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Original Article

Organic fertilizer modulates IAA and ABA levels and biochemical reactions of date palm *Phoenix dactylifera* L. Hillawi cultivar under salinity conditions

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Abstract

Organic fertilizer can be viewed to diminish the negative effect of saltiness on the plant. Foliar application of yeast (4 g l^{-1}) and algae (4 ml l^{-1}) extracts on Hillawi offshoots, watered with salt or freshwater examined. The outcomes demonstrated that yeast extracts improved both the hormone levels and the date palm biochemical reactions. Salinity reduced all examined growth parameters (leaf area, plant height, and leaves number). Likewise, chlorophyll content in leaves decreased. Organic fertilizers improved date palm growth. Yeast application increased chlorophyll content, organic solutes, and substances growth included indole-3-acetic acid, zeatin, and gibberellin. However, the yeast extracts increased amino acids and ascorbic acid. The organic fertilizer lowered the accumulation of sodium in the leaf. It increased uptake of potassium, bringing about a higher K^+/Na^+ ratio. Improving plant development under saltiness conditions was identified with an enhancement in the content of amino acids and carbohydrate content. The use of yeast extract could induce the recovery of the plant exposed to salinity. Organic fertilizers (yeast and seaweed extracts), which are economically and environmentally friendly, can be recommended to farmers for alleviating salinity.

Keywords: GA3, Auxins, Algae extracts, Yeast extracts

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Introduction

Date palm (*Phoenix dactylifera* L.) is a typical tree in arid and semi-arid locales. It is one of the standard fundamental substances in Iraq. Also, it takes a substantial part of financial and public activity. Hillawi date palm cultivar is one of the economically most critical soft-date cultivars in south Iraq. It is considered to be more susceptible to abiotic stress than other varieties. The high salinity of soil in the south of Iraq occurred from lack of drainage. As well as, increased water salinity due to sea-level rise, and the high temperature (Shareef, 2010). Salinity induces osmotic stress, ionic imbalance, and ionic harmfulness (Munns, 2002). Mainly the offshoots planted in the soil are more sensitive to salinity than adult plants (Shareef et al., 2017). The cultivar is salinity tolerant by maintaining a high proportion of K^+/Na^+ and controlling dimensions of abscisic acid (ABA) and indole-3-acetic acid (IAA) increased (Jasim et al., 2016). ABA and IAA are activated to maintain the plasticity of growth and adapt to extreme ecological conditions (Contreras-Cornejo et al., 2014). ABA enhanced various cellular physiological responses and adaptive (Danquah et al., 2013). Auxins are involved in the regulation of many signaling pathways, such as cell viability, cell cycle progression, and programmed cell death (Xu and Leskovar, 2015). Also, enhancing the building of plant hormones, such as cytokine (CK) and Gibberellin (GA3), can improve metabolic engineering needs to improve plant tolerance for brutal natural conditions (Luo et al., 2018).

Organic fertilizer reduces the negative influence of salinity conditions (Abdel Ghany et al., 2013). Recently, the use of commercial algae or seaweed extracts (SWE) (*Ascophyllum nodosum*) the products used in the form of liquid extracts and a foliar application. SWE is biodegradable and non-polluting to the environmental surroundings and has become popular as organic fertilizer (Xu and Leskovar, 2015). SWE contains minor and major nutrients, antioxidants, organic acids, vitamins, and natural hormones (Ahmed et al., 2015). The fact that bioactive substances produced from SWE provide stress resistance and enhance the plant's performance. The bioactive compounds in algae and the impact of intensifies that gave this resistance are generally obscure, the anti-stress effects of algae extracts are due to the activity of CK (Sharma et al., 2014). CK attenuates stress-induced free radicals by scanning and stopping the formation of reactive oxygen species (Fike et al., 2001).

The organic fertilizer of the dry yeast (*Saccharomyces cerevisiae*) is advantageous for controlling the development and improving the productivity and quality of the natural product (Abd El-Rahman and Mansour, 2015). Yeast extract (YE) is considered a natural source of IAA, CK, amino acids, minerals, nucleic acids, glutathione, coenzymes, vitamin B, and precursor of chlorophyll (Barnett and Rayne, 1990). YE is considered a natural source of cytokines that stimulate cell division and growth, just as the combination of proteins, nucleic acids, and chlorophyll (Al-Khayri, 2011). Darwesh (2016) indicated an increase in growth parameters like plant height, leaves number/plantlet, root length, and root number/plantlet, N, P content, and IAA. With tow treatments of yeast 20 and 40 cm^3 l^{-1}, improved date palm plantlets of Medjol cultivar.

No study has conducted yet on the effects of yeast extract and algae applications on date palm offshoots in salinity conditions. Accordingly, the experiment intended the efficacy of yeast extract and Kelpak as a commercial seaweed extract at the IAA, CK, GA3, and ABA levels and the biochemical responses of date palm in saline and non-saline conditions.

Material and Methods

Practical experiment

The analysis directed in the general specialist of Palm Station in the Hartha-Basrah locale of Iraq (30° 37'52.68 "N and 47° 45'8.15" E), in 2017 growing season, the experiment was performed with 30 offshoots of the vigorous "Hillawi" date palm of ± 10 cm diameter, uniform. The selected juveniles planted at a distance of 5×5 m in silty clay loam soil with a dribble water system framework. The ordinary EC soil was five dS m^{-1}, and the customary EC water was 4.5 dS m^{-1} to saltwater and one dS m^{-1} to freshwater. On the 1st of March and repeated on the 1st of May. The offshoots exposed to foliar treatments with one plant for each replicate irrigated with saltwater or with fresh water. Extract of seaweed (Klepak) (4 ml l^{-1}); Yeast (4 g l^{-1}) applied as a foliar spray in addition to the treatment of control (only freshwater).

Organic fertilizer compounds

Yeast treatment: Treatments of dry yeast (*Saccharomyces cerevisiae*) performed on 4 g l^{-1}. Starches 33%, nucleic acid 8%, protein content 47%, lipids 4%. It contains starches 33%, nucleic acid 8%, protein content 47%, lipids 4%. Vitamins such as thiamin, biotin, and B12. Folic acid and tryptophan. As well as hormones. Different minerals 8%, for example, Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Li Products by Özmaya San.A.S. (an organization of Lesaffre), Turkey.

Seaweed (Kelpak): Kelpak treatments were performed with 4 ml l^{-1} containing cytokines (11 mg l^{-1}). Kelpak Products, South Africa.

After 180 days of treatment, the following data recorded:

Plant height (cm)

The third leaf was measured entirely from the bottom to the top of the blade by tape measurement

New leaves number

New leaves number = number of leaves during a sampling – the number of leaves before treatment

Leaf area (m^2)

It was determined, according to Ahmed and Morsy (1999) at the middle part of each leaf for four leaflets taken according to the following equation:

Leaf area (m^2) = (0.37 (length x width) + 10.29 x number of pinnae)/ 1000

Chlorophyll total content

Small pieces of the leaf (0.5 g) were blended with 80% of acetone then centrifuged. Chlorophyll evaluated according to the strategy of Lichtenthaler and Wellburn (1983) at 645, and 663 nm has utilized the spectrophotometer.

Carbohydrate analysis

New pinnae examples (0.2 g) were gauged and homogenized with 70% ethanol. At that point, they separated, and the pigments evacuated with benzene. Leaf extricate (0.2 ml) was added to 1.0 ml (5% phenol + 5 ml of 95% H_2SO_4) to respond with the water bath at 100°C for 10 min. Soon after that, the test tube cooled in an ice bath, and the absorbance read as utilized by Yemm and Willis (1954) at 620 nm. Soluble carbohydrates were determined by contrasting the example take-up with a standard glucose bend extending from 0-100 µg ml^{-1}.

Amino acids concentrations

Amino acids were measured utilizing the ninhydrin calorimetric strategy by using ninhydrin reagent. In a word, 0.5 g tissue treated in 70% ethanol medium-term at room temperature and afterward washed with twofold refined water. 1.5 ml of 55% glycerol and 0.5 ml ninhydrin arrangement included, bubbled at 100°C for 20 min and chilled off. The last volume was made up to 6 ml utilizing twofold refined water, trailed by the estimating of the spectrophotometer at 570 nm glycine used for the generation of the standard bend (Hwang and Ederer, 1975).

Determination of proline concentration

The dried leaves (0.5 g) of date palm blend in 5 ml of 95% ethanol. Separated the upper layer of the filtrate, and its pellets were washed thrice with 5 ml of 70% ethanol. Their best layer added to the anterior chamber. The blend for 10 minutes at 6,000 rpm at 4°C centrifuged, and the supernatant gathered, and the alcoholic concentrate was put away at 4°C in the fridge (Paquin and Lechasseur, 1979). One ml of liquor remove weakened with 10 ml of distilled water, and 5 ml ninhydrin (0.125 g ninhydrin, 2 ml 6 mM NH_3PO_4, 3 ml icy acetic acid) and the blend set in a holder. Exposure to a temperature of 100°C for 45 min. The response halted by putting it in a high water temperature. The samples blended with 10 ml of benzene. The benzene retention stage assessed at 520 nm. Proline focus was resolved to utilize a standard bend. The convergence of free proline communicated in mg g^{-1} DW of leaves (Irigoyen et al., 1992).

Ascorbic acid content (AsA)

The substance of ascorbic acid was resolved to utilize (Luwe and Takahama, 1993) technique. Green leaf plant (0.5 g) was grounded in a fluid nitrogen mortar and afterward was blended in 1% super cold trichloroacetic acid. After homogenate did. The centrifuged at 12,000 xg for 20 minutes at 4°C did. Moreover, the supernatant (50 µl) was blended with100 mM Potassium Phosphate buffer. Furthermore, ascorbate estimated at 265 nm.

Hormones analysis

Tests of leaves gathered from date palm were surface dried and cleaned with a paper towel, promptly was gauged and solidified in liquid nitrogen, and put away at -20°C. Tests (around one g new fresh mass) were ground in liquid nitrogen, homogenized. Afterward, extricated medium-term with 30 ml of 80% cold methanol in murkiness at 4°C. The concentrate was centrifuged at 5,000 r min^{-1}, under 4°C for 15 min, and the supernatant gathered. At that point, new cold methanol filled was the remainder, extricated multiple times with the strategies above. The methanolic separate was united in a rotary evaporator. It broke up in 10 ml methanol. The infusion of the concentrate estimated IAA, ABA, GA3, and zeatin by HPLC With a methanol angle in 0.6% acetic acid as per (Tang et al., 2011).

Potassium and sodium concentration

The method described by Cresser and Parsons (1979) used in estimating both potassium and sodium. Then the solution was made transparent and used to determine flame photometers with K emissions and Na concentrations.

Chloride concentration
Chloride (Cl) estimated by potentiometric titration. Dry leaf tissue 0.2 g with the ground, and the addition of 50 ml of 2% acetic acid stirred for 30 minutes. Potassium chromate used as the endpoint indicator (Kalra, 1998).

Statistical analysis
Randomized completely block design and Duncan multiple comparison tests performed at $P \leq 0.05$ were conducted using a statistical program, SPSS 18.

Results and Discussion

The utilization of organic fertilizers can modulate the content of IAA, GA3, and CK to enhance the biosynthesis of natural osmolytes with reaction to salinity effects. The salinity caused a critical abatement in plant height, leaf area, and leaves number (Fig. 1A, B, C). The decrease in the growth rate of offshoots through the salinity because of the trouble of absorption and transport or conveyance of components in the plant (Jasim et al., 2016). In contrast, the growth increased with organic fertilizer applications (Fig. 1).

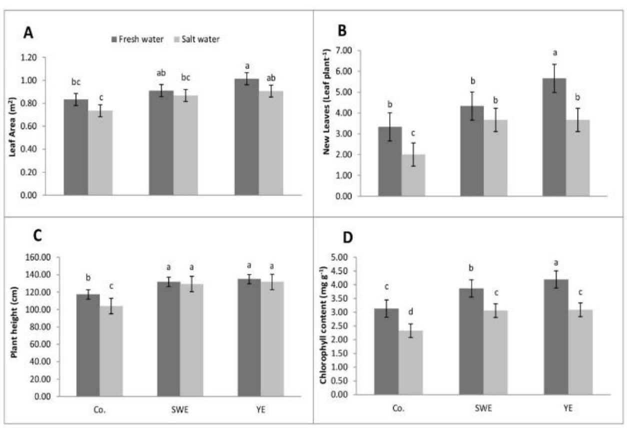

Fig-1: Effect of organic fertilizer as of control (Co.), extract spray seaweed at 4 ml l^{-1} (SWE), and yeast at 4 g l^{-1} (YE), under irrigation with salt or freshwater on leaf area (A), leaves number (B), plant height (C) and chlorophyll content (D) of plant. The means of five replicates ±SE. Bars with different letters are significantly different at *p* ≤ 0.05 after a Duncan correction.

The effect of YE and SWE extracts on growth improving attributed to their hormonal action, which reflected in an improvement in cell division and the stimulation of carbohydrate biosynthesis (Fig. 2A).
The salinity significantly ($P \leq 0.05$) lowered the chlorophyll content when contrasted with the control of fresh water, whereas fertilizers improved chlorophyll content. However, the use of YE demonstrated a noteworthy increment ($P \leq 0.05$) in chlorophyll content compared to the use of SWE with salt water (Fig. 1D). The abatement in chlorophyll content with saltiness cleared that the demolition of the chlorophyll pigments and the destruction of the protein complex due to the collection of particles, for example, Na^+ and Cl^- (Singh et al., 2015a). The positive effect of organic fertilizer applications on the diminished adverse impact of salinity on discards and the improvement of growth and chlorophyll substance may be required to the updated antioxidant agent framework just as the osmotic adjustment mediator due to the increase in amino acids and ascorbic acid (Fig. 2B, D). Subsequently, a possible correlation between the aggregations of amino acids with the rise of the chlorophyll content proposed. Amino acids play a promising role in the protection and promising function in the insurance of photosynthetic proteins. This use against the action of protease under salinity conditions (Choudhary et al., 2009).

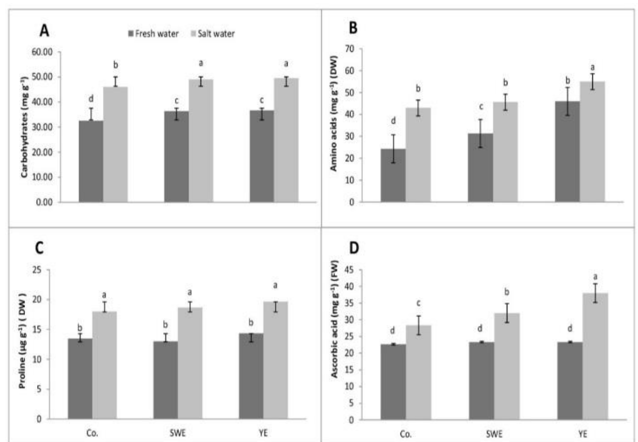

Fig-2: Effect of organic fertilizer as of control (Co.), extract spray seaweed at 4 ml l^{-1} (SWE), and yeast at 4 g l^{-1} (YE), under irrigation with salt or freshwater on carbohydrates content (A) amino acids concentration (B), proline concentration (C), and ascorbic acid content (D) of plant leaves. The means of five replicates ±SE. Bars with different letters are significantly different at *p* ≤ 0.05 after a Duncan correction.

The carbohydrate content increased significantly ($P \leq 0.05$) by organic fertilizer under saline conditions (Fig. 2A). The increase in soluble carbohydrate content with organic fertilizers due to the expansion in sugars, which are perfect solutes that gather in the tissues of plants presented to abiotic stress. The gathering of sugars and sucrose may assume a fundamental function in defense mechanisms of osmoregulation and energy conservation (Singh et al., 2015b). The sucrose molecule protects biological macromolecules (DNA and RNA) from the deleterious effects of salinity (Kurz, 2008).

The organic fertilizers significantly ($P \leq 0.05$) enhanced the concentration of the amino acid (Fig. 2B). However, the increase in amino acids company to the salinity was more significant with the use of YE than with SWE.

Proline concentration in the leaves with saltwater increased contrasted with normal water conditions. The proline concentration was not significant ($P \leq 0.05$) influenced by the application of organic fertilizer in both environmental conditions (Fig. 2C).

Organic fertilizer improved offshoot's osmoprotectants system. The gathering of amino acids and sugars is essential to direct the osmotic exercises and to shield the cell structure from extreme conditions by keeping up water parity and membrane stability. In any case, changes in sugars and amino acids brought about by salt stress related to the union of polyamines (glutamate, arginine, and proline) (El-Bassiouni and Sadak, 2015). However, the metabolism of proline is highly regulated. Moreover, its accumulation during salinity is not a stress symptom or consequence of passive collection caused by a reduction in growth (Bhaskara et al., 2015).

Ascorbic acid increased under saline effect contrasted with the non-saline state. The application of organic fertilizer under non-saline conditions was not significant ($P \leq 0.05$) in ascorbic acid content. AsA was significantly higher with YE than with SWE under saline conditions (Fig. 2D). The increase in AsA during the application of organic fertilizer with salinity might be to the contribution in resistance to oxidative stress. Also, As a participates in different physiological procedures in plants by mediating tolerance to abiotic stress (Khan et al., 2011). AsA saved the metabolic systems from H_2O_2 and other harmful oxygen subordinates that influence numerous enzymatic exercises. It limits the harm brought about by oxidative procedures through synergistic capacities with different proteins such as the enzymes (Yan et al., 2013).

IAA, CK, and GA3 decreased under salinity significantly ($P \leq 0.05$) compared to the non-saline conditions. In contrast, abscisic acid increased (Fig. 3A, B, C, D). The application of YE and SWE on endogenous phytohormones (IAA, CK, GA3) levels showed that all increased. Whereas abscisic acid level constant in both environmental conditions. YE raised CK and GA3 content significantly compared with SWE in both environmental conditions (Fig. 3 C, D). IAA, GA3, and CK increase with organic fertilizer applications of treated plants presented to saltiness (Fig. 3A, C, D). Similar results obtained for the date palm of Darwesh (2016), who revealed that the yeast extracts increased the auxin content in the leaves of the date palm. Therefore, an increase in the ABA level might be one of the crucial factors for saltiness resistance in date palm.

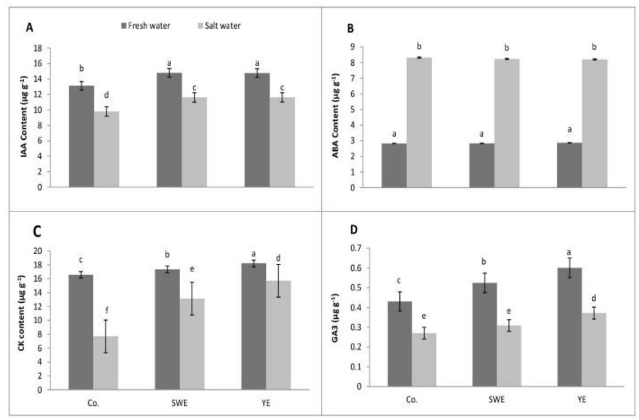

Fig-3: Effect of organic fertilizer as of control (Co.), extract spray seaweed at 4 ml l^{-1} (SWE), and yeast at 4 g l^{-1} (YE), under irrigation with salt or freshwater on IAA levels (A), ABA levels (B), CK levels (C) GA3 levels (D) of plant leaves. The means of five replicates ±SE. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

In date palm offshoot, a progressively remarkable collection of Na^+ and K^+ observed under salinity (Fig. 4A, B). Application of organic fertilizers has contributed to reducing the increase of sodium concentration Na^+ and Cl^-, and increase the ratio of K^+ and K^+/Na^+. Increasing the K^+ content help to reduce the sodium uptake, K^+ required to maintain osmotic balance. However, in a saline environment, the osmotic adaptation could occur in plants presented to saltiness due to the collection of inorganic ions or low molecular weight ions (Munns, 2002). The K^+/Na^+ ratio is an essential indicator of the plant's tolerance to salinity. The tolerant degree usually associated with an efficient K^+ selective absorption system (Wu et al., 2013). However, organic fertilizer decreased chloride concentration in leaf (Fig. 4C). The moderately more prominent resilience of the plant to saltiness identified with gather the required amount of amino acids and keep up the high K^+/Na^+ proportion and control endogenous phytohormones.

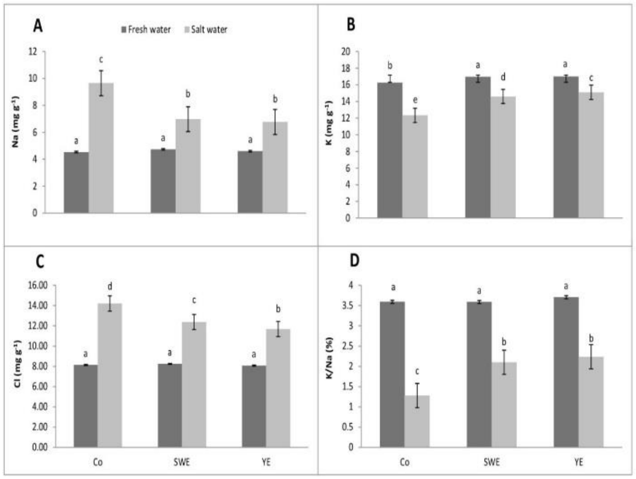

Fig-4: Effect of organic fertilizer as of control (Co.), extract spray seaweed at 4 ml l^{-1} (SWE), and yeast at 4 g l^{-1} (YE), under irrigation with salt or freshwater on Na concentration (A), K concentration (B), Cl concentration (C) K/Na ratio (D) of plant leaves. The means of five replicates ±SE. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

Conclusion

The application of organic fertilizer reduces the adverse impacts of salinity in the date palm through GA3, IAA, and CK concentrations modulated. As well as it improves the biosynthesis of natural osmolytes. Response to osmoregulation of salinity increased the production of carbohydrates, ascorbic acid, and amino acids. The proportion of K^+/Na^+ was raised with the application of organic fertilizer, allowing plants to overcome the toxic effects of saline. Organic fertilizers (yeast and seaweed extracts), which are economical and environmentally friendly, can be recommended to farmers to use in their orchids to enhance the stress-tolerant of the plant.

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