Available online at http://bajas.edu.iq
https://doi.org/10.37077/25200860.2023.36.1.10
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 36(1), 120-130, 2023 E-ISSN: 2520-0860

The Impact of Salicylic and Jasmonic Acid in Mitigating Salinity Stress on Date Palm *Phoenix dactylifera* L. Barhi Cv.

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Received 18th June 2022; Accepted 3rd February 2023; Available online 27th June 2023

Abstract: Date palm is one of the most important trees for economic and social development in many countries and its fruits with high nutritional value. This aimed to determine the role of salicylic (SA) and jasmonic acids (JA) as antioxidants against salt stress. Salt stress was applied with water irrigation to two-year-old date palm offshoots by using 200 mM NaCl alone or in combination with foliar sprays of JA and SA at 1, 2 and 3 mgL^{-1}. Results indicate that salinity at 200 mM NaCl remarkably increased the content of osmolytes (e.g., proline, glycine betaine and soluble sugars) in date palm leaves. Moreover, with the combination of 2 and 3 $mg.L^{-1}$ SA and 1 $mg.L^{-1}$ JA with salinity, the osmolyte content was remarkably higher than in salinity treatment alone. When date palm was exposed to salinity alone, the levels of oxidative markers, Malondialdehyde as a lipid peroxidation marker and H_2O_2 as a ROS accumulation marker, substantially increased compared with the control. Importantly, the levels of these oxidative markers remarkably decreased when plants were subjected to combined salinity and treatment with at 2 and 3 $mg.L^{-1}$ SA and 3 $mg.L^{-1}$ SA compared with the salinity treatment alone. In addition, spraying 2 and 3 $mg.L^{-1}$ SA and 3 $mg.L^{-1}$ JA on leaves combined with salinity treatment remarkably decreased the salinity effect on membrane stability index. Moreover, when 2 or 3 $mg.L^{-1}$ were sprayed, no remarkable difference was detected for any investigated characteristics, and SA had a greater effect than JA in alleviating the salinity effect.

Keywords: Abiotic stress, Glycine betaine, Lipid peroxidation, ROS.

Introduction

Salinity stress is one of the most significant environmental stresses, affecting more than 20% of cultivated land worldwide (Arora, 2019). Plants must not only grow and develop, but also tolerate harsh environmental conditions to survive and reproduce, because they are frequently subjected to both biotic and abiotic stresses (Atkinson & Urwin, 2012). The changes in Phytohormones levels in response to stress form part of the early defence response of plants (Zeng *et al*., 2019). Plants respond to salinity stress by regulating osmotic and ionic re-equilibrium, the detoxifying reactive oxygen species, and cell development and division (Zhao *et al*., 2020). Phytohormones are essential regulators of

plant growth and development (Ku *et al*., 2018), and they can effectively alleviate plant stress and reduce stress damage (Yan *et al*., 2015). Jasmonic acid (JA) is an important hormone that participates in various agricultural plant growth and development processes (Wasternack, 2014).

It can also regulate plant activities and prevents biotic and abiotic stresses in crops (Yan *et al*., 2015). Exogenous JA helps with salt toxicity by increasing plant's antioxidant activity through the activation of ROS scavenging enzymes and ion absorption because foliar spraying JA on various plants decreases the adverse effects of salt toxicity and increases plant performance and production (Anjum *et al*., 2011). Salicylic acid (SA) is a naturally occurring plant hormone that belongs to the phenolic acid group, and SA is required for plant growth and development and for important physiological activities such as stress tolerance (e.g., biotic and abiotic; Simaei *et al*., 2012). Endogenous and exogenous SA affect the production of secondary plant metabolites, antioxidant activity and plant tolerance to stress conditions such as salt (Kowalska & Smolen, 2012; Miura & Tada, 2014; Linic *et al.*, 2021). Nahrjoo & Sedaghathoor (2018) found that SA application improves plant growth, proline and antioxidant enzymes under salinity stress. Choudhury & Panda (2004) reported that SA can modify plant responses to oxidative stress, regulate antioxidant enzyme activity and enhance plant tolerance to a biotic stress.

The date palm tree is a member of the Arecaceae family (angiosperms, monocotyledon), which includes over 2,500 species and 200 genera (Ali & Fhaid, 2019). Phoenix is a genus of 14 species native to the tropical and subtropical areas of southern Asia and Africa, including *Phoenix dactylifera* L. (Eoin, 2016). As NaCl concentration increases, date palms respond to salt stress by increasing H2O2 content, peroxidase activity and malondialdehyde (MDA) content, whereas the membrane stability index shows the opposite tendency (Suhim *et al*., 2017). Jasim & Ati (2020) found that treatment of date palm plantlets with 1.5 mM SA under salt conditions increased the leaf chemical composition and antioxidant enzyme (catalase and peroxidase) activity compared with salt stress treatment and controls. Salinity weakened shoot and root weight and the number of tillers in Kentucky bluegrass (Arghavani *et al*., 2017). By contrast, progesterone and SA application mitigated the harmful effects of salinity (Sabzmeydani *et al*., 2020).

The date palm is the most common fruit tree in Iraq, especially in the province of Basrah, where the number of date palm trees is approximately two million. Most of the palm orchards in this province suffer from the problem of salinity, resulting in significant economic losses in productivity and affecting growth. This study aimed to investigate how foliar sprays of SA and JA affect the alleviation of oxidative and osmotic stress in date palm plants caused by salt toxicity.

Materials & Methods

Plant material

Offshoots of date palm Barhi cultivar obtained from one of the private orchards in Basrah province similar as possible in size, growth and age. Plants were cultured in plastic pots, and all pots (18 cm diameter and 15 cm depth) were placed in a greenhouse with natural light. The properties of culture soil were as follow: pH= 7.54; Ec=2.31 ds.m-1; content of organic matter 5.48%; Exchange Cationic Capacity (CEC) = 21.51 $Cmol.kg^{-1}$, as well as the soil texture was silty clay, which the particle size distribution were: Sand =8.12%, clay= 49.32% and 42.56%. Twenty four offshoots have been

selected and were divided into eight groups every group included three offshoots. Then were subjected to the following conditions for 120 days:

1. Group one: irrigated with tap water (control conditions; without any addition)

2. Group two: irrigated with NaCl at 200 mM (salinity conditions)

3. Group three: irrigated with NaCl at 200 mM and sprayed with Salicylic acid at 1 $mg.L^{-1}$

4. Group four: irrigated with NaCl at 200 mM and sprayed with Salicylic acid at 2 $mg.L^{-1}$

5. Group five: irrigated with NaCl at 200 mM and sprayed with Salicylic acid at 3 $mg.L^{-1}$

6. Group six: irrigated with NaCl at 200 mM and sprayed with jasmonic acid at 1 $mg.L^{-1}$

7. Group seven: irrigated with NaCl at 200 mM and sprayed with jasmonic acid at 2 mgL^{-1}

8. Group eight: irrigated with NaCl at 200 mM and sprayed with jasmonic acid at 3 $mg.L^{-1}$

Measurement of osmolytes concentrations

Proline

The proline content of date palm leaves was measured using the Bates *et al*. (1973) method. A sample of 500 mg of leaf was first ground in 5 ml of 3% sulfosalycylic acid with mortar and pestle. Then 2 ml of the extracted sample were placed in a plastic tube. Add 2 ml of glacial acetic acid and 2 ml of ninhydrin. The samples were heated for 1 hour at 100 ^{o}C, cooled to room temperature and the mixture was extracted with 4 ml toluene, with the upper phase's absorbance measured at 520 nm. The content of proline in leaves was measured using a pure proline (Himedia, India), calibration curve and expressed as mg g^{-1} fresh weight (FW).

Glycine betaine content

In a 20 ml plastic tube, 500 mg of leaf samples were crushed with mortar and pestle and then mixed with 5 ml of toluene-water combination (0.05 % toluene). At room temperature (25°C), all of the tubes were shaken for 24 hours. To 0.5 ml of prepared sample, 1 ml of 2 N HCl and 0.1 ml of potassium tri-iodide solution (15.7 g of iodine and 20 g of potassium iodide dissolved in 100 ml of distilled water) were added and agitated for 90 min in an ice cold water bath. The upper aqueous layer was removed, and the organic layer's optical density was measured at 365 nm. The amount of glycine betaine was expressed in $mg.g^{-1}$ DW (Grieve & Grattan, 1983).

Soluble sugar content

The soluble sugar content of leaves was determined using the phenol sulphuric acid method (Kochert, 1978). A calibration curve of pure glucose (phytotechlab, USA) was used to calculate the soluble sugar content and was expressed as $mg.g^{-1}$ DW.

Oxidative markers

Malondialdehyde (MDA)

MDA was employed as a membrane lipid peroxidation marker. MDA was extracted at 5% (w/v) using trichloroacetic acid (TCA). The absorbances at 532 and 600 nm were read, and MDA content was calculated using the extinction coefficient of 155 (Heath & Packer, 1968).

H_2O_2

H_2O_2 content was determined according to the protocol of Sergiev *et al*. (1997) calorimetrically at 390 nm and created a standard curve with H_2O_2 (38 %, Evonik, Germany).

Membrane stability index (MSI)

The MSI was calculated using the Lutts *et al* (1996) method. Leaf discs (0.2g) were cleaned

in double distilled water before being put in two sets of test tubes containing 10 ml of double distilled water. One set was heated in a water bath at 40°C for 30 min, another set was heated for 10 minutes at 100°C, and for both samples the electrical conductivity (EC1) of solutions was measured with an electrical conductivity meter. The MSI was computed using the following formula:

$$MSI(\%) = \left[1 - \frac{EC1}{EC2}\right] * 100$$

Statistical analysis

The experiment was set up as a completely randomized block design, with three replicates of each measurement and data subjected to one-way analysis of variance using 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. The total number of plants used in the experiment was 24 plants (date palm offshoots) with three replicates for each treatment. Three biological samples (leaves) were taken from each replicate.

Results

Accumulation of osmolytes

Proline

The results in fig. (1) reveal that proline content in date palm plants increased significantly when subjected to salinity treatment (NaCl at 200mM, 4.1mg.g^{-1}) compared with the control (2.8mg.g^{-1}). However, foliar spray of plants subjected to salinity with 2 and 3 mg.L^{-1} SA remarkably increased proline content to 6.53 and 6.36 mg.g^{-1}, respectively, while JA at 3mg.g^{-1} remarkably increased proline content to 5.5. mg.g^{-1}. As shown in fig. (1), foliar spray of plants subjected to salinity with SA at 1 and JA at 1 and 2 mg.g^{-1} did not affect proline content compared with plants subjected to salinity treatment alone.

Glycine betaine (GB) content

The results revealed that date palm plants under control treatment had the lowest content of betaine glycine, as shown in fig. (2) (1.3 mg.g^{-1}), whereas date palm plants subjected to salinity and sprayed with SA at 3 mg.L^{-1} had the highest content of GB (5.2 mg.g^{-1}). In comparison with salinity treatment alone, JA at all tested levels and 1 mg.L^{-1} SA did not affect GB content substantially. Moreover, when plants were sprayed with 2 mg.L^{-1} SA, GB content reached 3.7 mg.g^{-1}, which was remarkably higher than all other treatments, except 3 mg.L^{-1} SA.

Soluble sugar content

The results shown in fig. (3) indicate that the soluble sugar content in the leaves of date palm plants subjected to control treatment was 6.8 mg.g^{-1}, which remarkably increased to 10.2 mg.g^{-1} when subjected to salinity treatment. The results also revealed that the soluble sugar content was not affected significantly when SA and JA were spraying at all tested levels on the leaves of plants subjected to salinity treatment compared with salinity treatment alone.

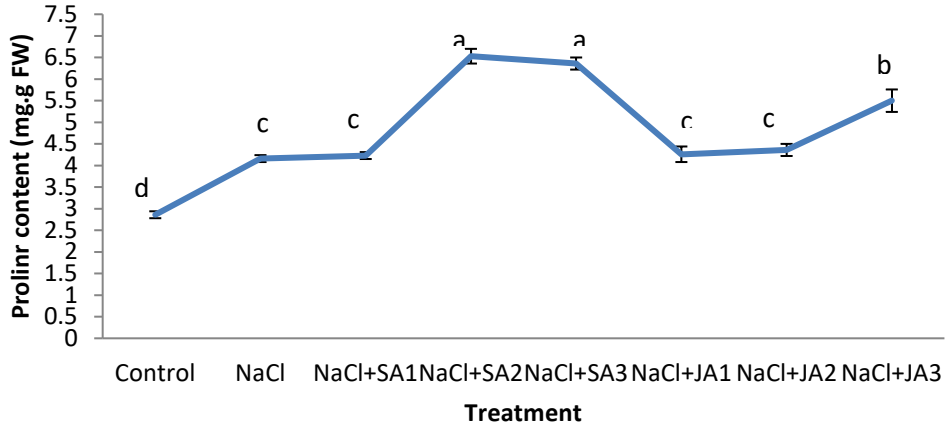

Fig. (1): Effect of SA and JA versus salinity stress on date palm content of proline.

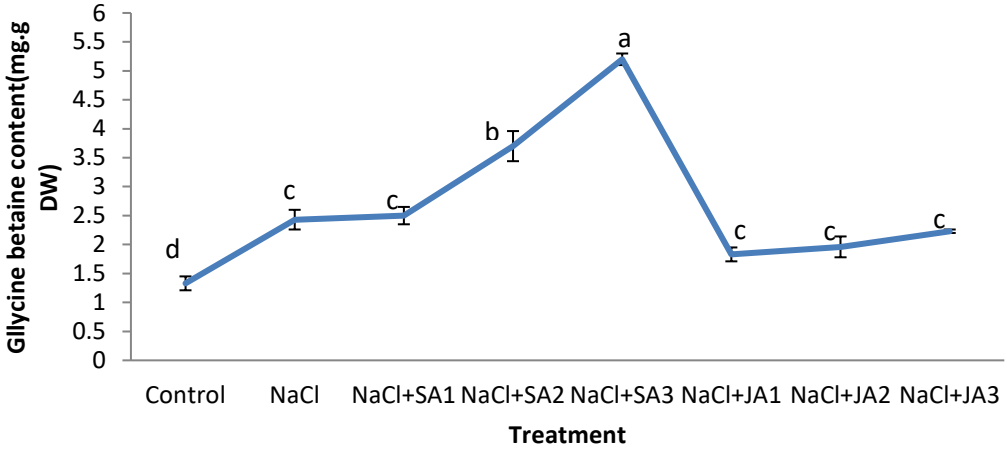

Fig. (2): Effect of SA and JA versus salinity stress on date palm content of glycine betaine.

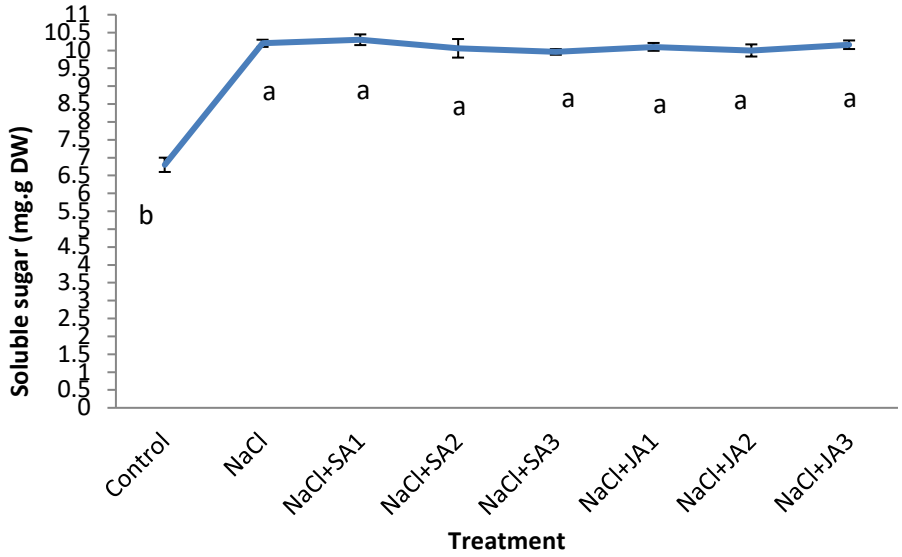

Fig. (3): Effect of SA and JA versus salinity stress on date palm content of soluble sugars.

Oxidative markers

Malondialdehyde (MDA)

The results in fig. (4) show that the MDA content of date palm leaves remarkably increased from 1.36 µmol.g^{-1} in plants subjected to control treatment to 5.96 µmol.g^{-1} in plants subjected to salinity treatment. However, MDA content remarkably decreased to 3.86, 2.30 and 2.53 µmol.g^{-1} in plants subjected to salinity treatment when the leaves were sprayed with 1, 2 and 3 mg.L^{-1} SA, respectively, compared to those subjected to salinity treatment only. MDA content remarkably decreased to 3.26 µmol.g^{-1} when plants were subjected to salinity treatment and sprayed with 3mg.L^{-1} JA, whereas no significant difference was observed in MDA content in plants subjected to salinity treatment alone or in combination with foliar spray of 1 and 2mg.L^{-1} JA.

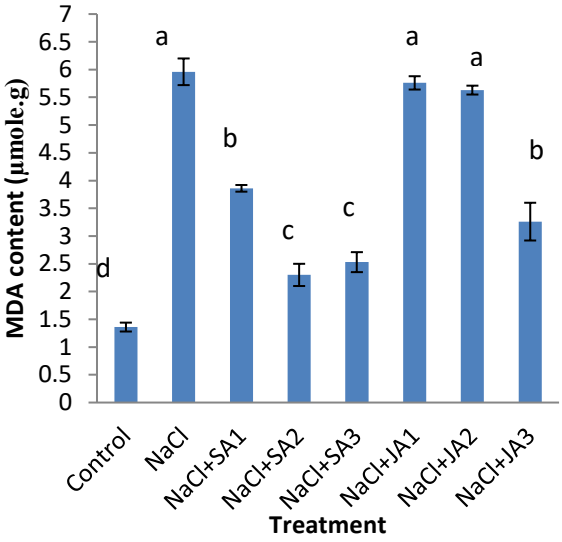

Fig. (4): Effect of SA and JA versus salinity stress on date palm content of MDA.

H_2O_2

The results in fig. (5) show that the H_2O_2 content of date palm leaves remarkably increased from 0.96 µmol.g^{-1} in plants subjected to control treatment to 4.20 µmol.g^{-1} in plants that were subjected to salinity treatment. However, H_2O_2 content remarkably decreased to 2.26, 1.66 and 1.76 µmol.g^{-1} in plants subjected to salinity treatment and those in which the leaves were sprayed with 1, 2, and 3 mg.L^{-1} SA, respectively. H_2O_2 content remarkably decreased to 2.33 µmol.g^{-1} when plants were subjected to salinity treatment and sprayed with 3mg.L^{-1} JA, whereas no significant difference was observed in the H_2O_2 content in plants subjected to salinity treatment alone or combined with foliar spray of 1 and 2 mg.L^{-1} JA.

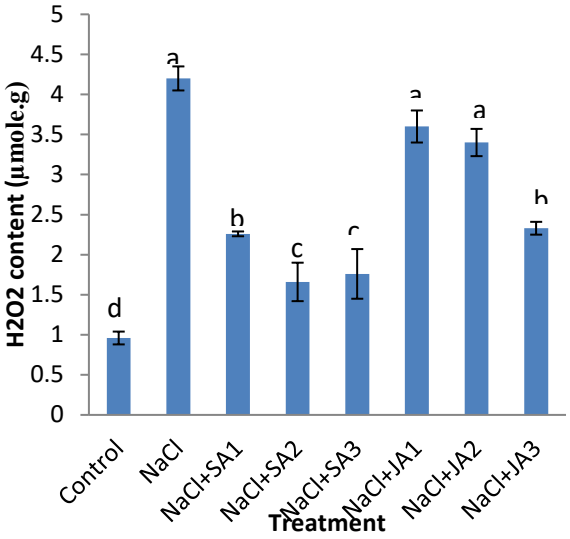

Fig. (5): Effect of SA and JA versus salinity stress on date palm content of H_2O_2.

Membrane stability index (MSI)

The results in fig. (6) show that the MSI value of date palm leaves remarkably decreased from 86.66% in plants were subjected to control treatment to 60.66% in plants that subjected to salinity treatment. However, MSI increased significantly to 81.33% and 78.33% in plants subjected to salinity treatment and in those with their sprayed with 2 and 3 mg.L^{-1} SA, respectively, compared with those subjected to salinity treatment only. MSI value remarkably increased to 70.33% when plants were subjected to salinity treatment and sprayed with 3 mg.L^{-1} JA, whereas no significant difference was observed in MSI value in plants

subjected whether to salinity treatment alone or combined with foliar spray of 1 and 2 mg.L-1 JA.

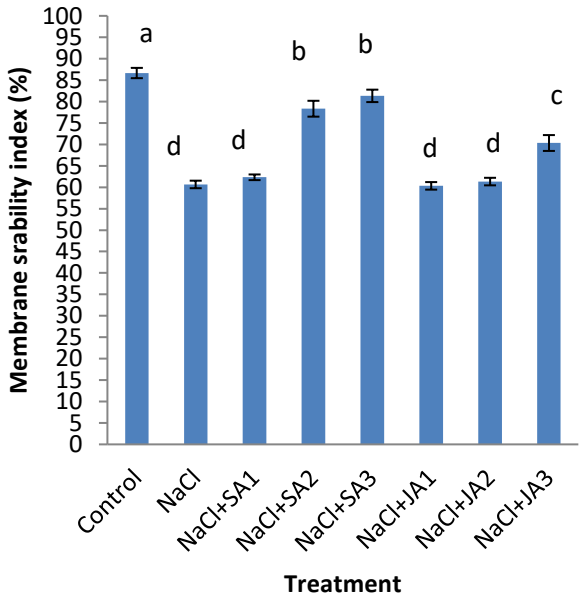

Fig. (6) Effect of SA and JA versus salinity stress on membrane stability index (MSI) of date palm leaves.

Discussion

Osmotic stress and ion toxicity are the two processes by which salt harms plants; as a secondary stress, oxidative stress is also brought on by salinity by increasing the production of ROS. Due to the adverse effects of salt, this research aimed to improve the tolerance of the date palm plant with the application of hormones (SA and JA) under salinity stress. The study results indicated that date palm plants showed enhanced accumulation of proline, glycine betaine and soluble sugar content under salinity stress. These results were consistent with the findings of Yaish (2015) and Abd *et al.* (2020) on date palm. Our results also revealed that the foliar application of SA and JA caused remarkably enhanced osmolytes accumulation in date palm plants subjected to salinity stress. The potential use of SA and JA in the alleviation of salt stress in plant has been reported by many researchers in various plants, such as date palm (Darwesh, 2014; Al-Qurainy *et al*., 2020; Jasim & Ati, 2020), tomato (Ahmad *et al*., 2018) and maize (El-Hakem, 2020). When plants are challenged by salt stress, they frequently accumulate osmolytes or compatible solutes, such as prolineand GB sugars to mitigate the effects of the stressor (Gao *et al*., 2021). Osmolytes act as ROS detoxifiers (antioxidants), photosynthesis protectants, osmoprotectants, macro-biomolecule stabilisers and protein folding enhancers (Giri, 2011). In addition, the stomatal opening is controlled by osmotic regulation, which involves the flow of solutes termed osmolytes, and it regulates water transport between cells; this water flow has various functions in plants that are related to cell hydration, such as stomatal opening and increased water absorption through the root system (Gupta & Huang, 2014). The accumulation of osmolytes is further modulated by phytohormones such as SA and JA (Sharma *et al*., 2019).

According to the results of this study, salt stress caused oxidative damage in date palm, as indicated by the increase of oxidative marker (MDA and H_2O_2) content and reduced MSI in date palm plants subjected to salinity treatment compared with the control, and these results were consistent with results of Suhim *et al*. (2017) and Shareef & Al-Khayri (2021) on date palm. Our findings proved that SA and JA have a beneficial effect on date palm under salt stress, as indicated by the reduced content of MDA and H_2O_2 and increased MSI in plants subjected to salinity and sprayed with SA and JA. The effects of salt stress have been mitigated by the application of SA foliar spray on some plants other than date palm, such as *Brassica juncea* L. (Hayat *et al*., 2012) and maize (Sultan *et al*., 2021). Moreover, foliar spray of JA alleviated oxidative damage

resulting from salinity stress in wheat (Qiu *et al*., 2014).

Conclusion

Salinity at a concentration of 200 mM caused an increase in MDA and H_2O_2 levels and a decrease in the index of membrane stability indicating the exposure of date palm plants to oxidative stress. Foliar spray of SA at 2 and 3 and JA at 3 $mg.L^{-1}$ mitigated oxidative stress via increasing the content of proline, soluble sugars and glycine betaine. SA was more effective than JA and also no significant difference between SA at 2 and 3 $mg.L^{-1}$ on all studied traits.

Acknowledgements

We would like to thank the University of Basrah, College of agriculture and date palm research center for offering facilities that supported this research.

Contributions of authors

A.A.S.: Carried out the experiment in the field and collected data.

K.M.A.: Analyzed the results statistically and contributed to the writing of the manuscript.

O.N.J.: Developed the idea and research plan and contributed to writing the manuscript and collecting sources.

M.H.A.: Writing the draft of manuscript.

All authors reviewed the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All ethical guidelines related to Fish and care issued by national and international organizations were implemented in this report.

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تأثير حامضي السالسيلك والجاسمونيك في تخفيف اجهاد الملوحة على نخيل التمر *Phoenix* L. صنف البرحي *dactylifera*

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المستخلص: يعتبر نخيل التمر من أهم الأشجار للتنمية الاقتصادية والاجتماعية في العديد من البلدان وثماره ذات قيمة غذائية عالية. يهدف هذا البحث إلى تحديد دور حامضي الساليسيليك (SA) والجاسمونيك (JA) كمضادات الأكسدة ضد الإجهاد الملحي. تم اضافة الملح مع مياه الري فسائل نخيل التمر البالغة من العمر عامين بتركيز 200 ملي مولار من كلوريد الصوديوم وحده أو بالاقتران مع الرش الورقي للسالسيلك او الجاسمونيك بالتراكيز 1 و 2 و 3 ملغم.لتر$^{-1}$. تشير النتائج إلى أن الملوحة عند 200 ملي مولار زادت بشكل ملحوظ من محتوى المركبات الاسموزية (البرولين، الجلايسين البيتين والسكريات القابلة للذوبان) في أوراق النخيل. علاوة على ذلك ، عندما اقترنت الملوحة مع الرش بالسالسيلك بتركيز 2 و 3 ملغم.لتر$^{-1}$ والجاسمونيك بتركيز 1 ملغم.لتر$^{-1}$ كان محتوى المركبات الاسموزية أعلى معنويا منه في معاملة الملوحة وحدها. كما اشارت النتائج الى تعرض نخيل التمر للملوحة وحدها, كانت زادت مستويات مؤشرات الأكسدة (MDA كمؤشر لأكسدة دهون الاغشية و بيروكسيد الهيدروجين كمؤشر لتراكم انواع الاوكسجين التفاعلية) معنويا مقارنة مع معاملة السيطرة. الأهم من ذلك، انخفضت مستويات هذه العلامات بشكل ملحوظ عندما تعرضت النباتات للملوحة مجتمعة مع الرش بالسالسيلك بتركيز 2 و 3 ملغم.لتر$^{-1}$ والجاسمونيك بتركيز 1ملغم.لتر$^{-1}$ مقارنة مع معاملة الملوحة وحدها. وبالإضافة إلى ذلك ، الرش بالسالسيلك بتركيز 2 و 3 ملغم.لتر$^{-1}$ والجاسمونيك بتركيز 1 ملغم.لتر$^{-1}$ على الأوراق جنبا إلى جنب مع معالجة الملوحة خفض بشكل ملحوظ تأثير الملوحة على مؤشر استقرار الغشاء. وعلاوة على ذلك ، الرش بالسالسيلك بتركيز 2 و 3 ملغم.لتر$^{-1}$ لم يتم الكشف عن فروقات معنوية لأي من الصفات المدروسة ، وكان تأثير السالسيلك أكبر من تاثير الجاسمونيك في التخفيف من تأثير الملوحة.

الكلمات المفتاحية: اجهاد لاحيوي، جلايسين بيتاين، اكسدة الدهون، انواع الاوكسجين التفاعلية.

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