

Available online at http://bjas.bajas.edu.iq https://doi.org/10.37077/25200860.2024.37.1.18 College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

Basrah J. Agric. Sci., 37(1), 236-246, 2024

E-ISSN: 2520-0860

# Biochemical Responses of Date Palm *Phoenix dactylifera* L. to Combined Stress of Salinity and Nickel

# Hassanain M. Gabash<sup>1\*</sup>; Ahmed Z. Resan<sup>1</sup>; Khairullah M. Awad<sup>2</sup>; Aqeel A. Suhim<sup>1</sup>& Ammar H. Abdulameer<sup>2</sup>

<sup>1</sup>Department of Horticulture and Land Scape, College of Agriculture, University of Basrah, Iraq

<sup>2</sup>Date Palm Research Center, University of Basrah, Iraq

\*Corresponding author email: H.M.G.: hassanain.gabash@uobasrah.edu.iq; A.Z.R.: ahmed.resan@uobasrah.edu.iq; K.M.A.: khearallah.awad@uobasrah.edu.iq; A.A.S, aqeel.suhaim@uobasrah.edu.iq; A.H.A.: ali781130@gmail.com

Received 26<sup>th</sup> June 2023 Accepted 29<sup>th</sup> October 2023; Available online 18<sup>th</sup> June 2024

Abstract: This study examined the effects of nickel (Ni) at 0, 25, and 50 mg.L<sup>-1</sup> concentrations, applied alone or in combination with salinity (represented by NaCl at 0, 100, and 200 mM concentrations), on the biochemical traits of date palm. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde (MDA), membrane stability index (MSI), peroxidase (POD) activity, superoxide dismutase (SOD), and proline content were among the parameters evaluated. The results revealed significant effects of nickel and salinity on the studied biochemical markers. Nickel at 50 mg.L<sup>-1</sup> significantly increased H<sub>2</sub>O<sub>2</sub> (0.87 µmol. L<sup>-1</sup>) and MDA (2.46 nmol.g<sup>-1</sup>) levels, while decreasing MSI (75.85%). Moreover, it enhanced POD (25.09 U.min<sup>-1</sup>.g<sup>-1</sup>) and SOD (3.78 U. min<sup>-1</sup>.g<sup>-1</sup>) activity, as well as proline content (4.35  $\mu$ mol.g<sup>-1</sup>). Salinity at 200 mM significantly increased H<sub>2</sub>O<sub>2</sub> (0.90  $\mu$ mol L<sup>-1</sup>) and MDA (2.54 nmol.g<sup>-1</sup>) levels, decreased MSI (77.69%), and increased POD (27.61 U. min<sup>-1</sup>.g<sup>-1</sup>) and SOD (3.77 U.min<sup>-1</sup>.g<sup>-1</sup>) activity, along with increased proline content (4.54 µmol.g<sup>-1</sup>). Additionally, the combined application of nickel and salinity, particularly at higher concentrations, resulted in significantly increased biochemical responses compared to individual treatments. The findings highlight the interactive effects of nickel and salinity on the oxidative and antioxidant mechanisms in date palm plants. This study contributes to our understanding of plant responses to abiotic stressors and provides insights for optimizing date palm cultivation under challenging environmental conditions.

Keywords: Antioxidants, hydrogen peroxide, Malondialdehyde, Membrane stability index, Peroxidase, Proline, Superoxide dismutase.

## Introduction

Date palm (*Phoenix dactylifera* L.) is an economically significant crop cultivated in arid and semiarid regions worldwide due to its nutritional value and commercial importance (Al-Alawi *et al.*, 2017; Al-Aradi *et al.*, 2020).

However, date palm growth and productivity are often hindered by various abiotic stresses, including salinity and heavy metal contamination (Akensous *et al.*, 2022). Among heavy metals, nickel (Ni) is known to adversely affect plant physiology and biochemical processes (Kastori et al., 2022). Additionally, salinity, primarily caused by sodium chloride excessive (NaCl) concentrations in soil and irrigation water, poses a significant threat to date palm al., cultivation (Dghaim et 2021). Understanding the interactive effects of Ni and salinity on date palm biochemical characteristics is crucial for developing effective strategies to mitigate their adverse impacts. Previous studies e shown that Ni and salinity can induce oxidative stress in plants, leading to the accumulation of reactive oxygen species (ROS) and lipid peroxidation (Shahzad et al., 2018; Hasanuzzaman & Fujita, 2022). Antioxidant enzymes, such as peroxidase (POD) and superoxide dismutase (SOD), play a crucial role in scavenging ROS and maintaining redox homeostasis (Gill & Tuteja, 2010). Proline, an important osmolyte, acts as an antioxidant and osmoprotectant under stress conditions (Mahdi et al., 2022; Spormann et al., 2023). Despite the significance of Ni and salinity in date palm cultivation. limited research has been conducted to investigate their combined effects on the biochemical characteristics of this crop. Therefore, this study aimed to assess the impact of Ni applied alone or in combination with salinity on selected biochemical parameters, including hydrogen peroxide, malondialdehyde, membrane stability index, peroxidase activity, superoxide dismutase activity, and proline content.

# Materials & Methods

# Plant material and growth conditions

Date palm (*Phoenix dactylifera* L.) offshoots of the Hillawi cultivar were used for this study. Healthy and uniformly sized seedlings at two years old were selected and acclimatized in a greenhouse at the Department of Horticulture and Landscape, College of Agriculture, University of Basrah. The plants were grown in plastic pots filled with soil. The properties of the soil are shown in table (1).

Table (1): Characteristics of the soil used       Image: solution of the soil used					
in the experiment.					
Soil characteristic	Values	Unit			

Soil characteristic	Values	Unit
pН	7.10	
EC	5.86	ds m <sup>-1</sup>
OM	0.58	%
Sand	18.13	%
Silt	42.74	%
Clay	39.13	%
Soil texture	Silty loam	
pH water	8.22	

## Chemicals and solutions

Nickel chloride hexahydrate (NiCl<sub>2</sub>.6H<sub>2</sub>O) and sodium chloride (NaCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the study were of analytical grade. Stock solutions of NiCl<sub>2</sub>.6H<sub>2</sub>O and NaCl were prepared separately and diluted to the desired concentrations.

# Experimental design

A randomized complete block design (RCBD) with three replicates was employed in this study. The treatments included three concentrations of nickel (0, 25, and 50 mg. L<sup>-</sup> <sup>1</sup>) applied alone and in combination with three salinity levels represented by sodium chloride (NaCl) concentrations (0, 100, and 200 mM). The experiment comprised a total of 9 treatment combinations, and each treatment combination was assigned to a separate pot. The pots were arranged randomly in a controlled greenhouse.

## Nickel and salinity treatments

For the nickel treatments, nickel sulfate  $(NiCl_2.6H_2O)$  was dissolved in distilled water to prepare stock solutions of desired concentrations (25 and 50 mg.L<sup>-1</sup>). The control treatment received distilled water without nickel sulfate. The nickel solutions were applied by carefully pouring the desired volume around the base of each plant.

To apply salinity, sodium chloride (NaCl) was dissolved in distilled water to prepare stock solutions of desired concentrations (100 and 200 mM). The control treatment received distilled water without NaCl. Salinity treatments were applied by uniformly irrigating the pots with the respective NaCl solutions.

# Data collection and analysis

After 180 days of treatment application, biochemical analyses were performed on the leaf samples. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content was determined following the method described by Velikova et al. (2000). Malondialdehyde (MDA) content was measured according to Heath & Packer (1968). Membrane stability index (MSI) was determined using the electrolyte leakage method (Singh et al., 2008). Peroxidase (POD) and superoxide dismutase (SOD) activities were determined according to the protocols described by Chance & Maehly (1955) and Giannopolitis & Ries (1977), respectively. Proline content was determined using the ninhydrin reagent method (Bates et al., 1973). The experiment was set up as a completely randomized block design(CRBD), with three replicates of each measurement and data were subjected to analysis of variance (ANOVA) using appropriate statistical software SPSS-22 software (SPSS In., Chicago, IL., USA). The least significant difference (LSD) was used to evaluate significant differences between means. Statistical significance was defined as a P value of less than 0.05.

# **Results & Discussion**

# Hydrogen peroxide (H2O2) accumulation

The results table (2) obtained from the study show significant differences in the content of hydrogen peroxide  $(H_2O_2)$  of date palm Hillawi cv. leaves with different treatments. The highest H<sub>2</sub>O<sub>2</sub> concentration was observed in treatment with a nickel at 50 mg.L<sup>-1</sup>, reaching  $0.87 \mu mol.L^{-1}$ . This finding is consistent with previous studies showing elevated H<sub>2</sub>O<sub>2</sub> levels in plants exposed to heavy metals such as nickel (Khan et al., 2016; Kumar et al., 2022). Nickel is known to induce oxidative stress by generating reactive oxygen species (ROS) in plant cells (Manna et al., 2021). The elevated H<sub>2</sub>O<sub>2</sub> concentration in this study suggests that the application of nickel at 50 mg.L<sup>-1</sup> increased the production of ROS, which caused oxidative damage to date leaves. Regarding the salinity treatments, the highest H<sub>2</sub>O<sub>2</sub> content was recorded at a salinity level of 200 mM, measuring 0.90 µmol.L<sup>-1</sup>. Salinity stress has been widely reported to cause oxidative stress in plants due to the accumulation of ROS (Gill & Tuteja, 2010; Suhim et al., 2023). The observed increase in H<sub>2</sub>O<sub>2</sub> concentration in the current study is consistent with previous results in other plant species under salt stress conditions (Munns & Tester. 2008: Hasanuzzaman & Fujita, 2022). Higher saline levels are thought to have encouraged the generation of reactive oxygen species (ROS), resulting in the accumulation of H<sub>2</sub>O<sub>2</sub> in date palm leaves. Furthermore, the interaction between nickel and salinity showed a synergistic effect on H<sub>2</sub>O<sub>2</sub> accumulation. The highest H<sub>2</sub>O<sub>2</sub> content was observed at a nickel concentration of 50 mg. L<sup>-1</sup> and a salinity

level of 200 mM, measuring 0.98  $\mu$ mol.L<sup>-1</sup>. This interaction may have enhanced ROS production, surpassing the individual effects of nickel or salinity alone. Similar interactive effects of nickel and salinity on oxidative stress have been reported in other plant species (Georgiadou *et al.*, 2018; Awad *et al.*,

2019; Dghaim *et al.*, 2021).The control treatment exhibited the lowest  $H_2O_2$  content, amounting to 0.71 µmol.L<sup>-1</sup>. This reflects the normal physiological state of date palm leaves under optimal growth conditions, where ROS production is maintained at a basal level (Apel & Hirt, 2004).

Table (2): The impact of salinity and nickel on the H<sub>2</sub>O<sub>2</sub> content (µmol L<sup>-1</sup>) of Hillawi date nalm leaves.

	paini icaves.		
	Ni (mg L <sup>-1</sup> )		Mean
0	25	50	
0.71±0.01 f*	0.76±0.02 e	0.79±0.01 de	0.75±0.03C
0.80±0.01 cde	0.82±0.01 cd	0.84±0.01 c	$0.82{\pm}0.01\mathrm{B}$
0.82±0.01 cd	0.89±0.01 b	0.98±0.01 a	$0.90{\pm}0.07A$
$0.78 \pm 0.05 \mathrm{C}$	$0.82{\pm}0.05\mathrm{B}$	$0.87 {\pm} 0.07 A$	
	0.80±0.01 cde 0.82±0.01 cd	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$

\*The same letter above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

## Malondialdehyde (MDA)

The results presented in table (3) demonstrate the impact of different treatments on the malondialdehyde (MDA) content in date palm Hillawi cv. leaves, reflecting the level of lipid peroxidation and oxidative damage. The most significant MDA level was found when treated with a nickel concentration of 50 mg. L<sup>-1</sup>, reaching 2.46 nmol.g<sup>-1</sup>. This results indicates that nickel-induced stress enhances lipid peroxidation in date palm leaves, resulting in the production of MDA, a measure of oxidative damage (Manna et al., 2021). Similar findings have been reported in other plant species exposed to nickel stress (Amjad et al., 2019; Kumar et al., 2022). The elevated MDA content in the present study highlights the role of nickel as a potent inducer of oxidative stress in date palm leaves. In addition, the salinity treatment at 200 mM produced the highest MDA level, reaching 2.54 nmol g<sup>-1</sup>. Salinity stress has been shown to affect cellular homeostasis and initiate lipid peroxidation processes in plants,

MDA resulting in elevated levels (Hasanuzzaman & Fujita, 2022; Mahdi et al., 2022). The observed increase in MDA content indicates the occurrence of oxidative damage in date palm leaves under high salinity conditions. Moreover, the interaction between nickel and salinity exhibited a significant effect on the MDA content. The highest MDA content was recorded at a nickel concentration of 50 mg.L<sup>-1</sup> and a salinity level of 200 mM, reaching 2.69 nmol.g<sup>-1</sup>. This interaction between nickel and salinity likely intensified oxidative stress and lipid peroxidation processes, resulting in elevated MDA levels. Similar interactive effects of nickel and salinity on MDA accumulation have been reported in other plant species (Yusuf et al., 2012; Awad et al., 2019; Naheed et al., 2022). In contrast, the control treatment displayed the lowest MDA content, amounting to 1.96 nmol.g<sup>-1</sup>. This signifies the absence of significant oxidative damage and lipid peroxidation in date palm leaves under optimal growth conditions.

			paini icaves.		
Salinity (mM)			Ni (mg L <sup>-1</sup> )		Mean
	Samily (mvi)	0	25	50	-
	0	1.96±0.02 d*	2.23±0.02 c	2.29±0.01 bc	2.16±0.15 C
	100	2.32±0.03 bc	2.36±0.02 bc	2.41±0.01 bc	2.36±0.85 B
	200	2.42±0.02 bc	2.51±0.01 ab	2.69±0.24 a	2.54±0.13 A
	Mean	2.23±0.20 B	2.37±0.13 A	2.46±0.17 A	

Table (3): The impact of salinity and nickel on the MDA content (nmol. L<sup>-1</sup>) of Hillawi date nalm leaves.

\*The same letter above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

#### Membrane stability index (MSI)

The findings from table (4) reveal the effects of different treatments on the Membrane Stability Index (MSI) in date palm Hillawi cv. leaves, which serves as an indicator of membrane integrity and stability under various stress conditions. The lowest MSI value was observed when treated with a nickel concentration of 50 mg. L<sup>-1</sup>, measuring 75.85%. This indicates that nickel stress adversely affects membrane stability in date palm leaves, leading to increased membrane damage (Zaid et al., 2019; Suhim et al., 2023). Previous studies reported similar outcomes, highlighting the disruptive effects of nickel on membrane function in various plant species (Kumar et al., 2022; Naheed et al., 2022). Furthermore, the salinity treatment at a concentration of 200 mM resulted in the lowest MSI content, amounting to 77.69%. Salinity stress imposes osmotic and ionic imbalances on plant cells, leading to

membrane dysfunction and reduced stability (Mahdi *et al.*, 2023). The decreased MSI value in the present study indicates compromised membrane integrity under high salinity conditions. Moreover, the interaction between nickel and salinity exhibited a significant effect on MSI.

The lowest MSI content was recorded at a nickel concentration of 50 mg.L<sup>-1</sup> and a salinity level of 200 mM, measuring 75.85%. This interaction between nickel and salinity intensified membrane damage, suggesting a synergistic effect of these stressors on membrane stability. Similar interactive effects of nickel and salinity on MSI have been reported in other plant species (Amjad *et al.*, 2019; Al-Qatrani *et al.*, 2021). In contrast, the control treatment displayed the highest MSI content, reaching 86.66%. This signifies the normal membrane stability and integrity in date palm leaves under optimal growth conditions, where no stressors are present.

Table (4): The impact of salinity and nickel on the Membrane stability index (MSI) (%) ofHillawi date palm leaves.

Salinity (mM)		Ni (mg .L <sup>-1</sup> )		Mean
Salinity (mM)	0	25	50	-
0	86.66±1.02 a*	83.13±1.00 b	80.20±0.20 bc	83.33±2.89 A
100	82.22±0.10 bc	80.13±0.13 bc	77.68±0.10 cd	80.01±1.97 B
200	79.31±0.20 c	77.91±0.10 cd	75.85±0.18 d	77.69±1.51 C
Mean	82.73±3.24 A	77.91±2.32 B	75.85±1.90 C	

\*The same letter above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

## Peroxidase (POD) activity

The results from table (5) indicated the activity of the Peroxidase (POD) enzyme in date palm Hillawi cv. leaves under different treatments. POD is an important enzyme involved in various physiological and defense responses in plants. The findings indicate notable variations in POD activity in response to nickel and salinity stressors. Firstly, when treated with a nickel concentration of 50 mg L<sup>-1</sup>, the highest activity of POD enzyme was observed, reaching 25.09 U.min<sup>-1</sup>.g<sup>-1</sup>. Previous studies reported similar increases in POD activity under nickel stress, indicating its role in scavenging reactive oxygen species and maintaining cellular redox balance (Gajewska et al., 2006; Khan et al., 2016). Furthermore, the salinity treatment at concentration of 200 mM resulted in the highest activity of POD enzyme, measuring 27.61 U.min<sup>-1</sup>.g<sup>-1</sup>. Salinity stress induces the production of reactive oxygen species, leading to oxidative damage in plant cells.

The increase in POD activity in response to salt stress indicates its role in reducing

oxidative stress by catalysing hydrogen peroxide detoxification (Nahar et al., 2022). Furthermore, the combination of nickel and salinity had a significant impact on POD enzyme activity. The interaction between a nickel concentration of 50 mg.L<sup>-1</sup> and a salinity level of 200 mM produced the highest activity for this enzyme, reaching 27.90 U. min<sup>-1</sup>.g<sup>-1</sup>. This results suggested that nickel and salt have a synergistic impact on the activation of POD activity, increasing the plant's antioxidative defence systems. Other plant species have shown similar interaction effects (Amjad et al., 2019). Conversely, the control treatment displayed the lowest activity of the peroxidase (POD) enzyme, with a measurement of 21.21 U. min<sup>-1</sup>.g<sup>-1</sup>. This indicates the typical baseline activity of POD in date palm leaves under optimal growth conditions without any external stress factors. Additionally, this baseline activity serves as a reference to assess the influence of stress treatments on the enzyme's activity.

	.g ) 01 1	imawi uate pami	Icaves.	
Salinity (mM)		Ni (mg.L <sup>-1</sup> )		Mean
Samily (mvi)	0	25	50	
0	21.21±0.10 d*	21.67±0.20 cd	22.94±0.30 bcd	21.94±0.79 C
100	23.11±0.10 bcd	23.85±0.10 bc	24.44±0.20 b	23.80±0.59 B
200	27.17±0.07 a	27.77±0.20 a	27.90±0.20 a	27.61±0.36 A
Mean	23.83±2.63 B	24.43±2.68 AB	25.09±2.21 A	

Table (5): The impact of salinity and nickel on the peroxidase enzyme activity (POD) (U.min<sup>-1</sup>.g<sup>-1</sup>) of Hillawi date palm leaves.

\*The same letter above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

### Superoxide dismutase (SOD) enzyme

Table (6) shows the activity of the Superoxide Dismutase (SOD) enzyme in date palm Hillawi cv. leaves in response to different treatments. SOD is an important enzyme in the defense against oxidative stress because it catalyses the dismutation of superoxide radicals. The results show that SOD enzyme activity varies significantly in response to nickel and salt stresses. To begin, the maximum activity of SOD enzyme was seen when treated with a nickel at 50 mg L<sup>-1</sup>, reaching 3.78 U.min<sup>-1</sup>.g<sup>-1</sup> fresh weight. This suggests that the antioxidant defense mechanism in plant leaves is being activated

in response to nickel-induced oxidative stress (Gajewska *et al.*, 2006; Ryan *et al.*, 2015). Previous studies reported similar increases in

SOD activity under nickel stress, highlighting its role in scavenging superoxide radicals and maintaining cellular redox homeostasis (Zaid et al., 2019). Furthermore, the salinity treatment at 200 mM resulted in the highest SOD enzyme activity, recorded 3.77 U.min<sup>-1</sup> .g<sup>-1</sup> fresh weight. Salinity stress caused the formation of reactive oxygen species, which resulted in oxidative damage in plant cells. SOD activity increased in response to salt stress, indicating that it was involved in the detoxification of superoxide radicals, hence alleviating oxidative stress (Georgiadou et al., 2018; Dghaim et al., 2021). Moreover, the interaction between nickel and salinity exhibited a significant effect on the activity of the SOD enzyme. The highest activity was recorded in the interaction between a nickel concentration of 50 mg L<sup>-1</sup> and a salinity level of 200 mM, measuring 4.19 U.min<sup>-1</sup>.g<sup>-1</sup> fresh weight. This indicates a synergistic effect of nickel and salinity on the induction of SOD activity, enhancing the plant's antioxidant defense mechanisms. Such interactive effects have been observed in other plant species as well (Amjad *et al.*, 2019; Al-Qatrani *et al.*, 2021).

The control treatment, on the other hand, had the lowest SOD enzyme activity of 2.44 U .min<sup>-1</sup>.g<sup>-1</sup> fresh weight. This is the baseline SOD activity in date palm leaves under ideal development conditions with no stresses. It is used as a baseline for assessing the effect of stress treatments on enzyme activity.

(C.min .g ) of finative date pain reaves.				
Salinity (mM)	Concentration Ni (mg. L <sup>-1</sup> )			Mean
Salinity (mM)	0	25	50	-
0	2.44±0.20 f*	3.01±0.01 de	3.25±0.25 cd	2.90±0.39 C
100	2.74±0.20 ef	3.56±0.20 bc	3.90±0.10 ab	3.40±0.53 B
200	3.12±0.10 de	4.00±0.20 a	4.19±0.10 a	3.77±0.50 A
Mean	2.77±0.33 C	3.52±0.45 B	3.78±0.44 A	

Table (6): The impact of salinity and nickel on the Superoxide dismutase (SOD) enzyme activity (U.min<sup>-1</sup>.g<sup>-1</sup>) of Hillawi date palm leaves.

\*The same letter above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

**Proline content:** The results in table (7) showed that nickel and salinity treatments had an effect on the proline content of date palm leaves. When the plants were treated with nickel at a concentration of 50 mg.L<sup>-1</sup>, the proline content reached 4.35  $\mu$ mol.g<sup>-1</sup>. Similarly, the salinity treatment at 200 mM increased proline content significantly, with a value of 5.45  $\mu$ mol.g<sup>-1</sup>. When the plants were subjected to 50 mg.L<sup>-1</sup> nickel and 200 mM salt, the interaction between nickel and salinity produced the highest proline content, with a value of 5.87  $\mu$ mol.g<sup>-1</sup>. The control

treatment, on the other hand, had the lowest proline concentration, measuring 2.25 µmol .g<sup>-1</sup>. A well-known adaptation process in plants is the accumulation of proline in response to stress conditions, such as heavy and salt. Proline metals acts as an osmoprotectant and antioxidant, aiding in the maintenance of cellular osmotic balance and the scavenging of reactive oxygen species. The observed increase in proline level in the treated plants showed that stress response mechanisms were activated to deal with the negative effects of nickel and salt. These

findings are similar with prior research on other plant species exposed to similar stress situations (Gajewska *et al.*, 2006; Dghaim *et*  *al.*, 2021; Mahdi *et al.*, 2022; Suhim *et al.*, 2023).

Table (7): The impact of salinity and nickel on the proline content (µmol.g <sup>-1</sup> ) of Hillawi date
palm leaves.

		Pulli leuves.		
Salinity (mM)		Ni (mg.L <sup>-1</sup> )		Mean effect
Samity (mivi)	0	25	50	-
0	2.25±0.11 e*	2.43±0.20 de	2.66±0.18 de	2.45±0.43 C
100	2.98±0.10 d	4.00±0.24 c	4.51±0.10 bc	3.83±0.38 B
200	5.07±0.20 b	5.42±0.20 ab	5.87±0.33 a	5.45±0.68 A
Mean	3.43±0.48 C	3.95±0.36 B	4.35±0.51 A	

\*The same letters above bars represents no significant difference between treatments, based on the range test at  $p \le 0.05$ .

# Conclusion

The findings of the experiments revealed that nickel and salt had a substantial influence on numerous biochemical parameters of date leaves. At 50 mg.L<sup>-1</sup>, nickel treatment enhanced the accumulation of hydrogen  $(H_2O_2)$ malondialdehyde peroxide and (MDA) while decreasing the membrane stability index (MSI), suggesting oxidative stress. Increases in POD and SOD enzyme activity, as well as proline accumulation, indicate that antioxidant defense mechanisms have been activated. The same findings were obtained with salt treatment at 200 mM. The interaction of nickel and salinity enhanced these effects. The control treatment showed lower values of these parameters, indicating the absence of stress-induced responses. These findings highlight the sensitivity of date palm leaves to nickel and salinity stress and emphasize the importance of antioxidant systems and osmoprotectants in mitigating the adverse effects. Understanding these responses is crucial for developing strategies enhance date palm tolerance to and productivity in challenging environments.

# Acknowledgment

We would like to thank College of Agriculture, University of Basrah and Date

Palm Research Centre for offering facilities that supported this research.

# **Contributions of authors**

H.M. G., Suggesting the research.

A.Z.R., designing the experimental approach.

**K.M.A.**, writing the manuscript;

**A.A.S. and A.H.A.;** Practical experiments and analyzing the data.

# ORCID

H.M.G. https://orcid.org/0009-0006-1208-0468

**A.Z.R.**, https://orcid.org/0000-0002-2907-7920 **K.M.A.**, https://orcid.org/0000-0003-2018-6474

A.A.S., https://orcid.org/0000-0002-3824-3097

A.H.A., https://orcid.org/0009-0009-2497-0881

# **Conflicts of Interest**

The authors declare no conflicts of interest.

# References

- Akensous, F.-Z. & Meddich, A. (2022).
  Biostimulants as Innovative Tools to Boost Date Palm (*Phoenix dactylifera* L.) performance under Drought, Salinity, and Heavy Metal (Oid)s' Stresses: A Concise Review. *Sustainability*, 14(23), 15984.
  https://doi.org/10.3390/su142315984
- Al-Alawi, R. A., Al-Mashiqri, J. H., Al-Nadabi, J. S. M., Al-Shihi, B. I., & Baqi, Y. (2017). Date palm

tree (*Phoenix dactylifera* L.): Natural products and therapeutic options. *Frontiers in Plant Science*, *8*, 845. https://doi.org/10.3389/fpls.2017.00845

- Al-Aradi, H. J., Al-Najjar, M. A., Awad, K. M., & Abass, M. H. (2020). Combination effect between lead and salinity on anatomical structure of date palm *Phoenix dactylifera* L. seedlings. *Agrivita Journal of Agricultural Science*, 42(3). https://doi.org/10.17503/agrivita.v42i3.2511
- Al-Qatrani, M. K. J., Al Khalifa, A. A. S., & Obaid, N. A. (2021). Effect of Jasmonic acid on stimulating the growth and development of date palm callus (*Phoenix dactylifera* L.) cultivar Shukar *in vitro* under salt stress conditions. *IOP Conference Series. Earth and Environmental Science*, 923(1), 012017.https://doi.org/10.1088/1755-1315/923/1/012017
- Amjad, M., Raza, H., Murtaza, B., Abbas, G., Imran, M., Shahid, M., Naeem, M. A., Zakir, A., & Iqbal, M. M. (2019). Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (*Zea mays* L.) hybrids. *Plants, 9*(1), 5. https://doi.org/10.3390/plants9010005
- Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of *Plant Biology*, 55(1), 373–399. https://doi.org/10.1146/annurev.arplant.55.03190 3.141701
- Awad, K. M., Salih, A. M., Khalaf, Y., Suhim, A. A., & Abass, M. H. (2019). Phytotoxic and genotoxic effect of Aluminum to date palm (*Phoenix dactylifera* L.) in vitro cultures. Journal, *Genetic Engineering* & *Biotechnology*, 17, 7. https://doi.org/10.1186/s43141-019-0007-2
- Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for waterstress studies. *Plant and Soil*, 39(1), 205–207. https://doi.org/10.1007/bf00018060
- Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. Pp. 764–775. In *Methods in Enzymology*. Volume 2, Elsevier. http://doi.org/10.1016/S0076-6879(55)02300-8
- Dghaim, R., Hammami, Z., Al Ghali, R., Smail, L., & Haroun, D. (2021). The mineral composition of date palm fruits (*Phoenix dactylifera* L.) under

low to high salinity irrigation. *Molecules (Basel, Switzerland)*, 26(23), 7361. https://doi.org/10.3390/molecules26237361

- Gajewska, E., Skłodowska, M., Słaba, M., & Mazur, J. (2006). Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. *Biologia Plantarum*, 50(4), 653–659. https://doi.org/10.1007/s10535-006-0102-5
- Georgiadou, E. C., Kowalska, E., Patla, K., Kulbat, K., Smolińska, B., Leszczyńska, J., & Fotopoulos, V. (2018). Influence of heavy metals (Ni, cu, and Zn) on Nitro-oxidative stress responses, proteome regulation and allergen production in basil (*Ocimum basilicum* L.) *plants. Frontiers in Plant Science*, 9.

https://doi.org/10.3389/fpls.2018.00862

- Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, 59(2), 309–314. https://doi.org/10.1104/pp.59.2.309
- Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry,48(12),909-930. https://doi.org/10.1016/j.plaphy.2010.08.016
- Hasanuzzaman, M., & Fujita, M. (2022). Plant responses and tolerance to salt stress: Physiological and molecular interventions. *International Journal of Molecular Sciences*, 23(9), 4810. https://doi.org/10.3390/ijms23094810
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives* of Biochemistry and Biophysics, 125(1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1
- Kastori, R., R., Putnik-Delić, M., I., & Maksimović,
  I., V. (2022). Functions of nickel in higher plants:
  A review. *Acta agriculturae Serbica*, 27(53), 89-101. http://doi.org/10.5937/AASer2253089K
- Khan, M. I. R., Khan, N. A., Masood, A., Per, T. S., & Asgher, M. (2016). Hydrogen peroxide alleviates nickel-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in mustard. *Frontiers in Plant Science*, 7, 44. https://doi.org/10.3389/fpls.2016.00044

- Kumar, S., Wang, M., Liu, Y., Fahad, S., Qayyum, A., Jadoon, S. A., Chen, Y., & Zhu, G. (2022). Nickel toxicity alters growth patterns and induces oxidative stress response in sweetpotato. *Frontiers in Plant Science*, 13, 1054924. https://doi.org/10.3389/fpls.2022.1054924
- Mahdi, A. S., Abd, A. M., & Awad, K. M. (2022). Effect of foliar application of nano-selenium on the anatomical characteristics of date palm *Phoenix dactylifera* L. barhi cultivar under salt stress. *Basrah Journal of Agricultural Sciences*, 35(2), 313–325. https://doi.org/10.37077/25200860.2022.35.2.24
- Mahdi, A. S., Abd, A. M., & Awad, K. M. (2023). The role of nano-selenium in alleviating the effects of salt stress in date palm trees (*Phoenix dactylifera* L.): A Fourier transform infrared (FTIR) spectroscopy study. *BioNanoScience*, *13*(1), 74–80. https://doi.org/10.1007/s12668-022-01046-1
- Manna, I., Sahoo, S., & Bandyopadhyay, M. (2021).
  Effect of engineered nickel oxide nanoparticle on reactive oxygen species-nitric oxide interplay in the roots of *Allium cepa* L. *Frontiers in Plant Science*, *12*, 586509.
  https://doi.org/10.3389/fpls.2021.586509
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of *Plant Biology*, 59(1), 651–681. https://doi.org/10.1146/annurev.arplant.59.03260 7.092911
- Nahar, K., Rhaman, M. S., Parvin, K., Bardhan, K., Marques, D. N., García-Caparrós, P., & Hasanuzzaman, M. (2022). Arsenic-induced oxidative stress and antioxidant defense in plants. *Stresses*, 2(2), 179–209. https://doi.org/10.3390/stresses2020013
- Naheed, N., Abbas, G., Naeem, M. A., Hussain, M., Shabbir, R., Alamri, S., Siddiqui, M. H., & Mumtaz, M. Z. (2022). Nickel tolerance and phytoremediation potential of quinoa are modulated under salinity: multivariate comparison of physiological and biochemical Environmental attributes. Geochemistry and 1409-1424. Health, 44(4), https://doi.org/10.1007/s10653-021-01165-w
- Ryan, K. C., Guce, A. I., Johnson, O. E., Brunold, T. C., Cabelli, D. E., Garman, S. C., & Maroney, M.

J. (2015). Nickel superoxide dismutase: structural and functional roles of His1 and its H-bonding network. *Biochemistry*, 54(4), 1016–1027. https://doi.org/10.1021/bi501258u

- Shahzad, B., Tanveer, M., Rehman, A., Cheema, S. A., Fahad, S., Rehman, S., & Sharma, A. (2018). Nickel; whether toxic or essential for plants and environment A review. *Plant Physiology and Biochemistry*, 132, 641–651. https://doi.org/10.1016/j.plaphy.2018.10.014
- Singh, A., Kumar, J., & Kumar, P. (2008). Effects of plant growth regulators and sucrose on postharvest physiology, membrane stability and vase life of cut spikes of gladiolus. *Plant Growth Regulation*, 55(3), 221–229. https://doi.org/10.1007/s10725-008-9278-3
- Spormann, S., Nadais, P., Sousa, F., Pinto, M., Martins, M., Sousa, B., Fidalgo, F., & Soares, C. (2023). Accumulation of Proline in Plants under Contaminated Soils—Are We on the Same Page? *Antioxidants*, 12(3), 666. https://doi.org/10.3390/antiox12030666
- Suhim, A. A. ., Awad, K. M. ., Jaffer, O. N. ., & Abass, M. H. (2023). The impact of salicylic and jasmonic acid in mitigating salinity stress on date palm *Phoenix dactylifera* L. Barhi Cv. *Basrah Journal of Agricultural Sciences*, 36(1), 120-130. https://doi.org/10.37077/25200860.2023.36.1.10
- Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Science: An *International Journal of Experimental Plant Biology*, 151(1), 59–66. https://doi.org/10.1016/s0168-9452(99)00197-1
- Yusuf, M., Fariduddin, Q., Varshney, P., & Ahmad, A. (2012). Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. *Environmental Science and Pollution Research International, 19*(1), 8–18. https://doi.org/10.1007/s11356-011-0531-3
- Zaid, A., Mohammad, F., Wani, S. H., & Siddique, K. M. H. (2019). Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. *Ecotoxicology and Environmental Safety, 180*, 575–587.

https://doi.org/10.1016/j.ecoenv.2019.05.042

الاستجابة الكيموحيوية لنخيل التمر . Phoenix dactylifera L لاجهاد الملوحة والنيكل المشترك

حسنين محمد غباش<sup>1</sup> وإحمد زاير رسن<sup>1</sup> وخير الله موسى عواد<sup>2</sup> وعقيل عبود سهيم<sup>1</sup> وعمار حسن عبد الامير<sup>2</sup> <sup>1</sup>قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة البصرة، العراق <sup>2</sup>مركز ابحاث النخيل، جامعة البصرة، العراق

**المستخلص**: تمت دراسة تأثير النيكل (Ni) بتراكيز 0 و 25 و 50 ملغم.لتر<sup>-1</sup>، بشكل مفرد او متداخل مع الملوحة (الممثلة بتراكيز 0 و 100 و 200 مليمول من كلوريد الصوديوم)، على الصفات الكيموحيوية لنخيل التمر. تم تقييم عدة مؤشرات بينها بيروكسيد الهيدروجين (H<sub>2</sub>O<sub>2</sub>) ومالونديالدهايد (MDA) ومؤشر ثباتيه الاغشية (MSI) وفعالية انزيمي بيروكسيديز (POD) ومعتوى البرولين. أظهرت النتائج تأثيرات معنوية للنيكل والملوحة على الصفات الكيموحيوية المدروسة. (POD) ومالونديالدهايد (MDA) ومؤشر ثباتيه الاغشية (MSI) وفعالية انزيمي بيروكسيديز (POD) والسوبر أكميد ديسموتيز (SOD) ومحتوى البرولين. أظهرت النتائج تأثيرات معنوية للنيكل والملوحة على الصفات الكيموحيوية MDA والسوبر أكميد ديسموتيز (SOD) ومحتوى البرولين. أظهرت النتائج تأثيرات معنوية للنيكل والملوحة على الصفات الكيموحيوية MDA والمورف. في من معانوية النيكل والملوحة على الصفات الكيموحيوية MDA والمورف. في من معانوية النيكل والملوحة على الصفات الكيموحيوية MOA (MAC) عومول. لتر<sup>-1</sup>) و MDA (2.00) دائومول. غم<sup>-1</sup>)، في حين انخفض مؤشر ثبات الاغشية ISM (75.85%). علاوة على ذلك، زاد من فعالية ODA (2.00) وحدة . في 2.00 (2.00) وحدة . في 2.00 (2.00) معرومول. لتر<sup>-1</sup>)</sup> و ADA (2.00) دوحة . في 200 (2.00) معانوي المولين (2.00) معنوي من مستويات 200 (3.00) ميكرومول. لتر<sup>-1</sup>) و MDA (2.00) مولول. غم<sup>-1</sup>)، بالإضافة إلى زيادة محتوى البرولين (2.00) معكرومول. لتر<sup>-1</sup>) و ADA (2.00) معرومول. غم<sup>-1</sup>). زادت الملوحة بتركيز 200 مليمول بشكل معنوي من مستويات 20.00 (4.00) معكرومول. لتر<sup>-1</sup>) و ADA (2.00) معرومول. غم<sup>-1</sup>). زادت الملوحة بتركيز العالية المول بشكل معنوي من مستويات 20.00 (4.00) معكرومول. لتر<sup>-1</sup>) و ADA (2.00) مولور فعالي الومول. غم<sup>-1</sup>). واندفضت ISO (3.00) موال مول. موالي الموحة بتركيز العالية الي إلى وزاد فعالية ADA (2.00) معرومول. فم<sup>-1</sup>)</sup>. والمول فم<sup>-1</sup> مول فم<sup>-1</sup>). والموحة بلي وزادة محتوى البرولين (4.50 معرومول. فم<sup>-1</sup>). بالإضافة إلى ذلك، أدت معاملة التداخل وحدة.دقيقة<sup>-1</sup>. غم<sup>-1</sup>) والموحة، عام<sup>-1</sup> مول في زادة محتوى البروليين (4.50 معرومول. فم<sup>-1</sup>). بالإضافة إلى ذلك، أدت معاملة التداخل وحدة.دقيقة<sup>-1</sup>. في مارة المول في زيادة محتوى البروليين (4.50 معكرومول. فم<sup>-1</sup>). بالإضافة إلى ذلك، أدل معام (2.00) مول في مكرومول.

الكلمات المفتاحية: مضادات الاكسدة، بيروكسيد الهيدروجين، مالونديالديهايد، مؤشر ثباتية الاغشية، بيروكسيديز، برولين، سوبر اوكسيد ديسميوتيز.