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Effect of Iron on Morpho-physiological and Biochemical attributes in *Vigna radiata* (L.)

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ABSTRACT

An attempt was made to study the influence of ferrous sulphate on seed germination, growth and biochemical parameter of Vigna radiata var. PDM-139. Increases of metal ions in the environment are absorbed by the roots of plants. Metal ion translocated to the different parts of the plants leading to reduced growth. Iron is considered as essential micronutrient, elevated level causes toxicity on metabolic activity of the plant. This paper reports the effect of iron ($FeSO_4.7H_2O$) at 30, 60 and 90ppm concentration. Vigna radiata var. PDM-139 at 30 and 60ppm concentration a significant increase was observed in all parameters, but at 90ppm concentration an inhibitory effect was observe on some parameters.

Key words: *Iron, Growth, Essential, Vigna radiate.*

INTRODUCTION

Iron plays a key role in various metabolic mechanisms but it can be toxic when the iron concentration in tissues is slightly higher than its optimal level. In present time, heavy metals have received considerable attention at the globel level due to their adverse effect on plants growth and metabolism (Dhankhar and Solanki, 2011). Some of the plant species have capacity to tolerate metals whereas some are highly sensitive (Akinola and Ekiyoyo, 2006). Although metals are required as structural and catalytic parts of enzymatic proteins involved in many physiological processes but they also can be toxic to a plant if present at supraoptimal concentrations (Sharma et al. 2010). When heavy metals are accumulated in excess in the soil environment, these may affect the seed germination, growth and alter the level of biomolecules in the cells (Zhang et al. 2009). Iron is essential micronutrient for plant growth but at high concentration these are highly toxic to plants. Generally iron is required for N2 fixation in plants, enhances the production of Nitrogenase, Leghaemoglobin, Ferrodoxin, Hydrogenase and Cytochromes. The objective of present study work is to investigate in laboratory the effect of Fe on metal accumulation in plant organs.

MATERIALS AND METHODS

The experiments were conducted at Department of Botany C.C.S. University Campus Meerut (Latitude 29^0 01'N; Longitude 77^0 43' E; 730 feet asl) in Uttar Pradesh, India. To determine the effect of iron on *Vigna radiata* var. PDM-139, the studies were carried out in laboratory conditions at room temperature. The healthy seeds were disinfected with 0.1% $HgCl_2$ solution for 5 minutes and washed 5-6 times with distilled water to remove its traces. The 30, 60, and 90ppm ferrous sulphate solutions were prepared in pure distilled water by using ferrous sulphate ($FeSO_4.7H_2O$). 30 Surface sterilized seeds were sown in 3 replicates (10 in each replicate) in plastic bowls filled with washed and oven dried sand and were irrigated on alternate days with 30, 60 and 90ppm concentration of ferrous sulphate solution at room temperature inside the laboratory. Distilled water treatment was used as control. The seed germination, (%) was calculated after counting the difference between germinated and ungerminated seeds. After 10 days of seedling growth, morpho-physiological and biochemical attributes were recorded. Seedling root length, shoot length, fresh weight per seedling and moisture % was measured on 10^{th} day after radical emergence. Seedling shoot and root were separated and dried at 80ºC for 48h and their dry weight determined. Moisture percentages were calculated as per the following formula:

$$\% \text{ Moisture content} = \frac{\text{Fresh Wt.} - \text{Dry Wt.}}{\text{Fresh Wt.}} \times 100$$

Germination speed was calculated according to ISTA (1976).

$$\text{Germination Speed} = \frac{\% \text{ Germination}}{\text{Day of completion of germination}} \times 100$$

Vigour Index was calculated according to the method adopted by Abdul Baki and Anderson (1973).

$$\text{Vigour Index (V.I.)} = \text{Root length} + \text{Shoot length of seedling} \times \% \text{ germination}$$

Chlorophyll content of the shoot was measured following the method of Arnon, 1949. Lowry *et al.*, 1951 method was used to determine the total protein content of root and shoot. The peroxidise activity was measured according to Maehly and Chance, 1967. The total activity was expressed as ΔA_{475} min $^{-1}$gfwt^{-1}.

RESULT AND DISCUSSION

Morpho-physiological Analysis:

Seed Germination: When *Vigna radiata* var. PDM-139 grown with the different concentrations of ferrous sulphate germination % increased at all the concentrations, as compared to control, so the stimulatory effect was appeared with the increasing concentration of iron. Seed germination was noticed maximum at 90ppm of concentrations of ferrous sulphate (**Table 1**). The increment in germination percent it might be because of the release of more free Fe ions by its hydrolyzing enzymes from its organic complexes (Obizoba and Atie, 1994; Bahram, 2012).

Seedling vigour: After 10 days of radical emergence in the seedlings of *Vigna radiata* var. PDM-139 root length, shoot length, vigour index, and germination speed increased at up to by 60ppm concentrations of ferrous sulphate, while fresh weight, dry weight and moisture % increased up to 30ppm concentration, as compared to control. Seedling treated with low level (30ppm and 60ppm) showed a significant increase in shoot and root length (**Table 1**). At higher concentration 90ppm there was a decrease in shoot length and root length as compare to control. The reduction in growth at

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higher concentration 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, 2000). An increase in the growth is may be due to the fact that iron is required by the plants in trace amount. The inhibitory effect 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 weight, dry weight and moisture percent content of seedlings treated with 30ppm concentration showed a gradual increase. At 60ppm concentration there was still higher fresh and dry weight as compared to control but not as much at 30ppm concentration. But at 90ppm there was a reduction in fresh and dry matter production (**Table 1**).

Biochemical Analysis:

Chalorophyll content: Result on the effect of ferrous sulphate on chlorophyll content of the leaf of *Vigna radiata var.* PDM-139 is showed in Table 2. Iron is an important content required by chlorophyll. Low levels of iron (30ppm) increase the amount of chlorophyll in crop plants over the control. Further the increase in the iron level (60ppm) amount of chlorophyll increase as compare to control but are less than in 30ppm concentration. The amount of chlorophyll decreased with further increase iron level (90ppm).

Protein content: A stimulatory effect of iron was observed on protein content of the root and shoot of *Vigna radiata* var. PDM-139. It is apparent from Table 5 that at 30 and 60ppm concentration there was showed an enhancement protein content but at 90ppm there was a decrease was recorded. The reduction in protein content might be due to enhance rate of denaturation of protein (Tripathi and Gautam, 2007). The enhance protein denaturation and breakdown of existing protein to amino acid is the main cause of protein content (Slatni et al. 2011).

Peroxidase activity: While peroxidase activity in shoot increased and in root it decreased with increasing concentration of ferrous sulphate solution concentrations of iron, as compared to control. POD was highest at 30ppm concentration of iron in both root and shoot. Peroxidase activity in root and root were recorded to decrease gradually at 90ppm concentration of iron, as compared to control.

Table No. 1: Morpho-physiological attributes of *Vigna radiata* var. PDM-139 seedlings at different concentrations of iron.

Treatment	% Germination	% Moisture	Vigour index	Germination speed
Control	55.5±5.59	94.38	1779.13	13.44
30ppm	76.25±5.23	94.46	3043.69	19.06
60ppm	78.75±9.48	94.61	3340	19.69
90ppm	81±6.85	94.23	2153.25	20

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

Table No. 2: Root length, shoot length (cm) and fresh weight, dry weight (gm) of *Vigna radiata* var. PDM-139 seedlings at different concentrations of iron.

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)
Control	7.95±1.90	25.05±2.93	0.392±0.012	0.0219±0.0009
30ppm	11.45±1.30	27.2±1.69	0.449±0.010	0.0242±0.0007
60ppm	13.4±1.52	28.35±1.55	0.449±0.010	0.0231±0.0002
90ppm	7.91±1.20	21.8±1.86	0.318±0.008	0.0201±0.0004

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

Table No. 3: Chlorophyll and protein content of 10 days old seedlings of *Vigna radiata* var. PDM-139 grown in different concentrations of iron.

Treatments	Chl.a (mg/gfwt)	Chl.b (mg/gfwt)	Total Chl. (mg/gfwt)	Protein (mg casein eq./gfwt)		Peroxidase (ΔA_{475}/min/ gfwt)	
	Shoot	Shoot	Shoot	Root	Shoot	Root	Shoot
Control	0.346±0.013	0.121±0.001	0.467±0.011	135.28±1.52	146.93±2.32	11.68	5.53
30ppm	0.381±0.016	0.134±0.003	0.515±0.013	140.85±4.89	155.04±1.52	13.92	7.76
60ppm	0.355±0.006	0.124±0.023	0.505±0.006	137.31±2.32	150.48±4.02	12.24	6.65
90ppm	0.274±0.024	0.109±0.018	0.383±0.019	124.64±0.024	128.69±3.16	8.88	3.39

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

CONCLUSION

The application of iron promotes the growth of *Vigna radiata* var. PDM-139 because this metal is necessary component such as enzymes and other cofectors. From these observations it can be concluded that, low concentration (30ppm) had stimulatory effect on morpho-physiological and biochemical content of *Vigna radiata* var. PDM-139. But at high concentrations iron, was harmful and reduce the plant growth.

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