

EFFECT OF IRON TOXICITY ON GROWTH OF FENUGREEK

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Abstract: Effect of iron stress on fenugreek (*Trigonella foenum-graecum* L.) was investigated to understand the basis of metal tolerance. Growth parameters such as root and shoot length, germination percentage, moisture percentage, fresh and dry weight were analyzed. Seeds were cultured on blotting paper in petri dishes at 22^oC and supplemented with 0 (control), 100, 200, 300, 400 and 500 μ M concentrations of FeCl₃. All the parameters were recorded at regular intervals of 5 days. A significant reduction from 92 to 44% was observed in seed germination percentage. Simultaneously, a significant reduction in shoot and root length was observed with increase in iron concentration. The maximum and minimum shoot length were 3.56cm (control) and 1.37cm (500 μ M FeCl₃) respectively. Root length exhibits a variable pattern. At low iron concentration (200 μ M) root length increased whereas it decreased significantly at higher concentrations, thus, indicating that low concentrations can enhance root growth. The root length ranges from 0.38cm (500 μ M FeCl₃) to 0.85 cm (control). Similar decrease was observed in fresh and dry weight with respect to increased iron concentrations. No significant increase was observed in moisture percentage. On the basis of present investigation it is concluded that fenugreek is iron sensitive as it exhibited a decline in all the growth parameters studied.

Keywords: Biomass, germination percentage, growth parameters, metal stress, *Trigonella foenum-graecum*

INTRODUCTION

Iron (Fe) has been central to life since the beginning. It is an essential element for the growth of almost all living organisms (Harrison and Arosio, 1996). For all plants, it is an essential element as it performs various important biological roles in various processes (Marschner, 1995).

In India, due to poor irrigation practices such as use of industrial effluents and sewage sludge on agricultural land, iron was gradually transferred and concentrated in the soil. This has resulted in pollution of environment and a major health issue (Nagajyoti *et al.*, 2008). Presence of excess iron ions produces irreversible biochemical and physiological perturbations in plants. Iron concentrations not only inhibit plant growth (Wheeler and Power, 1995; Baratakova *et al.*, 2001) but also cause free radical (such as hydroxyl radical) production that impairs cellular structures irreversibly and damages membranes, DNA and proteins (Harrison and Arosio, 1996).

Fenugreek (*Trigonella foenum-graecum*) an important annual legume crop, is cultivated as a herb (for leaves) and as a spice (for seeds) in semi-arid regions of the world. It is a native of India and Southern Europe. For centuries it has grown wild in India, the Mediterranean and North America. Its seeds are used as spice and its leaves are used as vegetable, which are rich in vitamins and minerals. The seeds are rich in proteins. It is also an important source of diosgenin, saponins, mucilaginous fibre (galactomannans), pyridine-type alkaloids (trigonelline, choline, gentianine and carpaine) and free amino acids (arginine, histidine and lysine) (Sauvaire *et al.*, 1991; Sahalian, 2004). Being a legume crop it also enriches soil by fixing atmospheric nitrogen and reduces the need for

nitrogen fertilizers for subsequent crops. Thus it is useful in crop rotation. As a semi-arid crop its water requirement is low. The cultivation of fenugreek can reduce the cost of irrigation, save water and reduces eutrophication of surface waters and limit contamination of ground water resources (Basu *et al.*, 2004; Acharya *et al.*, 2008).

Fenugreek is cultivated for its leaves and seeds in Meerut region (situated in the Doab of Ganga and Yamuna) where soils have been affected due to metal toxicity and salinity as a consequence of low and random precipitations as well as wrong irrigation practices. Thus, the present investigation was undertaken to analyze the toxic effects of heavy metal-iron on the growth of this crop. The objectives of the present investigation are to assess the effects of iron on (1) seed germination and (2) plant survival and morphology.

MATERIAL AND METHOD

Experimental material

Fenugreek (*Trigonella foenum-graecum*) seeds were procured from local market of Meerut. The experiments were carried out at Department of Biotechnology, MIET, Meerut, during growing season of 2009.

Germination percentage

Seeds of Fenugreek were surface sterilized with sodium hypochlorite and rinsed thoroughly with sterilized distilled water. To evaluate the effect of FeCl₃ on germination, seeds were treated in a range of 100 to 500 μ M concentrations. Four replicates of 25 seeds for each treatment and for the control (distilled water) were placed on two layers of moistened filter papers in 90 mm petri plates. Water

or fresh metal solution was added periodically, for maintaining the filter paper's moistness during experiment. The petri plates were kept in dark at 22°C in the germination chamber. Number of germinated seeds was counted every 24 hrs for 5 days and the percentage of germination was calculated.

Growth parameters

Experiments were conducted in a conditioned room under artificial light with mean air temperature of $22 \pm 1^\circ\text{C}$, air relative humidity of 75 to 80 and photoperiod of 16/8 hr. To analyze various growth parameters such as moisture content, root and shoot length, fresh and dry weight, plants from each treatment were harvested and subjected to analysis on every fifth day from the start of addition of metal solutions of different concentrations. Three independent experiments were performed and the data presented are means of one representative experiment conducted in four or five replicates for various growth parameters.

RESULT AND DISCUSSION

Germination percentage

The results pertaining to effect of different concentrations of FeCl_3 on germination percentage of fenugreek seeds are depicted in Fig.1. It was observed that metal stress induced by 100 to 500 μM FeCl_3 led to gradual decrease in germination percentage with increasing concentrations as compared to control. It declines from 92 to 44% in 24 hrs of germination. Germination percentage reached maximum in 48 hrs. Different concentrations of FeCl_3 resulted in delayed germination. Similar observations have been made by Todd (2001) in beach pea, Ashraf and Orooj (2006) in ajwain, Abdel Haleem (2007) in mungbean and Nagajyoti *et al.*, (2008) in ground nut. Delay in seed germination due to increased salt concentration may be explained by the lower osmotic potential of the solution (Todd, 2001).

Shoot length

With the increasing iron concentration there is significant decrease in shoot length (Fig. 2). After fifth day of germination maximum (3.56cm) shoot length was observed in control and minimum in 500 μM concentrations. Similar reduction in shoot length was observed with respect to different time intervals. Control plants appeared green and healthy

as compared to the stressed plants. Wheeler and Power (1995), Abdel Haleem (2007) and Nagajyoti *et al.* (2008) while working with wheat, mungbean and groundnut respectively made similar observations.

Root length

Root length shows a similar pattern to shoot length (Fig. 3). Iron treated seeds exhibited gradual decrease in root length with increasing iron concentrations as compared to control. The decrease in root length varies from 1.58cm (in 100 μM) to 0.38 cm (in 500 μM) on fifth day after germination. Seeds treated with 100 μM and 200 μM FeCl_3 solutions exhibited greater root length in comparison to control. This shows that these concentrations have an inducing effect on root length. Metal toxicity also exhibit delayed in shoot and root induction and development as at 500 μM concentration no shoot and root development observed at 5th day (Fig. 2 and 3). A similar decline pattern was also observed with different time intervals. Hasni *et al.* (2009) reported that stress has no effect on root development in fenugreek. Iron toxicity in most cases is characterized by root blackening and root flaccidity (Laan *et al.*, 1991).

Fresh weight and dry weight

Addition of ferric chloride significantly reduced biomass of fenugreek (Fig. 4 and 5). It has the concentrations as compared to control. A significant decrease of 0.503gm (500 μM) in fresh weight and 0.32gm (500 μM) in dry weight has been observed as compared to control on fifth day. Similar results were observed by Hasni *et al.*, (2009) in fenugreek. According to Ashraf and Orooj (2006) high concentrations have detrimental effect on plant growth. Salinity inhibits plant growth for two reasons: firstly, water deficit and secondly salt specific or ion – excess effects (Munns *et al.*, 2006).

Moisture percentage

The effect of FeCl_3 on moisture percentage has been shown in Fig. 6. The moisture percentage increased to 93.24% (500 μM) as compared to control (92.24%) on fifth day of germination. After twenty five days of germination, the moisture percentage was 94.76% (500 μM) as compared to 93.72% in control. Although, this increase in moisture percentage was not significant yet it explains the deterioration of seed due to iron stress.

Fig. 1: Effect of FeCl₃ on germination percentage of Fenugreek

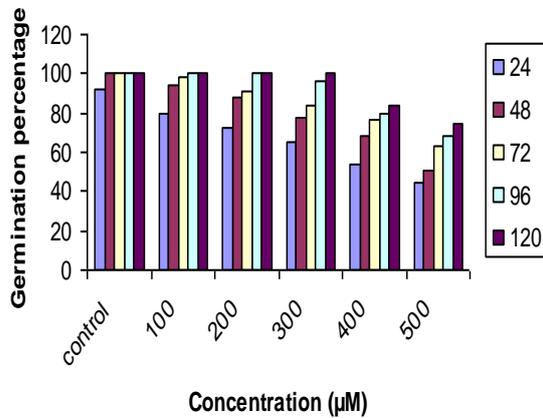


Fig. 2: Effect of FeCl₃ on Shoot Length of Fenugreek

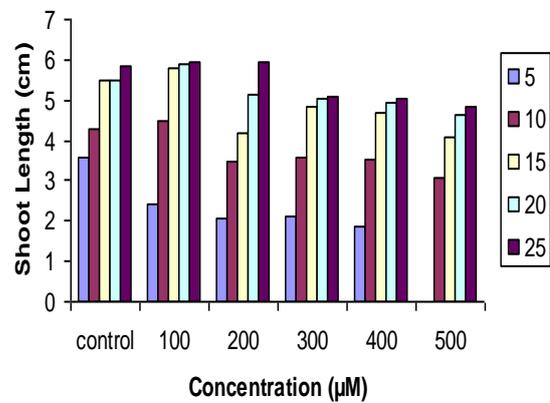


Fig. 3: Effect of FeCl₃ on Root Length of Fenugreek

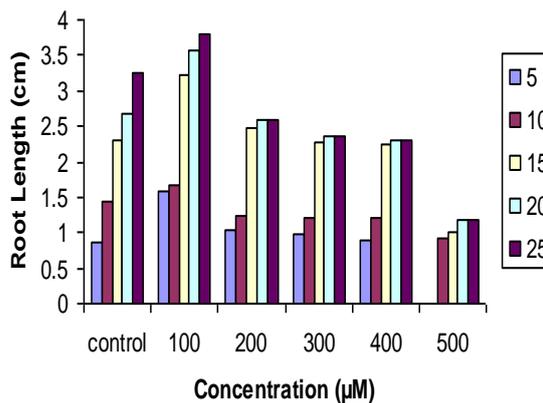


Fig. 4: Effect of FeCl₃ on Fresh Weight of Fenugreek

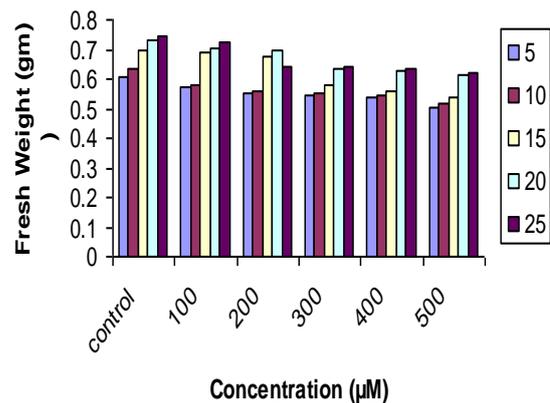


Fig. 5: Effect of FeCl₃ on Dry Weight of Fenugreek

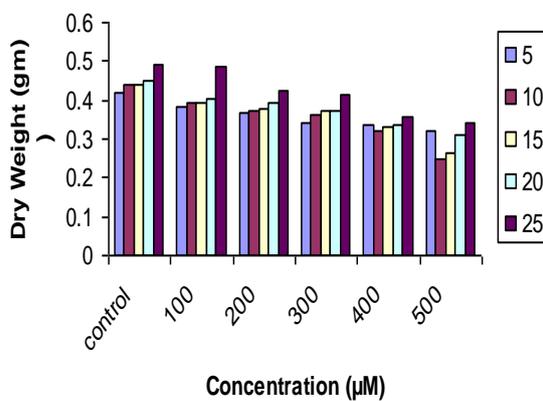
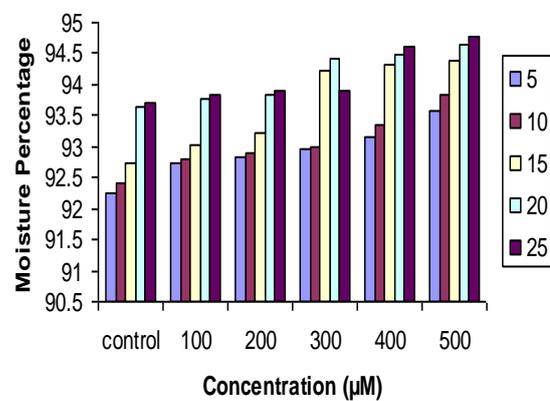


Fig. 6: Effect of FeCl₃ on Moisture Percentage of Fenugreek



CONCLUSION

Metal toxicity is one of the factors causing reduction in growth and productivity of almost all crops. It causes adverse effects on different physiological processes, which are responsible for decrease in plant growth. Therefore, increase in metal tolerance in crops is necessary to sustain food production in different regions. The present investigation undertaken to study the various growth parameters such as germination percentage, shoot and root length, fresh and dry weights and moisture percentage under different concentrations of iron stress on fenugreek clearly indicate that iron is toxic for this crop as it modifies the growth of the seedlings in different ways.

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