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Integrated role of nanofertilizer in reducing alternaria leaf spot severity and enhancing growth of date palm under saline conditions

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Abstract

This study used "LOENERGY PLUS" as a nanofertilizer to mitigate the negative effects of salinity on physiological functions, nutrient uptake, and disease spread in date palm offshoots of the Halawi cultivar. Three different levels of salinity water were added (5, 10, and 15) $dS.m^{-1}$, with nanofertilizer added in some treatments and without it in others. The leaf nitrogen, phosphorus, potassium, and chlorophyll content were reduced significantly by salinity, while the results showed an increase in proline levels and enhanced susceptibility to *Alternaria alternata*. Application of nanofertilizer markedly alleviated these effects by improving nutrient accumulation, reducing proline accumulation, and preserving chlorophyll content. Most notably, the disease severity was significantly reduced under nanofertilizer treatment, with the damaged area decreasing from 58.29% to 32.12% at 15 $dS.m^{-1}$. It is evident that nanofertilizers play an important role in protecting plants against diseases. This indicates that nanofertilizers play an important role in improving plant physiological functions and reducing damage under saline conditions.

Keywords: Alternaria alternata, fungi, pathogenicity, oxidative stress, proline accumulation, chlorophyll stability

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نقش تلفیقی نانوکود در کاهش شدت لکه‌برگی آلترناریا و بهبود رشد نخل خرما تحت شرایط تنش شوری

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چکیده

این پژوهش با هدف ارزیابی نقش نانوکود «LOENERGY PLUS» در کاهش اثرات منفی تنش شوری بر شاخص‌های فیزیولوژیک، جذب عناصر غذایی و گسترش بیماری در پاجوش‌های نخل خرما رقم حلاوی انجام شد. تیمارهای شوری در سه سطح ۵، ۱۰ و ۱۵ دسی‌زیمنس بر متر اعمال گردید و اثر کاربرد نانوکود در مقایسه با تیمارهای فاقد نانوکود مورد بررسی قرار گرفت. نتایج نشان داد که افزایش شوری موجب کاهش معنی‌دار غلظت نیتروژن، فسفر، پتاسیم و کلروفیل برگ گردید، در حالی‌که میزان تجمع پرولین افزایش یافته و حساسیت گیاهان به قارچ *Alternaria alternata* تشدید شد. با این حال، کاربرد نانوکود به‌طور معنی‌داری اثرات نامطلوب شوری را تعدیل نمود؛ به‌گونه‌ای که با بهبود جذب و تجمع عناصر غذایی، کاهش انباشت پرولین و حفظ پایداری کلروفیل همراه بود. افزون بر این، شدت بیماری در تیمارهای حاوی نانوکود به‌طور قابل توجهی کاهش یافت، به‌طوری‌که درصد سطح آسیب‌دیده در شوری ۱۵ دسی‌زیمنس بر متر از ۵۸/۲۹ درصد به ۳۲/۱۲ درصد کاهش پیدا کرد. در مجموع، نتایج حاکی از آن است که نانوکودها با بهبود عملکردهای فیزیولوژیک و افزایش مقاومت گیاه، نقش مؤثری در کاهش خسارت ناشی از تنش شوری و عوامل بیماری‌زا ایفا می‌کنند.

کلید واژه‌ها: ***Alternaria alternata*، بیماری‌زایی، پایداری کلروفیل، تجمع پرولین، تنش اکسیداتیو، قارچ‌ها**

دبیر تخصصی: دکتر سعید عباسی

Introduction

Date palms (*Phoenix dactylifera* L.) are among the most important fruit trees cultivated in arid and semi-arid regions, playing a significant economic and environmental role in Arab countries, including Iraq (Chao & Krueger, 2007). Date palm trees are cultivated in Iraq on an area estimated at 1,250,000 dunams, and date production has reached more than 735,000 tons (Khalil & Jassim, 2025). Nowadays, Iraq ranks first in date palm numbers, with 22 million, followed by Iran with 21 million, Saudi Arabia with 12 million, and Algeria with 9 million. It also ranks fourth among date palm producers globally (Aziz, 2025; Khalil & Jassim, 2025). However, date palm cultivation faces several challenges, most notably the adverse effects of soil and irrigation water salinity, which lead to reduced growth and productivity (Al-Rawi, 1998). Salinity negatively affects plants by disrupting nutrient balance, inducing osmotic stress, and causing ionic toxicity (Challa & Van Bensichem, 2004). In date palm, high salinity markedly reduces fruit yield, with losses reaching up to 67% at 15 $dS.m^{-1}$ compared to low salinity (5 $dS.m^{-1}$), and yield reduction thresholds varying between 8–12 $dS\ m^{-1}$ depending on the cultivar (Arif, et. al., 2020). Also, plant resistance to diseases is weakened by salinity, and exposure to fungal pathogens increases. One example is leaf spot disease (Mahdi et al., 2025; El-Meleigi, 2015) and black scorch disease (El-Meleigi, 2015).

Traditional fertilizers are likely to fail in providing the necessary nutrients for plants in saline conditions and may amplify ion disorder. In response, nanofertilizers have overcome these issues. They show properties that improve nutrient uptake, enhance photosynthetic efficiency, and support the physiological and biochemical processes of plants (Al-Hchami & Alrawi, 2020; Higa, 2006). According to a few studies, nano-fertilizers strengthen plant immune responses and reduce the incidence and severity of diseases (Tarafdar et al., 2014; Elbasuney et al., 2022; Eid et al., 2025).

Lots of studies have stated that applying nanofertilizers can help with plant diseases. Due to their nanoscale properties, they can absorb nutrients more effectively. In turn, this enhances plant health and builds its resilience against pathogens (Chand et al., 2025). Being made from minerals, synthetic particles, and biomass, nanofertilizers release nutrients in a controlled way. Therefore, this allows the plant to defend itself against diseases (Yadav et al., 2023). Also, plant production is increased, while the use of chemicals is decreased. Using this method results in sustainable production and agriculture.

Also, antifungal and antibacterial activities have been demonstrated in nanomaterials, specifically silver nanoparticles (AgNPs). AgNPs can enhance the health of the plant in stressful conditions (Abasi et al., 2022). They also inhibit soil fungi. These fungi include *Monilinia fructicola* and *Fusarium solani* (Mirbakhsh, 2023). According to the results, nanofertilizers can improve plant resistance to pathogens and enhance nutrient absorption. This increases the crop's ability to survive under stressful conditions (Abasi et al., 2022).

It has been shown in recent studies that nanofertilizers can increase nutrient use efficiency by up to 30% and improve crop yields by 20%, while reducing the environmental footprint associated with excessive conventional fertilizer use (Khundi et al., 2025). Their controlled-release mechanisms and improved bioavailability allow for more sustainable, precise nutrient delivery, making them an important advancement for cleaner and more resilient agricultural systems (Saurabh et al., 2024). In addition, nanofertilizers are composed of

engineered nanoparticles containing essential macro- and micronutrients (e.g., N, P, K, Fe, and Mn) that are either encapsulated or adsorbed onto nanoscale carriers. These features allow for the targeted delivery of nutrients to the plant rhizosphere and their gradual release in accordance with plant demand, reducing nutrient losses and environmental contamination (Nongbet et al., 2022), resulting in improved plant growth in normal environments, but also in stress, such as salinity, where specific management of nutrients is important for overall plant strength, as well as preserving cellular balance.

To that end, the goal of this study was to test the effect of the nanofertilizer "LOENERGY PLUS" on the growth and performance of offshoots in date palms and to investigate its effectiveness at different levels of salinity, lowering the incidence of leaf spot disease. An agricultural method that will be long-lasting and improve the strength of date palms under stressful conditions needs to be identified, highlighting the importance of this study.

Materials and Methods

This study was conducted at two locations in the Basra Governorate: Abu Al-Khaseeb (30°27′19″N, 47°58′45″E) and the Palm Research Center Station at the University of Basra in Karmat Ali (30°35′01″N, 47°45′27″E), during the 2024 agricultural season. Twenty-four six-year-old, uniform, healthy, and disease-free Halawi date palm offshoots were selected. The offshoots were digitally marked for identification and divided into two treatment groups. The experiment followed a factorial randomized complete block design (RCBD) with two factors: fertilizer application (two levels: with and without LOENERGY PLUS) and irrigation water salinity (three levels: 5, 10, and 15 $dS.m^{-1}$). Each treatment combination was replicated four times, giving a total of 24 experimental units. The first group consisted of 12 offshoots without fertilizer, while the second group included 12 offshoots treated with LOENERGY PLUS nanofertilizer at a concentration of 150 mL per 200 L of water. All offshoots were irrigated using three salinity levels: 5, 10, and 15 $dS.m^{-1}$. The fertilizer treatment was applied once a month following the manufacturer's recommendations. LOENERGY PLUS, a liquid fertilizer produced by Agrisens (Turkey), contains 10% nitrogen (N), 12% water-soluble phosphorus pentoxide, 12% water-soluble potassium oxide, and micronutrients. It is a concentrated liquid formulation produced via salt electrolysis technology, characterized by its ability to enhance nutrient absorption by plants.

Measuring leaf chemical properties

Some chemical properties of plant leaves were analyzed according to the method of Cresser and Parsons (1979). A 0.2 g portion of dry, ground plant sample was mixed with 5 mL of concentrated sulfuric acid and left overnight. The sample was heated until white fumes appeared. Then, 3 mL of a digestion mixture (96 mL H_2SO_4 + 4 mL $HClO_4$) was added, and the solution was further heated until it became clear. After cooling, 50 mL of distilled water was added.

Nitrogen content was measured using a micro Kjeldahl apparatus following the method of Page et al. (1982). Phosphorus was determined using the method of Murphy and Riley (1962) with a spectrophotometer at 700 nm after the development of a blue color. Potassium was measured using a flame photometer according to Page et al. (1982).

Estimation of Proline Content

The proline content in the leaf tissue samples was calculated based on Bates et al. (1973). The upper colored layer was used to measure proline concentration using a spectrophotometer to detect absorbance at 520 nm. The standard proline curve was utilized to calculate the proline content,

which was then represented in units of (µM.g^{-1} dry weight) using the following equation:

$$Proline\ Content = \frac{Proline\ (\mu g) \times Toluene\ Volume\ (ml) \times 5}{Sample\ Weight \times 115.5}$$

Where 115.5 is the molecular weight of proline.

Total chlorophyll Content of Leaves

Total chlorophyll content in leaves was determined using the Holden method as described by Howrtiz (1975). Absorbance was measured at wavelengths of 645 and 663 nm using a spectrophotometer. The content of chlorophyll was calculated using the following equation:

$$\text{Total chlorophyll (mg/L)} = 20.2 \times OD_{645} + 8.02 \times OD_{663}$$

Evaluation of disease development

In this experiment, the fungus *Alternaria alternata* (accession number: PP330024) was used. The spore suspension of *A. alternata* was prepared following the method described by Cheng et al. (2025).

The concentration of conidia was adjusted to 1×10^6 spores/mL using a hemocytometer. This suspension was used for spraying in the pathogenicity testing on date palm leaves using a manual atomizer. The pathogenicity test was conducted using a factorial arrangement consistent with the salinity treatments. Each treatment consisted of four replicates, with one plant per replicate. On each plant, three leaves of similar physiological age were selected and sprayed with the conidial suspension. For the control treatment, leaves were sprayed with sterile distilled water containing only 0.01% Tween 20. Spraying with the spore suspension was carried out after two weeks of salt stress application. The development of leaf spot disease under salt stress conditions was evaluated 30 days after inoculation by measuring the damaged area using ImageJ at the end of the experiment (Alasadi, 2025).

Statistical Analysis

Data were analyzed using SPSS (version 21). Treatment means were compared using the least significant difference (LSD) test at a significance level of $p \leq 0.05$ to determine significant differences among treatments.

Results

Leaf Nitrogen Content

The outcomes showed that increasing salinity in irrigation water led to a slow decrease in nitrogen content across all treatments. However, applying nanofertilizer helped maintain higher nitrogen levels compared to treatments without nanofertilizer at all salinity levels.

At a salinity level of 5 dS.m^{-1}, the nitrogen content reached 2.45% with nanofertilizer, the highest recorded value, while it was only 1.15% without nanofertilizer. At 10 dS.m^{-1}, the nitrogen content decreased to 1.75% with nanofertilizer and 0.75% without it. At the highest salinity level 15 dS.m^{-1}, the content further declined to 1.35% with nanofertilizer, compared to 0.45% without it (Figure 1).

The results showed that increasing the salinity of irrigation water led to a gradual and significant decrease in phosphorus content in plants, both in treatments with and without nanofertilizer.

At a salinity level of 5 dS.m^{-1}, the treatment with nanofertilizer recorded the highest phosphorus content at 2.75%, while the content decreased to 1.75% in the absence of nanofertilizer. As salinity increased to 10 dS.m^{-1}, phosphorus content declined to 2.45% in treated plants and 1.35% in untreated ones. At the highest salinity level, 15 dS.m^{-1}, phosphorus content was 1.90% with nanofertilizer and 1.05% without it (Figure 2).

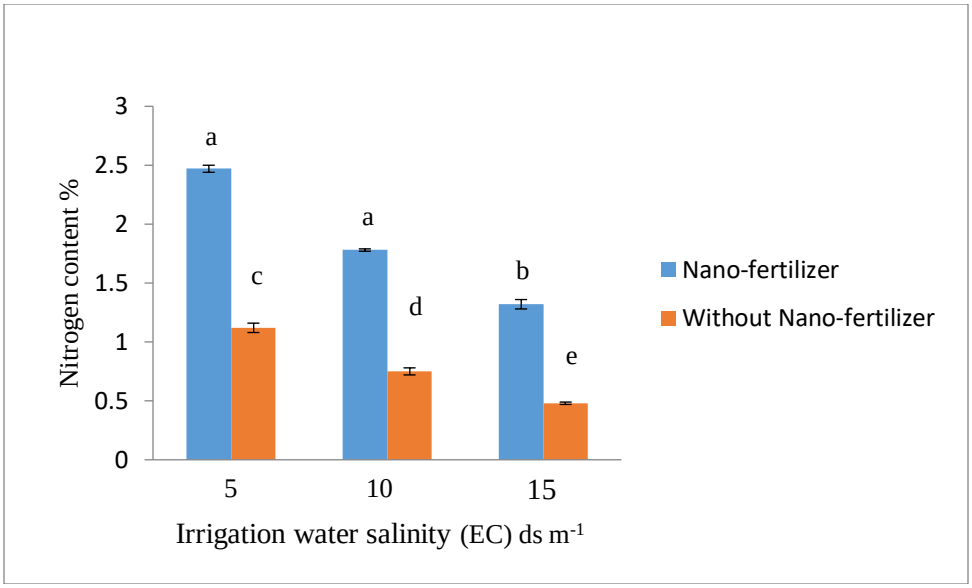


Figure 1. Interactive effects of saline irrigation and nanofertilizer on leaf nitrogen concentration (%). Columns sharing the same letter are not significantly different at $p \leq 0.05$. EC stands for electrical conductivity. Bars indicate the standard error.

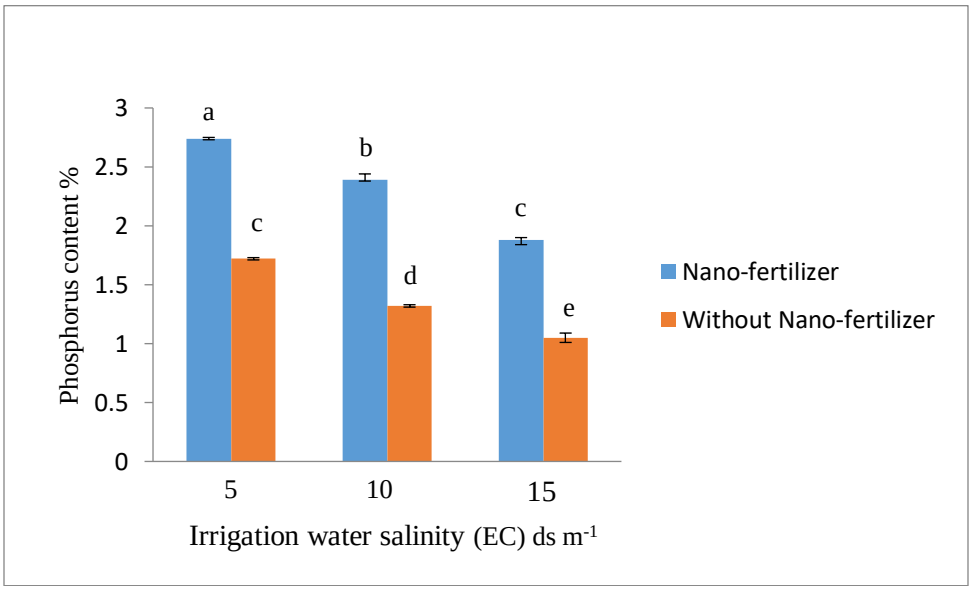


Figure 2. Interactive effects of saline irrigation and nanofertilizer on leaf phosphorus concentration (%). Columns sharing the same letter are not significantly different at $p \leq 0.05$. EC stands for electrical conductivity. Bars indicate the standard error.

Leaf Potassium Content

The outcomes showed that an increase in the salinity of irrigation water led to a gradual decrease in potassium content across all treatments. However, plants treated with nanofertilizer maintained higher potassium levels than those without it.

At a salinity level of 5 $dS.m^{-1}$, potassium content reached 8.85% in plants treated with nanofertilizer, compared to 6.15% in untreated plants. At 10 $dS.m^{-1}$, the content decreased to 7.75% and 5.35%, respectively. At the highest salinity level, 15 $dS.m^{-1}$, treated plants recorded 6.45% sodium content, while the content did not exceed 4.15% in untreated plants (Figure 3).

Leaf Proline Content

The outcomes showed that increasing salinity led to a slow increase in proline content in both nanofertilizer-treated and untreated plants. However, the values were significantly higher in untreated plants.

At a salinity level of 5 $dS.m^{-1}$, proline content reached 0.47 $\mu g.g^{-1}$ in treated plants compared to 0.72 $\mu g.g^{-1}$ in untreated ones. At 10 $dS.m^{-1}$, the content increased to 0.56 and 0.81 $\mu g.g^{-1}$, respectively. At the highest salinity level, 15 $dS.m^{-1}$, proline content reached 0.68 $\mu g.g^{-1}$ in treated plants and 1.08 $\mu g.g^{-1}$ in untreated plants (Figure 4)

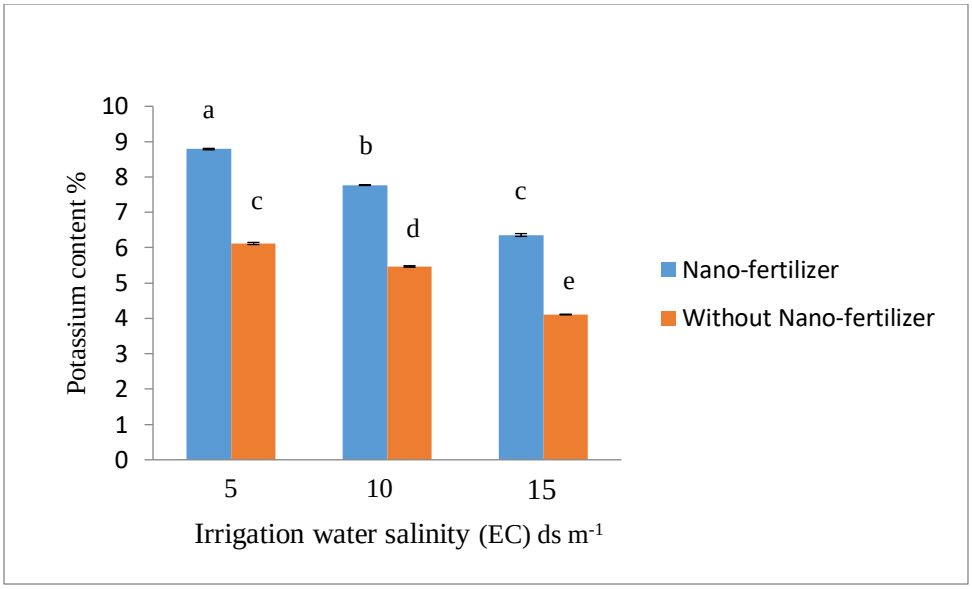


Figure 3. Effect of irrigation water salinity and nanofertilizer application on potassium content in date palm leaves. The same letter is not significantly different according to the least significant difference (LSD) test at $p \leq 0.05$. EC stands for electrical conductivity. Bars indicate the standard error.

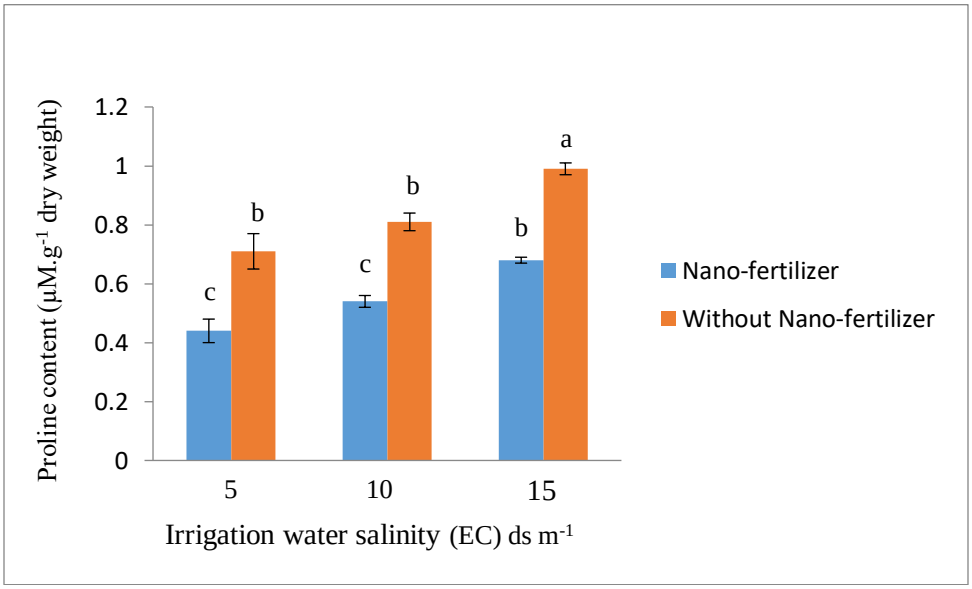


Figure 4. Effect of irrigation water salinity and nanofertilizer application on proline content in date palm leaves. Columns sharing the same letter are not significantly different at $p \leq 0.05$. EC stands for electrical conductivity. Bars indicate the standard error.

The Content of Chlorophyll

The results showed a gradual decrease in chlorophyll content with increasing irrigation water salinity in both nanofertilizer-treated and untreated plants. However, plants treated with nanofertilizer maintained higher chlorophyll levels than untreated plants at all salinity levels.

At a salinity level of 5 dS.m^{-1}, chlorophyll content reached 24.8 mg L^{-1} in treated plants, compared to 11.6 mg L^{-1} in untreated plants. At 10 dS.m^{-1}, it decreased to 18.1 mg L^{-1} with nanofertilizer and 10.2 mg L^{-1} without it. At the highest salinity level, 15 dS.m^{-1}, chlorophyll content was 13.4 mg L^{-1} in treated plants and 8.7 mg L^{-1} in untreated ones (Figure 5).

Disease Development

The results presented in Table 1, it was observed that increasing salinity levels led to a corresponding increase in the damaged area. The largest damage area, 58.29 %, was recorded at the highest salinity level of 15 dS.m-1 without fertilizer application, while this percentage decreased to 32.12% with fertilizer application at the same salinity level.

At 5 dS.m^{-1}, the damage area reached 38.07% without fertilizer, while it decreased significantly to 12.98% with fertilizer application. At 10 dS.m^{-1}, the damage area was 45.40 % without fertilizer compared to 19.91% with fertilizer (Figure 6).

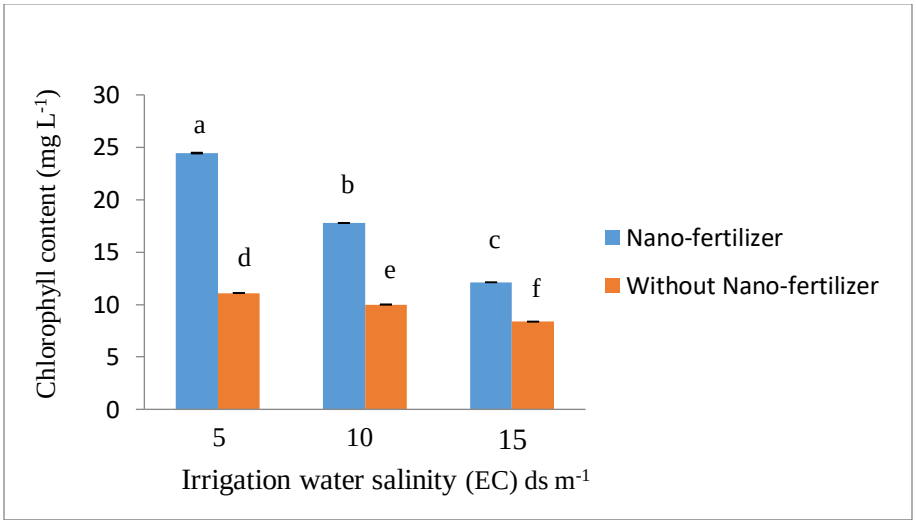


Figure 5. Interactive effects of saline irrigation and nanofertilizer on chlorophyll. Columns sharing the same letter are not significantly different at $p \leq 0.05$. EC stands for Electrical conductivity. Bars indicate the standard error.

Table 1. Leaf damage area in date palm (*Phoenix dactylifera* L. cv Halawi) under the effect of irrigation water salinity, nanofertilizer application, and treatment with *Alternaria alternata*.

Irrigation water salinity (dS.m-1)	Nanofertilizer "LOENERGY PLUS" application	Damage area %
5	No	$38.07^{c} \pm 0.85^{*}$
5	Yes	$12.98^{e} \pm 0.42$
10	No	$45.40^{b} \pm 0.93$
10	Yes	$19.91^{e} \pm 0.55$
15	No	$58.29^{a} \pm 1.10$
15	Yes	$32.12^{d} \pm 0.74$

*Standard error



Figure 6. Damaged leaf area (%) of date palm treated with fertilizers under different irrigation water salinity levels and inoculated with *Alternaria alternata*. (A, B, C) not treated with nanofertilizer. (D, E, F) treated with nanofertilizer. (A, D) is irrigated with 5 dS.m^{-1}; (B, E) is irrigated with 10 dS.m^{-1}, and (C, F) is irrigated with 15 dS.m^{-1}.

Discussion

An increase in irrigation water salinity reduced essential nutrients (nitrogen, phosphorus, and potassium) and chlorophyll content, while it increased proline levels and disease severity. However, the application of nanofertilizers helped decrease these negative outcomes by improving plant physiology and boosting stress tolerance under saline conditions.

Nutrient uptake is lowered by salinity due to root zone competition and ionic stress. Plants can stop absorbing important nutrients like nitrate (NO_3^-), calcium (Ca^{2+}), phosphate (PO_4^{3-}), and potassium (K^+) because of too much chloride (Cl^-) and sodium (Na^+). Sodium competes with potassium, while chloride limits nitrate uptake. Salinity also reduces phosphorus availability by affecting its solubility in the soil (Niste et al., 2014).

Ehtaiwesh (2020) reported that salinity lowers the uptake of important macronutrients such as phosphorus, potassium, and nitrogen. Also, salinity can negatively affect the nutrient balance in plants by lowering the ratio of K^+/Na^+, which is important for the growth of plants and their function. The low absorption of potassium in particular was attributed to competition with sodium ions at the membrane transport level, resulting in nutrient imbalances and metabolic inefficiencies.

The decrease in nutrient concentration in plants without treatment was shown clearly in the study. For example, nitrogen content was only 1.15% at 5 $dS.m^{-1}$ salinity with no nanofertilizer, compared to 2.45% with nanofertilizer. Similar trends were observed for phosphorus and potassium, where nutrient levels were consistently higher in plants treated with nanofertilizer at all salinity levels. The outcomes confirmed that salinity-induced nutritional imbalances can be alleviated by nanofertilizers. This happens by enhancing nutrient solubility and transport, as well as by mitigating ionic antagonism in the root zone. It may help the plant stay balanced under saline conditions by lowering harmful sodium and keeping nutrients stable.

Using ultra-small particles that dissolve quickly, nanofertilizers enhance nutrient availability. The small particles are absorbed easily and move rapidly within plants. Also, even under stress, they enhance enzyme activities and ion transport. As stated by ul Ain et al. (2023), nanofertilizers not only increase the efficiency of nutrient absorption but also promote root development, chlorophyll synthesis, and the activity of key enzymes under saline conditions. Their outcomes showed that nano-formulations help plants use nutrients better when stressed. In agreement with this, Madlala et al. (2024) emphasized that the physical characteristics of nanofertilizers, such as particle size, surface charge (zeta potential), and dispersion capacity, play a pivotal role in determining their bioavailability and transport across plant tissues.

Chlorophyll content declined progressively with rising salinity, a response commonly associated with the disruption of the photosynthetic apparatus and oxidative stress. High salinity levels can lead to the disintegration of chloroplast membranes, inhibition of chlorophyll biosynthesis enzymes such as δ-aminolevulinic acid dehydratase, and accumulation of reactive oxygen species (ROS), which degrade chlorophyll molecules and impair photosystem II activity. This mechanism has been confirmed by Al-Gaadi et. al. (2024), who reported that salinity causes a marked decrease in chlorophyll a and b due to oxidative degradation and impaired synthesis pathways.

That being said, higher levels of chlorophyll remained with nanofertilizer-treated plants. It reached 24.8 mg L^{-1} at 5 $dS.m^{-1}$ compared to only 11.6 mg L^{-1} in untreated plants. Nanofertilizers did the

protection of chloroplasts and support of chlorophyll production.

Salinity stress, especially in untreated plants, is increased by proline buildup and reflects the plant's response to osmotic imbalance. Proline acts as an osmoprotectant, stabilizing proteins and membranes, and as a ROS scavenger. The lower proline levels in nano-fertilizer-treated plants indicate reduced stress, suggesting these plants were better able to maintain osmotic and cellular balance without relying on stress-related metabolites.

Yaish (2015) found that proline buildup *in Phoenix dactylifera* is a core response to both short-term and prolonged salt stress, and protects cells from oxidative stress and osmotic shock. The study indicates that a localized defense tactic against salt buildup in the rhizosphere results from proline accumulation.

Also, the study by Khan et al. (2025) showed that proline protects the cellular redox balance and photosynthetic machinery. Their work focused on maize under drought conditions, but the physiological mechanisms are comparable: proline buildup enhanced antioxidant enzyme activities. Superoxide dismutase and catalase kept chlorophyll content, and stabilized cellular membranes. It is known from the outcomes that proline acts as a protective agent under different abiotic factors, including salinity and drought.

Thus, the reduced proline buildup in nanofertilizer-treated date palm plants indicates that these plants experienced less osmotic and oxidative stress, thanks to the cellular protection mechanisms provided by the nanofertilizers. The treated plants remained more balanced through stress regulation and proactive nutrient use, rather than relying on secondary metabolite buildup.

Higher salinity levels were connected to more leaf damage by *A. alternata*, as shown when it comes to disease development. The most damaged area has a percentage of (58.29%), and it was recorded at 15 $dS.m^{-1}$ with no nanofertilizer. On the other hand, the damage was reduced to 32.12% by applying nanofertilizer at the same level of salinity. Hence, using nanofertilizers makes the plants more resilient and lowers the disease severity.

Ghazy et al. (2024) reported that the application of certain nanomaterials, including nano-zinc oxide (ZnO-NPs) and nano-copper oxide (CuO-NPs), reduced the severity of Alternaria leaf spot in sugar beet, which agrees with the outcomes. Their study showed that nanomaterials decreased disease by raising the defenses of plants and inhibiting the growth of fungi. And it increased the productivity and resulted in higher content of chlorophyll.

Conclusion

Irrigation water with high salinity lowered chlorophyll levels, nutrient uptake, and the tolerance of stress in date palm offshoots, and made them more at risk to *Alternaria alternata*. Nanofertilizer was applied to help with these negative effects and help plants grow better. Treated plants experienced high levels of phosphorus, nitrogen, chlorophyll, and potassium. This also results in lower proline buildup in saline conditions.

Importantly, nanofertilizer substantially reduced disease severity, indicating enhanced plant defense under combined abiotic and biotic stress. It may be connected to increasing antioxidants' activity, enhancing plant defense, and balancing nutrition. Nanofertilizers help to reduce the effects of salinity and diseases. That being said, to understand the mechanism of nanofertilizers, a genetic test is needed.

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