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RESEARCH PAPER

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MITIGATION OF SALINITY STRESS ON PISTACHIO (*Pistacia vera* L.) SEEDLINGS THROUGH THE APPLICATION OF CARBON NITRIDE MODIFIED WITH IRON (FE/C_3N_4) NANOSTRUCTURES

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ABSTRACT

Salinity has a global impact on plants by inducing biochemical and metabolic changes that lead to oxidative stress, impairing growth, yield, and productivity. The pistachio tree (*Pistacia vera* L.) is a salt-tolerant species. This study aims to investigate the effectiveness of carbon nitride nanostructures modified with iron (Fe/C_3N_4) on the Akbari pistachio variety under salinity stress levels (0, 50, 100, and 150 mM) and foliar applying (distilled water as a control, Fe_2O_3 (0.2 g L^{-1}), C_3N_4 (0.2 g L^{-1}), and carbon nitride modified with iron or Fe/C_3N_4 (0.2 g L^{-1}). The findings showed that salinity decreased relative water content (RWC), SPAD index, membrane stability index (MSI), maximum fluorescence (Fm), and variable fluorescence (Fv), and increased hydrogen peroxide (H_2O_2). However, foliar application with Fe_2O_3, or Fe/C_3N_4, improved all traits. Nevertheless, there was no significant interaction between the applied mitigating treatments and salinity levels on RWC, MSI, SPAD index, Fm, Fv, and H_2O_2. Salinity stress increased malondialdehyde (MDA), phenol, and flavonoid levels, and reduced leaf number, height, photosynthetic pigments, vitamin C, and total protein. The application of foliar treatments, especially Fe/C_3N_4, improved the influence of salinity stress. Additionally, the activity of antioxidant enzymes increased under salt stress and foliar application. Fe/C_3N_4-treated seedlings consistently exhibited higher growth and photosynthetic traits and lower oxidative damage than untreated controls across salinity levels, indicating a stable physiological benefit rather than a salinity-specific effect.

Keywords: antioxidant enzymes, carbon nanomaterials, environmental stresses, growth index

INTRODUCTION

Pistacia vera L. is a significant crop in arid and semi-arid regions. This species is also tolerant to soil salinity, making it a suitable alternative for farming in orchards with salinity-influenced soils [Hajiboland et al. 2014]. Despite this, it is imperative to mention that the resistance of pistachio trees to salinity stress varies significantly based on the specific genotype and rootstock employed [Rahneshan et al. 2018]. The pistachio is one of the most valuable tree nuts worldwide and is deeply integrated into the economic and nutritional systems of semi-arid regions. Global pistachio production surpassed 1.2 million tons annually in the 2020s, with Iran, the United States, Turkey, and China leading, collectively accounting for over 90% of the world's exports. In Iran, pistachio orchards cover approximately 273881 ha, contributing more than 25% of total global pistachio yield and providing livelihoods to hundreds of thousands of smallholder farmers [FAO 2023]. Globally, the pistachio industry is a model for sustainable perennial crop management in salty landscapes [Akhavan and Gonçalves 2021].

Plant cultivation is subject to several stress environments, with salinity reflected as the most restricting factor. According to Kamran et al. [2019], salinity critically impacts many plant processes, especially yield. Industrial and agricultural activities, along with modern living, have increased soil and water salinity, leading to a decline in their quality worldwide [Ahmadi and Souri 2020]. Over the past decades, the rapid increase in the world's population has put significant pressure on soil fertility and agricultural productivity. High application rates of chemical fertilizers to achieve high yields have considerably promoted soil salinity [Ahmadi and Souri 2018].

Soil salinity is one of the principal abiotic stresses that reduces crop yields worldwide. It impairs crop growth, yield, and quality, particularly in arid and semi-arid areas. Recent global assessments indicate that salinity affects more than 20% of irrigated land and is spreading further due to climate change, unsustainable irrigation, industrial waste, and intensive fertilizer application [Demo et al. 2025, FAO 2023]. Increasing human activities, such as industrial effluents, over-irrigation, and excessive fertilizer use, have raised saline levels in arable soils, reducing their fertility and crop yields [Demo et al. 2025]. Munns and Tester [2008] pointed out that a plant's salinity tolerance relies on keeping low cytosolic Na^+ levels through methods such as selective ion exclusion, vacuolar sequestration, and the production of compatible osmolytes like proline and glycine betaine to maintain osmotic balance. Salinity also causes osmotic stress and ionic toxicity, mostly by causing too much Na^+ and Cl^- ions to build up, which messes up water relations, nutrient uptake, metabolic processes, and photosynthesis [Shafi et al. 2025]. Plants have developed complex physiological and molecular mechanisms to deal with salinity stress [Zhou et al. 2024]. These include selective ion exclusion, vacuolar sequestration of Na^+, accumulation of osmolytes (e.g., proline and glycine betaine), and activation of antioxidant defense systems [Mendis et al. 2025]. However, these natural processes do not always function effectively when salinity is high or persists for extended periods. This can cause oxidative damage, chlorophyll breakdown, and CO_2 assimilation to slow down, and less biomass to build up [Karpinska and Foyer 2024]. As a result, developing long-term, practical strategies to improve plant salt tolerance has become a top priority for researchers worldwide.

By enhancing nutrient balance and physiological resilience, foliar and soil nutrient application has long been acknowledged as a successful strategy to reduce damage caused by salinity [Kaya and Ashraf 2024]. When these elements are applied to plant leaves, they can enter via various pathways, including stomata, cuticular cracks, epidermal cells, and leaf hairs, as reported by Abdoli et al. [2020]. One significant problem in saline and alkaline soils is iron deficiency due to their high pH [Askary et al. 2017]. The effectiveness of Fe-containing solutions is primarily determined by evaluating the rate at which Fe is absorbed by plant tissues, the speed at which it is translocated from treated organs to other tissues, and the extent to which it enhances chlorophyll synthesis. Accurate assessment of these factors is crucial to ensure that Fe-supplemented plants are healthy and exhibit optimal growth [Fernández et al. 2008]. Nanotechnology has emerged as a promising approach in sustainable agriculture, offering innovative methods for precise nutrient delivery, enhanced bioavailability, and reduced environmental impact [An et al. 2022]. Recently, the use of nanotechnology in horticulture has been widely investigated through nanoscale manipulation of materials to improve fertilizer delivery, nutrient uptake, and crop fortification [Ma et al. 2020]. According to recent research, Fe-based nanomaterials can improve nutrient signaling and redox active interactions to control ionic homeostasis, increase chlorophyll content, and reduce oxidative stress under salinity [Ghosh et al. 2025, Singh et al. 2024]. Iron oxide nanoparticles (Fe_2O_3-NPs) are known to enhance chlorophyll content, antioxidant activity, and growth under stressful conditions, thereby supporting sustainable farming, as highlighted by Khanizadeh et al. [2024]. The use of Fe_2O_3 nanoparticles in nanofertilizers has the potential to advance the horticultural industry. By enhancing nutrient absorption and reducing soil toxicity,

these nanofertilizers can significantly promote sustainable agricultural practices and ensure food security [Verma et al. 2022]. Fe_2O_3 nanoparticles serve as both micronutrient sources and redox modulators, thereby affecting photosynthetic efficiency in saline environments, antioxidant enzyme activities, and reactive oxygen species (ROS) scavenging [Feng et al. 2022, Zhang et al. 2025]. Polymeric carbon nitride (CN), is composed of the earth-abundant elements carbon (C) and nitrogen (N) [Chen and Song 2017]. Graphitic carbon nitride nanosheets (C_3N_4) have attracted significant attention recently due to their distinctive structure and remarkable catalytic properties. C_3N_4, which consists solely of C and N, can be produced simply via low-cost N-N-supplemented composites such as melamine and urea via heat condensation [Inagaki et al. 2019]. The potential of C_3N_4-based nanostructures for biological and agro-environmental systems has been highlighted by recent international research, and these nanostructures have been extensively studied for photocatalysis, environmental remediation, and nanozyme applications [Zhuang et al. 2024].

Recent advances in agricultural nanotechnology indicate that nano-enhanced micronutrient formulations can improve nutrient-use efficiency and enhance plant stresnanos tolerance [Al-Dossary et al. 2025, Lalitha et al. 2025]. The Organization for Economic Co-operation and Development (OECD), the European Food Safety Authority (EFSA), and FAO recommend testing these nanomaterials based on observable biological indicators such as growth rate, photosynthetic traits, and oxidative stress markers to verify their safety and efficacy [EFSA et al. 2021, FAO 2023, OECD 2020]. New research shows that iron-based nanoparticles support plant growth, increase chlorophyll levels, and boost antioxidant enzyme activity under salinity stress, with reduced MDA and H_2O_2 levels and better membrane stability [Rico et al. 2015, Verma et al. 2022].

Although the positive impacts of iron-based nanoparticles like Fe_2O_3 and Fe_3O_4 on salinity tolerance, such as enhanced leaf water content, chlorophyll levels, membrane integrity, and antioxidant enzymes, have been well documented since the mid-2010s, research on iron-loaded graphitic carbon nitride (Fe/C_3N_4) nanocomposites in plants is still scarce. Specifically, there is a lack of *in vivo* physiological and biochemical data on the effects of Fe/C_3N_4 in woody perennial crops, such as pistachio, under salinity stress. This study explores the potential of combining iron-based nanoparticles and graphitic carbon nitride into a single nanocomposite (Fe/C_3N_4) to enhance protection against salinity stress in plants. Given their distinct yet potentially complementary roles in plant stress responses, we examined whether Fe/C_3N_4 confers greater benefits than Fe_2O_3 or C_3N_4 alone. We hypothesized that foliar application of Fe/C_3N_4 would more effectively reduce salinity-induced damage in pistachio seedlings by improving growth, leaf water content, membrane integrity, photosynthetic pigment levels, and efficiency. Additionally, we expected it to reduce oxidative stress by more strongly activating antioxidant enzymes (SOD, APX, and GPX) and by decreasing levels of MDA and H_2O_2. To evaluate this, pistachio seedlings were exposed to varying salinity levels and treated with Fe_2O_3, C_3N_4, or Fe/C_3N_4, and their physiological and biochemical responses were carefully compared.

MATERIALS AND METHODS

Plant material and growth conditions

The trial was conducted in the Faculty of Agriculture research greenhouse at the University of Maragheh in Iran. The study was a factorial experiment in a completely randomized design (CRD) with three replications. It included four foliar treatments (distilled water as control, Fe_2O_3, C_3N_4, and Fe/C_3N_4 at 0.2 g L^{-1}) combined with four levels of salinity (0, 50, 100, and 150 mM NaCl). One-year pistachio seedlings (Akbari cultivar) with the same height and growth were transplanted in 7-liter pots with a combination of soil and peat moss (1:1) as the culture medium. The soil was a loam-sandy clay, air-dried, sieved through a 2 mm mesh, and free of visible plant residues. According to routine laboratory analyses conducted before the experiment, the soil had a pH of 7.4, an electrical conductivity (EC) of 0.62 dS m^{-1}, potassium of 438.12 mg kg^{-1}, phosphorus of 8.67 mg kg^{-1}, nitrogen of 0.174%, and approximately 1.4% organic matter. Each pot contained one seedling. Following a 2-week establishment period under non-saline conditions, salinity treatments were applied using NaCl solutions at the designated concentrations. To avoid osmotic shock, NaCl concentration was increased gradually over three consecutive irrigations until the target salinity level was reached. Foliar treatments were applied three times to the leaves at three-day intervals, beginning two weeks after the initiation of stress. The materials (C_3N_4 and Fe/C_3N_4) used for this work were identically prepared according to the protocols mentioned by Heidarpour et al. [2020]. A concentration of 0.2 g L^{-1} for Fe_2O_3, C_3N_4, and Fe/C_3N_4 foliar applications was chosen based on prior research showing it provides physiological benefits without causing phytotoxicity in crop plants exposed to abiotic stress. Previous studies

have demonstrated that concentrations between 0.1 and 0.5 g L^{-1} can boost antioxidant activity, photosynthesis, and stress resilience, while avoiding growth suppression or oxidative damage [Kokina et al. 2020, Ma et al. 2020]. Additionally, initial observations during the study's establishment phase revealed no visible phytotoxic effects, such as leaf chlorosis or necrosis, at this concentration. Hence, 0.2 g L^{-1} was deemed both effective and safe for pistachio seedlings.

At the end of the experiment, morphological attributes, such as plant height and leaf number, were quantified. Fully developed leaves were sampled to study morphological, physiological, and biochemical traits, as well as total antioxidant enzyme activity.

Relative water content (RWC)

To determine the RWC of leaves, we followed the technique by Kumar et al. [2020]. Fresh leaf samples were collected, and their initial weight (FW) was noted immediately. The leaves were then soaked in distilled water at room temperature for 4 hours to reach turgid weight (TW). Subsequently, the samples were oven-dried at 65 °C for 24 h until their weight stabilized, and the dry weight (DW) was recorded. The relative water content was determined using the following formula:

$$RWC\% = (FW - DW) / (TW - DW) \times 100$$

Membrane stability index (MSI)

Membrane stability index (MSI) was measured according to the method described by Sairam et al. [1997]. Fresh leaf samples (0.1 g) were placed in 10 mL of double-distilled water and incubated at 40 °C for 30 minutes. The electrical conductivity (C_1) of the solution was then measured. Next, the samples were boiled at 100 °C for 10 minutes, cooled to room temperature, and the final conductivity (C_2) was recorded. MSI was calculated using the formula: 0.1 g leaf samples from each pot were used to measure electrical conductivity at 40 °C (EC_1) and 100 °C (EC_2).

$$MSI\% = [1 - (C_1 / C_2)] \times 100$$

Chlorophyll index (SPAD) determination

The chlorophyll index or greenness index was quantified in young leaves via a portable Chlorophyll Meter and expressed as SPAD (Instruments SPAD-502, Japan).

Photosynthesis pigments

Chlorophylls and carotenoids were measured using a spectrophotometer and Arnon [1949]. To extract pigments, 0.5 g of fresh leaf tissue was crushed with liquid nitrogen and mixed with 5 mL of 80% acetone. The mixture was then analyzed for absorbance at 664 nm, 647 nm, and 470 nm wavelengths through a spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as mg g^{-1} FW.

Chlorophyll fluorescence parameters

The pulse amplitude modulation fluorometer (PAM-2500, Walz, Effeltrich, Germany) was exploited to record chlorophyll fluorescence parameters on fully expanded young leaves, following the technique depicted in detail by Chen et al. [2011]. The parameters measured included the minimum chlorophyll fluorescence (F0), the maximal fluorescence (Fm), the variable fluorescence (Fv), and the maximal quantum yield of PS II (Fv/Fm).

Vitamin C

To determine the amount of vitamin C, 1 g of pistachio leaves was blended with 3 mL of 1% metaphosphoric acid and centrifuged at 4 °C and 6000 rpm for 15 minutes. The resulting extract was mixed with 180 µL of 2,6-dichloroindophenol sodium hydrate, and the optical density (OD) was measured at 520 nm using a spectrophotometer. Finally, the concentration of vitamin C was measured based on a pure ascorbic acid standard and expressed as mg per 100 g^{-1} FW [Bor et al. 2006].

Total phenol content (TPC)

To measure TPC, 0.5 g of fresh leaves was extracted with acidic methanol and centrifuged at 10000 rpm for 15 min. The reaction mixture comprised water, 10% Folin-Ciocâlteu reagent, the extract, and 7.5% sodium carbonate. The OD was noted at 765 nm using a UV spectrophotometer. TPC was estimated as mg gallic acid g^{-1} FW [Singleton and Rossi 1965].

Total flavonoids content (TFC)

To determine the total flavonoid content (TFC), 1 g of the fresh pistachio sample was mixed with 80% methanol. The resultant mixture was centrifuged at 16000 rpm for 15 min to attain the supernatant. A solution was prepared by mixing 95% methanol, 10% aluminum chloride (Merck, DarmStasdt, Germany), 1 M potassium acetate, and distilled water with the supernatant. The resulting OD was quantified at 415 nm, and the TFC was calculated as mg quercetin g^{-1} FW [Chang et al. 2002].

Malondialdehyde (MDA)

To analyze MDA content in the leaf sample, 0.5 g of fresh pistachio leaves was digested in 0.1% trichloroacetic acid. After centrifugation, 0.1% thiobarbituric acid, containing 20% trichloroacetic acid, was added. The mixture was heated in a bain-marie, then placed on ice to stop the reaction. After centrifugation, the OD sample was read at 532 and 600 nm, as expressed by [Heath and Packer 1968] MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol MDA g^{-1} FW.

Hydrogen peroxide (H_2O_2)

To perform an H_2O_2 analysis on a plant sample, a mixture was prepared by combining 10 mM KH_2PO_4 buffer (pH 6.8) and 1 M KI. The plant sample was then extracted with 0.1% w/v trichloroacetic acid. The extracted plant sample was added to the prepared combination, and the OD was measured at 390 nm using a spectrophotometer. H_2O_2 concentration was calculated using a standard calibration curve and expressed as µmol H_2O_2 g^{-1} FW [Sinha et al. 2005].

Total soluble protein content

Fresh leaf tissue (0.5 g) was homogenized in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 12,000 × g for 15 minutes at 4 °C. A 0.1 mL aliquot of the supernatant was combined with 5 mL of Bradford reagent, and absorbance was read at 595 nm with a spectrophotometer. Finally, the total soluble protein content was determined using a BSA standard curve and expressed as mg g^{-1} FW [Bradford 1976].

Enzymatic antioxidant activity

Ascorbate peroxidase activity (APX). The activity of the APX was assessed via creating a reaction combination that included 250 mM KH_2PO_4 buffer with a pH of 6.8, 1 mM H_2O_2, 0.5 mM ascorbic acid, and 0.1 mM EDTA. The reaction was initiated by adding hydrogen peroxide to the mixture. The peroxidation of ascorbic acid decreased light absorption at 290 nm, which was measured over two minutes using a spectrophotometer. At the end APX activity was calculated as µmol min^{-1} mg^{-1} FW [Yoshimura et al. 2000].

Guaiacol peroxidase activity (GPX). To determine GPX activity, 0.5 g of leaf tissue was pulverized using KH_2PO_4 buffer (100 mM, pH = 6.8), EDTA (4 mM), and 1% PVP. The mixture was subsequently centrifuged at 10,000 rpm for 20 minutes. The GPX activity was determined by measuring the amount of tetraguaiacol at 470 nm for 1 minute. Finally GPX activity was defined as µmol min^{-1} mg^{-1} FW [Yoshimura et al. 2000].

Superoxide dismutase activity (SOD). To determine SOD activity, we used the Nakano and Asada [1981] method. The reaction mixture included 1.5 mM sodium carbonate, 0.2 mM methionine, 3 mM EDTA, 0.1 M sodium phosphate buffer, and 2.25 mM NBT. Distilled water was added, and the mixture was incubated for 15 min at 24 °C under light, after which the absorbance was measured at 560 nm. SOD activity was calculated as µmol min^{-1} mg^{-1} FW.

STATISTICAL ANALYSIS

All data were analyzed using analysis of variance (ANOVA) with MSTAT-C (version 2.1; Michigan State University, East Lansing, MI, USA). Before conducting ANOVA, residuals were checked for normal distribution via the Shapiro–Wilk test and for equal variances using Levene's test. Mean comparisons were made using the least significant difference (LSD) test at $P \leq 0.05$. All results are presented as means ± standard deviation (SD) from three biological replicates.

RESULTS

Morphological parameters

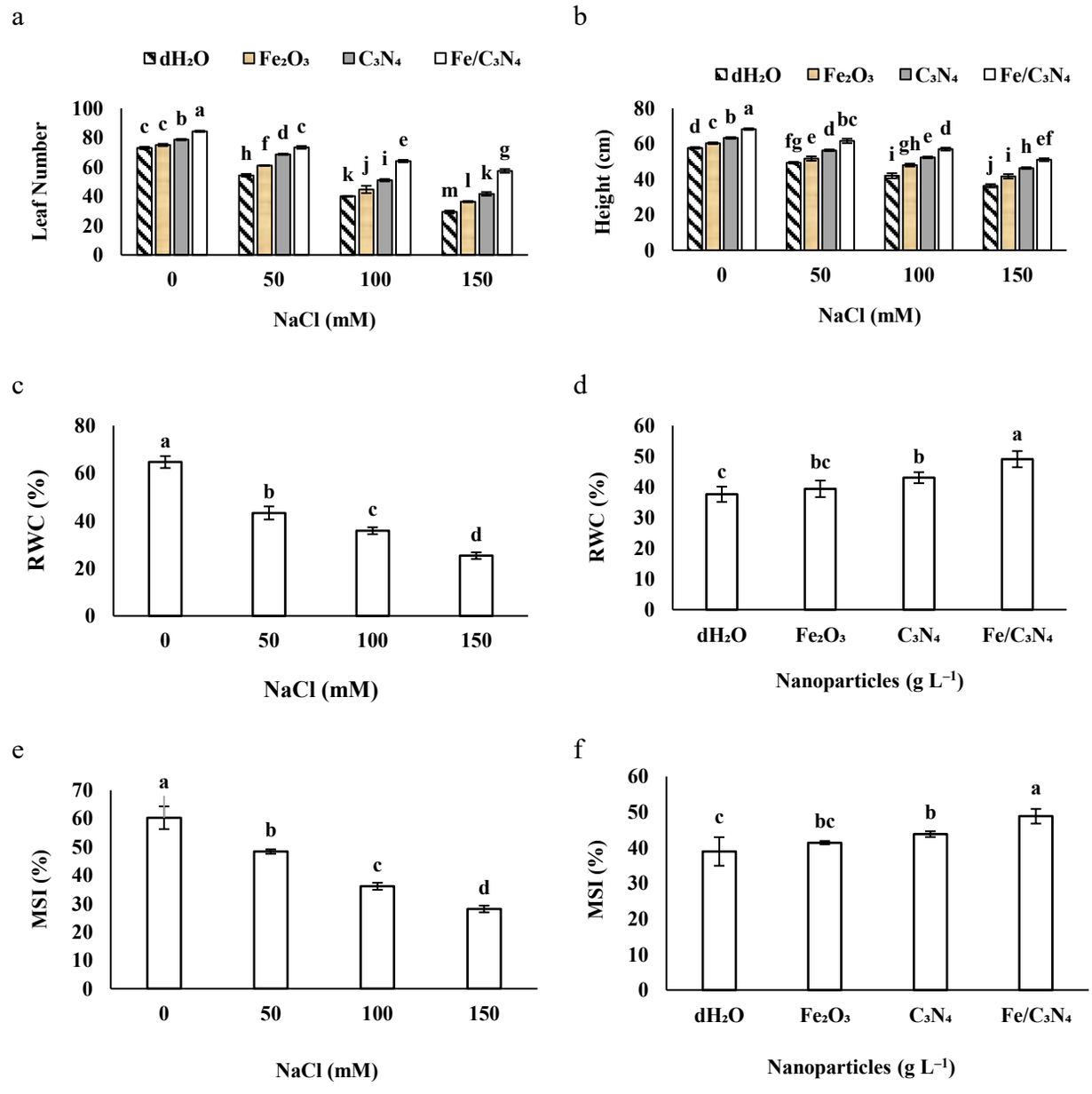
Salinity significantly ($P \leq 0.05$) reduced the number of leaves. Under both stress and non-stress conditions, all foliar treatments increased leaf number compared to the control pistachio. The uppermost leaf number was obtained under no stress and treated with Fe/C_3N_4, and the lowest was obtained in the control pistachio seedlings and under 150 mM stress. Also, the application of salt stress resulted in a 59% diminution in the number of leaves

compared to the control (Figure 1a). Salt stress reduced height, and all foliar treatments mitigated the effects of stress and increased height compared with the no-foliar-treatment condition. Based on these results, the tallest seedlings were observed in the Fe/C_3N_4 treatment and in the no-saline application condition, whereas the lowest were in control pistachio plants subjected to 150 mM salinity stress (Figure 1b).

Relative water content of leaf (RWC)

The salinity stress caused a significant lessening ($P \leq 0.05$) in RWC, and the most significant decrease was achieved with 60% at the highest salinity stress treatment compared to the control pistachio plants (Figure 1c). Foliar application of the treatments showed a 24% increase in RWC compared to the control pistachio seedlings, with no significant difference between the Fe_2O_3 and C_3N_4 treatments (Figure 1d).

Figure 1. The effects of soil salinity and iron, carbon nitride, and carbon nitride modified with Fe_2O_3 treatment (mg L^{-1}) on the number of leaves (a), height (b), RWC (c and d), and MSI (e and f) of pistachio seedlings. If the letters used to represent the data points differ, it indicates significant differences among the data points at the 5% level of significance according to the LSD test. The error bars indicate standard deviation (SD)



Membrane stability index (MSI)

Salinity stress led to a meaningful diminution in MSI, with the most significant decrease observed at 53% at 150 mM stress compared to the pistachio seedlings grown in normal situations (Figure 1e). The foliar application treatments increased MSI, with the highest increase of 20% observed in Fe/C_3N_4 compared with pistachio seedlings receiving any treatment (Figure 1f).

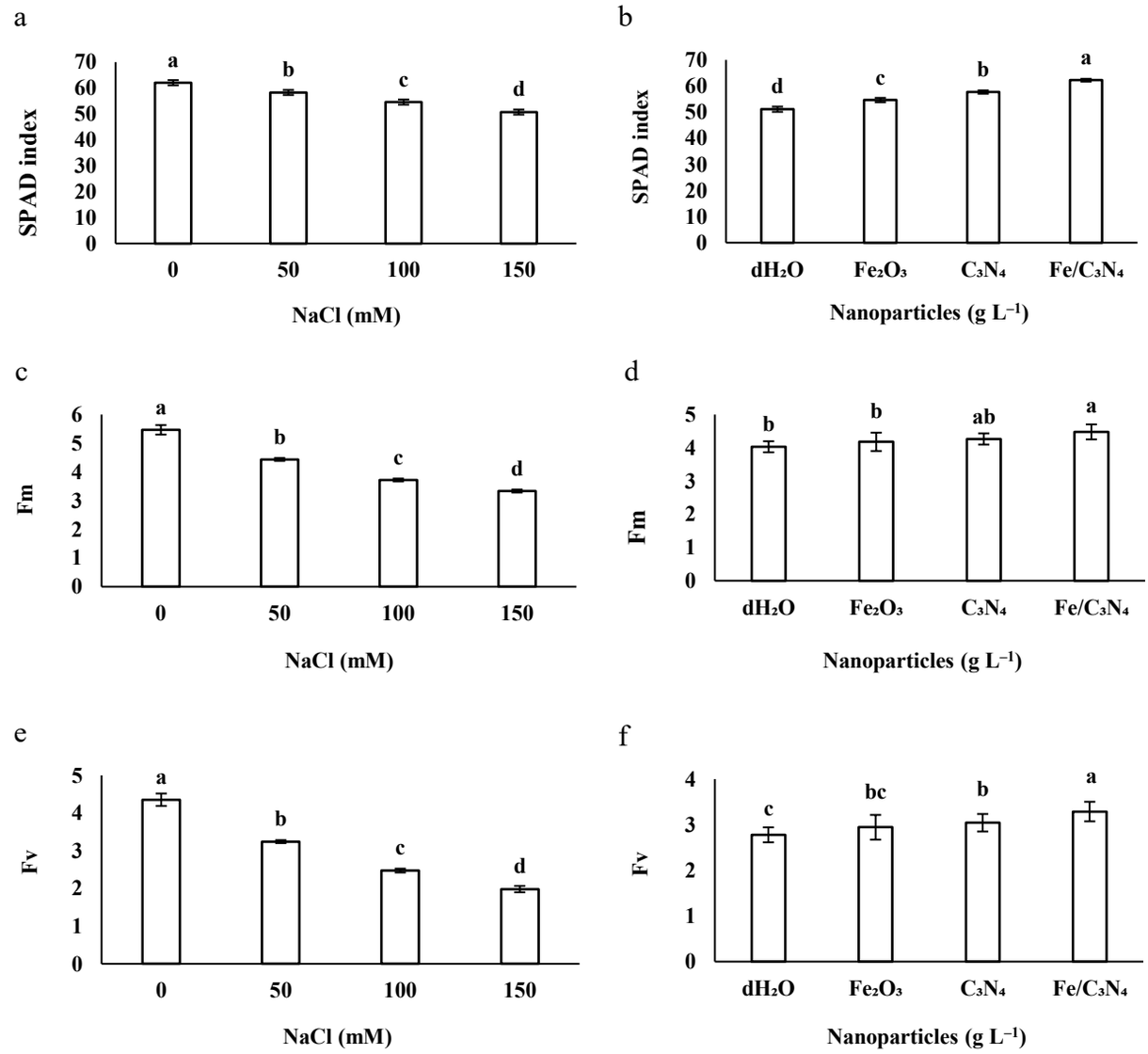
SPAD index

The chlorophyll index declined meaningfully ($P \leq 0.05$) through enhancing salinity stress. There was an 18% reduction at the highest stress as compared to the pistachio control (Figure 2a). Also, the results exhibited that foliar spray treatments significantly increased the chlorophyll index and led to a 21% increase compared to the pistachio seedlings control (Figure 2b).

Photosynthetic pigments

The concentrations of Chl a, b, total chlorophyll, and carotenoids lessened with the increase in salinity level. A significant increment ($P \leq 0.05$) in Chl a, b, total chlorophyll (Tchl), and carotenoid content was observed follow-

Figure 2. The effects of soil salinity and iron, carbon nitride, and carbon nitride modified with iron treatment on SPAD index (a and b), Fm (c and d), and Fv (e and f) of pistachio seedlings. If the letters used to represent the data points differ, it indicates significant differences among the data points at $P \leq 0.05$, as determined by the LSD test. The error bars indicate standard deviation (SD)



ing Fe_2O_3, C_3N_4, and Fe/C_3N_4 treatments in stressed and non-stressed conditions. The highest concentrations of Chl a, b, Tchl, and carotenoids were obtained in the foliar treatment of Fe/C_3N_4 without applying salinity stress. The lowest concentrations were observed at 150 mM NaCl without foliar treatments. Salt stress led to a 55% decrease in Chl a and b, a 68% decrease in Tchl, and a 50% decrease in carotenoid content (Table 1).

Fm. The salinity stress significantly decreased Fm, and the maximum decrease was achieved at 39% at the highest stress level (Figure 2c). The outcomes also exhibited that the utilization of foliar treatments increased Fm, but the foliar treatments of C_3N_4 had no significant difference with Fe_2O_3, with the control, as well as C_3N_4 and Fe/C_3N_4 with Fe_2O_3 (Figure 2d).

Table 1. Effect of Fe_2O_3, Carbon nitride, and Carbon nitride modified with iron foliar treatments on photosynthetic pigments, F0 and Fv/Fm of pistachio seedlings under salinity stress (mean ±SD)

NaCl (mM)	Treatment (g L^{-1})	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	F0	Fv/Fm
0	ddH_2O	41.43 ±0.088 c	24.55 ±1.282 de	65.98 ±1.284 c	1.502 ±0.013 bc	1.127 ±0.001 fg	0.784 ±0.007 b
	Fe_2O_3	42.10 ±0.032 bc	25.69 ±0.463 cd	67.7 ±0.439 c	1.531 ±0.006 ab	1.127 ±0.006 fg	0.789 ±0.010 b
	C_3N_4	42.90 ±0.040 ab	27.44 ±0.446 b	70.34 ±0.453 b	1.548 ±0.006 ab	1.129 ±0.047 f	0.795 ±0.012 b
	Fe/C_3N_4	43.51 ±0.138 a	29.24 ±0.042 a	72.76 ±0.176 a	1.570 ±0.019 a	1.076 ±0.026 g	0.813 ±0.007 a
50	ddH_2O	33.98 ±0.456 f	21.65 ±0.679 f	55.63 ±0.707 e	1.362 ±0.012 e	1.217 ±0.011 cd	0.710 ±0.001 e
	Fe_2O_3	36.05 ±0.478 e	23.70 ±0.671 e	59.75 ±1.084 d	1.417 ±0.008 d	1.197 ±0.001 de	0.726 ±0.002 de
	C_3N_4	39.93 ±0.735 d	25.95 ±0.567 c	65.88 ±0.609 c	1.441 ±0.005 d	1.188 ±0.004 de	0.737 ±0.003 cd
	Fe/C_3N_4	41.75 ±0.017 bc	28.17 ±0.478 ab	69.92 ±0.487 b	1.454 ±0.003 cd	1.161 ±0.003 ef	0.750 ±0.004 c
100	ddH_2O	25.43 ±0.370 kb	16.96 ±0.247 j	42.39 ±0.616 i	1.231 ±0.002 gh	1.251 ±0.004 c	0.644 ±0.006 h
	Fe_2O_3	26.84 ±0.804 ij	18.89 ±0.437 gh	45.73 ±0.745 gh	1.269 ±0.002 fg	1.255 ±0.039 c	0.668 ±0.004 fg
	C_3N_4	27.65 ±0.949 i	18.43 ±0.633 hi	46.08 ±1.582 g	1.294 ±0.007 f	1.238 ±0.002 cd	0.663 ±0.004 g
	Fe/C_3N_4	32.49 ±0.604 g	21.66 ±0.402 f	54.14 ±1.006 e	1.421 ±0.017 d	1.235 ±0.001 cd	0.683 ±0.012 f
150	ddH_2O	18.52 ±0.865 m	11.01 ±0.454 l	29.53 ±0.656 k	0.751 ±0.044 k	1.420 ±0.041 a	0.553 ±0.008 k
	Fe_2O_3	24.34 ±1.017 l	15.56 ±1.393 k	39.91 ±2.289 j	0.997 ±0.057 j	1.375 ±0.006 ab	0.577 ±0.001 j
	C_3N_4	26.27 ±0.710 jk	17.52 ±0.473 ij	43.79 ±1.183 hi	1.095 ±0.016 i	1.338 ±0.011 b	0.602 ±0.007 i
	Fe/C_3N_4	29.72 ±0.689 h	20.15 ±0.311 g	49.87 ±0.837 f	1.186 ±0.039 h	1.269 ±0.015 c	0.640 ±0.006 h
LSD at $P \leq 0.05\%$		1.22	1.32	2.07	0.05	0.05	0.01
S.O.V.		–	–	–	–	–	–
NaCl		825.82**	298.378**	2111.262**	0.620**	0.118**	0.091**
Treatment		105.699**	246.508**	374.087**	0.081**	0.008**	0.005**
NaCl × treatment		9.561**	31.079**	22.235**	0.017**	0.003**	0.0001**
Error		0.545	20.399	1.555	0.001	0.001	0.0001
C.V. (%)		2.22	3.69	2.27	2.09	2.05	4.20

** indicates significant at $P \leq 0.01$. If the letters used to represent the data points are different from each other, it means there are significant differences between those data points at $P \leq 0.05$ of significance as per the LSD test in each column

Chlorophyll fluorescence parameters

F_0. Salinity stress significantly increased F0 and moderated its effects, whereas foliar application treatments did not. The highest F0 was observed under 150 mM saline stress without foliar application, and the lowest was observed in the control and the Fe/C_3N_4 foliar treatment (Table 1).

Fv. Salinity stress may result from a notable reduction in Fv (Table 2), with the highest decrease of 54% observed relative to the control (Figure 2e). The foliar treatments indicated an increment of Fv, and the highest value was obtained in the pistachio seedlings via the foliar treatment of Fe/C_3N_4 with 15% (Figure 2f).

Fv/Fm. Based on the results, salinity stress drastically reduced Fv/Fm ($P \leq 0.05$)/Fm, but foliar treatments partially alleviated this effect. The highest increase, up to 29%, was obtained at the highest stress level compared to the control (Table 1).

Vitamin C

Based on the results, the interaction between foliar treatments and salinity on vitamin C content in the studied variety was determined. In the absence of salinity stress, foliar application with Fe_2O_3, C_3N_4, or Fe/C_3N_4 increases vitamin C content compared to the control. Additionally, salinity stress reduced the highest level by 78% relative to the control seedlings, and foliar spray treatments mitigated its effects (Table 3).

Table 2. Result of the analysis of variance on the studied characteristics in pistachio seedlings

S.O.V.	df	Mean square											
		Number leaf	Height (cm)	SAPD	RWC (%)	MSI (%)	Fm	Fv	MDA (nmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)	APX activity (µmol min^{-1} mg^{-1} FW)	GPX activity (µmol min^{-1} mg^{-1} FW)	SOD activity (µmol min^{-1} mg^{-1} FW)
NaCl	3	3114.139**	741.806**	283.903**	3327.383**	2390.402**	10.573**	12.822**	3.459**	4.219**	4.877**	2.575**	1677.497**
Treatment	3	935.250**	382.139**	267.157**	306.011**	213.500**	0.419**	0.546**	0.238**	0.720**	2.070**	0.522**	136.740**
NaCl × treatment	9	33.454**	3.565**	0.501ns	6.913ns	4.125ns	0.010ns	0.010ns	0.048**	0.127**	0.045**	0.053**	33.230*
Error	32	1.458	1.063	1.331	5.375	3.782	0.021	0.021	0.006	0.087	0.006	0.001	12.869
C.V (%)	–	2.07	1.96	2.04	5.48	4.50	3.39	4.76	6.51	4.37	7.84	4.24	4.36

*, ** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns indicates non-significant differences

Total phenol and flavonoid content

Salinity significantly increased the total phenolic and flavonoid content in pistachio leaves. Under control and foliar treatment conditions, the contents of phenols and flavonoids decreased. The highest amount of phenol and flavonoid was related to the salinity stress of 150 mM without applying foliar treatments, and the lowest was related to the foliar treatment of Fe/C_3N_4 without applying salt stress (Table 3).

Malondialdehyde (MDA)

The results indicated that, under non-stress conditions, Fe_2O_3, C_3N_4, and Fe/C_3N_4 applications reduced the MDA content in pistachio seedlings. Additionally, salinity stress increased MDA accumulation by 2.9-fold in pistachio leaves compared to the pistachio control, and foliar application treatments mitigated the effects of stress. The utmost MDA content was found at 150 mM NaCl use and with no application of spraying treatments (Figure 3a).

Hydrogen peroxide (H_2O_2)

The data showed that salinity stress increased H_2O_2 accumulation, with no significant difference between the 50- and 100-mM levels (Figure 3b). The outcomes revealed that the use of Fe_2O_3, C_3N_4, and Fe/C_3N_4 foliar treat-

ments reduced the concentration of H_2O_2 compared to the pistachio seedling in normal conditions, and no significant alteration was perceived among the mentioned foliar treatments (Figure 3c).

Total Soluble protein content

Total soluble protein content decreased under NaCl stress, with the greatest reduction observed at the highest stress level (39% compared to the control seedlings). Under no-stress conditions, the application of foliar treatments significantly increased protein content and improved it across salinity treatments. The highest total soluble protein content was obtained with foliar treatment with Fe/C_3N_4 and without salinity application; the lowest was at 150 mM salinity stress without foliar treatment (Table 3).

Figure 3. The effects of soil salinity and iron, carbon nitride, and carbon nitride modified with iron treatment on MDA (a), H_2O_2 (b and c), APX (d), GPX (e), and SOD (f) of pistachio seedlings. If the letters used to represent the data points differ, it indicates significant differences among the data points at $P < 0.05$, as determined by the LSD test. The error bars indicate standard deviation (SD)

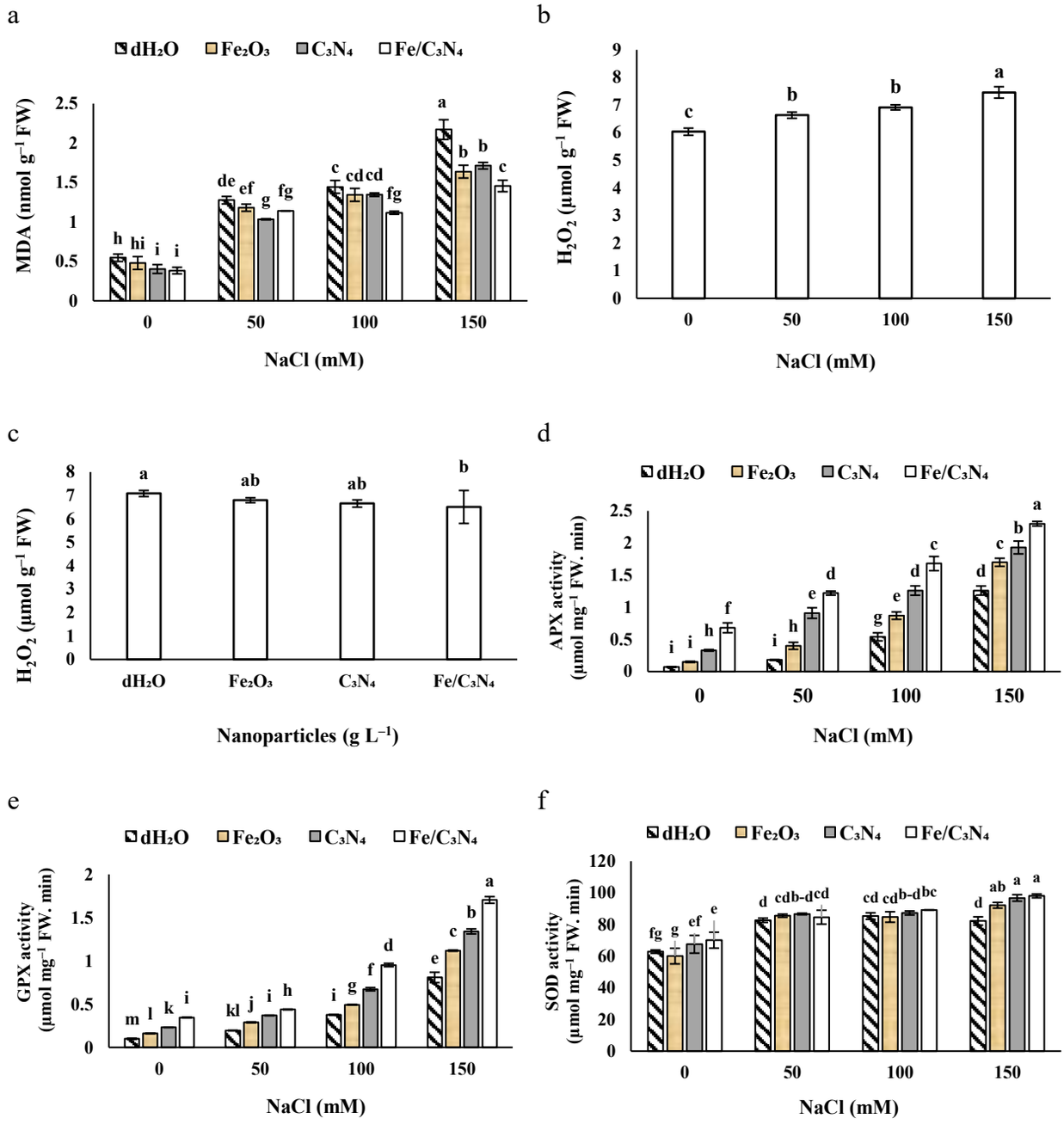


Table 3. Effect of Fe_2O_3, Carbon nitride (C_3N_4), and Carbon nitride modified (Fe/C_3N_4) with iron foliar treatments on vitamin C, Total phenol and Flavonoid content, and Total Soluble protein of pistachio seedlings under salinity stress (mean ±SE)

NaCl (mM)	Treatment (g L^{-1})	Vitamin C (mg 100 g^{-1} FW)	Total phenol content (mg g^{-1} FW)	Flavonoid content (mg g^{-1} FW)	Total Soluble protein (mg g^{-1} FW)
0	ddH_2O	14.07 ±0.585 d	681.3 ±7.716	249.9 ±8.716 gh	140.7 ±1.856 f
	Fe_2O_3	17.88 ±0.419 b	640.6 ±8.315	188.7 ±4.775 i	167.6 ±2.079 c
	C_3N_4	17.14 ±0.192 bc	617.2 ±2.400	156.2 ±5.734 j	153.9 ±0.944 de
	Fe/C_3N_4	21.15 ±1.895 a	550.3 ±10.791	125.7 ±5.835 k	194.1 ±2.283 a
50	ddH_2O	10.40 ±1.018 fg	778.3 ±11.544	347.6 ±16.462 d	132 ±1.732 g
	Fe_2O_3	12.31 ±1.166 e	734.3 ±6.687	263.2 ±6.682 g	148.8 ±0.227 e
	C_3N_4	14.07 ±0.536 d	691.2 ±15.877	251.5 ±10.113 gh	156 ±0.000 d
	Fe/C_3N_4	15.64 ±0.822 c	594.8 ±15.402	190.3 ±10.610 i	179.8 ±4.813 b
100	ddH_2O	5.292 ±0.247 ij	866.2 ±10.745	462.8 ±10.709 b	120.5 ±2.045 h
	Fe_2O_3	11.15 ±0.347 e–g	797.6 ±13.118	384.7 ±5.614 c	124.9 ±0.454 h
	C_3N_4	9.721 ±0.679 g	756.5 ±2.788	306.5 ±5.412 f	141.8 ±3.341 f
	Fe/C_3N_4	11.65 ±0.751 ef	697.8 ±8.342	236.5 ±5.027 h	170.4 ±1.487 c
150	ddH_2O	3.035 ±0.208 k	976.1 ±20.021	513.3 ±3.075 a	85.51 ±9.344 j
	Fe_2O_3	3.857 ±0.360 jk	925.5 ±6.677	448.8 ±15.386 b	123 ±0.524 h
	C_3N_4	6.113 ±0.117 hi	868.4 ±12.913	382.4 ±2.850 c	104.5 ±1.048 i
	Fe/C_3N_4	7 ±0.289 h	803.4 ±11.198	326.2 ±5.158 e	151.7 ±5.073 de
LSD at 0.05%		1.52	22.89	17.48	6.68
S.O.V.		–	–	–	–
NaCl		342.170**	161027.804**	127310.512**	5200.797**
Treatment		65.489**	57544.18**	65145.362**	6095.306**
NaCl × Treatment		3.549**	524.962*	1443.771**	190.664**
Error		0.842	189.427	110.422	16.151
C.V (%)		8.13	3.84	3.48	2.80

** indicates significance at $P \leq 0.01$. If the letters used to represent the data points are different from each other, it means there are significant differences between those data points at a 5% level of significance, as per the LSD test in each column

Enzymatic antioxidant activity

The results (Figure 3d) showed that salinity and different foliar applications of nanocomposite significantly ($P \leq 0.05$) improved the APX activity. The highest APX activity was observed with 150 mM NaCl and Fe/C_3N_4, approximately 3-fold higher than in control pistachio seedlings, which exhibited the lowest APX activity.

Also, GPX activity was considerably influenced by various levels of NaCl application and C_3N_4, Fe_2O_3, and iron-modified carbon nitride treatments ($P \leq 0.05$), see Figure 3e. The highest and lowest GPX activity was detected in Fe/C_3N_4-treated pistachio plants under 150 mM salinity stress and in control pistachio plants, respectively.

The enhancement in salinity level and the concentration of the treatments used improved the activity of SOD ($P \leq 0.05$), see Figure 3f, and the highest and lowest activity of SOD was revealed, respectively, in the salinity stress of 150 mM with Fe/C_3N_4, and the Fe_2O_3 solution treatment was obtained without applying stress.

DISCUSSION

Salinity can reduce leaf number by restricting leaf development, as salt stress hampers growth [Shahid et al. 2020]. The osmotic stress arises from the difference between the saline solution outside the cell and the internal

cellular solution within the root [Munns 2002]. Salinity-induced osmotic stress results in plasmolysis, inhibition of cell enlargement, and cell death in young leaves, stems, and roots [Arif et al. 2020]. Additionally, this stress causes stomata to close [Kiełkowska 2017]. Exposure to osmotic stress can adversely affect plants, such as inhibiting the expansion of young leaves, reducing new leaf production, and reducing stomatal conductance [Kiełkowska et al. 2019]. However, Kokina et al. [2020] found that applying nano-Fe_2O_3 increased leaf production in *Medicago falcata* L. When irrigated with saline water, plants may accumulate high concentrations of NaCl in cell walls and cytoplasm, negatively affecting photosynthesis rates, carbohydrate content, and some growth hormones [Barbieri et al. 2012]. Salinity stress has long-term effects on plant growth, reducing growth rates owing to osmotic and ionic stresses. Turgor pressure is reduced, decreasing wall extensibility and growth yield thresholds, particularly in stems and leaves. Height decreases due to reduced photosynthesis, and growth reduction is an adaptation that enhances plant endurance under stressful conditions [Bistgani et al. 2019]. Rui et al. [2016] report that specific concentrations of iron nanoparticles can increase the growth index, likely due to increased antioxidant activity and regulation of hormone levels. Ma et al. [2020] found that treating rice crops with C_3N_4 under heavy metal pollution reduced toxicity and improved plant growth. The Fe/C_3N_4 composite likely boosted antioxidant activity and maintained redox balance, reducing ROS-mediated suppression of cell division in shoot tips [Kaliyaperumal et al. 2025, Saleem et al. 2022]. Additionally, the polymeric N-rich structure of C_3N_4 offers strong coordination sites for metal ions, promoting better Fe retention and controlling its release via surface complexation and redox interactions [Chen and Song 2017, Ong et al. 2016].

Plants grown in saline environments exhibit a significant reduction in RWC. This decrease can be directly attributed to salt's impact on the plasma membrane's electrical potential. This impact not only impedes the ions' absorption but also significantly hinders the absorption of water, causing a state of water stress [Babaei et al. 2017]. Plants have various osmotic mechanisms to respond to water shortages in cells. Osmotic adjustment is one of them, helping plants maintain turgor pressure by adjusting the solute concentration in their cells. As osmotic potential decreases, leaves' RWC declines. RWC is a key parameter for regulating a plant's water status. It's calculated by comparing the weight of fresh tissue to fully hydrated tissue. Higher RWC leads to better growth, improving nutrient transport and metabolic processes [Ghadakchi asl et al. 2019]. In the current study, the use of C_3N_4, Fe_2O_3, and Fe/C_3N_4 boosted the RWC. Habibi and Sarvary [2015] found that adding iron to lemon balm plants under salinity stress helps them maintain stability. Fe application is associated with thicker collenchyma cells in plant stems, which help accumulate water and, hence, yield a higher and more consistent RWC.

The first signs of salinity stress are visible in the cell membranes; therefore, MSI is a crucial parameter for plant defense against this stress [Azarmi-Atajan and Sayyari-Zohan 2020]. Measuring electrolyte leakage in leaf samples can help estimate the impact of salinity on cell membrane stability. Previous studies by Akrami and Arzani [2018] have shown that conserving the integrity of the cell membrane is crucial for salinity tolerance. Azarmi-Atajan and Sayyari-Zohan [2020] unequivocally demonstrated that MSI content in lettuce decreased significantly under salt stress. However, reported with confidence that applying nano-Fe_2O_3 effectively mitigated the adverse effects of stress. Furthermore, various treatments with C_3N_4, Fe_2O_3, and Fe/C_3N_4 significantly improved MSI in pistachio leaves under salt stress. However, foliar Fe_2O_3, C_3N_4, and Fe/C_3N_4 nanoparticles significantly restored these parameters. The improvement in RWC and MSI can be attributed to multiple mechanisms, including enhanced cuticular and stomatal uptake of Fe nanoparticles, which improve ionic regulation and osmotic balance in leaf tissues [Ghosh et al. 2025]. Additionally, Fe and C_3N_4 facilitate redox buffering at the leaf surface, reducing lipid peroxidation and preserving membrane phospholipids [Cai et al. 2021]. Improvements in RWC and MSI show that Fe/C_3N_4 strengthens cellular integrity in saline environments [Gholami et al. 2024]. While Fe_2O_3 partially restored these traits, likely through improved Fe nutrition and osmotic regulation, its protective effect decreased at higher salinity levels [Gholami et al. 2024, Zhang et al. 2025, Zhou et al. 2024]. Conversely, the present findings showed that Fe/C_3N_4 maintained significantly higher RWC and MSI across all salinity levels, suggesting a combined physiological and chemical mechanism.

The current study found that the chlorophyll index decreased in pistachio seedlings as salinity stress increased, likely due to damage caused by free radicals produced under saline conditions, which degrade chlorophyll. Salinity significantly affects photosynthetic capacity, and chlorophyll content can be measured to evaluate this impact [Sim et al. 2015]. In addition, Karimi and Sadeghi-Seresht [2018] reported that salinity reduces photosynthetic rate in pistachio seedlings by increasing stomatal resistance. Chlorophyll content is a crucial indicator of a plant's health, which is influenced by water availability and nutrient levels. Sodium toxicity often displaces essential

cations from chloroplast membranes, thereby decreasing chlorophyll stability and light-harvesting ability [Hassanpouraghdam et al. 2025]. Salinity stress adversely affected the photosynthetic pigment content of pistachio plants, and chlorophyll fluorescence features were observed in this study. The absorption of essential nutrients, for instance, Fe and Mg, was also impeded in salinity environments, thereby hindering chlorophyll biosynthesis. The decrease in chlorophyll content under salt stress primarily reduces the potential for photosystem II activity [Hassanpouraghdam et al. 2020]. Salinity reduces chlorophyll levels and impairs photosynthesis by disrupting gas transmission and stomatal conductance [Ashraf and Harris 2004, Barbieri et al. 2012]. However, the higher content of photosynthetic pigments in the present trial is consistent with previous studies on cabbage and water dropwort (*Oenanthe javanica*) [Jamil et al. 2007, Kumar et al. 2020]. Carotenoids are a class of antioxidants that help plants develop tolerance to salt stress by diminishing free oxygen radicals [Ali et al. 2017]. Salinity stress reduces carotenoid content, consistent with reports by Azarmi-Atajan and Sayyari-Zohan [2020]. The contents of Chl a, b, and Total Chl show opposing responses to salinity stress. Although salinity decreases chlorophyll concentration, the application of iron nanoparticles can mitigate the adverse effects of salt stress and increase chlorophyll concentration [Ghadakchi asl et al. 2019]. The observed restoration of chlorophyll and carotenoids after Fe/C_3N_4 spraying is due to Fe, which functions as a cofactor for protochlorophyllide reductase, a crucial enzyme in chlorophyll production [Pushnik et al. 1984]. The Fe/C_3N_4 nanocomposite gradually releases Fe in ionic form, enhancing bioavailability. The C_3N_4 polymeric matrix functions as a photoprotective shield, reducing photobleaching and providing photoelectron storage for chloroplast repair reactions. The synergy between Fe and C_3N_4 enhances the scavenging of singlet oxygen and hydroxyl radicals generated under high-salt conditions, thereby preventing pigment degradation [Ali et al. 2017].

Chlorophyll fluorescence parameters help study the impacts of various environmental stresses on photosynthesis [Kumar et al. 2020]. High salt accumulation can damage chloroplasts, affecting membrane permeability and thylakoid function. This leads to a progressive decline in photosystem activity and chlorophyll fluorescence [Tsai et al. 2019]. Salinity reduced the photosynthetic rate by reducing leaf chlorophyll content and the maximum quantum yield of PS II (Fv/Fm) [Wang et al. 2018]. Under stressful conditions, electron flux from PS II to the quinone receptor decreases significantly, reducing Fv/Fm [Rasouli et al. 2022]. According to Torabian et al. [2017], foliar application with nano-Fe_2O_3 can help sunflower plants recover from salt stress by altering chlorophyll fluorescence. Foliar Fe/C_3N_4 reversed these declines by using Fe-based nanostructures that act as extra electron carriers and help stabilize redox reactions [Kwon et al. 2017]. Maintaining thylakoid structure is associated with lower ROS production and a better chloroplast lipid composition [Foyer and Hanke 2022]. Restoring Fe–S cluster activity is essential for proper PS II photochemistry [Tiwari et al. 2016].

Vitamin C (ascorbic acid) is a key antioxidant in plants, scavenging ROS, protecting photosynthesis, and maintaining redox balance under stress. In this study, salinity stress significantly reduced vitamin C levels in pistachio leaves compared with control seedlings. This noticeable decline aligns with earlier findings that high salinity levels interfere with ascorbate biosynthesis and accelerate its oxidation via excessive ROS production, ultimately damaging the ascorbate–glutathione cycle [Hasanuzzaman et al. 2020]. Under non-stress conditions, foliar application of Fe_2O_3, C_3N_4, and especially Fe/C_3N_4 significantly increased vitamin C content compared with the untreated control. This improvement results from enhanced metabolic activity and redox balance in leaves provided with micronutrients and nanostructured materials [Elemike et al. 2019]. Enhanced Fe availability increases carbohydrate availability, which is necessary for ascorbate biosynthesis via the L-galactose pathway [Samuolienė et al. 2019]. In parallel, carbon-based nanomaterials such as C_3N_4 may contribute to redox stabilization by moderating ROS production at the chloroplast level, thereby reducing ascorbate consumption [Bi et al. 2025, González-García et al. 2019]. Reducing ROS pressure with foliar Fe_2O_3 and Fe/C_3N_4 decreases ascorbate oxidation, enabling plants to sustain elevated vitamin C levels during stress [Gholami et al. 2024]. Furthermore, Fe/C_3N_4 could offer a dual advantage by maintaining continuous Fe availability and aiding redox buffering at the leaf surface, thereby helping preserve the functionality of the ascorbate–glutathione cycle [Gholami et al. 2024, Li et al. 2024].

Plant cells are protected by phenolics, which act as water-soluble antioxidants by quenching ROS and free radicals [Ashraf et al. 2010]. Phenolics can prevent ROS production and accumulation, thereby inhibiting oxidative stress. This plays a principal role in reducing the negative effects induced by ROS [Rico et al. 2015]. Apel and Hirt [2004] found that plants increase total phenolic content under stress to activate defense mechanisms against oxidative damage caused by ions, protecting cytoplasmic structures and chloroplasts. Phenols chelate iron ions, preventing ROS and decreasing superoxide levels from the Fenton reaction [Sakihama and Yamasaki

2002]. The decline in Fe/C_3N_4-treated pistachio plants likely resulted from stress relief, reducing the need for phenolic accumulation. Similarly, flavonoid variation is associated with the normalization of oxidative status, as excess Fe can inhibit the phenylpropanoid pathway via feedback inhibition [Yin et al. 2012]. According to Moradbeygi et al. [2020], treating plants with salinity leads to the accumulation of phenolic compounds, thereby increasing antioxidant activity, findings consistent with this report. However, Hassanpouraghdam et al. [2020] showed that foliar application of iron caused a reduction in phenol content under salt stress. Determining the optimal iron level is crucial for regulating plant flavonoid levels [Chung et al. 2019]. Nourozi et al. [2019] found that increased flavonoid content can induce salt tolerance in pistachios. Fe_2O_3 nanoparticle spraying also increased flavonoid and phenolic levels in *Dracocephalum kotschyi* by upregulating genes involved in the phenylpropanoid pathway.

Salinity stress markedly increased oxidative stress in pistachio leaves, as indicated by elevated H_2O_2 and MDA levels, reflecting excessive ROS production and membrane lipid damage [Rahneshan et al. 2018]. High salinity, especially at 150 mM NaCl without foliar treatment, triggers accumulation of phenols and flavonoids, indicating activation of secondary metabolism to boost antioxidant defenses and compensate for enzymatic ROS scavenging. In contrast, foliar application of Fe_2O_3, C_3N_4, and especially Fe/C_3N_4 significantly reduced phenolic and flavonoid contents under both non-stress and salinity conditions, suggesting mitigation of oxidative stress and a reduced need for secondary antioxidant accumulation. This protective effect is closely linked to enhanced antioxidant capacity and improved redox regulation, which limit ROS-driven membrane degradation. The Fe/C_3N_4 treatment seems to encourage a stress-avoidance strategy rather than stress tolerance. Instead of building up high levels of secondary antioxidants, plants treated with Fe/C_3N_4 kept ROS levels lower, experienced less lipid peroxidation, and maintained more stable physiological states [Guo et al. 2023]. This change enables the plants to conserve carbon resources for primary metabolism and cell maintenance, thereby improving overall physiological performance in saline conditions [Arif et al. 2020].

Salinity had a significant influence on the leaf's protein content. Total soluble protein content decreased in plants exposed to salt treatments. It is widely recognized that salinity affects protein biosynthesis, the principal process in plants. Both increment and decrease in protein content are commonly observed in salt-susceptible and salt-tolerant plants, respectively [Ashraf and Harris 2004]. Protein content declined by roughly 39 % under salinity in the current research, primarily due to oxidative denaturation, protease activation, and impaired amino acid metabolism [Cao et al. 2022, Saed-Moucheshi et al. 2014]. Salinity stress can reduce protein content due to oxidative damage to protein structure. Free radicals react with proteins, altering their structures, increasing the activity of degrading enzymes, and reducing amino acid synthesis. These factors contribute to the diminution of the soluble protein content in stressful situations [Saed-Moucheshi et al. 2014]. Plants treated with nano-Fe_2O_3 and subjected to salinity exhibited better levels of soluble protein and glycine betaine, suggesting a potential role of Fe in the biosynthesis of proteins [Pain and Dancis 2016]. Foliar nanoparticles enhanced protein levels through Fe-assisted enzyme activation involved in amino acid synthesis and decreased oxidative proteolysis [Pain and Dancis 2016].

The antioxidative system maintains a balance between ROS generation and scavenging to regulate signaling levels [Ahmed et al. 2013]. Plants have evolved an antioxidant system that includes APX, SOD, and POD enzymes to combat oxidative damage triggered by harsh environmental conditions [Alam et al. 2021]. Tolerant plant varieties exhibit higher antioxidant enzyme activity than sensitive varieties under different abiotic stresses [Jamshidi Goharrizi et al. 2020]. APX and CAT are similar enzymes that use ascorbate to remove H2O2 in the glutathione-ascorbate cycle [Gharsallah et al. 2016, Rao and Shekhawat 2016]. SOD defends against ROS by converting superoxide radicals to H2O2, which is detoxified by PODs with electron donors [Gharsallah et al. 2016]. Nanoparticles may reduce Na+ uptake and oxidative stress while enhancing the antioxidant system, including enzyme activity. K+ involvement in enzyme activation may explain how NPs can increase enzyme content by lowering Na+ concentration at salinity [Khan et al. 2017]. Nanocarbons, such as graphene oxide, can affect the activities of antioxidative enzymes, including SOD, APX, and POD. Nanocarbon particles can react with specific sites on enzymes at high concentrations, thereby rendering them ineffective. Nanomaterials' small size and high permeability effectively protect plants from stressors. This is why nanomaterials are often considered beneficial [Bacakova et al. 2020]. Therefore, Fe/C_3N_4 acts as a nanobiochemical enhancer, reducing oxidative damage while enhancing nutrient metabolism [Li et al. 2025].

CONCLUSION

Salinity stress significantly affected the morphology, physiology, and biochemistry of *P. vera* seedlings, leading to reduced growth, water content, photosynthetic efficiency, and protein levels, along with increased oxidative damage and secondary metabolite accumulation. The adverse effects of rising NaCl levels confirm that, despite being relatively salt-tolerant, pistachio seedlings are strongly affected by salinity-induced osmotic and oxidative stress. Foliar application with Fe_2O_3, C_3N_4, and Fe/C_3N_4 markedly enhanced most traits measured under different salinity conditions, as shown by significant main effects in the variance analysis. These improvements included higher RWC, MSI, chlorophyll pigments, and fluorescence parameters, whereas H_2O_2, MDA, total phenols, and flavonoids decreased. The decline in oxidative markers and secondary metabolites suggests better overall physiological stability rather than stress defense. Although the interaction between salinity level and foliar treatment was not statistically significant, seedlings treated with Fe/C_3N_4 consistently exhibited higher growth and photosynthetic traits and lower oxidative stress indicators compared to untreated plants. This indicates that Fe/C_3N_4 offers a general, stable physiological benefit across both saline and non-saline conditions, rather than a specific response to salinity. The improved performance of Fe/C_3N_4-treated plants can be explained by enhanced membrane integrity, redox control, and photosynthetic function, which together reduce oxidative signaling and secondary metabolite build-up. The combination of iron and carbon nitride likely supports sustained nutrient availability and redox buffering, helping maintain cellular stability under stress. In conclusion, the study indicates that using Fe-based and C-based nanomaterials as foliar treatments can effectively mitigate the adverse effects of salinity on pistachio seedlings. The current statistical analysis shows that Fe/C_3N_4 provided the most consistent positive outcomes among the treatments. These results endorse the application of Fe/C_3N_4 as a foliar supplement to enhance the stability and growth of pistachio seedlings in saline soils, and they also establish a foundation for future field studies and long-term research in orchard environments.

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AUTHOR CONTRIBUTION STATEMENT

S.M.Z. and M.A.A.: supervised the experiment. S.G., S.M.Z., and F.R.: wrote and performed the experiments. S.G., F.B., F.R. and S.K.: analyzed data and wrote the paper. S.E., N.E.K., M.A.A., A.A. and J.M.: reviewed and checked all the details. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

The data and other information will be available on request.

CONFLICT OF INTEREST

The authors have reviewed the journal's policies and have confirmed that there are no conflicts of interest to declare.

COMPETING INTERESTS

The authors declare no competing interests.

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