.8610	10.16 ± 1.5204
		50	9.77 ± 1.5868	11.92 ± 1.6758
		100	9.93 ± 1.5973	13.44 ± 0.9961
		500	10.61 ± 1.6029	13.11 ± 1.5088
		1000	10.14 ± 2.0571	14.84 ± 2.3458
4	8th	Control	8.1 ± 1.6519	10.44 ± 1.1645
		10	9.89 ± 1.7565	11.95 ± 1.4812
		50	10.51 ± 2.0058	13.18 ± 1.3456
		100	10.63 ± 1.2284	14.46 ± 1.2730
		500	11.24 ± 1.2860	15.87 ± 0.8832
		1000	11.4 ± 2.2993	17.41 ± 0.9315
5	10th	Control	8.78 ± 1.2541	11.7 ± 1.1547
		10	10.53 ± 1.7839	13.53 ± 0.7379
		50	11.21 ± 1.8181	14.6 ± 0.9463
		100	13.39 ± 1.0225	15.78 ± 0.7177
		500	14.3 ± 1.1528	17.15 ± 0.7517
		1000	15.39 ± 0.8774	19.46 ± 0.9675
6	12th	Control	10.51 ± 1.2991	12.43 ± 1.1166
		10	11.86 ± 1.4331	14.37 ± 0.8883
		50	12.98 ± 1.4680	15.25 ± 1.0814
		100	14.3 ± 0.9055	16.48 ± 1.0020
		500	15.26 ± 0.8540	17.32 ± 0.9004
		1000	16.32 ± 1.0152	20.19 ± 1.3650
7	14th	Control	12.06 ± 1.1918	13.57 ± 1.0371
		10	13.24 ± 0.9501	15.33 ± 0.9238
		50	14.33 ± 1.0457	16.05 ± 0.9652
		100	15.37 ± 1.0698	17.45 ± 0.9083
		500	16.36 ± 1.1520	18.37 ± 0.6767
		1000	17.19 ± 1.1160	21.81 ± 1.5536

Measurement of total Chlorophyll

Chlorophyll plays indispensable role in light harvesting and energy transfer during photosynthesis. Iron is an essential element for synthesis of chlorophyll. Iron oxide can promote chlorophyll content to some extent at a suitable dose. Plants absorbed iron from nano-ferric oxide but large doses can reduce the effects. In pigment estimation, variations occurred. At 7th day, chlorophyll a, b and carotenoids was maximum at 10 mg/l. Meng Wang *et al*. (2015) [13] studied the effect of nano-ferric oxide on watermelon by taking 0,20,50,100 mg/l of nanoparticles and found that as the nanoparticle concentration increased, chlorophyll content decreased and 20 mg/l concentration had higher content of chlorophyll. At 15th day, chlorophyll a,b was maximum at 50 mg/l and carotenoids content was found maximum at 10 mg/l so effects were found to be concentration dependent. Total chlorophyll was increased at 15th day as compared to 7th day.As the nanoparticles concentration increased from 10 mg/l -1000 mg/l, total chlorophyll content decreased (Fig.3).

Table 2: Showing pigment contents of *Vigna radiata* on 7th day and 15th day

Sr. No.	Concentration of nanoparticles (mg/l)	Chlorophyll contents	7th day (pigment calculated in material)	15th day (pigment calculated in material)
1	Control	Chl a	5.548	7.77
		Chl b	3.28	4.48
		Carotenoids	1.05	1.77
2	10	Chl a	5.647	10.7
		Chl b	3.21	5.29
		Carotenoids	0.95	3.56
3	50	Chl a	4.506	11.26
		Chl b	3.02	6.67
		Carotenoids	0.681	3.38
4	100	Chl a	3.14	9.48
		Chl b	1.83	5.95
		Carotenoids	0.35	1.81
5	500	Chl a	4.46	6.74
		Chl b	2.626	3.99
		Carotenoids	0.54	1.37
6	1000	Chl a	4.69	6.44
		Chl b	2.621	3.36
		Carotenoids	9.87	1.4

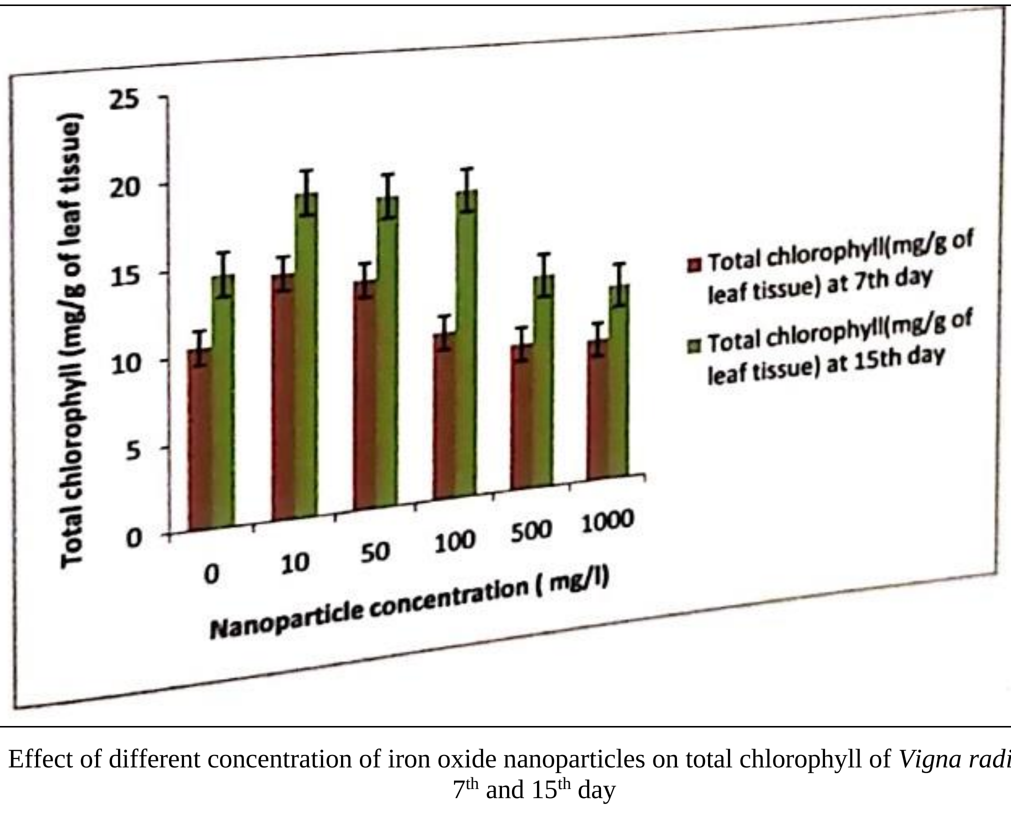

Fig 3: Effect of different concentration of iron oxide nanoparticles on total chlorophyll of *Vigna radiata* plant at 7th and 15th day

Measurement of total protein content

Protein variation is an essential part of plant response to stress as well as for adaptation to environmental conditions. Content of soluble protein was highly correlated with the age of plant i.e. content of soluble protein decreases with the increasing of the age (Hong- Xuan Ren *et al* (2011). E.Nadi *et al*. (2013) studied the effect of nano-iron chelate fertilizer on faba bean and found increasing of nano-iron concentration had positive and significant on protein content.Content of soluble protein was found higher at 7th day in comparison to 15th day. But as the nanoparticle concentration increased from (10 mg/1 - 1000 mg/l), content of soluble protein was increased (Fig.4). So, iron oxide nanoparticles might have stimulatory effect on total protein content of plants.

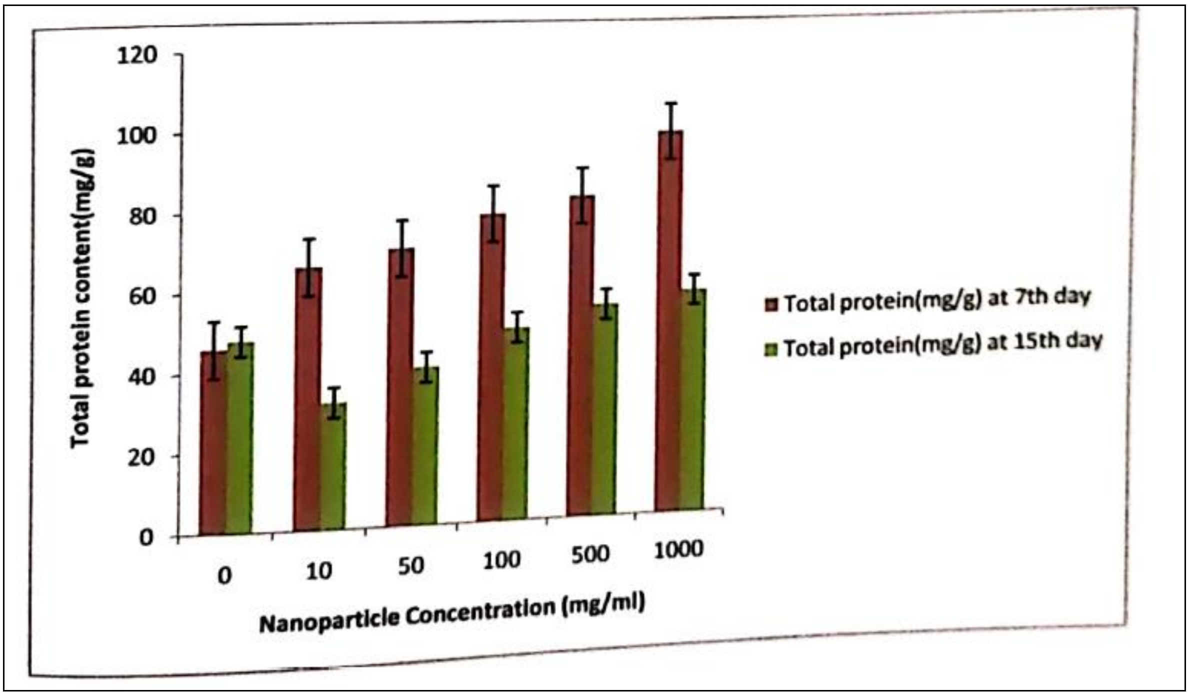

Fig 4: Effect of different concentration of iron oxide nanoparticles on total protein content of *Vigna radiata* plant at 7^{th} and 15^{th} day

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