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Investigating the Effects of Soluble Iron Nutrients on the Growth and Morphological Characteristics of Calendulla Officinalis Under Salt Stress

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ABSTRACT

This study was conducted to investigate the effects of foliar application of iron on the growth and morphological characteristics of *Calendula officinalis* under salinity stress conditions. Soil salinity is one of the most important abiotic stresses that limits plant growth and productivity, especially in arid and semi-arid regions. To evaluate the response of *Calendula officinalis* to salinity stress and iron application, a factorial experiment was carried out based on a completely randomized design with three replications under greenhouse conditions. Treatments consisted of four salinity levels (0, 30, 50, and 80 mM NaCl) and three iron foliar application levels (0, 200, and 400 mg L^{-1}). Growth and morphological traits such as plant height, stem diameter, number of leaves, number of flowers, flower diameter, number of lateral branches, and relative water content were measured. The results indicated that increasing salinity levels significantly reduced all measured traits. However, foliar application of iron, particularly at moderate concentrations, significantly improved plant growth and reduced the adverse effects of salinity stress. The findings of this study highlight the important role of iron foliar application in improving the growth performance of *Calendula officinalis* under saline conditions.

Keywords- *Calendula officinalis*; Salinity stress; Iron foliar application; Morphological characteristics.

I. INTRODUCTION

Calendula officinalis L. is an important ornamental and medicinal plant belonging to the Asteraceae family. This plant is widely cultivated for decorative purposes in gardens and landscapes and is also valued for its medicinal properties. Due to its economic and medicinal importance, improving the growth and quality of *Calendula officinalis* under unfavorable environmental conditions has received considerable attention. Soil salinity is one of the major environmental constraints affecting agricultural production worldwide. Salinity stress is particularly prevalent in arid and semi-arid regions, where excessive accumulation of soluble salts in the soil negatively affects plant growth and development. High salinity reduces water availability to plants, causes ionic imbalance, and disrupts nutrient uptake, ultimately leading to reduced growth, poor morphological development, and decreased productivity. Salinity stress affects plants at different stages of growth by limiting cell expansion, reducing photosynthetic activity, and altering physiological and biochemical processes. As salinity levels increase, plants experience osmotic stress and ion toxicity, which result in reduced plant height, leaf number, flowering, and overall biomass production. Ornamental plants such as *Calendula officinalis* are particularly sensitive to salinity stress, as growth reduction and poor flowering directly affect their ornamental value. Iron is an essential micronutrient required for normal plant growth and development. It plays a vital role in chlorophyll synthesis, photosynthesis, respiration, and various enzymatic reactions. Iron deficiency leads to chlorosis, reduced photosynthetic efficiency, and impaired plant growth. Under saline conditions, the availability and uptake of iron by plants are often reduced, which further aggravates the negative effects

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of salinity stress. Foliar application of micronutrients has been reported as an effective method to improve nutrient availability and enhance plant tolerance to environmental stresses. Foliar application of iron can rapidly correct iron deficiency and improve physiological functions, particularly under stress conditions such as salinity. Therefore, the use of iron foliar application may be a suitable strategy to mitigate the adverse effects of salinity stress on ornamental plants. The present study was conducted to investigate the effects of different levels of salinity stress and iron foliar application on the growth and morphological characteristics of *Calendula officinalis*. The main objective of this research was to evaluate whether foliar application of iron could reduce the negative effects of salinity stress and improve plant growth under saline conditions.

II. MATERIALS AND METHODS

This experiment was conducted under greenhouse conditions at the Faculty of Agriculture, Paktika University. The study was carried out during the growing season using a factorial experiment arranged in a completely randomized design (CRD) with three replications. The experiment aimed to evaluate the effects of salinity stress and foliar application of iron on the growth and morphological characteristics of *Calendula officinalis*. The experiment consisted of two factors. The first factor was salinity stress applied at four levels: 0, 30, 50, and 80 mM sodium chloride (NaCl). The second factor was foliar application of iron at three concentrations: 0, 200, and 400 mg L^{-1}. The salinity treatments were applied through irrigation water, while iron was applied as a foliar spray. Seeds of *Calendula officinalis* were used as plant material in this study. The seeds were initially sown in seed trays filled with a suitable growth medium. After germination and establishment of seedlings, uniform plants were selected and transplanted into plastic pots containing prepared soil. The pots were placed in the greenhouse under controlled environmental conditions. Throughout the experiment, standard agronomic practices were applied uniformly to all treatments. Plants were irrigated regularly, and necessary cultural practices were performed to ensure normal plant growth. Salinity treatments were applied gradually to avoid sudden osmotic shock to the plants. Iron was applied as a foliar spray at the designated concentrations according to the experimental treatments. Foliar spraying was carried out at specific growth stages to ensure proper absorption of iron by plant leaves. Control plants were sprayed with distilled water. Care was taken to spray plants uniformly until leaf surfaces were fully wetted. Growth and morphological characteristics of *Calendula officinalis* were evaluated during the experiment. The measured traits included plant height, stem diameter, number of leaves per plant, number of flowers per plant, flower diameter, number of lateral branches, and relative water content. Measurements were taken using standard procedures commonly employed in plant growth studies. The collected data were subjected to statistical analysis using appropriate software programs, including Excel, SAS, and Minitab. Analysis of variance (ANOVA) was performed to determine the effects of salinity stress, iron foliar application, and their interaction on the measured traits. Mean comparisons were carried out using Duncan's multiple range test at a specified probability level.

III. RESULTS AND DISCUSSION

The results of variance analysis showed that the effects of salinity stress (induced by sodium chloride) and the interaction effect of foliar application of iron nutrient element were significant on the morphological traits of marigold (Table 1).

Sources of Change	Degree of freedom	Mean Squares				
		Height	Flower diameter	Number of Leaves	Number of Flowers	Relative Water Content (RWC)
Salinity (Mm)	3	1222.63**	632.593**	18290.9**	83.1852**	377.954**
Fe foliar application	2	322.33 **	13.444**	2245.7**	2.6944*	16.778**
(Salinity*Fe foliar)	6	100.85**	41.704**	556.1**	11.1019**	28.815**
Errors	24	37.14	2.528	128.7	0.8333	4.472
CV (%)		11.46	6.55	7.44	9.31	3.72

1: Height

The results of variance analysis in Table (1) show that the main and interaction effects of iron foliar application and salinity stress on the height of marigold were significant at the 1% probability level. Meanwhile, the results in Figure (1) indicate that salinity stress caused a decrease in plant height, whereas foliar application of iron increased this trait. The

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highest height was observed under 0 mM salinity stress and 400 mg/L foliar application, while the lowest height was recorded under 80 mM salinity stress and 0 mg/L foliar application.

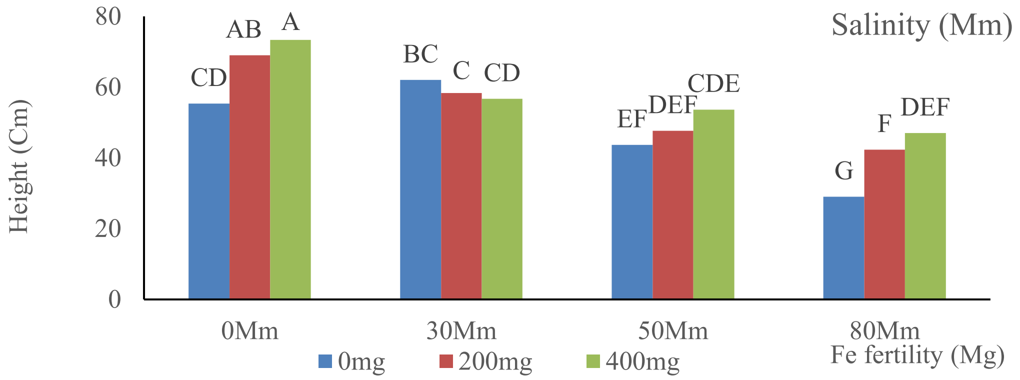

Figure (1)

2: Flower diameter

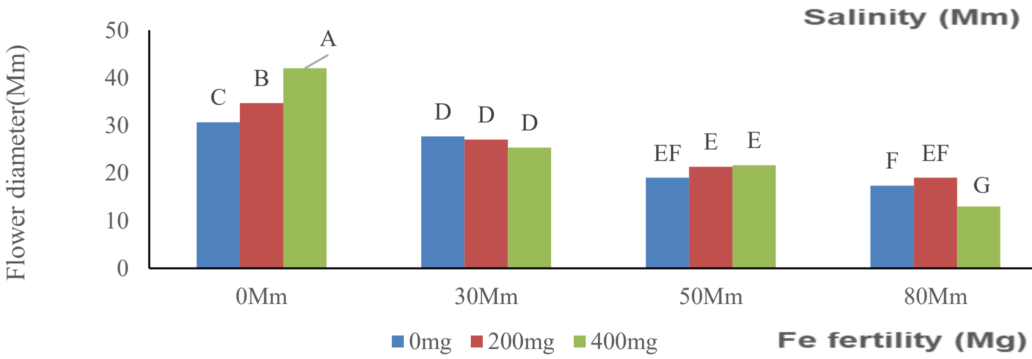

Figure (2)

The results of the variance analysis in Table (1) show that the main and interaction effects of iron foliar application and salinity stress on the flower diameter of marigold were significant at the 1% probability level. Meanwhile, the results in Figure (2) indicate that salinity stress caused a decrease in flower diameter, but in contrast, foliar application of iron increased this trait. The highest value was related to 0 mM salinity stress and 400 mg/L foliar application, while the lowest value was related to 80 mM salinity stress and 400 mg/L foliar application of iron fertilizer.

3: Number of Leaves

The results of the variance analysis in Table (1) show that the main and interaction effects of iron foliar application and salinity stress on the number of leaves of marigold were significant at the 1% probability level. Meanwhile, the results in Figure (3) indicate that salinity stress caused a decrease in the number of leaves of the plant, but in contrast, foliar application of iron increased this trait. The highest number of leaves was related to 0 mM salinity stress and 400 mg/L foliar application, while the lowest number was related to 80 mM salinity stress and 0 mg/L foliar application.

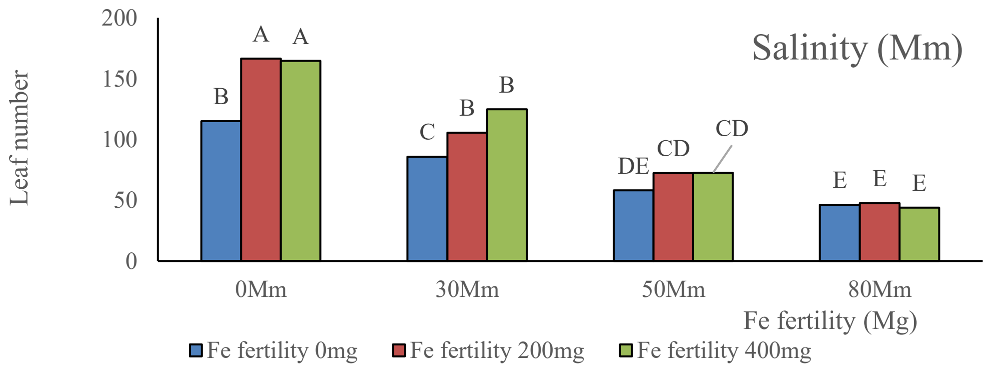

Figure (3)

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4: Number of Flowers

The results of the variance analysis in Table (1) show that the main effect of iron foliar application was significant at the 5% probability level, and the main and interaction effects of salinity stress on the number of flowers of marigold were significant at the 1% probability level. Meanwhile, the results in Figure (4) indicate that salinity stress caused a decrease in the number of leaves of the plant, but in contrast, foliar application of iron increased this trait. The highest number of flowers was related to 0 mM salinity stress and 400 mg/L foliar application, while the lowest number was related to 80 mM salinity stress and 400 mg/L foliar application of iron fertilizer.

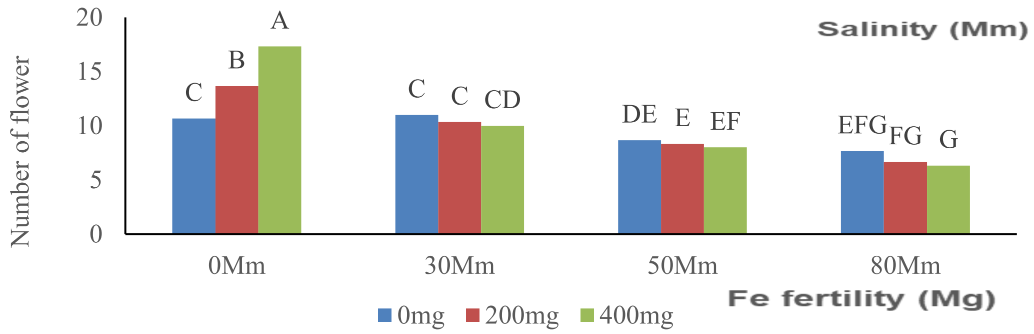

Figure (4)

5: Relative Water Content (RWC)

The results of the variance analysis in Table (1) show that the main and interaction effects of iron foliar application and salinity stress on the relative water content of marigold were significant at the 1% probability level. Meanwhile, the results in Figure (5) indicate that salinity stress caused a decrease in the relative water content of marigold, but in contrast, foliar application of iron increased this trait. The highest relative water content was related to 0 mM salinity stress and 400 mg/L foliar application, while the lowest value was related to 80 mM salinity stress and 0 mg/L foliar application of iron fertilizer.

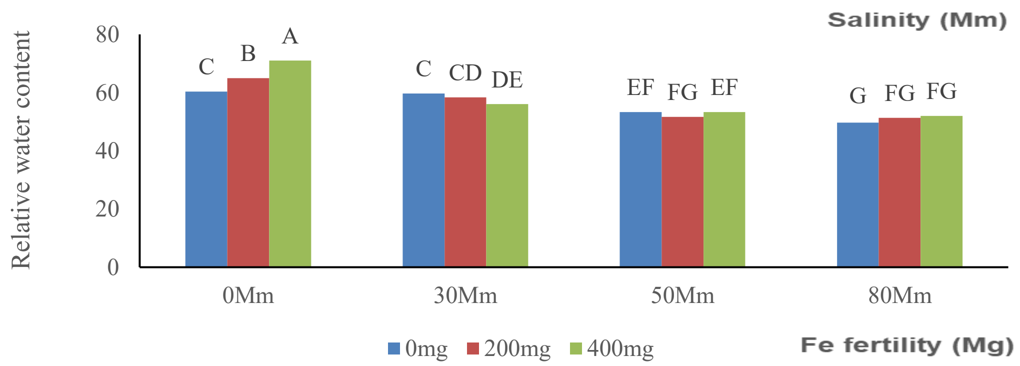

Figure (5)

IV. DISCUSSION

This study, which investigated the effects of foliar application of iron on the growth and morphological traits of marigold under salinity stress, was conducted in the greenhouse of the Faculty of Agriculture at Paktika University. The results showed that salinity stress caused a decrease in the growth of the morphological traits of marigold. However, in contrast, foliar application of iron increased the morphological traits of the plant such as plant height, fresh weight, number of leaves, number of fruits, number of lateral branches, and also the relative water content. Under saline conditions, it improved plant performance and reduced the negative effects of salinity stress. The results of the study by Moradi et al. (1395) indicate that foliar application of iron or the use of iron fertilizer increased plant height, which is similar to the results of this study. Iron increases plant growth in such a way that it contributes to the formation of chlorophyll and increases the photosynthetic content, which consequently leads to increased plant growth. Majidi et al. (1402) reported that the foliar application of iron nanoparticles resulted in a significant difference in the height of marigold compared to the control. In addition, foliar application of iron and zinc elements caused increased production of carbohydrates. Furthermore, foliar application of iron resulted in an increase in stem diameter, which is similar to the results of this study. Iron plays a key role in electron transfer, which consequently increases photosynthesis, and its deficiency leads to reduced plant growth and a decrease in stem diameter (Fang et al., 2009). According to the reports of Riahi Nia et al. (1401), foliar application of iron increased the number of leaves and fruits in plants such as Salicornia and pine (Pirzad et al., 1392), which is similar to the results of the above studies. Foliar application of iron plays a key role in the synthesis of sugars and ultimately leads to the development of plant green cover. As a result, it increases plant diameter, number of leaves, and number of lateral or secondary branches. Iron increases cell division, and as the amount of carbohydrates increases, plant cell division becomes faster, which leads to an increase in the number of lateral branches and leaves of the plant. Likewise, foliar application of iron increases the relative water content. The results of the study by Majidi et al. (1402) show that the use of iron under saline conditions increased the relative water content of marigold leaves, which is similar to the results of the present study. This is because iron plays a key role in creating ionic balance in vacuoles and in the production of certain specific proteins in order to reduce the osmotic potential of the cell (Kakhki and Goldani, 1397). Also, foliar application of iron increased the flower diameter of marigold (Mahdi Nejad et al., 1397). Iron and zinc elements stimulate the biosynthesis of the cytokinin hormone, which results in the development of buds on branches and ultimately leads to an increase in flower diameter (Markovic et al., 1397).

V. CONCLUSION

During this research, salt stress caused a decrease in some morphological traits of marigold plants. For example, salt stress reduced the height of the plant. However, with the foliar application of the iron fertilizer, the negative effects of this stress were reduced and it helped improve plant height. Similarly, salt stress also caused a decrease in other traits such as plant weight, number of lateral branches, number of leaves and fruits, and flower diameter. It also reduced the relative water content in the leaves of the plant. However, with the foliar application of iron, these traits improved and the negative effects of salt stress that reduce plant growth and yield were alleviated. Salt stress showed greater negative effects at higher concentrations, such as 50 millimolar, and more severe damage was observed at 80 millimolar. However, the foliar application of the micronutrient iron at a concentration of 400 milligrams per liter showed the most positive result, and at this concentration it improved many traits of the marigold plant.

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