



The Effect of Fe₃O₄ Nanoparticle Applications on Seedling Development in Water-Stressed Strawberry (*Fragaria × ananassa* ‘Albion’) Plants

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Abstract

In this study, the effects of iron nanoparticles (NP) applied under water stress conditions on strawberry plant developments were investigated. The study was conducted using a split-plot trial design consisting of three replications. The main plots in the trial had three distinct irrigation water levels (I) (i.e., 50% [I50], 75% [I75] and 100% [I100] of the plant's required water), whereas the subplots contained four different concentrations (0 mg L⁻¹ Fe₃O₄ [NP0], 0.01 mg L⁻¹ Fe₃O₄ [NP1], 0.1 mg L⁻¹ Fe₃O₄ [NP2], and 1 mg L⁻¹ Fe₃O₄ [NP3] applied parcels) of Fe₃O₄ nanoparticles. The effects of irrigation water level, NP level, and their interactions on seedling development were found to be statistically significant. With increasing irrigation water and NP levels, increases in the number of seedlings, leaf area and number of leaves were observed. The highest average number of seedlings was obtained from the I100 and NP3 subjects, at 145.2 and 183.0 plants, respectively. In terms of the interaction between irrigation water level and iron nanoparticles, the highest value was 207.7 plant-1 from the I50 × NP3 interaction. With increasing iron nanoparticle concentration, the number of leaves per plant also increased, and the greatest increase was detected for the I50 × NP3 interaction. Iron nanoparticle application also had a positive effect on the relative water content (RWC), leaf relative water content (LRWC), and chlorophyll stability index (CSI), especially under water stress conditions, which increased significantly compared to those under control conditions. After analyzing the data, it was determined that, when applied under water stress conditions, iron nanoparticles increased the plant's resilience to stress and had a beneficial effect on strawberry plant development.

Keywords Chlorophyll content · Iron oxide · Plant water content · Stolon · Deficit irrigation

Introduction

The diminishing availability of clean water intensifies the demand for irrigation in agriculture and results in reduced accessibility to irrigation water. This leads to an increase in water stress throughout the plant growth season. Drought or an inadequate water supply during crucial stages inhibits plant water absorption and photosynthesis, leading

to a detrimental impact on plant metabolism and resulting in reduced crop output and quality (Mpandeli et al. 2015; Lin et al. 2023). Hence, the significance of agriculture's future, the sustainable administration of water resources, and the efficient utilization of water cannot be overstated. This goal will be accomplished by employing effective irrigation methods and adopting innovative strategies that will mitigate water scarcity.

Nanotechnology (NT) is a newly implemented approach. Nanotechnology is the utilization of materials engineered at the atomic and molecular scales and is often regarded as the industrial revolution of the contemporary era (Woldemanuel et al. 2021). Nanotechnology, which has a wide range of uses, is a versatile field of technology that includes various activity sectors and scientific disciplines, such as biomedicine, chemistry, agriculture, food, medicine, optics, pharmaceuticals, cosmetics, textiles, the environment, and electronics (Rai and Ingle 2012; Judy and Bertsch 2014).

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Recent studies indicate that nanotechnology has significant promise for promoting sustainability in agriculture (Usman et al. 2020; Shah et al. 2022). Nanoparticles (NPs) form the basis of nanotechnology. Metallic nanoparticles that enhance plant growth and development are composed of various metal elements (such as Ag, Cu, Zn, and Fe) or their oxide complexes (such as CuO, ZnO, Fe₂O₃, and TiO₂) (Manikandan et al. 2017). The use of nanoparticles, specifically Mn, Fe, Zn, Cu, Ag, Au, Se, and TiO₂, in plants has been found to have a beneficial impact (Aqeel et al. 2021). Iron oxide (Fe₂O₃, Fe₃O₄) nanoparticles, particularly those with dimensions less than 10–20 nm, have a beneficial impact on plant growth and productivity and enhance plant resilience under challenging environmental conditions (Sheng et al. 2014; Karpagavinayagam and Vedhi 2019). Iron oxide nanoparticles enhance chlorophyll production by facilitating regular stomatal aperture (Adrees et al. 2020). They also decrease the presence of stress indicators during drought conditions and exhibit improved morphological characteristics in plants. Additionally, these nanoparticles mitigate oxidative stress by boosting the activity of antioxidant enzymes in plant cells. Consequently, plants have become more resilient to drought stress (Rui et al. 2016; Siva and Benita 2016, Mozafari et al. 2018). Furthermore, it has been established that nanoparticles encourage the growth of plant root systems and enhance water utilization efficiency. These findings indicate that nanoparticles could serve as a potent tool for addressing drought in agriculture (Adrees et al. 2020; Linh et al. 2020; Morales-Espinoza et al. 2019). It is crucial to investigate the impacts of nanoparticles through empirical investigations conducted in real-world environments for these reasons.

Strawberry (*Fragaria × ananassa* Duch.) is a highly significant berry that can be cultivated in various ecologies and is economically viable on a global scale. Strawberries are abundant in vitamins, minerals, and sugars, as well as in bioactive substances such as flavonoids, anthocyanins, and phenolic acids (Giampieri et al. 2014). Its production and consumption have increased rapidly worldwide in the last 20 years due to its high adaptability, consumption as a fresh or processed food, its importance for human health, and its economic profitability with the return of investments from the first year (Öztürk Erdem and Çekic 2017). According to the FAO, the worldwide strawberry market had a value of €14 billion in 2020. Europe accounted for approximately €3.5 billion of this market share. Furthermore, the market has continued to progress since then (FAO 2022). Climate change can have an impact on the quality of strawberries and the amount of strawberries that can be grown, similar to other plant products. Currently, drought is regarded as the primary determinant of crop productivity. During extreme water intolerance, a plant's water consumption efficiency is adversely affected (Gholami and Zahedi 2019). Effective

irrigation is crucial for achieving optimal yield and superior quality in strawberries. Physiological impacts, such as reduced water use efficiency, stomatal closure, and the accumulation of substances such as proline and polyamine occur (Kaman et al. 2023a). Drought stress induces metabolic interactions, such as the buildup of osmoprotectants, which have a detrimental impact on both the quantity and quality of plant development. Reducing drought stress is crucial due to several of these factors. Global research efforts are underway to devise strategies that include the creation of drought-resistant plant types, the alteration of crop planting schedules, and the use of advanced agricultural water management techniques (Venkateswarlu and Shanker 2009). Recent studies indicate that nanotechnology has significant promise for promoting sustainability in agriculture (Dimkpa et al. 2017; White and Gardea-Torresdey 2018; Morales-Espinoza et al. 2019; Xiao et al. 2019; Adrees et al. 2020; Linh et al. 2020).

The initial and crucial phase in strawberry production, for achieving an efficient output and superior quality, is to commence production with high-quality seedlings to ensure healthy seedlings and good yield and quality production, which is one of the important problems of strawberry cultivation. Plants, which are plant materials, must be produced in special seedling production centers using high-yielding and high-quality varieties that are free from diseases and pests, and these baby plants must be used in production. Among the various techniques for vegetative reproduction in strawberries, stolon propagation is the most favored. The relationships between stolon development and photoperiod and temperature are well established (Hancock 2020).

Abiotic stressors, such as extreme temperatures, drought stress, and salinity, exert detrimental effects on plants. The continuous utilization of water resources by horticulture crops is becoming increasingly worrisome in terms of its environmental implications (Terry et al. 2007; Grant et al. 2012). As a result of global warming, almost 45% of the world's agricultural fields are subjected to both drought stress and salinity. Additionally, drought is a problem in 6% (Kuşvuran 2010) of agricultural areas. These pressures have a substantial impact on horticultural plants (Shao et al. 2008; Lopez et al. 2016).

The primary objective of this study was to utilize nanoparticles to enhance the efficiency of water usage. This study aimed to examine the impact of a foliar spray containing Fe₃O₄ NPs on strawberry plants subjected to water shortages and stressful circumstances. This study also assessed the impacts of these applications on the growth parameters of strawberry plants.

Materials and Methods

Plant Material and Treatments

The research was conducted in a high tunnel located at the Agricultural Application and Research Center of Bilecik Şeyh Edebali University in 2023 (40°11'00.6"N, 30°00'60.3"E). The 'Albion' strawberry variety utilized in this study is a day-neutral variety that is highly suitable for cool and temperate locations, and frigo strawberry plants were planted in March (Shaw and Larson 2006). The flowers that emerged after planting were plucked.

The study was conducted using a split-plot trial design, consisting of three replications and 10 plants in each replication. The main plots in the trial had three distinct irrigation water levels (I50, I75, and I100), whereas the subplots contained four different concentrations of Fe₃O₄ (0, 0.01, 0.1, and 1 mg/L). The plants were planted with a row spacing of 100 cm and a within-row spacing of 30 cm. Additionally, a gap of 2 m was maintained between each experimental plot.

The investigation involved preparing Fe₃O₄ NP (Sigma–Aldrich, CAS: 1317-61-9) at concentrations of 0, 0.01, 0.1, and 1 mg/L. These concentrations were then applied directly to the leaves of the main plant until saturation was reached (Wu et al. 2021). The first treatment commenced when the plants reached the stage of having five leaves, followed by a further series of eight applications at intervals of 2 weeks.

Irrigation was performed by monitoring soil moisture, and when the usable water holding capacity decreased by 30–40%, the drip irrigation method was applied, and three restrictions were applied (Table 1).

A PCE-Smm 1 soil moisture sensor (PCE Deutschland GmbH, Meschede, Deutschland) was used to measure soil moisture. The quantity of water to be administered in each irrigation treatment was determined using Eqs. 1 and 2:

$$dn = \frac{(P.C - E.M) \times \gamma t \times D}{100} \tag{1}$$

$$I = dn \times A \times k \tag{2}$$

Similarly, dn is the amount of irrigation water to be applied for each irrigation (mm), P.C is the pot capacity, E.M is the existing moisture in the soil before irrigation, γt is the potting soil bulk weight, D is the effective root depth (mm), I is the amount of water to be applied in each irrigation (m³), A is the pot area (m²), and k is the water restriction value to be applied.

Morphological and Physiological Measurements

The plants were harvested at the conclusion of the vegetative period in November. The number of stolons per plant was determined by dividing the total number of stolons by the number of plants. The number of seedlings was determined by collecting the number of plants that formed stolons. The number of leaves per plant was obtained by counting the leaves of the parent plants and dividing the total number of leaves by the number of plants. The leaf area was quantified in cm² by scanning mature leaves obtained from the parent plant and utilizing Adobe Photoshop software (Ipek et al. 2014). The relative leaf relative water content (LRWC) in the leaf, fresh weight (FW), turgor weight (TW) and dry weight (DW) of the leaves taken from the mother plants were determined according to Eq. 3 (Smart and Bingham 1974), and the relative water content (RWC) was measured according to Nie et al. (2016) (Eq. 4). Chlorophyll stability analysis (CSI) was performed by Sairam et al. (1997) according to Eq. 5.

$$LRWC(\%) = \frac{FW - DW}{TW - DW} \times 100 \tag{3}$$

$$RWC(\%) = \frac{FW - DW}{FW} \times 100 \tag{4}$$

Table 1 Experimental irrigation (I) and Fe₃O₄ nanoparticle (NP) applications

	Subject	Applications
<i>Irrigation (I)</i>	I50	Irrigation involves providing 50% of the water that is used up when 30–40% of the water holding capacity at the effective root depth is used up
	I75	Irrigation involves providing 75% of the water that is used up when 30–40% of the water holding capacity at the effective root depth is used up
	I100	Irrigation involves providing 100% of the water that is used up when 30–40% of the water holding capacity at the effective root depth is used up
<i>Fe₃O₄ Nanoparticle (NP)</i>	T1	Subject excluding Fe ₃ O ₄
	T2	0.01 mg L ⁻¹ Fe ₃ O ₄ NP applied subject
	T3	0.1 mg L ⁻¹ Fe ₃ O ₄ NP applied subject
	T4	1 mg L ⁻¹ Fe ₃ O ₄ NP applied subject

$$CSI(\%) = \frac{TCUS}{TCC} \times 100 \quad (5)$$

In the equations, *FW*: fresh weight, *DW*: dry weight, *TW*: total weight, *TCUS*: total chlorophyll content under stress condition, *TCC*: total chlorophyll content under control condition.

Statistical Analysis

All data were analyzed with Minitab 19 and graphed with ORIGIN 2022. The data were evaluated by one-way ANOVA and Duncan's multiple range tests, where differences were considered significant at $P < 0.05$.

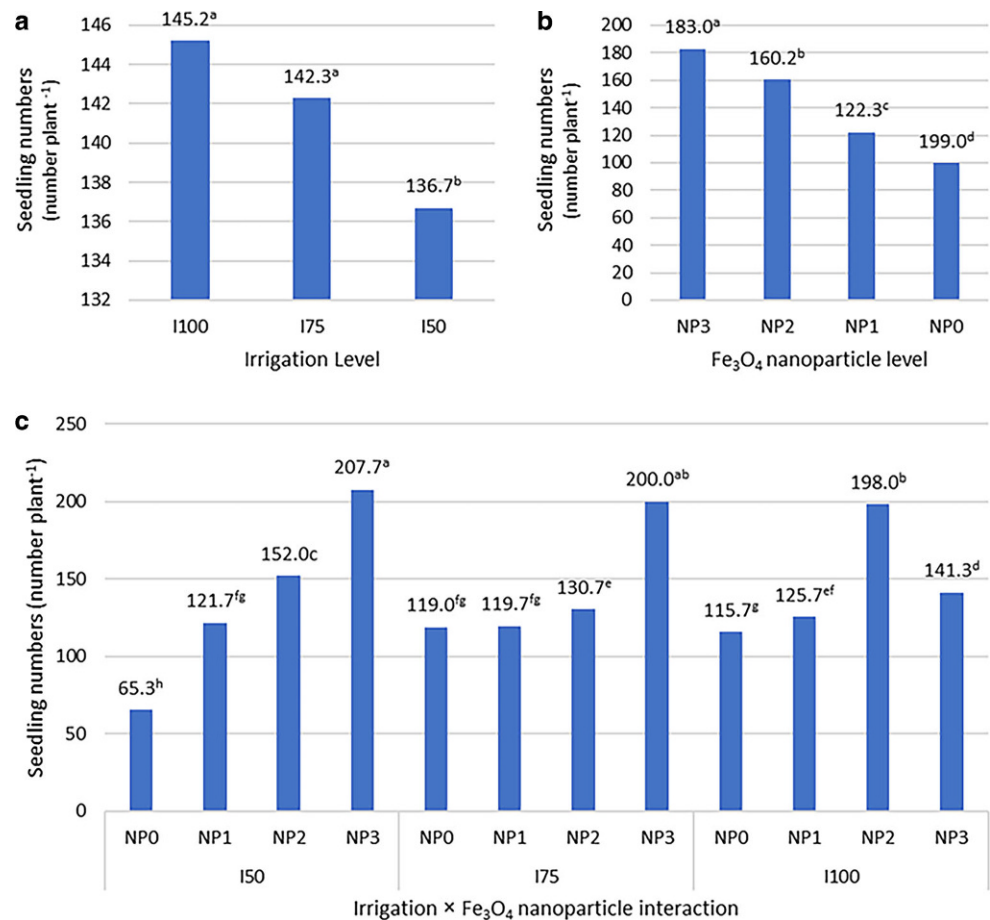
Results and Discussion

The study revealed that the irrigation water level, iron nanoparticles, and their interactions had a statistically significant impact on the quantity of strawberry seedlings. The results indicate that subjects I100 and NP3 had the highest average number of seedlings. Upon examining the interaction values between iron nanoparticles and irrigation

water levels, it was noted that the number of seedlings ranged from 65.3 to 207.7 per plant. The I50×NP3 interaction has the highest value, while the I50×NP0 interaction has the lowest value (Fig. 1). The results obtained show that there is a positive relationship between irrigation water and plant seedling number (Kaman et al. 2023b). The findings demonstrated a positive correlation between the utilization of iron nanoparticles and the augmentation of seedling quantity. The application of iron nanoparticles under stress conditions has been determined to enhance plant development without any negative impact.

The statistical significance of the effects of varying irrigation water levels and Fe₃O₄ nanoparticle treatments on leaf area and number of leaves was determined. Significant reductions in both the area and quantity of leaves in the plants were found as a result of the decreased application of irrigation water. The I100 irrigation treatment had the greatest average leaf number, with 21.27 per plant, while the I50 treatment had the lowest average leaf number, with 13.49 per plant. Upon analyzing the impact of iron nanoparticle application on leaf number, we observed that while the average leaf number of NP0 plants was high, the I100×NP2 interaction yielded the greatest number of leaves, with 22.83 per plant. Based on these findings, it can

Fig. 1 The effect of irrigation level (a), Fe₃O₄ nanoparticle (NP) doses (b), and irrigation level×Fe₃O₄ NP interaction (c) on seedling number



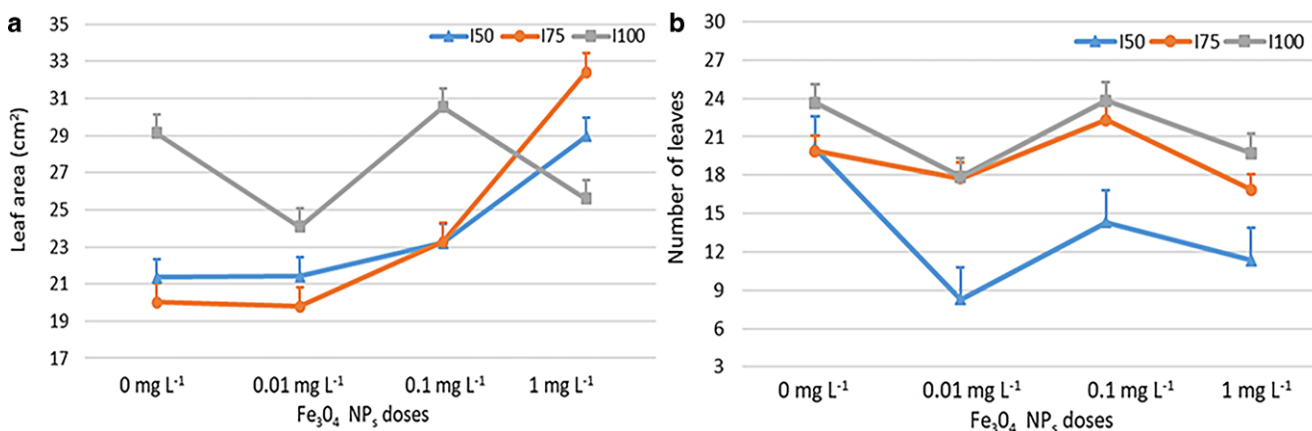


Fig. 2 The effect of irrigation level × Fe₃O₄ nanoparticle (NP) doses interaction on leaf area (cm) (a) and number of leaf (b)

be inferred that Fe₃O₄ nanoparticles have a significant impact on chlorophyll synthesis and photosynthesis in strawberry plants, resulting in an increase in leaf number. Similar results were obtained for the leaf area, as shown in Fig. 2a. The highest average leaf area was obtained from the I100 (27.33 cm²) irrigation application, and the lowest was obtained from the I50 (23.74 cm²) irrigation application. A decrease in leaf area under low water conditions leads to a reduction in the plant’s water requirements for photosynthesis and transpiration, hence protecting its water resources (Al-Khayri et al. 2023). The application of Fe₃O₄ nanoparticles to strawberry plants cultivated under limited watering had a statistically significant impact on leaf area, as shown in Fig. 2a. The highest leaf area, measuring 32.41 cm², was achieved from the I75 × NP₃ interaction. Previous research yielded comparable findings. Mozafari et al. (2018) showed that the use of iron nanoparticles had a beneficial impact on the leaf area of strawberry plants when they were exposed to drought stress. Additionally, the use of iron nanoparticles improved plant growth. Research has shown that iron particles enhance the ability of plants to withstand unfavorable environmental conditions, such as drought, and can mitigate the detrimental impacts of stress on plant growth, including a decrease in leaf area caused by drought stress (Şener et al. 2023). The utilization of nanoparticles leads to an increase in the leaf number. Sharf-Eldin et al. (2023) revealed that the presence of nanoparticles, specifically silicon dioxide (SiO₂), can enhance leaf production in plants during drought conditions. Razavi et al. (2008) highlighted that water stress has a substantial impact on both the area and number of leaves in plants.

The results obtained at the conclusion of the study demonstrated that the impacts of irrigation water level, iron nanoparticles, and their interactions on the number of primary plant leaves were statistically significant. As the irrigation water level increased, the leaf number likewise increased. The I100 irrigation treatment exhibited the great-

est average values, while the I50 irrigation treatment had the lowest. Upon analyzing the impact of iron nanoparticle application on leaf number, we observed that while the NP₀ group yielded high results, the I100 × NP₂ interaction resulted in the greatest number of leaves, with an average of 22.83 per plant. Among the I75-treated plants, those subjected to NP₂ had the greatest number of leaves (Fig. 2b). Iron nanoparticles are believed to have a significant impact on chlorophyll production and photosynthesis in strawberry plants, leading to an increase in the leaf count. In their investigation, Kumar et al. (2017) reported a positive correlation between the application of iron oxide and the number of leaves on plants.

Statistically significant results were obtained at the conclusion of the study on the impact of irrigation water level, iron nanoparticles, and their interactions on the number of runners on the mother plant. An examination of the interactions between iron nanoparticles and irrigation water levels revealed that the number of runners on the mother plant ranged from 9.10 to 5.83 units per plant. The I50 × NP₃ interaction had the highest value, whereas the I100 × NP₃ interaction had the lowest value. The results demonstrated

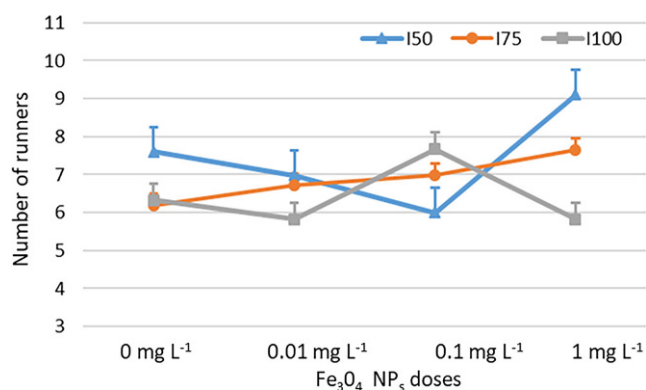
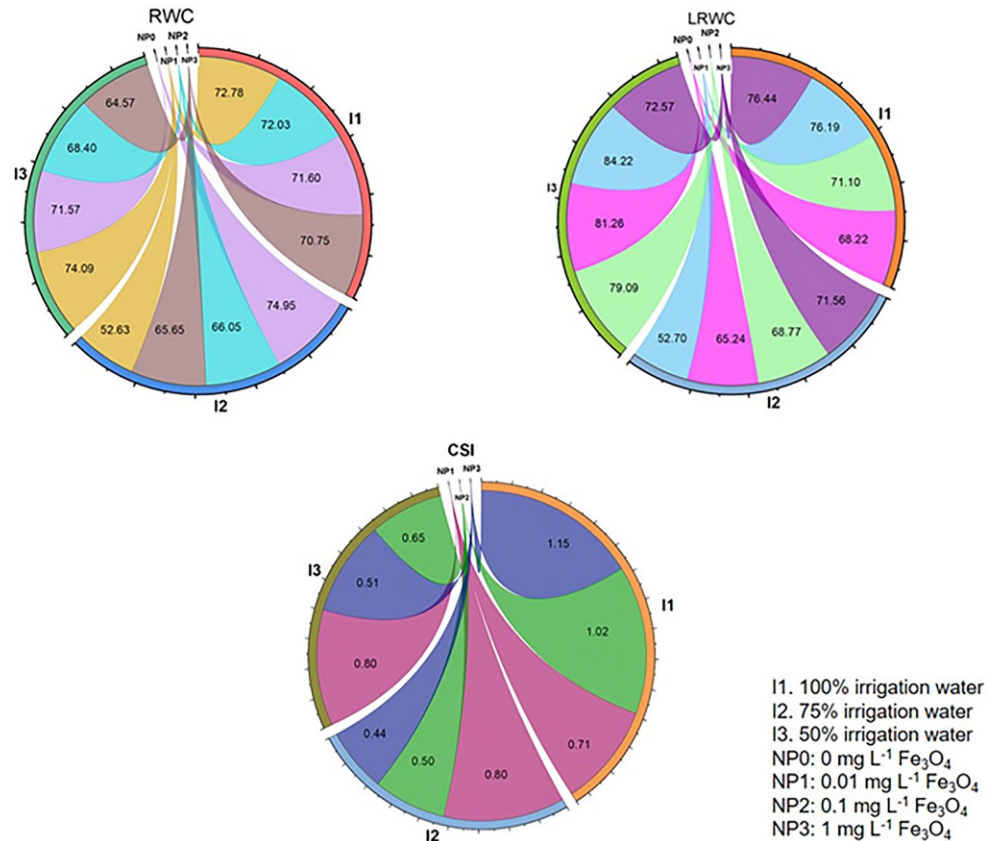


Fig. 3 The effect of irrigation level × Fe₃O₄ nanoparticle (NP) dose interaction on number of runners

Fig. 4 Effect of iron nanoparticles (*NP*) (Fe_3O_4 NPs) on relative water content (*RWC*), leaf relative water content (*LRWC*), and chlorophyll stability index (*CSI*) of strawberries grown under different water deficit conditions (irrigation levels)



that the utilization of iron nanoparticles led to an increase in the number of runners on the parent plant under stressful circumstances but had an adverse impact at elevated water levels (Fig. 3).

The biomarkers frequently employed to evaluate the water status or amount of dehydration in plants are RWC and LRWC. The application of Fe_3O_4 nanofertilizers at various doses to strawberry plants under limited irrigation conditions had a statistically significant impact on both the RWC and LRWC (Fig. 4). Furthermore, it has been noted that applying nanofertilizers to the leaves of strawberry plants can mitigate the impact of water scarcity. The RWC was greatest when 100% full irrigation (74.09%) and 50% limited irrigation (72.78%) conditions were used with a nanofertilizer treatment of 0.01 mg L⁻¹. The elevated RWC in comparison to that of the control suggests that the application of nanofertilizer to the leaves enhances water accessibility. In another study, Deepa et al. (2015) reported that the use of iron and copper nanoparticles resulted in a greater proportion of water in the treated group than in the control group. Taran et al. (2017) reported that the application of copper and zinc nanofertilizers resulted in an 8–10% increase in the number of leaves of plants. The increase in relative water content is accomplished through the external application of nanoparticles, which are then transferred from the site of application to heterotrophic cells via phloem ves-

sels and plasmodesmata (40 nm in diameter) (Knoblauch and Oparka 2012). Nanofertilizers have been identified as a viable and dependable means of transferring nutrients and water (Deepa et al. 2015). The chlorophyll stability index (CSI) is a significant instrument used to assess plants' reactions to drought and their ability to tolerate stress. It does so by tracking variations in the chlorophyll levels of plants. The maximum CSI was achieved with the application of 1 mg L⁻¹ (1.15) and 0.1 mg L⁻¹ (1.02) Fe_3O_4 nanofertilizer under 50% limited watering conditions. A high CSI implies that plants have greater resistance to drought stress and is correlated with elevated chlorophyll levels. The elevated CSI value, which was below 50% under limited watering conditions, indicates that the administration of iron nanofertilizer enhances the efficiency of chlorophyll utilization and aids plants in mitigating stress during stressful conditions. Furthermore, a high CSI enhances the availability of chlorophyll, resulting in an elevated rate of photosynthesis, enhanced dry matter production, and greater productivity (Mohammadkhani and Heidari 2008).

Conclusion

This study investigated the notable impacts of limited water and the use of iron nanoparticles (NP) on the growth

and development of strawberry seedlings. The quantity of seedlings increased in direct proportion to the increase in both the irrigation water level and the dosage of iron nanoparticles applied. The I100 and NP3 treatments yielded the highest average seedling numbers. The I50×NP3 interaction yielded the greatest effect on the relationship between irrigation water level and iron nanoparticle interactions. Iron nanoparticle use under water stress conditions has been demonstrated to mitigate the adverse impact on seedling quantity. A similar situation was noted for the leaf area and number of leaves. As the irrigation water level decreased, both the leaf area and the number of leaves decreased. However, under water stress conditions, the application of iron nanoparticles resulted in greater values. The subjects I50 and NP3 had the largest average number of runners per plant. Under water stress conditions, the number of runners per plant increased in comparison to the other parameters. This is thought to be so that the plant can expand its shoots under stress conditions and get water from a larger area. When we look at the irrigation water level and iron nanoparticle interaction, the highest value was determined from the I50×NP3 interaction.

The relative water content (RWC) and leaf relative water content (LRWC) are often employed as measures to assess the water status and degree of dehydration of plants. Gaining a comprehensive grasp of how plants react to water stress and regulate their water levels is of utmost importance. Low RWC and LRWC values indicate that plants are exposed to water stress and are losing water content. The results of our study showed that iron nanoparticle applications applied under water stress conditions achieved higher RWC and LRWC values than those of control plants.

The chlorophyll stability index is a test method used to quantify the resilience of chlorophyll in plants to different stressors. This measure is utilized to assess the resilience of plants to environmental challenges. The chlorophyll stability index often shows a positive correlation with higher values, indicating that plants exhibit more resistance to stress. That is, the higher the chlorophyll stability index is, the better the plant is considered to respond and be resistant to environmental stresses. In terms of the interaction between irrigation water level and iron nanoparticles, the greatest difference was detected for the I50×NP3 interaction. These results show that iron nanoparticle application increases plant resistance to water stress conditions.

Upon evaluating the data, we discovered that the use of iron nanoparticles enhanced the growth and development of the seedlings. Additionally, despite a decrease in the amount of water applied, there were significant improvements in the monitored parameters with the use of iron nanoparticles. These findings are also significant for the sustainable utilization of water resources.

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Conflict of interest S. Öztürk Erdem, M. Karakoyun Mutluay, M. Karaer and H.T. Gültaş declare that they have no competing interests.

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