EFFECT OF IRON ON SEED GERMINATION, PLANT GROWTH AND NODULATION IN *Vigna radiata* R. Wilczek

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This paper reports the effect of iron ($FeSO_4.7H_2O$) as a micronutrient at 25, 50 and 100 ppm concentration on some growth and biochemical parameters of *Vigna radiata.* At 25 and 50 ppm concentration, a significant increase was observed in all parameters but at 100 ppm concentration an inhibitory effect was observed on some parameters

Key words: *:* Inhibitory effect, *Vigna radiata*

Now a days iron and zinc have attracted considerable attention as these minerals affect the nutrient value of the vegetarian diet. It is a well established fact that iron is required for N_2 fixation in plants. It enhances the production of the nitrogenase, leghaemoglobin, ferrodoxin, hydrogenase and cytochromes. Deficiency of iron decreases the nodule formation and nitrogen metabolism. The deficiency results in low concentration of N_2 in the shoots of some legumes. N_2 fixation relies more on the supply of iron rather than the minerals. Decrease in iron supply affects the initial stage of nodulation. Iron deficiency also limits the root cortical cell division and rhizobia escalation. Due to its physio-chemical properties, iron is recognized as one of the most important plant nutrients (Stephan 2002). At the same time, the physio-chemical properties inhibit iron to be extracted from soil and its movement within the plant symplast. Many soil factors (pH, free $CaCO_3$, phosphorous, Mn, HCO_3) affect the availability of iron to the plant. Insufficient supply of iron causes chlorosis and decreases activities related to certain enzymes (Leidi *et al.* 1987, Balakrishnan 2000).

MATERIAL AND METHODS

Plant material and treatment

The present work was carried in the experimental plots of Botany Department, C.C.S. University, Meerut, during 2011-2012. The experiments were carried out under natural conditions in a randomized block design with three replicates of each treatment. Experiments were carried in four plots, three plots for treatment and one for control. *Vigna radiata* seeds were sawn for the field experiments. Seeds of *Vigna radiata* were obtained from Seed Certification Office, Meerut. Healthy seeds of uniform size were selected and washed with distilled water. Standard agronomic practices were adopted and no fertilizer was applied to the field. Ferrous sulfate solution of 25, 50 and 100 ppm in water, and tap water was used in control. The experiments were carried out in triplicate.

Hundred healthy seeds of *Vigna radiata* were sown in each plot. Control plots were irrigated with tap water and others with three concentrations of ferrous sulfate solution. The seed germination percentage was calculated after counting the difference between germinated and ungerminated seeds. Nodules were detached from the plant root with the help of forceps from plants. Fresh weight of nodules was measured immediately, followed by drying of nodules at 60ºC for 24 h to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. Leghemoglobin quantity was measured

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Table 1: Germination percentage of *Vigna radiata* (L.) with different concentration of Fe.

Fe concentration (ppm)	Seed Germination Percentage
0	60. 66⊥5.131
25	73.3_3.055
50	80.66_5.859
100	88.33_2.516

Above all values are averages (Mean) of three replicates with SD.

Table 2: Shoot length and root length of *Vigna radiata* (L.) with different concentration of Fe at different developmental stages.

Fe concentration (ppm)	Shoot Length (cm)			Root Length (cm)		
	25 Days	**45 Days**	**60 Days**	**25 Days**	**45 Days**	**60 Days**
0	28.72+2.366	39.5+2.402	52.98−1.942	14.24+2.203	22.36−3.282	25.08+1.668
25	31.13±1.706	42.52±3.367	57.31_2.070	18.08±2.148	25.95_3.100	28.53±1.224
50	30.15±1.527	40.33±2.483	54.16=1.804	15.71±1.381	22.9±2.198	26.07±2.193
100	25.72|2.213	34.99|1.195	49.65 1.762	11.52|2.311	20.78|3.341	22.08|1.383

Above all values are averages (Mean) of three replicates with SD.

Table 3: Shoot and root fresh weight of *Vigna radiata* (L.) with different concentration of Fe at different developmental stages.

Fe concentration (ppm)	Shoot Fresh weight (mg)			Root Fresh weight (mg)		
	25 Days	**45 Days**	**60 Days**	**25 Days**	**45 Days**	**60 Days**
0	6.54±1.239	10.76±1.692	15.45±1.116	2.06=0.718	3.14=0.891	4.18±1.273
25	7.11=0.894	12.29±0.835	17.37±1.036	2.76=0.570	3.74=1.469	4.88±1.273
50	6.75+0.863	11.38−0.835	16.89+1.507	2.46−0.703	3.21−1.093	4.26+1.262
100	6.33±0.974	8.92±1.037	13.71±1.507	1.16_0.598	2.84_0.757	3.1±1.483

Above all values are averages (Mean) of three replicates with SD.

Table 4: Shoot and root dry weight of *Vigna radiata* (L.) with different concentration of Fe at different developmental stages.

Fe concentration (ppm)	Shoot Dry weight (mg)			Root Dry weight (mg)		
	25 Days	**45 Days**	**60 Days**	**25 Days**	**45 Days**	**60 Days**
0	1.55−0.670	2.61−0.515	2.88+0.470	0.271+0.117	0.386+0.084	0.403+0.119
25	1.92=0.527	3.19=1.032	3.57±0.087	0.271±0.117	0.442±0.141	0.521±0.087
50	1.64_0.604	2.79_0.582	2.97±0.132	0.301±0.118	0.416±0.144	0.503±0.132
100	1.37−0.468	2.48−0.677	2.74+0.082	0.225+0.070	0.403+0.119	0.566+0.088

Above all values are averages (Mean) of three replicates with SD

Table 5: Nodule number and nodule fresh and dry weight of *Vigna radiata* (L.) with different concentration of Fe at different developmental stages.

Fe concentration (ppm)	Nodule Number	Nodule fresh weight (mg)	Nodule dry weight (mg)	Nodule Number	Nodule fresh weight (mg)	Nodule dry weight (mg)
	25 Days			45 Days		
0	19.6±1.516	185±2.607	38±2.607	33.6±3.049	308±3.209	58±3.1
25	30.6±2.701	286±2.073	81±2.387	42.2±4.147	453±3.049	91±3.3
50	24.4±2.549	214±2.387	52±2.774	36.4±3.577	334±2.073	72±3.2
100	15.4±2.302	168±2.645	32±2.736	29.8±3.834	260±2.683	51±2.3

Above all values are averages (Mean) of three replicates with SD.

Table 6: Yield parameter of *Vigna radiata* with different concentration of Fe at harvest stage.

Fe concentration (ppm)	Number of fruits	Number of seed plant	100 seed weight (gm)
0	19.4±1.673	124.8±2.280	31.6±2.073
25	26.8±2.167	155.4±1.140	55.8±2.588
50	24.2±1.788	146.8±2.387	48.6±2.073
100	9.6±1.870	67.2±1.643	22.8±1.923

Above all values are averages (Mean) of three replicates with SD

Table7. Leghaemoglobin and total protein content of *Vigna radiata* (L.) with different concentrations of Fe.

Fe concentration (ppm)	Lb mg/g fresh weight	Protein mg/g fresh weight
0	34.81	67.93
25	53.59	77.84
50	45.90	70.99
100	31.82	54.98

spectrophotometrically as hemochromogen following the method of Hartee (1957).

RESULT AND DISCUSSION

Seed germination

The stimulatory effect was apparent with the increasing concentration of ferrous sulphate. Maximum germination was noticed at 100 ppm of concentration of ferrous sulphate. The rate of seed germination gradually increased with increasing concentration of ferrous sulphate (Table 1). The increase in germination percent might be due to release of more free Fe ions by its hydrolyzing enzymes from its organic complexes (Obizoba and Atie 1994, Bahram 2012).

Shoot and root length

The shoot and root length were measured at different stages of plant growth after 30, 45 and 60 days. Plants treated with 25 ppm solution showed a significant increase in shoot and root length (Table 2). At 50 ppm concentration there was higher growth in terms of shoot length and root length as compared to control but it was slightly lower than at 25 ppm concentration. A decrease in shoot and root length as compared to control was recorded at 100 ppm. The reduction in shoot and root growth at higher concentration of micronutrients may be due to the fact that germinating seeds under higher concentrations would get less amount of oxygen which might have restricted the energy supply and retarded the growth and development (Kumar *et al.* 2000). The percentage of phyto-toxicity of root was found to be more than shoot, as metal accumulation in root binds the cell wall of root (Wool house 1983).

Significant increase in the growth is possibly due to the fact that iron is required by the plants in trace amount. The inhibitory action of excess iron in root and shoot length may be due to the reduction in cell division, toxic effects on photosynthesis, respiration and protein synthesis (Kupper *et al.* 1996, Sonmez *et al.* 2006).

Fresh and dry matter yield Plants treated with 25 ppm concentration showed a significant increase in fresh and dry matter production of root and shoot. At 50 ppm concentration there was still higher fresh and dry weight as compared to control but less than at 25 ppm concentration. A decline in fresh and dry matter production was recorded at 100 ppm (Table 3 and 4). Application of Fe increased number of nodules plant which may be due to higher nitrogenase activity (Rao *et al.* 1984). This consequently enhanced N_2 fixation, which led to increase in plant dry matter accumulation and total plant dry weight.

Nodule number

Iron is an important nutrient required by legume nodules. The demand for this micronutrient increases during the symbiotic establishment, where the metal is utilized for the synthesis of various iron-containing proteins in both the plant and bacteroid. Low levels of iron (25 ppm) increased the number of nodules in *Vigna radiata* over the control (Table 5). Further the increase in the iron level (50 ppm) increased number of nodules as a compared to control, but were less than in 25 ppm concentration. The number of nodules decreased with further increase in iron level (100 ppm). A decrease in the number of nodules due to elevated level of iron can be attributed to the reduction in the development of root system as well as direct toxicity of iron on soil microbes. Several studies have shown that most metal ions are toxic to soil microorganisms, even in small quantities (Tyler *et al.* 1991, Ginn *et al.* 2006). The lowest rhizobial population was found in the soil with the highest extractable metal concentration (Angle and Chaney 1991).

Fresh and dry weight of nodules

Fresh and dry weight of nodules showed an increase from pre-flowering 25 DOS (Days after sowing) to post-flowering 60 DOS. The treated plants showed an increase in number of nodules except in 100 ppm concentration where a decrease was recorded in comparison to control (Table 5).

Plant yield

The yield parameters such as number of fruits per plant, number of seeds per fruit and their economic yield were higher in 25 ppm concentration of iron but it decreased at 100 ppm concentration (Table 6). Pragasam *et al.* (2001) reported that the high yield of plants at lower concentrations might depend on the enhanced biosynthesis of pigments, carbohydrates and proteins. Present observations of increase and decrease in yield parameters are in agreement with those of Kumar and Bhargava (1998). The present study reveals that micronutrient at lower concentrations promotes growth and yield but at higher concentrations they posed inhibitory effect.

Protein content

A general promotory effect of iron was observed on protein content of the nodules of *Vigna radiata.* It is apparent from Table 7 that 25 and 50 ppm concentration enhanced the protein content but at 100 ppm there was a decrease. The reduction in protein content might be due to enhanced rate of protein denaturation (Tripathi and Gautam 2007, Prasad and Inamdar 1990). The enhance protein denaturation and breakdown of existing protein to amino acid may be the main cause of protein content (Slatni *et al.* 2011).

Leghaemoglobin content

The Lb concentrations significantly increased except for 100 ppm (Table 7). Leghaemoglobin content was high at lower concentration of iron at 25 ppm, which increased at 50 ppm. At 100 ppm it also increased as compared to control but was less than in 50 ppm concentration. Iron is an important nutrient in nitrogen fixing legume root nodule. Supplied to the nodule it is used by the plant for the synthesis of leghemoglobin, as it regulates oxygen content within cells (Singh *et al.* 1995, Pooladvand *et al.* 2012). While in the bacteroidal fraction, it is used as an essential cofactor for the bacterial nitrogenase and iron contaning proteins of the electron transport chain. The supply of iron to the bacteroid membrane, which physically separates bacteroids from the infected plant cells cytosol. Magid and Chedly (2003) also reported that in cereal nodules iron is necessary for leghemoglobin biosynthesis.

Conclusion

The application of iron promotes the growth of *Vigna radiata* because this metal is necessary component such as enzymes and other cofectors. From these observations it can be concluded that, low concentration (25 ppm) had stimulatory effect on growth and protein content of *Vigna radiata*. But iron was harmful in high concentration and harmful to crop production.

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