University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 543-553

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/utjagr.v13i1.323

Effect of Melatonin on Some Indicators of Vegetative and Biochemical Growth in *Washingtonia filifera* Trees Under Salinity Stress

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Abstract

The experiment was conducted at the Agricultural Research Station of the College of Agriculture, University of Basrah, during the 2023-2024 growing season. The objective was to study the effect of different levels of irrigation water salinity (2, 6, and 12 dS.m^{-1}) and melatonin treatment (0, 50, and 100 mg.L^{-1}) on the vegetative and biochemical growth indicators of Washingtonia filifera seedlings. The experiment was carried out as a factorial experiment with three replicates, following the Randomized Complete Block Design (RCBD). The results were analyzed using the GenStat program, and means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level. The results showed a significant decrease in growth with increasing salinity levels, with the 2 dS.m^{-1} treatment outperforming in most traits, including plant height, leaf number, leaf area, and chlorophyll and carbohydrate content, and a significant decrease in proline content. Additionally, the results indicated that plants sprayed with 100 mg.L^{-1} of melatonin outperformed those with other treatments in most of the studied traits. Regarding the interaction effect between the study factors, there was a significant impact on most of the studied traits.

Keywords: Washingtonia filifera, salinity, melatonin, Biochemical

*Part of PhD dissertation of the first author

I. Introduction

Washingtonia filifera is one of the ornamental palm species belonging to the Arecaceae family, which holds significant aesthetic value in streets, parks, roads, and gardens. Its native origin is the United States of America, and there are two types: *Washingtonia filifera* and *Washingtonia robusta*. These species have been introduced to most Arab regions, including Iraq (Johnson, 1998). The Washingtonia palm is a monocotyledonous, bisexual, and monoecious tree, meaning it bears both male and female flowers on the same tree. It produces small, spherical fruits that are inedible (Al-Batal, 2005). Washingtonia palms are evergreen trees that grow vertically, with heights ranging from 15 to 30 meters. They have a large crown, a tall, upright, and thick trunk that swells at the base and is covered with leaf bases. Its leaves are fan-shaped, and the edges of the leaflets dangle in the form of large threads. It grows excellently under local environmental conditions and can also withstand unfavorable environmental conditions such as high temperatures, as well as being tolerant to wind, salinity, and drought (Askar, 2011).
Plants, being stationary organisms, are exposed to a variety of environmental stresses such as heat stress (both low and high), ionic stress, salinity, and drought stress. These environmental stresses cause harmful effects on plant growth and development, and approximately 90% of agricultural lands are subjected to one of these environmental stresses (Altaf et al., 2023). Salinity stress is one of the most common abiotic environmental stresses that plants face, and it negatively affects the growth of plants, including Washingtonia trees. Salinity stress can cause nutrient imbalances, ion toxicity, and oxidative stress, leading to reduced growth and fruit quality (Zhou-Tsang et al., 2021). Due to the harmful effects these

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stresses have on plant growth and productivity, it has become necessary to employ certain methods to increase plants' tolerance to stresses and reduce their damaging effects. One of these methods includes the use of plant hormones such as melatonin, which helps protect plants from oxidative stress caused by salinity stress (Altaf et al., 2020).

Melatonin is a natural compound that plays a role in reducing the harmful effects caused by salinity stress. It has a similar effect to auxin in plants, contributing to seed germination and plant growth by enhancing photosynthesis efficiency and osmotic regulation within the plant (Jan et al., 2022). It is a small molecule that acts as a powerful antioxidant, promoting plants' resistance to stress caused by various environmental factors (Hoque et al., 2021). Studies have indicated that Washingtonia trees can tolerate salinity stress. In a study conducted by Simón et al. (2010), the response of two ornamental tree species, *Chamaerops humilis L.* and *Washingtonia robusta H. Wendl*, was compared using four concentrations of salts (2, 4, 6, 8 dS m^{-1}). The results showed a significant effect of salinity on *W. robusta* compared to *C. humilis*, with a significant decrease in plant height and leaf area at a concentration of 8 dS m^{-1}, and fewer leaves in the treatments with salt concentrations of 6 or 8 dS m^{-1}. In another study conducted by Daroui et al. (2013) on *Washingtonia filifera* trees in vitro under the influence of salinity, seeds were planted in a solid medium (agar-agar: 7g L^{-1}) containing NaCl (0, 1, 3, and 5 g L^{-1}). The results showed that salinity stress had a significant effect only on stem height, primary root length, stem and root biomass. In a study by Kamiab (2020) on pistachio seedlings (*Pistacia vera cv. Badami-Zarand*) grown under different levels of salinity (25, 50, 100, and 150 mmol L^{-1}) and treated with different levels of melatonin (0, 25, 50, 75, 100, 125, and 150 µmol L^{-1}) twice to mitigate the harmful effects of salinity, the results indicated that the level of 100 µmol/L melatonin provided the best protective effect against salinity stress at 150 mmol/L. Additionally, melatonin significantly contributed to alleviating the harmful effects of salinity on root and stem growth, chlorophyll content, as well as increasing the activity of antioxidant enzymes and proline content.

The study conducted by Hu et al. (2022) aimed at investigating the response of *Citrus (Poncirus) trifoliata L.* seedlings to external melatonin treatments (0, 50, 100, 150 µmol L^{-1}) under salinity stress (0 and 150 µmol L^{-1} NaCl) for 4 weeks. The results showed that spraying with 100 µmol/L melatonin produced the best results, leading to improved plant height, increased dry matter, and enhanced photosynthesis rates. this study was conducted to determine the effect of melatonin and salinity in irrigation water on the growth of Washingtonia trees, both individually and in combination.

II. Materials and Methods

The experiment was conducted in the greenhouse affiliated with the Department of Horticulture and Landscape Engineering at the College of Agriculture, University of Basra, from October 1, 2023, to May 30, 2024. The objective was to study the effect of melatonin on Washingtonia trees under salinity stress. The Washingtonia trees used in the experiment were obtained from a private nursery in Basra and were one and a half years old. The seedlings were transplanted into pots with a diameter of 30 cm, containing a growth medium composed of 2 parts loam soil and 1 part peat moss. Several random soil samples were taken before planting, and some chemical and physical properties of the pot soil were analyzed (Table 1), as well as the chemical properties of the peat moss used in planting (Table 2) and the irrigation water used in the experiment, which was tested at the central laboratory of the Directorate of Agriculture in thi Qar (Table 3).

Table (1) Some physical and chemical properties of the soil used in the experiment

Type of Analysis	Value	Unit of Measurement
Soil texture	Sandy loam	
Sand	785.5	gk . g^{-1}
Silt	61.48	
Clay	153	
Soil reaction (pH)	7.34	

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EC (Electrical Conductivity)	1.4	ds. m^{-1}
Organic matter	0.043	gk . g^{-1}

Table (2) Some chemical properties of the peat moss used in planting

Type of Analysis	Value	Unit of Measurement
pH (Soil reaction)	5.7 – 6.5	--
EC (Electrical Conductivity)	0.7 – 0.9	dS. m^{-1}
N (Nitrogen)	70 – 160	mg . m^{-1}
Available P_2O_5 (Phosphorus)	70 – 180	mg . m^{-1}
Available K_2O (Potassium)	80 – 190	mg . m^{-1}

Table (3) Characteristics of the irrigation water used in the experiment at different salinity levels

Property	Unit of Measurement	2 dS. m^{-1}	6 dS. m^{-1}	12 dS. m^{-1}
SAR	mmol	2.57	6.86	13.2
E.C (Electrical Conductivity)	dS. m^{-1}	2.2	6.4	12.3
pH		7.23	7.86	7.88
HCO_3^-	mg/L	1.54	3.82	4.92
CO_3^{2-}		0	0	0
K^+		0.131	0.463	0.896
Na^+		5.112	12.304	21.717
Ca^{2+}		3.890	9.046	16.218
SO_4^{2-}		5.019	10.714	18.045
Cl^-		26.958	55.048	73.802
Mg^{2+}		6.527	11.180	20.663

Experimental Design and Treatments

The experiment was conducted as a factorial experiment following a Randomized Complete Block Design (RCBD) with three replicates. It included two factors:

- The first factor was salinity treatments at three levels (2, 6, and 12 dS. m^{-1}), applied through weekly irrigation.
- The second factor was melatonin spraying at three levels (0, 50, and 100 mg. L^{-1}), with one spray every 15 days until complete wetting.

Thus, the total number of treatments was 3 x 3 = 9 treatments, and the total number of experimental units was 3 x 3 x 3 = 27 units, with each unit containing 3 seedlings, bringing the total number of seedlings to 81.

The data were statistically analyzed using the GenStat statistical software (dec-2008), and the Least Significant Difference (L.S.D) test at a probability level of 0.05 was used to compare the means (Al-Rawi and Khalaf Allah, 2000).

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Measured Parameters: Measurements were taken on 1/4/2024 as follows:

1. **Plant Height (cm plant^{-1}):** Plant height (cm) was measured using a measuring tape from the soil surface to the growing tip of the plant. The average height for each treatment was calculated by measuring the height of all seedlings in each replicate.
2. **Number of Leaves (Leaves plant^{-1}):** The total number of leaves per seedling was counted, and the average number of leaves for each experimental unit and treatment was calculated.
3. **Leaf Area (cm^2 plant^{-1}):** Leaf area was measured using the ImageJ software installed on a computer, following the method described by Sadik et al. (2011).
4. **Total Chlorophyll Content in Leaves (mg . 100 g-1 Fresh Weight):** Total chlorophyll content in the leaves was estimated using the method described by Goodwin (1976).
5. **Total Soluble Carbohydrates in Leaves (mg 100 g^{-1} dry weight):** The total soluble carbohydrates in the leaves were determined using the modified phenol-sulfuric acid colorimetric method described by Dubois et al. (1956).
6. **Free Proline Content (µg per g Dry Matter):** Proline content was estimated following the method of Troll and Lindslay (1955).

III. Results and Discussion

Plant Height (cm plant^{-1})

The results of the statistical analysis in Table (4) showed that both factors of the study had a significant effect on plant height. The seedlings irrigated with water at a salinity level of 2 dS. m^{-1} had the highest average plant height, reaching 60.90 cm, compared to those irrigated at 12 dS. m^{-1}, which showed the lowest average plant height of 39.54 cm. The same table also indicates that the plants sprayed with melatonin at a concentration of 100 mg. L^{-1} had the highest average plant height of 62.97 cm, compared to the other treatments.

Furthermore, the interaction between the two factors had a significant effect on plant height. The combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity produced the highest plant height of 71.67 cm, while the combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity resulted in the lowest plant height of 28.30 cm.

Table (4) The effect of melatonin spraying on Washingtonia palms growing under salinity stress on plant height (cm)

Melatonin (mg. L^{-1})	Salinity (dS. m^{-1})			Average Melatonin
	2	6	12	
0	49.93	39.67	28.30	39.30
50	60.20	58.33	35.17	51.23
100	71.67	62.07	55.17	62.97
Average Salinity	60.60	53.36	39.54	
L.S.D	Interaction		Salinity	Melatonin
	3.608		2.083	2.083

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Number of Leaves (Leaves .Plant^{-1})

The results in Table (5) indicate that both study factors significantly affected the number of leaves. The table shows that plants irrigated with water at a salinity level of 12 dS. m^{-1} had the lowest average number of leaves, at 5.867 leaves per seedling, while those irrigated with 2 dS. m^{-1} salinity had the highest average, at 8.907 leaves per seedling. Melatonin spraying also had a significant effect on this trait. The plants sprayed with melatonin at a concentration of 100 mg. L^{-1} had the highest average number of leaves, at 7.929 leaves per seedling, compared to the control treatment, which had the lowest average of 6.311 leaves per seedling. Moreover, the interaction between the two factors showed a significant effect on the number of leaves. The combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity resulted in the highest average number of leaves, at 9.589 leaves per seedling, compared to the combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity, which had the lowest average of 4.632 leaves per seedling.

Table (5) The effect of spraying melatonin on Washingtonia trees growing under salt stress on the number of leaves (leaves . plant^{-1}).

Melatonin (mg. L^{-1})	Salinity (dS. m^{-1})			Average Melatonin
	2	6	12	
0	8.967	5.333	4.632	6.311
50	8.167	6.000	5.857	6.674
100	9.589	7.088	7.111	7.929
Average Salinity	8.907	6.140	5.867	
L.S.D	Interaction		Salinity	Melatonin
	0.4207		0.2429	0.2429

Leaf Area (cm² plant $^{-1}$)

The results in Table (6) indicate that both melatonin spraying and saline irrigation had a significant effect on leaf area. The table shows that the highest average leaf area was observed in plants irrigated with water at a salinity level of 2 dS. m^{-1}, reaching 159.09 cm², compared to the lowest average leaf area of 76.38 cm² in plants irrigated with 12 dS. m^{-1} salinity. Additionally, melatonin spraying at a concentration of 100 mg. L^{-1} resulted in the highest average leaf area of 140.07 cm², while the control treatment had the lowest average leaf area of 102.69 cm².

The interaction between melatonin and water salinity also had a significant effect on leaf area. The combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity resulted in the highest average leaf area of 173.60 cm², compared to the combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity, which had the lowest average leaf area of 76.38 cm².

Table (6) The effect of spraying melatonin on Washingtonia trees growing under salt stress on leaf area (cm² plant $^{-1}$)

Melatonin (mg. L^{-1})	Salinity (dS. m^{-1})			Average Melatonin
	2	6	12	
0	143.34	88.34	76.38	102.69
50	160.33	111.88	102.25	124.82

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https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/utjagr.v13i1.323

100	173.60	120.46	126.14	140.07
Average Salinity	159.09	106.89	101.59	
L.S.D	Interaction		Salinity	Melatonin
	5.374		3.102	3.102

Chlorophyll Content in Leaves (mg . 100 g^{-1} Fresh Weight)

The statistical analysis results presented in Table (7) indicate that both study factors significantly affected the chlorophyll content in the leaves. Salinity had a significant impact on leaf chlorophyll content, with plants irrigated with water at a salinity level of 2 dS. m^{-1} showing the highest average chlorophyll content of 12.590 mg . 100 g^{-1} Fresh Weight. In contrast, plants irrigated with water at 12 dS. m^{-1} had the lowest average chlorophyll content of 6.444 mg . 100 g^{-1} Fresh Weight. Regarding melatonin, the treatment with 100 mg. L^{-1} resulted in the highest average chlorophyll content of 10.466 mg . 100 g^{-1} Fresh Weight, while the control treatment had the lowest average of 6.843 mg . 100 g^{-1} Fresh Weight.

The interaction between melatonin and water salinity also had a significant effect on chlorophyll content. The combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity resulted in the highest average chlorophyll content of 15.290 mg . 100 g^{-1} Fresh Weight, compared to the combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity, which had the lowest average of 4.732 mg . 100 g^{-1} Fresh Weight.

Table (7) The effect of spraying melatonin on Washingtonia trees growing under salt stress on leaf chlorophyll content (mg/100 g fresh weight)

Melatonin (mg. L^{-1})	Salinity (dS. m^{-1})			Average Melatonin
	2	6	12	
0	10.109	5.687	4.732	6.843
50	12.369	9.209	6.907	9.495
100	15.290	8.414	7.693	10.466
Average Salinity	12.590	7.770	6.444	6.843
L.S.D	Interaction		Salinity	Melatonin
	0.4912		0.2836	0.2836

Carbohydrate Content in Leaves (mg 100 g^{-1} dry weight)

The statistical analysis results shown in Table (8) indicate that both study factors significantly affected the carbohydrate content in the leaves. Salinity had a significant impact on leaf carbohydrate content, with plants irrigated with water at a salinity level of 2 dS. m^{-1} showing the highest average carbohydrate content of 4.206 mg 100 g^{-1} dry weight. In contrast, plants irrigated with water at 12 dS. m^{-1} had the lowest average carbohydrate content of 2.043 mg 100 g^{-1} dry weight. Regarding melatonin, the treatment with 100 mg. L^{-1} resulted in the highest average carbohydrate content of 3.247 mg 100 g^{-1} dry weight, while the control treatment had the lowest average of 2.464 mg 100 g^{-1} dry weight.

The interaction between melatonin and water salinity also showed a significant effect on carbohydrate content. The combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity resulted in the highest average carbohydrate content of 5.020 mg 100 g^{-1} dry weight, compared to the combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity, which had the lowest average of 1.898 mg 100 g^{-1} dry weight.

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Table (8) The effect of spraying melatonin on Washingtonia trees growing under salt stress on leaf carbohydrate content (mg100 g^{-1} dry weight)

Melatonin (mg. L⁻¹)	Salinity (dS. m⁻¹)			Average Melatonin
	2	6	12	
0	3.347	2.149	1.898	2.464
50	4.251	2.398	2.081	2.910
100	5.020	2.569	2.151	3.247
Average Salinity	4.206	2.372	2.043	
L.S.D	Interaction		Salinity	Melatonin
	0.2449		0.1414	0.1414

Proline Content in Leaves (µg g^{-1} dry weight)

Table (9) illustrates a significant effect of both study factors on the proline content in the leaves. It is evident from the table that plants irrigated with water at a salinity of 12 dS. m^{-1} showed a significantly higher proline content, averaging 13.19 µg g^{-1} dry weight, compared to plants irrigated with water at 2 dS. m^{-1}, which had an average of 5.98 µg g^{-1} dry weight. Additionally, there was a significant difference in proline content based on melatonin concentrations. Plants sprayed with 0 mg. L^{-1} melatonin had a higher proline content, averaging 12.50 µg g^{-1} dry weight, compared to plants sprayed with 100 mg. L^{-1} melatonin, which had an average of 8.11 µg g^{-1} dry weight.

The interaction between melatonin and water salinity also showed a significant effect. The combination of 0 mg. L^{-1} melatonin + 12 dS. m^{-1} salinity resulted in the highest proline content, averaging 16.62 µg g^{-1} dry weight, compared to the combination of 100 mg. L^{-1} melatonin + 2 dS. m^{-1} salinity, which had the lowest average of 4.45 µg g^{-1} dry weight.

Table (9) The effect of spraying melatonin on Washingtonia trees growing under salt stress on leaf proline content (µg g^{-1} dry weight)

Melatonin (mg. L⁻¹)	Salinity (dS. m⁻¹)			Average Melatonin
	2	6	12	
0	7.20	13.68	16.62	12.50
50	6.28	11.20	12.35	9.94
100	4.45	9.27	10.62	8.11
Average Salinity	5.98	11.38	13.19	
L.S.D	Interaction		Salinity	Melatonin
	0.900		0.520	0.520

The results presented in Tables (4, 5, and 6) indicate a significant reduction in vegetative growth parameters, such as plant height, number of leaves, and leaf area, with increasing concentrations of saline irrigation water. This reduction may be attributed to the effects of water salinity on physiological processes in plants, including stomatal behavior, water status, and ionic balance, as well as carbon assimilation efficiency (Munns, 2002). Salinity inhibits plant growth through two main mechanisms: it

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either impedes the provision of essential cellular components needed for normal cellular functions or disrupts the function of cellular components, rendering the cells incapable of performing their functions (Parihar et al., 2015). Growth inhibition may also result from the suppression of photosynthesis due to osmotic stress, which reduces water uptake by the plant, or it could be due to diminished transport of growth hormones and nutrients from the roots to other parts of the plant because of reduced water absorption (Tuteja, 2005). Consequently, this leads to an increase in osmotic stress and a decrease in water potential due to the elevated concentration of salts in plant tissues as a result of irrigation with high-salinity water (Ashraf and Foolad, 2005).

Moreover, a decrease in water potential results in reduced cell turgor, which adversely affects leaf area. Reduced cell turgor leads to less cell elongation, and consequently, a decrease in leaf area (Table 6) (Al-Shehawi, 2006). The observed reductions in plant height (Table 4), number of leaves (Table 5), and leaf area (Table 6) may be due to the direct effects of irrigation water salinity, which cause ionic imbalance, enzyme activity inhibition, and reduced chlorophyll content in the leaves (Table 7), as well as decreased carbohydrate content (Table 8), proteins, and plant hormones (Nikee et al., 2014). These findings are consistent with those reported by Salem et al. (2020), Jawad (2022), and Ekbic and Yorulmaz (2023), who observed reductions in plant height and leaf area due to irrigation with saline water compared to water with low salinity or fresh water.

Despite the significant reduction in the vegetative growth indicators of the studied plants due to the effect of salt stress, melatonin spraying was used to mitigate this decline as much as possible. A concentration of 100 mg/L of melatonin resulted in a significant increase in plant height (Table 4), the number of leaves (Table 5), and leaf area (Table 6). The reason for the increase in these indicators is attributed to the role of melatonin, which is similar to the role of the growth regulator auxin (IAA) indole-3-acetic acid. It enhances cell division and elongation, stimulates photosynthesis, delays leaf senescence, and helps maintain cellular membranes and chlorophyll pigments from degradation under saline stress conditions (Gong et al., 2017, and Zhang et al., 2015). Another possible reason for the improvement in vegetative growth indicators could be melatonin's role in reducing salt stress by scavenging reactive oxygen species (ROS) through the stimulation of enzymatic antioxidants such as Peroxidase (POD), Superoxide dismutase (SOD), and Catalase (CAT), as well as non-enzymatic antioxidants like glutathione, ascorbic acid, and flavonoids, thereby protecting enzymes from oxidation. Additionally, it enhances the efficiency of the electron transport chain and reduces its leakage in the mitochondria, thereby lowering the ROS content within the plant (Gao et al., 2022). Some studies have also indicated that spraying plants with melatonin increases carbon assimilation efficiency while inhibiting ROS activity, leading to an increase in plant height, number of leaves, and leaf area. Moreover, applying melatonin as a foliar spray encourages root growth, as resistance to salt stress is directly related to root growth strength, allowing plants to maintain water balance, increase chlorophyll production, accumulate carbohydrates, reduce growth inhibition, and improve carbon assimilation (Arnao and Ruiz Hernández, 2019). These results align with the findings of (Al-Mousawi, 2022) and (Hu et al., 2022).

The statistical analysis results in Tables 7 and 8 show that irrigation water salinity led to a reduction in leaf chlorophyll and carbohydrate content. This may be due to the fact that high salt concentrations reduce vital cellular activities such as respiration and photosynthesis, as a result of the osmotic effect caused by salt accumulation in the soil, which limits the absorption of water and nutrients by the plants (Golezani et al., 2011).

The reduction in leaf chlorophyll content (Table 7) can also be attributed to salt accumulation in the soil, which reduces the size of chloroplasts. Additionally, salinity accelerates chlorophyll degradation and slows down its formation by increasing the activity of the chlorophyll-degrading enzyme chlorophyllase (Sevengor et al., 2011). The decline in leaf chlorophyll content may also be due to the scarcity of essential elements for chlorophyll synthesis, such as water, carbohydrates, and minerals, as well as an increase in the plant hormone ABA, which accelerates chlorophyll degradation. Furthermore, salinity damages membranes, causing direct damage to photosynthetic pigments (Cha-Um et al., 2010).

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The plant's carbohydrate content is one of the important functional indicators that reflect the plant's growth and activity. The amount of carbohydrates in a plant exposed to stress reflects the extent of water stress caused by irrigation water salinity (Al-Wahaibi, 2009). As shown in Table 8, there was a decrease in carbohydrate levels in the leaves due to the increase in salt levels in plant tissues, which causes an increase in osmotic pressure and a decrease in water potential. This reduction in water potential leads to stomatal closure and an imbalance in gas exchange, which hinders photosynthesis (Ashraf and Foolad, 2005). The decline in carbohydrate content in the leaves at high salt levels may be due to the role of salt stress in reducing the number of leaves (Table 5), leaf area (Table 6), and chlorophyll content (Table 7). These results are similar to the findings of (Jawad, 2022, and Al-Harmoush, 2023).

The results show an increase in leaf proline content (Table 9) when irrigated with highly saline water. This increase in proline content is attributed to it being a defensive mechanism employed by the plant to eliminate accumulated ammonia in plant cells (Al-Hamishi, 2006). The accumulation of proline within plant cells enhances their ability to draw water from the saline medium, thereby reducing the toxicity of ions associated with the damage caused by high salinity (Chinnusamy et al., 2005). These results are consistent with the findings of (Mirás-Avalos and Intrigliolo, 2017, and Jawad, 2022).

The reduction in leaf chlorophyll and carbohydrate content (Tables 7 and 8) and the increase in proline content (Table 9) were addressed by spraying the plant with melatonin. The 100 mg/L level showed a significant increase in leaf chlorophyll and carbohydrate content and a reduction in proline. Melatonin acts as an antioxidant against various biotic stresses, including salt stress, by protecting plants from free radicals. In addition, melatonin serves as a plant growth regulator with effects similar to IAA, enhancing plant growth and mitigating the negative impact of sodium chloride (NaCl) on roots and the entire plant (Arnao and Ruiz Hernández, 2019). These results are in line with the findings of (Zhong et al., 2020, and Al-Mousawi, 2022).

IV. Conclusions:

1. Irrigating Washingtonia seedlings with water of different salinity levels resulted in a significant decrease in most of the studied traits as irrigation water salinity levels increased.

2. Spraying Washingtonia seedlings with melatonin increased the tolerance of seedlings irrigated with saline water and reduced the negative effects of these salts. This positively reflected in the improvement of vegetative and biochemical indicators of the plant, with the best treatment being 100 mg/L melatonin.

3. The interaction between the study factors showed a significant effect on most of the studied traits, with the interaction treatment (2 dS/m and 100 mg/L) yielding the best results.

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