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Araştırma Makalesi

Pamukta (*Gossypium hirsutum* L.) Tuzluluk Stresinin K-humat ve Demir Oksit Nanopartikülleri ile Azaltılması

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ÖZ

Tuzluluk stresi, bitki büyümesi ve gelişimi için büyük bir zorluk oluşturur; ozmotik stres, iyon toksisitesi ve besin dengesizlikleri gibi sorunlara ve sorunlar fotosentezin azalmasına ve bitkilerin erken yaşlanmasına neden olur. Bu çalışmada, potasyum humat (Kh) ve demir oksit nanopartiküllerinin (Fe (II,III) oksit pamuk bitkilerinin (*Gossypium hirsitum* L.) tuzlu koşullarla başa çıkmasına yardımcı olma potansiyelini araştırıldı. Bitki boyu, yaprak sayısı, yaprak ve köklerin taze ve kuru ağırlıkları, yaprak alanı, klorofil içeriği (SPAD değerleri) ve bağıl su içeriği (RWC) gibi çeşitli büyüme parametreler incelendi. Bulgular tuzluluk stresinin bitki boyunu, yaprak sayısını, taze yaprak ağırlığını, kuru yaprak ağırlığını, yaprak alanını ve RWC'yi önemli ölçüde azalttığını gösterdi. Ancak Fe (II,III) oksit'lerin ve Kh'nin uygulanması bu olumsuz etkilerin azaltılmasına yardımcı oldu. Özellikle Fe (II,III) oxide-NP'ler ve Kh kombinasyonu, tuzlu koşullar altında en yüksek bitki boyu saptandı. Tek başına Kh, stres altında bile yaprak sayısını ve taze yaprak ağırlığını arttırmada özellikle etkiliydi. Sonuç olarak, hem Fe (II,III) oxide'lerin hem de Kh'nin, pamuk bitkisinin tuzluluk stresine karşı direncini arttırmada etkili olduğu kanıtlandı ve bunların tuzlu ve kurak bölgelerde sürdürülebilir tarımda potansiyel kullanımlarının altı çizildi. Bu bilgiler, zorlu çevresel koşullar altında bitki büyümesini ve verimini artırmaya yönelik stratejiler geliştirmeye yardımcı olabilir.

Anahtar kelimeler: Nanopartiküller, demir oksit, potasyum humat, SPAD, Gossypium hirsutum L.

Mitigating Salinity Stress in Cotton (*Gossypium hirsutum* L.) with K-humate and Iron Oxide Nanoparticles

ABSTRACT

Salinity stress poses a major challenge to plant growth and development, causing problems like osmotic stress, ion toxicity, and nutrient imbalances. These issues lead to reduced photosynthesis and early aging of plants. In this study, we explored the potential of potassium humate (Kh) and iron oxide nanoparticles (Fe (II,III) oxide-NPs) to help cotton plants (*Gossypium hirsitum* L.) cope with saline conditions. We examined various growth parameters such as plant height, leaf number, fresh and dry weights of leaves and roots, leaf area, chlorophyll content (SPAD values), and relative water content (RWC). Our findings showed that salinity stress significantly decreased plant height, leaf number, fresh leaf weight, dry leaf weight, leaf area, and RWC. However, the application of Fe (II,III) oxide-NPs and Kh helped mitigate these negative effects. Notably, the combination of Fe (II,III) oxide-NPs and Kh resulted in the highest plant height under saline conditions. Kh alone was particularly effective in increasing leaf number and fresh leaf weight, even under stress. In conclusion, both Fe (II,III) oxide NPs and Kh proved to be effective in enhancing cotton plant resilience to salinity stress, highlighting their potential use in sustainable agriculture in saline and arid regions. These insights can help develop strategies to improve plant growth and yield under challenging environmental conditions.

Key words: Nanoparticles, iron oxide, potassium humate, SPAD, *Gossypium hirsutum* L.

INTRODUCTION

Saline stress, which causes various metabolic changes in the plant, is associated with a decrease in shoot growth as the initial symptom, as well as a general growth disorder. It also reduces the photosynthetic activity of the plant and accelerates its premature senescence (Munns, 2002). Khorsandi and Anagholi (2009) observed that germination and emergence of cotton seeds were delayed in response to salt stress, with reduced germination observed at a salt concentration of 10 ds m^{-1}. As a consequence of soil salinity, the amount of photosynthetic pigment in plants is reduced, which in turn results in a reduction in photosynthetic activity and a slowing down of the growth rate (Doğru and Canavar, 2020). As the concentration of salt in the soil increases, the levels of chlorophyll a, chlorophyll b and total chlorophyll decrease and reach their lowest point after 150 mm (İzci, 2009).

The area of study where plant science and nanotechnology intersect is referred to as phytonanotechnology, while studies related to agricultural sciences are designated as agronanotechnology. In the present era, agronanotechnology applications facilitate the efficacious utilization of intelligent application systems for the sustainability of agriculture. These applications assist in reducing the environmental impact of agriculture by enhancing agricultural productivity. In the present era, agronanotechnology applications facilitate the efficacious utilization of intelligent application systems for the sustainability of agriculture. The aforementioned applications assist in reducing the environmental impact of agriculture by enhancing agricultural productivity.

The versatility of nano-sized particles, as evidenced by their use in various fields such as life sciences and technology, is attributed to their physico-chemical properties (Jeevanandam et al., 2022). Nanoparticles are employed in a multitude of fields due to their elevated surface-to-volume ratio, despite their diminutive size (Roduner, 2006).

Nanoparticles can be transported via two distinct pathways: the apoplastic and symplastic pathways. Apoplastic transport occurs outside the plasma membrane, within the cell wall and in the extracellular space. Symplastic transport occurs between the pores of the plasmodesmata and phloem parenchyma, in conjunction with the movement of water and solutes within the cell cytoplasm (Etxeberria et al., 2006; Lv et al., 2019). The plant cell wall is the initial point of contact with nanoparticles. Nanoparticles or metal ions dissolved from nanoparticles enter the cell wall of root tissues, where they form a complex with the carboxyl groups of pectin (Yang et al., 2008). This binding can alter the symplastic or apoplastic mode of transport across the cell wall and membrane, which may result in the inhibition of root elongation (Horst et al., 2010).

Moreover, metallic nanoparticles, like silver nanoparticles derived from plant sources, have been found to regulate cell cycle processes and enzyme activities in plants, suggesting potential pharmacological applications in plant systems (Kuppusamy et al., 2016). Biogenic CuO (Copper(II) oxide nanoparticles) and Zinc oxide (ZnO) nanoparticles have also been explored as nano fertilizers for promoting sustainable growth in plants like *Amaranthus hybridus*, influencing the uptake of essential metals and impacting plant growth (Francis et al., 2022).

However, it is essential to consider the potential phytotoxicity of nanoparticles on plants. Research has shown that while lower concentrations of nanoparticles can enhance germination in plants, higher levels of exposure may have adverse effects, such as biomass reduction, especially in crops (Stampoulis et al., 2009). Nanoparticle toxicity can lead to oxidative stress in plants, causing physical damage and affecting physiological processes like stomatal closure (Wang et al., 2023).

Furthermore, the interactions between nanoparticles and edible crop plants are still under investigation. While some studies have contributed to understanding nanoparticle-plant interactions, knowledge gaps persist regarding the effects of nanoparticle exposure on edible crops and their implications for plant health and productivity (Thunugunta et al., 2018). In some research studies have been conducted in the field of plant sciences and nanotechnology. It is therefore necessary to conduct further research into effective alternative methods for seed germination, growth, protection of plants against biotic and abiotic stresses, and coping with these stresses (Wang et al., 2016).

Also, the targeted delivery of nucleotides, proteins, and other photoactive molecules via nanoparticles has the potential for genetic modification and regulation of plant metabolism (Scheringer, 2008). The use of nanotechnology for the control and release of agricultural chemicals has the potential to reduce the damage of plant protection products, minimize nutrient losses in fertilizers and increase crop yields through optimized nutrient management.

Furthermore, the utilization of nanoparticle-containing fertilizers enhances the nutrient utilization efficiency of plants. For instance, the utilization of fertilizers that facilitate a low phosphate (PO_4^{3-}) release in plants serves to safeguard the environment and enhance the productivity of agricultural regions by reducing the risk of eutrophication in water (Liu and Lal, 2015)

Consequently, in order to gain a detailed understanding of the biochemical and molecular processes involved in salt tolerance, it is necessary to consider the use of nanoparticles in cotton under salinity stress. By utilizing research on gene expression, nutrient uptake, and physiological responses to salinity stress, sustainable strategies to enhance cotton productivity in saline environments can be developed.

It is of paramount importance to minimize the negative effects of salt stress on plants and to prevent yield losses caused by this stress if genotypes resistant to salty soils are to be developed and used in agricultural production. The objective of this study was to investigate the effects of K-humate (Kh) and iron oxide nanoparticles (Fe (II,III) oxide-NPs) on cotton (*Gossypium hirsitum* L.) in order to minimize the impact of salt stress. Furthermore, the research aims to provide an accurate evaluation of the effects of salinity stress and nanoparticles by establishing optimal conditions for plant growth. The findings of this study will contribute to an enhanced understanding of the resilience of plants to salinity stress and the effects of nanoparticles on plant growth and development.

MATERIAL and METHOD

In order to ascertain the impact of salinity stress on plants and the role of nanoparticles, a two-factor randomized plots experiment was conducted. A suitable acclimatization environment was prepared for this experiment, and *Gossypium hirsitum* L. (Karizma) was selected as the plant material. The experiment was designed to observe the response of the plant material to salinity stress and the effects of Fe (II,III) oxide-NP and k-humate (Kh).

To determine the effects of salinity stress on plants and the role of nanoparticles, a two-factor randomized blocks experiment was conducted. The seedbed utilized in the study was prepared with a mixture of 50% perlite and 50% peat in transparent pots with a volume of 1.2 L. This mixture was selected to facilitate optimal growth and development of the plant seeds. The dimensions of the pots were 9 cm in width and 18 cm in length, with these dimensions chosen to ensure that the roots of the plant would have sufficient space and soil fertility.

Inside the grown chamber, lighting conditions were maintained with temperatures of approximately 24-26°C during the day and 18±2°C at night, with a photoperiod of 12 hours. Red light with a wavelength of 630 nm was used for illumination, as this wavelength supports the photosynthesis and growth processes of plants.

The sowing process was initiated on 23 November 2023, with three seeds planted at a depth of 2 cm in each pot. Each pot was initially irrigated with approximately 50 mL of water to create an optimal germination environment. Subsequent irrigations were carried out at two-week intervals to promote germination and seedling emergence.

Irrigation Water: For 10L of irrigation water; (Macro and micronutrients used were equal for both conditions. *[4g NPK (3x15)/ 2g urea/ 20 mL liquid fertiliser (Multimicro® Fluid)*

Calculation of NaCl Concentration

The irrigation water applied in the experiment was provided with tap water. The calculation of salt concentrations is given below

$$1\ dS\ m^{-1} = 10\ mM\ NaCl = 0.584\ g/\ 1\ lt$$

$$130\ mM = 13\ dS\ m^{-1} = 0.584 = 7.592\ g\ NaCl\ L^{-1}$$

On 30 November 2023, the experimental plant was established, comprising two groups: a saline and a non-saline group. The saline group was constituted by plants that were subjected to a saline stress, while the non-saline group was constituted by plants that were not subjected to a saline stress. On 13 December 2023, the sixth set of leaves from the plants in the non-saline conditions was harvested. Furthermore, a series of measurements were taken across all treatments. The irrigation water was provided in equal quantities of 100 mL on a two-day cycle.

Iron oxide nanoparticle dose concentration adjustment was calculated from Iron oxide NPs from Sigma ALDRICH (637106-25G) (Lot # MKBT3736V, particle size 50-100 nm) as 0.3 mg 100 mL^{-1}. K humate: A solution of 0.3 g per 1 liter was prepared.

Investigated Parameters

Plant Height (cm): Plant height was determined by measuring the vertical distance from the soil surface to the top of the plant. The measurement was made in centimeters (cm) using a ruler or meter.

Leaf Number: The total number of leaves on the plant was manually counted and recorded.

Leaf Fresh Weight (g): The fresh weight of the plant was measured in grams (g) after the plant was carefully removed from the soil and the excess soil was removed.

Leaf Dry Weight (g): After the fresh weight was measured, the plant was dried in a drying oven at 70-80°C and kept until it reached a constant weight. The dried plant was weighed again, and its weight was determined in grams (g).

Root Fresh Weight (g): The roots of the plant were carefully weighed after being cleaned from the soil. The fresh weight of the roots was measured in grams (g) using a recision scales

Root Dry Weight (g): After the fresh weight was measured, the roots were dried in oven and kept until they reached a constant weight. The dried roots were weighed, and their weights were determined in grams (g).

Leaf Area (cm²): Leaf area was measured using a leaf area meter (LI-3000C Portable Leaf Area Meter) using images taken from the leaves of the plant or the surface of the leaves. The measurement was made in square centimeters (cm²).

Leaf Chlorophyll Content (SPAD): It was measured with a "Konica Minolta SPAD-502 Plus" portable chlorophyll meter in the fully-developed flag leaves and determined as "SPAD value" (Pask et al.,2012). It was taken three averages of five leaves per plot, and they were done from 11:00h to 14:00h.
The analysis of variance (ANOVA) and comparison of means at the Student-t 0.05 level for the least significant difference (LSD) test were conducted using the JMP Pro16 software (SAS Institute, Cary, NC, USA).

Relative water content (RWC):It is a measure of the water status of a plant, reflecting the balance between water supply and water loss. It is commonly used to assess plant water stress and hydration levels. The formula to calculate RWC is as follows:

$$RWC = (\text{Fresh Weight} - \text{Dry Weight}) /(\text{Turgid Weight} - \text{Dry Weight})] \times 100$$

RESULTS

The results of the variance analysis of the plant height values of the experiment are presented in Table 1. Upon statistical evaluation of the data, a statistically significant difference (p-value of less than 0.01) was identified in the plant height values between the control and salt medium. When the media were analyzed, the highest plant height in control conditions were observed in the control- Fe (II,III) oxide-NPs with a value of 23 cm, while the lowest plant height was observed in the distilled-water treatment in the salinity medium, with a value of 9.25 cm. In saline conditions, the highest plant height was observed in the Fe (II,III) oxide-NPs +Kh treatment, with an average height of 14.55 cm. In contrast, the average plant height in control conditions was 21 cm, while in saline conditions it was 12.56 cm. Salinity had a negative effect on plant height compared to control conditions. When the treatments were compared, it was observed that Fe (II,III) oxide-NPs, Fe (II,III) oxide-NPs +Kh, and K-humate were in the same statistical group. The lowest value was observed in the distilled water treatment, with a height of 13.25 cm.

A statistical evaluation of the data obtained revealed a statistically significant difference in the number of leaves between the control and salt environments. Upon examination of the environments, it was observed that while the number of leaves in control conditions was 6.5 per plant, this value decreased to 5.56 per plant in the salinity environment. The highest number of leaves was observed in the control-K-humate interaction (7.75 pcs/plant), while the lowest number of leaves was observed in saline conditions (5.5 pcs/plant). It was found that the application of K-humate had a numerically positive effect on the number of leaves under salinity conditions.

Table 1. Variance analysis results of plant nutrients applied under control and salinity conditions

Source	DF	Mean Square								
		PL	LN	LFW	LDW	RFW	RDW	LA	SPAD	RWC
Enviroments	1	569.531**	7.031**	96.953**	5.586**	0.035	0.114**	799.698**	21.780	975.253*
Applications	3	47.115**	1.865	6.919*	0.103	1.799	0.243**	14.248	26.506	132.142
Rep.	3	2.531	0.615	0.462	0.181	0.386	0.017	38.116	14.546	146.343
Env.*Aplications	3	1.948	1.031	4.728	0.342*	0.556	0.416**	11.675	36.724	219.498
Error	21	65.150	0.829	1.980	0.079	0.644	0.014	37.560	16.83	129.466

*DF:Degrees of Freedom, PL: Plant Height, LN: Leaf Number , LFW: Leaf Fresh Weight, LDW: Leaf Dry Weight, RFW: Root Fresh Weight, RDW: Root Dry Weight, LA:Leaf Area, SPAD: Chlorophyll Content (SPAD meter reading) ,RWC: Relative Water Content, **:p-value of less than 0.01,*: p-value of less than 0.05. Enviroments: Saline condition or Control condition

A statistical evaluation of the data obtained revealed statistically significant differences in fresh leaf weight values between the control and salinity conditions, as well as between the treatments. The highest fresh leaf weight was observed in the control-K-humate conditions, with a value of 10.59 g, while the lowest fresh leaf weight was determined in the nonsaline-Zn application, with a value of 8.96 g. In saline conditions, the highest fresh leaf weight was found to be 4.86 g in salinity- Fe (II,III) oxide-NPs conditions. While the average of fresh leaf weight in control conditions was 8.67 g, it was observed to be 5.19 g in salty conditions. Salinity negatively affected fresh leaf weight compared to the control environment. Upon comparison of the averages of the applications, it was observed that the K-humate and Fe (II,III) oxide-NPs +Kh applications exhibited the highest fresh leaf weight.

When the data obtained from the variance analysis results of the leaf dry weight values of the experiment were evaluated, a statistically significant difference was found between the leaf dry weight values of Environments, Applications and Env.*Applications. When the results are examined, it appears that salinity stress has a negative effect on leaf dry weight (Table 2). Among the applications, the highest leaf dry weight value was obtained under FE_2O_3-NPs values. When the environment*application data were evaluated, the highest leaf dry weight in control conditions was control-K-humate 1.73 g, and the lowest leaf dry weight was salinity- K-humate was observed as 0.30 g.

Table 2. Mean values of applied plant nutrients under control and salinity conditions

Applications	Plant lenght (cm)			Leaf Number			Leaf Fresh Weight (g)			Leaf Dry Weight (g)		
	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean	Control	Salinity	Mean
Distilled Water	17.25	9.25	13.25 B	6	5.5	6.75	6.672	5.21	5.94 b	1.21 BC	0.71 DE	0.96 b
Fe (II,III) oxide-NPs	23	13.75	18.37 A	6.25	5.5	5.875	8.09	4.86	6.48b	1.51 AB	0.90 CD	1.2 a
Fe (II,III) oxide-NPs +Kh	22	14.75	18.37 A	6	5.5	5.75	9.34	5.16	7.3 ab	1.56 AB	0.76 D	1.16 a
K-humate	21.75	12.5	17.12 A	7.75	6.75	5.75	10.59	5.55	8.07 a	1.73 A	0.30 E	1.02 ab
Mean	21 a	12. 56 b		6.5 a	5.56 b		8.67 a	5.19 b		1.5 a	0.67 b	

*Letters indicate LSD (0.05) significance levels. Uppercase letters indicate significant differences between treatments while lowercase letters indicate significant differences between saline and control conditions.

The results of the analysis of variance of root fresh weight values are presented in Table 1. Upon statistical evaluation of the data, no significant differences were observed in terms of root fresh weight values, either in relation to the environment or the treatments. Upon analysis of the environments, the root fresh weight under control conditions was found to be 2.58 g, while that under salinity conditions was 2.51 g. When the treatments were compared, the highest root fresh weight was determined to be 3.17 g in the Fe (II,III) oxide-NPs +Kh treatment.

A statistically significant difference was found between the obtained root dry weight values between environments, applications and environments* applications. When evaluated statistically, a statistical difference was found between root dry weight values between control and salinity conditions, although the numerical difference is not significant. When the control and salinity conditions were compared, the root dry weight was determined to be 0.43 g in salinity conditions, while this value was determined to be 0.30 g under control conditions. This difference was observed. Considering the environmental and application interactions, the highest root dry weight was determined to be 1.02 g in salinity-K-humate conditions. The lowest root dry weight was determined to be 0.2 g under salinity-distilled water conditions.

Upon statistical evaluation of the variance analysis results of the leaf area values obtained from the experiment, a statistically significant difference was found between the control and salt environment. The average leaf area under control conditions was determined to be 35.45 cm^3, while under salinity conditions, this value was negatively affected and decreased to 28.22 cm^3. Upon numerical comparison of the values, the lowest leaf area was determined to be 26.39 cm^3 under salinity-K-humate conditions, while the highest leaf area was determined to be 40.22 cm^3 under control-K-humate conditions.

The results of the variance analysis of the SPAD values obtained in the experiment are presented in Table 1. Upon statistical evaluation of the data, no significant differences were observed in SPAD values, regardless of environmental conditions or application. Upon examination of the environments, the average provincial content of chlorophyll in control conditions was found to be 40.21, while it was determined to be 41.86 under salinity stress. When the applications were evaluated individually, the highest value was observed in the Fe (II,III) oxide-

NPs +Kh application, with a value of 43.7, while the lowest value was observed in the distilled-water application, with a value of 39.78.

When the relative water content values obtained in the experiment were compared statistically, a significant difference was detected in terms of environment. While the relative water content was 71.85% under salinity stress, this value was found to be 60.81% in control conditions. The highest RWC average among the applications was determined as 70.88% in the Fe (II,III) oxide-NPs application, while the lowest value was obtained in the K-humate application at 61.60%.

DISCUSSION

The study findings suggest that salinity stress generally reduces plant growth due to osmotic stress, ion toxicity, and nutrient imbalance (Vessal et al., 2020). In control conditions, the highest plant height was recorded in the control-Fe (II,III) oxide-NPs treatment, indicating that iron oxide nanoparticles may enhance plant growth by improving nutrient availability and stimulating physiological processes (Khan et al., 2019). Conversely, the lowest plant height under salinity stress was observed in the distilled-water treatment, highlighting the adverse effects of salinity without ameliorative treatments. Under saline conditions, the highest plant height was observed in the Fe (II,III) oxide-NPs + Kh treatment, suggesting a synergistic effect in mitigating salinity stress. Potassium humate, known for improving soil structure and enhancing nutrient uptake, played a crucial role in promoting plant growth under both control and stress conditions (Zeitelhofer et al., 2022). The reduction in plant height under salinity stress is consistent with existing literature on the negative impacts of salinity on plant growth (Badawy et al., 2023). Salinity-induced reductions in cell division and elongation can significantly affect overall plant height (Jameel et al., 2021).

The study demonstrates the potential of Fe (II,III) oxide-NPs and K-humate in mitigating the adverse effects of salinity on plant growth. Moreover, a significant difference in the number of leaves was observed between control and salt environments, with a decrease in leaf number under salinity stress. The control-K-humate treatment exhibited the highest leaf count under non-stress conditions, indicating the positive effects of K-humate on leaf production by enhancing nutrient availability and soil health (Hacisalihoglu, 2020). Even under saline conditions, K-humate positively impacted leaf numbers, suggesting its role in mitigating salinity stress and supporting plant growth (Ghiabi et al., 2013). Salinity stress negatively affects leaf production, but K-humate application can alleviate some of these effects, promoting better plant growth in both control and saline environments. Furthermore, significant differences were found in fresh leaf weight between control and saline conditions, with the highest fresh leaf weight observed in the control-K-humate treatment. Salinity stress led to reduced fresh leaf weight, consistent with previous research on the impact of salinity on plant biomass (Kumar et al., 2008). Treatments like K-humate and Fe (II,III) oxide-NPs + Kh showed promising results in mitigating the adverse effects of salinity on fresh leaf weight, potentially through improved soil fertility, nutrient availability, and enhanced physiological processes (Azim et al., 2022). While salinity stress reduces fresh leaf weight, the application of K-humate and Fe (II,III) oxide + Kh can help enhance plant growth under stressful conditions. In summary, the study underscores the importance of Fe (II,III) oxide-NPs and K-humate in mitigating the negative effects of salinity stress on plant growth. These treatments offer promising solutions for sustainable agriculture, particularly in challenging environments like saline and arid regions.

The analysis of leaf dry weight values in the study revealed significant differences between environments, applications, and their interactions. Salinity stress was found to have a negative impact on leaf dry weight, aligning with previous research indicating the detrimental effects of salinity on plant growth parameters, including biomass reduction Angon et al. (2022). Among the applications, Fe (II,III) oxide-NPs demonstrated the highest leaf dry weight values, suggesting that iron oxide nanoparticles may enhance plant resilience under drought stress by improving nutrient uptake and potentially enhancing antioxidant defenses (Benito et al., 2023). When considering the interaction between environment and application, the control-K-humate treatment exhibited the highest leaf dry weight under control conditions, emphasizing the positive effects of K-humate in promoting plant growth by enhancing soil structure, nutrient availability, and water retention capacity (Badawy et al., 2020). Conversely, the salinity-K-humate treatment recorded the lowest leaf dry weight, illustrating the compounded negative impacts of salinity and drought stress on plant biomass (Toraman et al., 2020). Overall, while salinity stress was shown to reduce leaf dry weight significantly, the applications of Fe (II,III) oxide-NPs and K-humate were found to mitigate some of these adverse effects, enhancing plant growth and resilience under stressful conditions. This highlights the potential of these treatments in sustainable agriculture, particularly in saline and arid environments.

The SPAD values obtained in the experiment did not show significant differences across different environmental conditions or applications. However, when examining the relative water content (RWC), a significant difference was observed between environments, with salinity stress resulting in a lower RWC compared to control conditions. The highest RWC average among the applications was found in the Fe (II,III) oxide-NPs application, while the lowest was observed in the K-humate application. These findings suggest that while SPAD values remained consistent, the RWC was influenced by environmental conditions, with salinity stress leading to reduced water content in the plants. This emphasizes the importance of considering multiple physiological parameters, such as RWC, alongside chlorophyll content, in assessing plant responses to different stressors like salinity and drought. The literature supports the notion that chlorophyll stability can serve as an indicator of drought tolerance in various plant species (Arunyanark et al., 2008). Additionally, studies have shown that the application of chelated nano zinc can significantly affect chlorophyll content in plants (Hasan et al., 2023). Furthermore, research on physiological and agrometeorology indices in chickpea genotypes has highlighted the importance of considering factors like planting density in relation to chlorophyll content (Patil et al., 2020). Moreover, investigations into the physiological and biochemical responses of sugarcane to salt stress have underscored the significance of chlorophyll in plant adaptation to stressful conditions (Simões et al., 2020.) These references shows relationship between chlorophyll content, water status, and plant responses to environmental stressors, supporting the findings of the experiment regarding RWC and SPAD values.

CONCLUSION

This study highlights the detrimental effects of salinity stress on various plant growth parameters, including plant height, leaf number, fresh and dry leaf weight, root fresh weight, and leaf area. Salinity stress significantly reduced these parameters, emphasizing the negative impact of saline conditions on plant growth. However, the application of Fe (II,III) oxide-NPs and K-humate showed promising results in mitigating these adverse effects. Fe (II,III) oxide -NPs enhanced plant growth by improving nutrient availability and physiological processes, while K-humate improved soil structure and nutrient uptake. The highest plant height and fresh leaf weight were observed in the control- Fe (II,III) oxide-NPs and control-K-humate treatments, respectively, underlining their potential to enhance plant growth even under stress conditions. Moreover, Fe (II,III) oxide-NPs and K-humate positively influenced drought resilience, as evidenced by higher leaf dry weights in their respective treatments. While salinity stress reduced RWC, the highest RWC was recorded in the Fe (II,III) oxide-NPs application, suggesting improved water retention capacity. These findings underscore the importance of Fe (II,III) oxide-NPs and K-humate in promoting sustainable agriculture, especially in saline and arid regions. The study demonstrates the potential of these treatments to enhance plant growth and resilience, providing valuable insights for developing strategies to combat salinity stress in agriculture.

Conflict of interest disclosure statement: The authors declare that they have no conflicts of interest relevant to this research.
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