Potassium mediated alleviation of iron toxicity in okra seedlings

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Abstract

Background: The objective of this study was to evaluate the effects of different concentrations of iron (Fe) on various growth parameters and to assess the effectiveness of different concentrations of potassium in mitigating the negative effect of iron toxicity in *A. esculentes* (L.) Moench. **Methods**: After 21 days of sowing, the soil was treated with ferric chloride ($FeCl_3$) solution of different concentrations (0.5, 1, 1.5, 2 and 2.5 g/kg soil) along with an untreated control and various growth parameters were measured. After fixing the stress concentration that imparting 50% of growth inhibition, the treatment with potassium in the form of potassium chloride (0.02, 0.04, 0.06 and 0.08 g/kg soil) was done in okra plants and various growth parameters were analyzed. **Findings**: Plant growth characteristics were significantly affected by application of excess iron in 21 days old okra seedlings. The potassium mediated Fe stress alleviation also evaluated in okra seedlings. The seedlings were treated with 0.04 g/kg soil K, reduced the toxic effects of iron and lead to the growth enhancement in terms of growth parameters in okra plants.

Keywords: Iron, Heavy metal, Potassium, Stress, Toxicity.

1. Introduction

Macronutrients are the nutrients required by the plants in large amounts and these include iron, zinc, boron etc. Iron (Fe) plays an important role in various physiological and biochemical processes in plants and it is important for the maintenance of chloroplast structure and function in plants. However, excess iron application or iron toxicity in plants induces various morphological and biochemical alterations. They include the root hair morphogenesis, yellowing of leaves and ultrastructural disorganization of mitochondria and chloroplast, antioxidants accumulation. Fe stress induces the generation of reactive oxygen species which reduces the activity of cytoplasmic enzymes and damage to cell structures [1,2]. The antioxidant defence system protecting the plant from heavy metal induced oxidative damage [3].

In plants, a balance of inorganic nutrients is required for adapting growth under stressful environments and thus the sufficient availability of nutrients may reduce the metal toxicity in plants. Potassium (K) plays a major role in numerous physiological functions related to plant health and resistance to biotic and abiotic stress. Potassium application reduces malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, enhanced protein, proline synthesis, secondary metabolites and enzymatic and non-enzymatic antioxidants in plant cells which is finally responsible for plant's survival [4]. In this study, field experiment was conducted in okra plants (*Abelmoschus esculentus*) grown in high concentrations of Fe treated soils. The objectives of the study were to explore the effects of different levels of Fe on the growth parameters and find the optimal K concentration for application to okra plants. This study could provide useful information for sustainable agricultural production and environmental management in Fe polluted soils.

2. Materials and methods

2.1 Plant material

Abelmoschus esculentus (L.) Moench also known as okra, is a hairy annual plant of the Malvaceae family. Seeds of Varsha uphar variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India. The experiment was carried out at botanical garden K. A. H. M. Unity Women's College, Manjeri, Malappuram, Kerala from January - June under natural conditions. The climate is generally hot and humid. The range of temperature varying between 32 and 28ºC. The average rainfall is 290 mm.

2.2 Experimental setup

The pot experiment was conducted under the green house conditions for 6 months. Okra was selected as experimental crop. 6-8 seeds were grown in plastic grow bags containing soil, weighing about 4 kg. After germination, the seedlings were thinned and these with best growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water.

2.3 Soil conditions

The soil analysis was conducted at District soil testing laboratory, Malappuram, Kerala. The conditions of soil that used for the experiment was presented below:

Parameters	Value/Content
PH value	5.30
Total soluble salt (TSS)	0.001
Carbon content (%)	0.29
Phosphorus (Kg/ha)	6
Potassium (Kg/ha)	197
Calcium (ppm)	0.0
Magnesium (ppm)	0.0
Sulphur (ppm)	12.5
Boron (ppm)	1.0
Iron (ppm)	25.1
Manganese (ppm)	13.3
Zinc (ppm)	4.8
Copper (ppm)	0.2

2.4 Iron (Fe) treatment

After 21 days of sowing, the soil was treated with ferric chloride ($FeCl_3$) solution of different concentrations (0.5, 1, 1.5, 2 and 2.5 g/kg soil) along with an untreated control. The experiment was conducted in triplicates. After iron treatment all pots including control were equally watered measuring 200 ml of tap water daily. The growth of plants was monitored daily to fix the day of stress showing severe conditions in plants. On the second day, toxic limits of iron were found in plants treated with 1.5, 2 and 2.5 g/kg soil. In the same day all treated plants including control were separated out from the experiment pots for analysis.

2.5 Fe stress alleviation by potassium treatment

After fixing the stress concentration that imparting 50% of growth inhibition, the treatment with potassium in the form of potassium chloride was done in okra plants. The soil was treated with potassium chloride solution (0.02, 0.04, 0.06 and 0.08 g/kg soil) along with an untreated control. The samples were collected and analysed after two days of potassium treatment with Fe.

2.6 Growth parameters

The shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage was recorded. For dry weight measurements, the weighed seedlings were kept in a hot air oven at 100ºC for one h and further kept in oven set at 60ºC. Drying and weighing were repeated at regular intervals (24 h) until the values of dry weight became constant. Tolerance index percentage is calculated according to the following formula:

2.7 Statistical analysis

$$TI = \frac{\text{observed value of root length in solution with metal}}{\text{observed value of root length in solution without metal}} \times 100$$

The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (S.E.) and oneway ANOVA, Tukey's studentized range test ($p \leq 0.05$) used for the statistical analysis.

3. Results

The present study has been aimed to investigate the protective effects of different concentrations of potassium (K) on iron (Fe) stress induced *Abelmoschus esculentus* (L.) Moench seedlings and evaluated the various morphological and biochemical parameters. The results obtained from various experiments were presented below.

3.1 Effect of different concentration of iron stress in okra plants

Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 2.5 g/kg soil Fe (14% as compared to control) than other stress treatments. However, the decrease of shoot length was less upon exposure to 0.5 g/kg soil Fe (3%) as compared to control plants. Moreover, the shoot length of the plants subjected to 1, 1.5, and 2 g/kg soil Fe stress also decreased as compared to control okra plants (5, 7 and 10% respectively). The root length was also decreased with increasing Fe concentrations in okra plants and maximum decrease (19%) was observed when subjected to 2.5 g/kg soil Fe as compared to control plants. In the case of okra plants subjected to various concentrations of Fe (0.5, 1, 1.5 and 2 g/kg soil), the number of leaves per plant was reduced (25%) as compared to control plants. Moreover, 50% reduction in the number of leaves was noticed in okra plants after subjected to 2.5 g/kg soil Fe treatment. Moreover, the reduction in leaf area was less in okra plants subjected to 0.5 and 1 g Fe stress (4-8%), as compared to control plants and it was maximum in plants subjected to 2 and 2.5 g/kg soil Fe (19-24%), as compared to control plants. Moreover, okra plants treated with 1.5 g/kg soil Fe showed 13% reduction in the leaf area as compared to control plants (Table1: Plate 1).

Likewise, the reduction in fresh weight was maximum in plants subjected to 2 and 2.5 g/kg soil Fe (53 and 66%, respectively) as compared to control plants. Moreover, okra plants treated with 1.5 g/kg soil Fe showed 44% reduction in fresh weight as compared to control plants. Dry weight of Fe stressed okra plants were decreased compared with the control plants and maximum decrease of dry weight was recorded in the plants treated with 1.5, 2 and 2.5 g/kg soil Fe (52, 62 and 72% respectively as compared to control plants). Also, there was significant difference in tolerance index (TI) of okra plants exposed to different Fe concentrations. Compared with the control, the TI was slightly decreased when okra plants were treated with 0.5 & 1 g/kg soil Fe (5-7% as compared to untreated control plants), while TI was decreases obviously after 1.5, 2 and 2.5 g/kg soil Fe treatments (10, 16 and 19% respectively) as compared to untreated okra plants (Table 1).

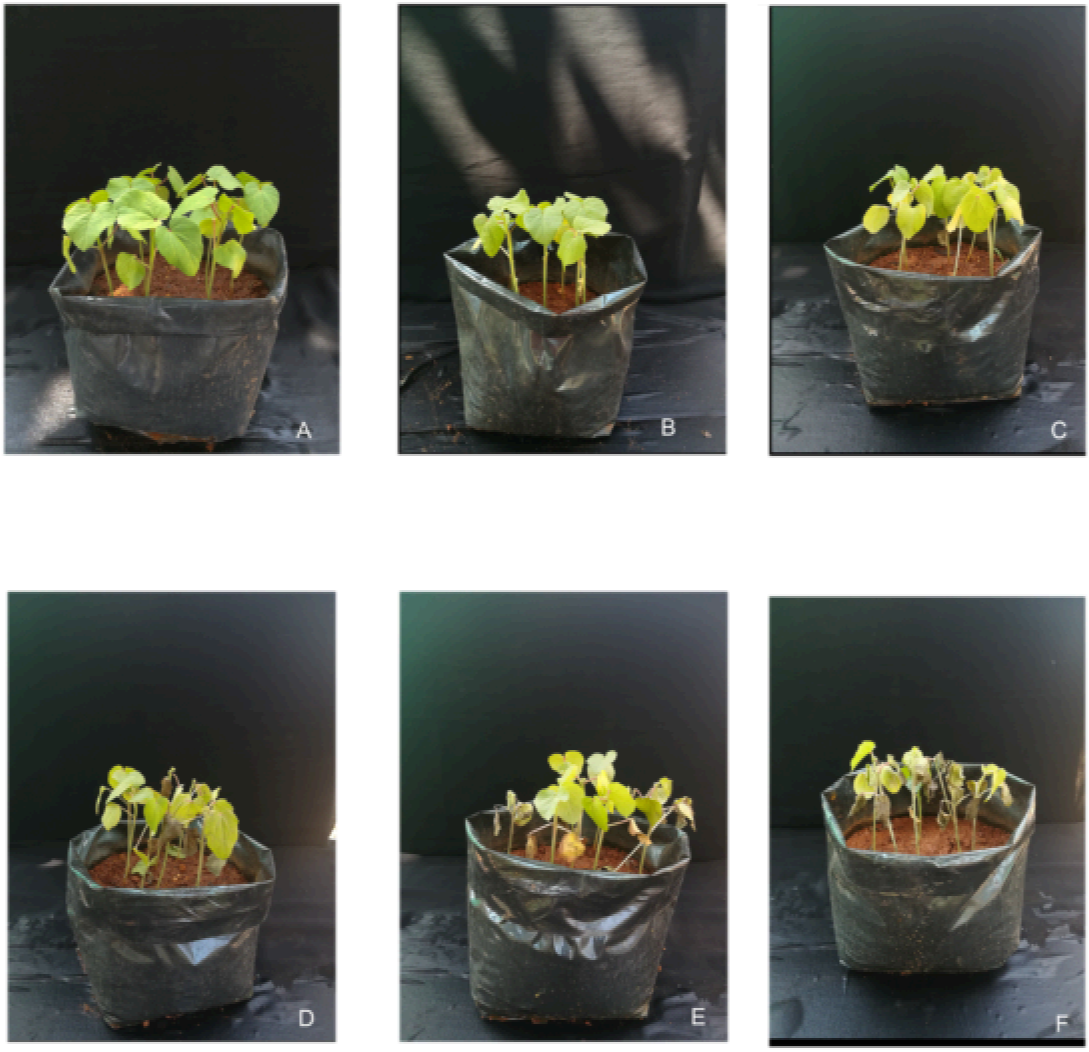

Plate 1: Effect of different concentrations of Fe (0.5, 1, 1.5, 2 and 2.5 g/kg soil) in in okra plants A – control, B – 0.5 g/kg soil, C,- 1 g/kg soil, D – 1.5 g/kg soil, E- 2 g/kg soil, F- 2.5 g/kg soil.

Table 1: Growth parameters of okra plants subjected to different concentrations of Fe (0.5, 1, 1.5, 2 and 2.5 g/kg soil). Different alphabetical letters indicate statistically significant differences at p≤0.05 following oneway ANOVA (Tukey's studentized range test).

Fe Treatments (g/kg soil)	Shoot length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Tolerance index (%)
Control	15.2±0.24^{a}	9.5±0.23^{a}	4±0.28^{a}	10.6±0.23^{a}	1.87±0.03^{a}	0.29±0.01^{a}	100±3.1^{a}
0.5	14.7±0.23^{b}	9±0.15^{a}	3±0.15^{b}	10.2±0.42^{a}	1.49±0.02^{b}	0.23±0.01^{b}	94.73±2.6^{2}
1	14.5±0.18^{c}	8.8±0.18^{b}	3±0.1b	9.7±0.25^{b}	1.13±0.12^{c}	0.17±0.11^{c}	92.63±1.2^{b}
1.5	14±0.22^{d}	8.5±0.26^{b}	3±0.21^{b}	9.2±0.16^{c}	1.05±0.14^{c}	0.14±0.01^{c}	89.47±1.4^{c}
2	13.6±0.37^{e}	8±0.14^{c}	3±0.18^{b}	8.6±0.29^{d}	0.88±0.04^{d}	0.11±0.01^{d}	84.21±2.4^{d}
2.5	13±0.83^{e}	7.7±0.18^{d}	2±0.08^{c}	8.1±0.17^{e}	0.64±0.33^{e}	0.08±0.01^{e}	81.05±1.7^{e}

3.2 Potassium (K) mediated modulation of iron (Fe) stress in okra plants

To analyze the alleviation potential of different concentrations of potassium (0.02, 0.04, 0.06 and 0.08 g/kg soil) towards iron (1.5 g/kg soil – standardized concentration of Fe which imparts 50% growth inhibition) induced stress effects in okra, various growth parameters were recorded.

After two days of iron treatment of fixed concentration (1.5 g/kg soil) and different concentrations of K treatments (0.02, 0.04, 0.06, 0.08 g/kg soil), iron stress (1.5 g/kg soil) reduced the shoot length in okra plants by 8% as compared to the control. However, 0.0 2 g/kg soil K treated Fe stressed plants, the reduction in shoot length was less relative to control plants. 0.04 g/kg soil of Potassium treated plants showed slight increase in shoot length as compared to control plants. However, higher concentrations of K treatments (0.06 and 0.08 g/kg soil) highly reduced the shoot length of okra plants than control plants (Figure 1: Plate 2).

In the case of root length also, there was a small reduction in decreasing the root length of plants treated with 0.02 g/kg soil (5%) concentration of potassium. Surprisingly, the root length was slightly increased upon subjected to 0.04 g/kg soil K as compared to untreated plants. However, the higher concentrations of K reduced the root length of okra plants which were treated with 1.5 g/kg soil Fe as compared to control plants. Maximum rate of alleviation of Fe stress induced decline in number of leaves among various concentrations of K treated okra plants was shown by plants treated with 0.04 g/kg soil K. Moreover, under Fe toxicity, 0.04 g/kg K treatment slightly increased the leaf area as compared to control okra plants. All other treatments of K under Fe stress reduced the leaf area of okra plants and maximum reduction was observed upon subjected to 0.08 g K (8%) (Figure 1: Plate 2).

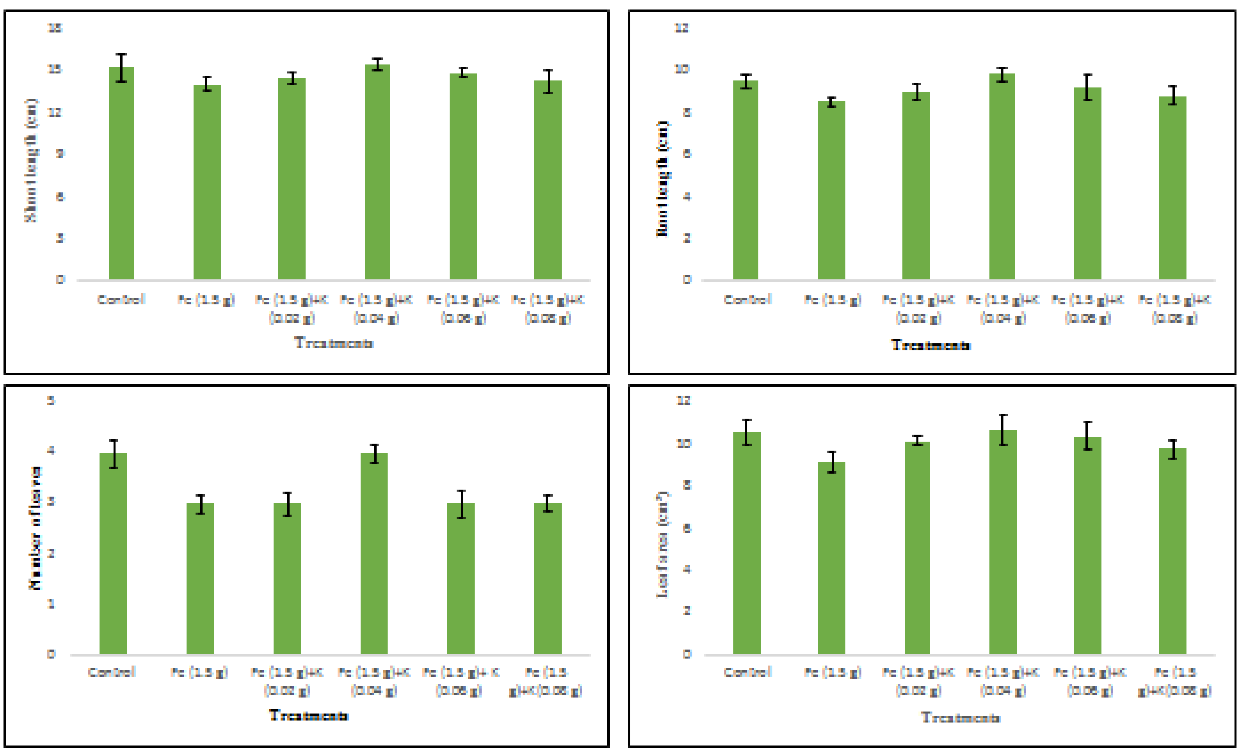

Figure 1: Shoot length, root length, number of leaves and leaf area of okra plants subjected to Fe and K treatments. The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

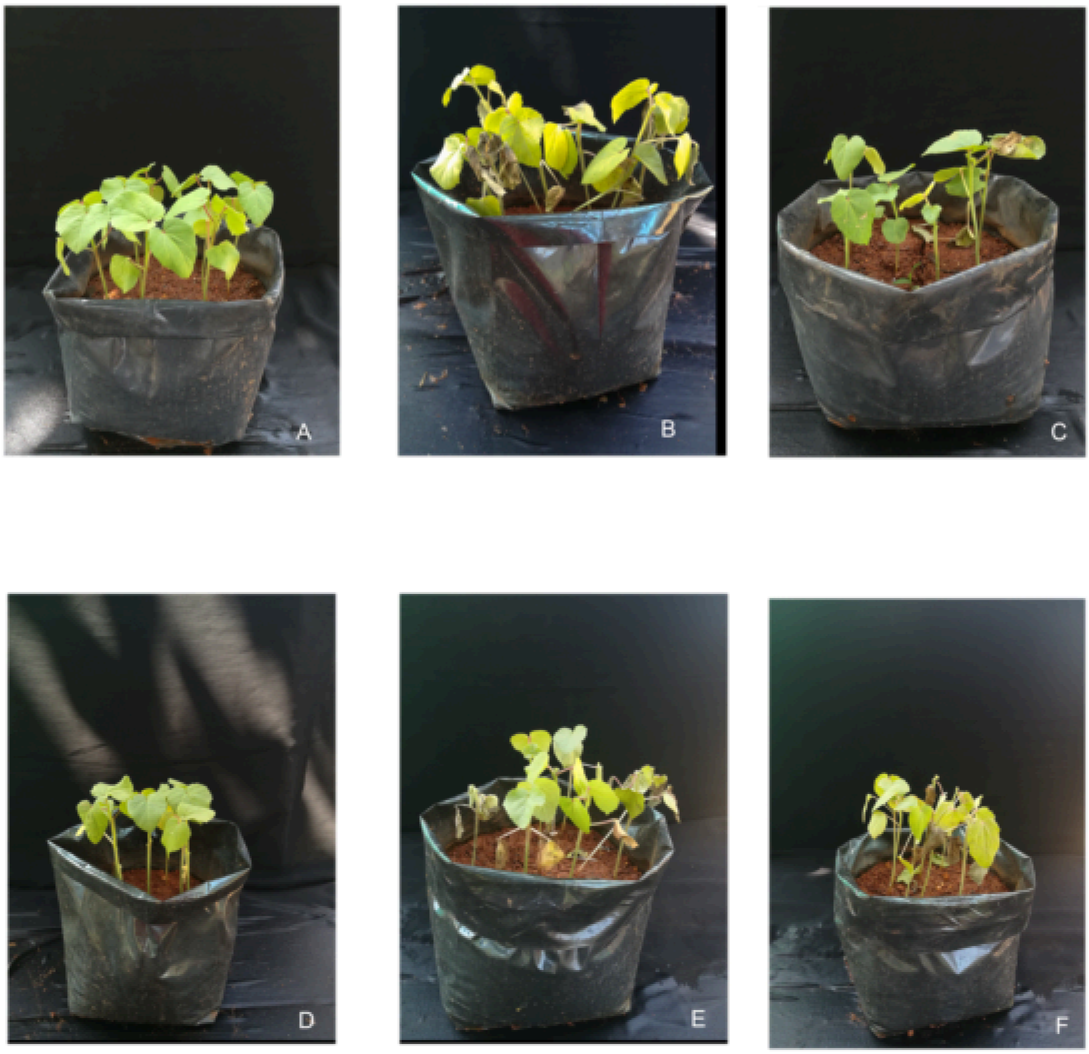

Plate 2: K mediated modulation of Fe stress in morphology of okra plants. A- control, B- Fe (1.5 g/kg soil), C- Fe (1.5 g/kg soil)+ K(0.02 g/kg soil), D – Fe(1.5 g/kg soil)+ K (0.04 g/kg soil), E- Fe(1.5 g/kg soil)+ K (0.06 g/kg soil), F- Fe(1.5 g/kg soil)+ K(0.08 g/kg soil).

Iron stress reduced the fresh weight in okra plants (44%) as compared to the control. Likewise, K treatment also reduced the fresh weight under iron toxicity, except upon subjected to 0.04 g K treatment in okra plants, where it was slightly enhanced as compared to control plants. The maximum reduction in fresh weight was recorded at 0.06 and 0.08 g/kg soil K treatments (10 and 29% respectively) under Fe stress. Relative to control plants, 0.04 g/kg soil K treated plants showed a 7% increase in dry weight under Fe toxicity as compared to control plants.

However, higher concentrations of K treatment (0.08 g/kg soil) slightly reduced the dry weight of okra plants than control plants. Moreover, different concentrations of K supply increased tolerance index of okra plants as compared to Fe stressed plants. However, the tolerance index was maintained by 0.04 g/kg soil K as in untreated plant. While tolerance index was slightly decreased in 0.06 and 0.08 g/kg K treatments (3 and 7% respectively) as compared to untreated okra plants (Figure 2).

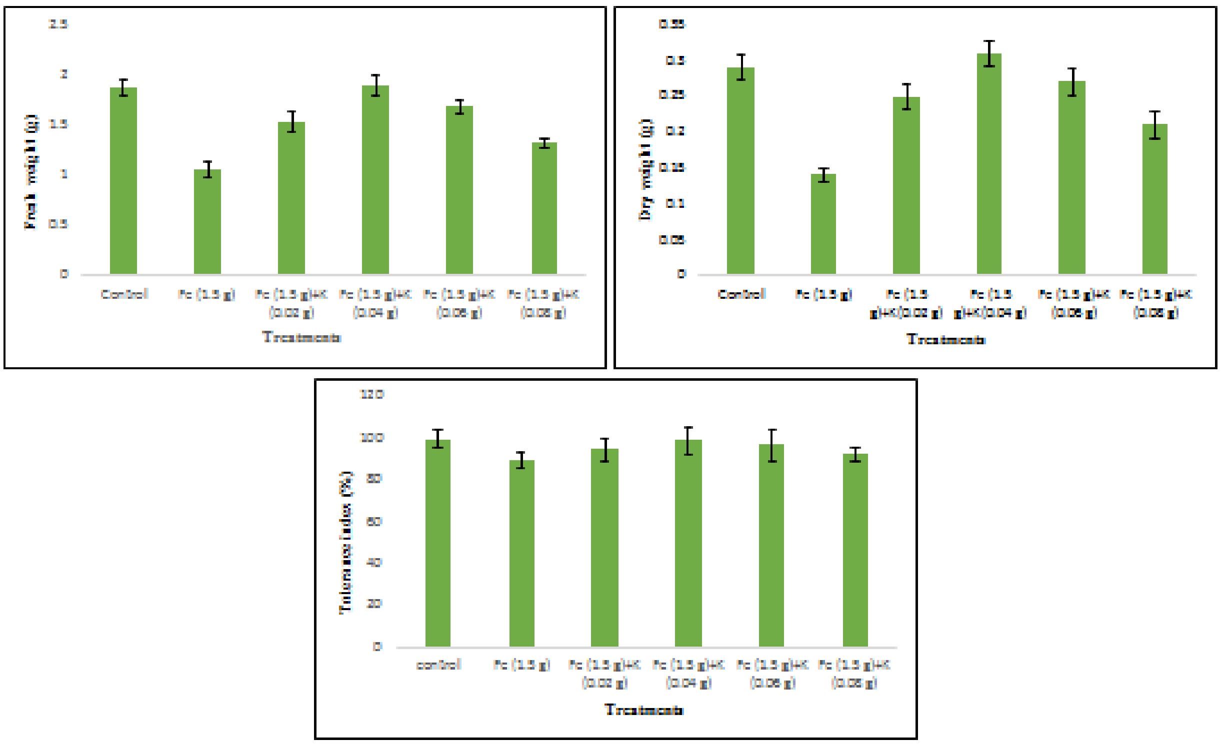

Figure 2: Fresh weight, dry weight and tolerance index of okra plants subjected to Fe and K treatments. The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

4. Discussion

The results showed that the plant growth characteristics were adversely influenced by application of excess iron in 21 days old okra seedlings. The effects of excess iron were measured by various growth parameters such as root length, shoot length, number of leaves per plant, dry weight, fresh weight and leaf area were found to be more sensitive to excess iron concentration. In the present study, it was found that the Fe toxicity severely affected the morphological parameters in okra plants and the maximum decrease of shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was clearly visible in the plants treated with 2 and 2.5 g/kg soil Fe than other stress treatments. However the decrease of shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was less upon exposure to 0.5, 1, 1.5 g/kg soil Fe and 1.5 g/kg soil Fe concentration fixed as 50% growth inhibition as compared to control in okra plants.

Previously it was experimentally proved that growth parameters were significantly affected by iron toxicity in rice plants. Extreme reduction of root growth occurred under iron treatments and the iron content in the root and shoot of rice plants increased significantly by increment of iron concentration in the root medium [5]. Moreover, excessive iron greatly decreased root and shoot length of the hybrid rice [6]. Likewise, Fe toxicity significantly reduced the growth and metabolism of four different varieties of rice plants and at severe stress level, decrease of root length is observed in all the varieties under excess Fe stress [7].

The protective effect of different concentrations of potassium on iron stress (0.5, 1, 1.5, 2, 2.5 g/kg soil) induced okra plants were evaluated and results showed that the plant growth characteristics were adversely influenced by application of excess iron in 21 days old okra seedlings. In the present study, it was found that the Fe toxicity severely affected the morphological parameters in okra plants and the maximum decrease was clearly visible in the plants treated with 2 and 2.5 g/kg soil Fe than other stress treatments. However the decrease of shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was less upon exposure to 0.5, 1, 1.5 g/kg soil Fe and 1.5 g/kg soil Fe concentration fixed as 50% growth inhibition as compared to control in okra plants.

Potassium supplementation induced marked increase in growth under critical iron concentration. The present study revealed that the potassium mediated modulation of okra seedlings under Fe toxicity reduced the toxic effects of iron stress. Different concentration of K (0.02, 0.04, 0.06 and 0.08 g/kg soil) applied towards Fe (1.5 g/kg soil) induced stress effect in *Abelmoschus esculentus*. Root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was slightly enhanced upon subjected to 0.04 g/kg soil K, revealing the alleviation potential of K towards Fe toxicity in okra plants. But high concentration of K (0.06 and 0.08 g/kg soil) reduced these growth parameters which were treated with 1.5 g/kg soil

Fe. However, 0.02 g/kg soil K treated Fe stressed plants the shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was slightly reduced.

Potassium participates in physiological and biochemical processes that can alleviate the toxicity induced by heavy metals. Naciri *et al* [8] experimentally proved that K significantly reduced heavy metal cadmium translocation from root to shoot and improved root and shoot growth parameters in tomato plants. Moreover, Shamsi *et al* [9] reported that K supplementation alleviated the reduction of growth and nutrients uptake in Cd treated soybean plants. In this study, the optimum concentration of potassium was 0.04g/kg soil under high concentrations of iron in soil for okra cultivation which can alleviate the toxic effects of iron. However, higher concentrations of potassium are negatively affected the morphological as well biochemical parameters of okra plants. Moreover, the application of K in soil could be generalized as promising strategy to alleviate Fe toxicity in okra at the initial crop establishment stage.

5. Conclusion

By analysing the morphological parameters through this investigation, it was observed the iron stress severely affected the plant growth and biomass, which can lead to plant death. The higher concentrations of iron lead to significant alterations in the normal plant growth there by causing chlorosis, leaf withering, defoliation and necrosis as visible symptoms. The seedlings treated with 2.5 g/kg soil Fe showed extreme leaf withering, chlorosis, defoliation and overall deformities of the plant and ultimately the death of plants. It helped to fix the concentrations of 1.5 g/kg soil iron which caused an average range of heavy metal toxicity and that can be reduced using appropriate control measures. The morphological parameters such as root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index are adversely affected by the application of iron stress in okra plants. The potassium mediated Fe stress alleviation also evaluated in okra seedlings. The seedlings were treated with 0.04 g/kg soil K, reduced the toxic effects of iron and lead to the growth enhancement in terms of root length, shoot length, leaf area, fresh and dry weight of okra plants.

6. Acknowledgement

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7. References

1. Arif N, Yadav V, Singh S, Singh S, Ahmad P, Mishra RK, Sharma S, Tripathi DK, Dubey NK, Chauhan DK. Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. Frontiers in Environmental Science. 2016;4:69.
2. Lapaz AD, Yoshida CH, Gorni PH, Freitas-Silva LD, Araújo TD, Ribeiro C. Iron toxicity: effects on the plants and detoxification strategies. Acta Botanica Brasilica. 2022;36.
3. Hossain MA, Piyatida P, da Silva JA, Fujita M. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany. 2012;2012.
4. Yasin NA, Zaheer MM, Khan WU, Ahmad SR, Ahmad A, Ali A, Akram W. The beneficial role of potassium in Cd-induced stress alleviation and growth improvement in Gladiolus grandiflora L. International Journal of Phytoremediation. 2018;20(3):274-83.
5. Mehraban P, Zadeh AA, Sadeghipour HR. Iron toxicity in rice (Oryza sativa L.), under different potassium nutrition. Asian Journal of Plant Science. 2008;7(3):251-9.
6. Li H, Yang X, Luo A. Ameliorating effect of potassium on iron toxicity in hybrid rice. Journal of Plant Nutrition. 2001;24(12):1849-60.
7. Onyango DA, Entila F, Dida MM, Ismail AM, Drame KN. Mechanistic understanding of iron toxicity tolerance in contrasting rice varieties from Africa: 1. Morpho-physiological and biochemical responses. Functional Plant Biology. 2018;46(1):93-105.
8. Naciri R, Lahrir M, Benadis C, Chtouki M, Oukarroum A. Interactive effect of potassium and cadmium on growth, root morphology and chlorophyll a fluorescence in tomato plant. Scientific Reports. 2021;11(1):1-0.

9. Shamsi IH, Jiang L, Wei K, Jilani G, Hua S, Zhang GP. Alleviation of cadmium toxicity in soybean by potassium supplementation. Journal of Plant Nutrition. 2010;33(13):1926-38.