Al-Mukhtar Journal of Basic Sciences 24 (1): 12-24, 2026
Doi: https://doi.org/10.54172/4y8kgh05

Research Article **Open Access**

The Synergistic Effects of Yeast and Humic Acid on Improving the Growth of Basil (*Ocimum Basilicum* L.) Under Salinity

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Received:
06 December 2025

Accepted:
27 February 2026

Published online:
30 April 2026

Abstract

Salinity is among the major abiotic stressors that limit plant productivity. Basil is an aromatic plant of economic importance, but it has low tolerance to salinity. Using organic compounds, such as humic acid (HA) and dry yeast (DY), can reduce the harmful effects of salinity. This environmentally friendly method mitigates the impacts of salinity. This research aimed to assess the influence of HA, DY, and their mixture on the development of basil. The experiment was conducted using a split-plot design in three replicates, where the primary units were two salinity concentrations (0, 80 mM NaCl), and HA and DY solutions were distributed in the sub-plots. The results showed that salinity stress decreased ($p<0.001$) the growth traits of basil. The results showed that spraying with HA, DY, and their mixture resulted in higher values of all studied traits compared with unsprayed plants. Furthermore, the results showed that the HA + DY mixture was the most effective on growth, as indicated by higher values of all studied traits compared with other treatments. The results showed that HA and DY were of great importance as environmentally friendly fertilizers in improving the productivity of plants under stress. This could contribute to sustainable bioactive herb cultivation.

Keywords: Salinity; *Ocimum basilicum* L.; Humic acid; Yeast; Growth

INTRODUCTION

Basil is an aromatic herb that has been cultivated for numerous millennia for its aromatic applications. It belongs to the Lamiaceae family, which includes various herbs that grow in diverse climates (Pushpangadan and George, 2012). It grows in many regions around the world for its essential oil, which is used as an ingredient in cooking and cosmetics (Heidari, 2012). The leaves are the useful parts of the basil plant, and therefore both fresh and dried leaves are used in the food and spice industries. Basil plants are used in traditional medicine as stimulants (Romano et al., 2022). However, the basil plant is sensitive to salinity and exhibits a marked physiological decline under saline conditions, including reduced water and nutrient absorption, impaired photosynthetic activity, ion balance disturbance, and increased oxidative stress (Meftahizadeh et al., 2025).

Environmental stressors such as heat, salinity, and drought are the main factors that affect crop production (Ehtaiwesh, 2016). It is predicted that by the year 2050, more than 50% of all agricultural land will be severely salt-affected land (Munns and Tester, 2008). Climate change and its consequences lead to increased salinization of soil or irrigation water, posing a challenge to various agricultural systems (Wang et al., 2025). Salinity stress reduces the ability of plants to utilize water and therefore results in a decrease in the rate of plant growth (Jadczak et al., 2021; Meftahizadeh et al., 2025). It is recommended to adopt integrated and compatible agricultural systems to overcome abiotic stressors. Some studies indicate the use of certain substances, such as algal biochar, spermine, salicylic acid, humic acid, and yeasts, as growth promoters (Ehtaiwesh, 2022; Abohbell et al., 2024; Dıraz Yıldırım et al., 2025).

Humic acid has hormone-like substances that improve the physical, chemical, and biological properties of the soil, positively impacting crop production. Humic acid is a natural polymeric organic compound and contains most of the micronutrients necessary for plant growth (Mackowiak et al., 2001). Humic acid improves seed germination, growth, and plant productivity in several plants (Shehata and Abdel-Wahab, 2018; Yıldız et al., 2022). Its use can significantly reduce the need for chemicals. In addition to the damaging effects of chemicals on the environment, the use of non-organic farming systems containing humic acid is essential for the clean production of medicinal plants (Ampong et al., 2022).

Yeast (*Saccharomyces cerevisiae*) is considered a natural and safe biofertilizer with effective and beneficial effects on plant productivity. It has potential use as a plant growth stimulant, as well as its ability to mitigate damage caused by salinity (Ehtaiwesh, 2023). Yeasts are a natural source of certain plant hormones, such as cytokinins, which stimulate cell division and cell elongation, along with the production of proteins, nucleic acids, and some vitamins (Nimsi et al., 2023; Dmytruk et al., 2025). The use of yeast has led to increased plant growth and productivity (Abdelaal et al., 2021; Ehtaiwesh and Abuiflayjah, 2024).

Carbon-based and bio-composts are now being applied instead of chemicals in sustainable farming systems. Consequently, they not only improve soil productivity but also contribute to environmental conservation and the production of high-quality crops (Balkrishna et al., 2025). The use of humate substances and dry yeast solutions may meet the essential nutritional needs of many economic and medicinal crops. The combined effects of yeast and humic acid on salinity stress are not fully understood. Most studies that dealt with organic fertilizers focused on monoculture systems. Therefore, the objective of this study was to assess the effect of humate, yeast, and their mixture on the development of basil.

MATERIALS AND METHODS

This study was carried out at one of the Jouddam farms, Zawia, Libya. A randomized complete block design with a split-plot arrangement was used with three replicates. The main plot included salinity levels (0, 80 mM NaCl), while the subplots consisted of humic acid, yeast, and HA + DY. Basil seeds were sown in plastic containers. Four weeks after sowing, uniform, healthy basil plantlets (8-10 cm) were planted in 20 cm diameter pots, with each pot filled with 4 kg of peat moss, and 4 seedlings of basil were planted in each pot. Before transplanting, 0.25 g/kg of urea (46% N) was added to each pot. Irrigation was performed at appropriate intervals. Two weeks after transplanting, seedlings were fertilized with (20:20:20 NPK) and micronutrients. One month after transplanting, the pots were divided into groups, each group representing one of the salinity treatments. The plants in the control group were irrigated with 1.5 dS/m^2. The salinity stress pots were irrigated with a 6.5 dS/m^2 saline solution for about one month twice a week. Subsequently, all plants were maintained under optimal irrigation conditions until the end of the experiment. Before, during, and after the salinity treatment period, all plants were subjected to foliar spraying with 1% humic, 3 g/L yeast, a mixture of HA and DY, and the control, which was foliar sprayed with tap water. Spraying with humic acid, yeast solution, the mixture, and tap water were applied four times during the growing period, biweekly.

Data collection: One shrub was collected from each replicate. Plant height (cm), shoot number and leaf number were recorded. The plants were cut above the ground surface, and the fresh weight of the shoots was recorded, then the shoots were dried in an oven at 60 C° and the dry weight was recorded. Leaf area (LA) was measured using leaf length and width according to Peksen (2007).

Leaf area (LA) = leaf length x width.

The relative growth rate (RGR) was estimated during two periods of plant growth at 45 and 90 days after planting using the equation of Meganid et al. (2015): $RGR = (W2 – W1/t2-t1)* 100$

where W is planting dry mass (g), t is the period (days), and the subscripts 1 and 2 refer to the first and second sampling of plant weight.

Relative water content (RWC) was determined according to Rady et al. (2012). Leaf samples were collected and weighed immediately to obtain fresh weight (FW), and then leaf samples were placed in fresh water and kept in darkness. After 24 h, the turgid weight (TW) was obtained. Leaves were dried in the oven for 24 h at 60°C and dry weight (DW) was taken, and the RWC was then calculated following this equation:

$$RWC \% = (FW – DW)/(TW-DW)* 100$$

Statistical analysis: Statistical analysis was performed with SPSS Software v. 27. The analysis of variance test (ANOVA) was applied in order to test the significance of salinity, humic acid and yeast solution on plant growth parameters. The means were compared by Duncan's multiple range test at $p \leq 0.05$.

RESULTS

Table 1 presents the probability values for evaluating the effect of salinity, yeast, humic acid, and the interaction between them on some growth traits of basil. The results showed that salinity had a significant effect (P<0.001) on all studied traits. Similarly, the application of yeast and humic acid had a highly significant effect (P<0.001) on all studied traits. The results also showed that the effect of the interaction between salinity, yeast, and humic acid was significant (P<0.05) for all growth traits included in this study.

Table (1). Probability values of effects of salinity (S), dry yeast (DY), humic acid (HA) and S x DY x HA interaction on various growth traits of basil.

	Variables			
Traits	Salinity (S)	Dry yeast (DY)	Humic acid (HA)	S x DY x HA
Shoot length (cm)	<.001	<.001	<.001	0.047
Branches number	<.001	<.001	<.001	0.035
Leaves number	<.001	<.001	<.001	0.042
Leaf area (cm^2)	<.001	<.001	<.001	0.041
Shoot fresh weight (g)	<.001	<.001	<.001	0.049
Shoot dry weight (g)	<.001	<.001	<.001	0.005
Relative growth rate	<.001	<.001	<.001	0.002
Relative water content	<.001	<.001	<.001	0.019

The results showed that salinity significantly decreased growth traits of basil plants, namely plant elongation, branches, leaves, leaf area, plant fresh mass, plant dry mass, relative growth rate and relative water content by (27, 44, 20, 45, 48, 27, 36, and 34%), respectively, compared to the control plants (Table 2).

Table (2). The main effect of salinity on various growth traits of basil.

Traits	0 mM NaCl	80 mM NaCl
Shoot length (cm)	68.6	50.2 (-27)
Branches number	9.6	5.4 (-44)
Leaves number	92.4	73.7 (-20)
Leaf area (cm^2)	31	17 (-45)
Shoot fresh weight (g)	38	19.6 (-48)
Shoot dry weight (g)	7.1	5.2 (-27)
Relative growth rate	8.4	5.4 (-36)
Relative water content	87	57 (-34)

*Individual value is the mean of 12 replicates under different salinity levels. Values in parentheses indicate the percent decrease from the control treatment (0 mM) to the (80 mM).

The application of yeast significantly improved the growth characteristics of basil grown under both saline and non-saline conditions. It was observed that the use of dry yeast solution significantly improved growth characteristics, plant height, branches, leaves, leaf area, fresh and dry mass of the plant, relative growth rate and relative water content by (5, 14, 5, 18, 15, 12, 14, and 7%), respectively, compared to plants not treated with dry yeast (Table 3).

Table (3). The main effect of dry yeast (DY) solution on various growth traits of basil.

Traits	-DY	+DY
Shoot length (cm)	58	61 (+5)
Branches number	7	8 (+14)
Leaves number	81	85 (+5)
Leaf area (cm^2)	22	26 (+18)
Shoot fresh weight (g)	27	31 (+15)
Shoot dry weight (g)	5.8	6.5 (+12)
Relative growth rate	6.4	7.3 (+14)
Relative water content	70	75 (+7)

*Individual value is the mean of 12 replicates under different yeast treatments. Values in parentheses indicate the percent increase from the control treatment (-DY) to the (+DY).

The results showed that the application of humic acid significantly enhanced some growth characteristics of basil under saline and non-saline conditions. Specifically, the use of humic acid significantly enhanced all the growth characteristics identified in this study, namely stem elongation, branches, leaves, leaf area, plant fresh and dry mass, relative growth rate and relative water content by (13, 24, 10, 40, 28, 20, 25, and 13%), respectively, compared to plants not treated with humic acid (Table 4).

Table (4). The main effect of humic acid (HA) on various growth traits of basil.

Traits	-HA	+HA
Shoot length (cm)	56	63 (+13)
Branches number	6.7	8.3 (+24)
Leaves number	79	87 (+10)
Leaf area (cm^2)	20	28 (+40)
Shoot fresh weight	25.4	32.4 (+28)
Shoot dry weight (g)	5.6	6.7 (+20)
Relative growth rate	6.1	7.6 (+25)
Relative water con-	67	76 (+13)

*Individual value is the mean of 12 replicates under different humic treatments. Values in parentheses indicate the percent increase from the control treatment (-HA) to the (+HA).

The results showed that (DY) and (HA) and their mixture significantly affected growth characteristics such as stem elongation and number of branches for basil plants. In non-saline conditions, it was observed that yeast, humic acid, and their mixture significantly improved plant height by (6, 12 and 23%), respectively, compared with the control group. Whereas, in saline conditions, plant height of basil increased by (2, 10, and 15%), respectively, compared with the control (Figure 1A). In non-saline conditions, it was observed that yeast, humic acid, and their mixture significantly increased the number of branches by (8, 21, and 50%), respectively, compared with the control. While, in saline conditions, the number of branches increased by (7, 21, and 29%), respectively, compared with the control (Figure 1B).

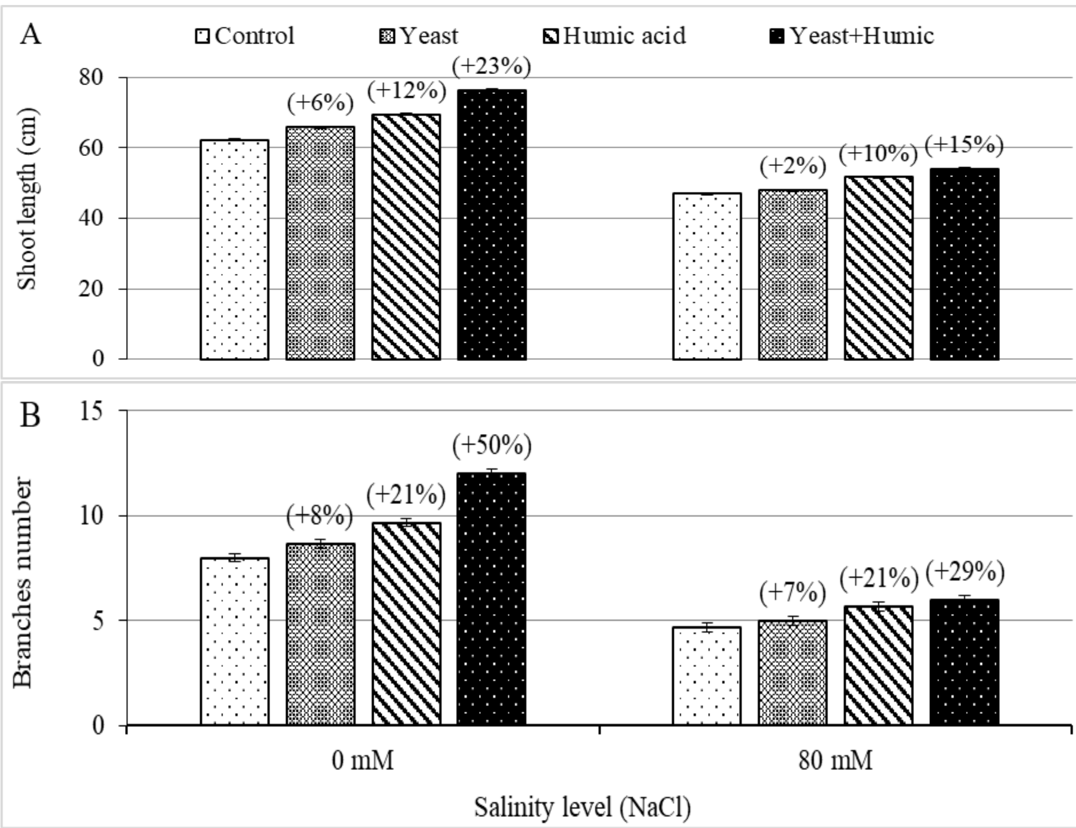

Figure: (1). The effects of (DY), (HA), and (DY+HA) on (A) shoot height (cm) and (B) number of branches plant-1 of basil plants grown under saline and non-saline environments. Values in parentheses indicate percentage increase from the control.

The results showed that the application of (DY), (HA), and their mixture significantly affected growth parameters, leaf number and leaf area for basil, under non-saline conditions, it was observed that yeast, humic acid, and their mixture significantly enhanced leaf number by (4, 8 and 9%), respectively, compared with the control group. Whereas, in saline conditions, leaf number increased by (14, 21, and 28%), respectively, compared with the control (Figure 2A). Likewise, in non-saline conditions, it was observed that yeast, humic acid, and their mixture significantly improved leaf area by (14, 22 and 45%), respectively, compared with the control. Whereas, in saline conditions, basil leaf area increased by (19, 62, and 91%), respectively, compared with the control (Figure 2B).

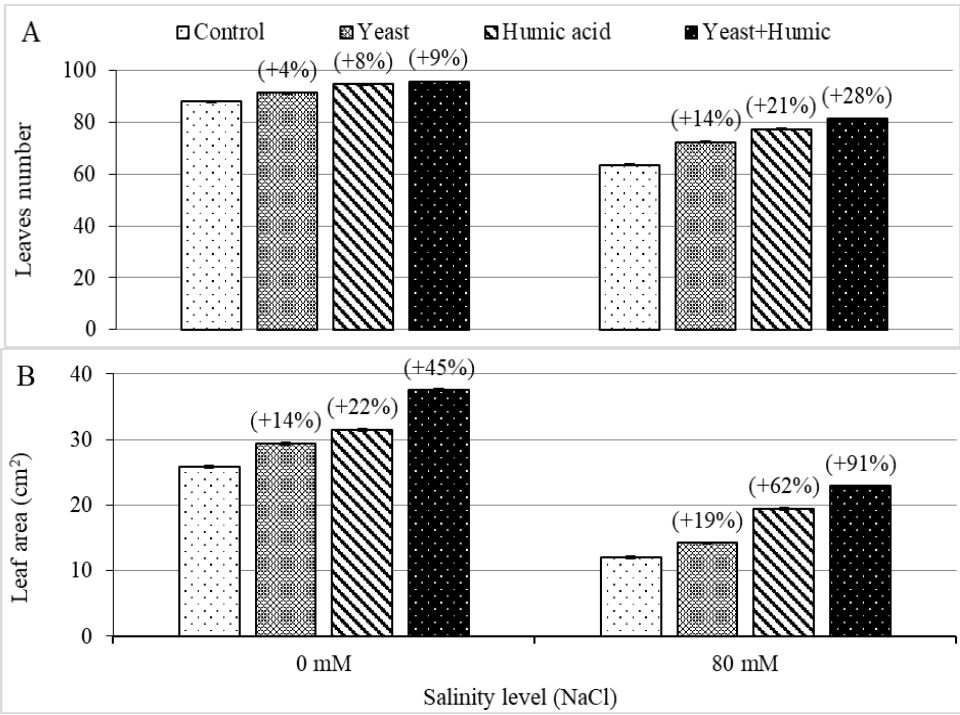

Figure: (2). The effects of (DY), (HA), and (DY+HA) on (A) leaf number plant^{-1} and (B) leaf area (cm^2) of basil plants grown under saline and non-saline environments. Values in parentheses indicate percentage increase from the control.

Spraying basil plants with (DY), (HA), and their mixture significantly affected growth characteristics, namely shoot fresh and dry weights. Under non-saline conditions, spraying with yeast, humic acid, and a mixture improved shoot fresh weight by (10, 23, and 43%), respectively. However, under saline conditions, spraying basil plants with yeast, humic acid, and a mixture increased shoot fresh weight by (6, 27, and 44%), respectively, compared with the control (Figure 3A). Similarly, applying yeast, humic acid, and a mixture significantly enhanced shoot dry weight by (6, 13, and 40%), respectively, under non-saline conditions and by (6, 13, and 25%) under saline conditions compared with the control (Figure 3B).

The single and combined application of yeast and humic acid improved the growth rate and water content of basil. The application of yeast and humic acid individually and synergistically increased the relative growth rate of the plant in the absence of salinity by (7, 18, and 45%), while the use of yeast, humic acid, and a mixture of both increased the relative growth rate of basil by (10, 24, and 35%), respectively, under salinity stress conditions compared with the control (Figure 4A). Correspondingly, the application of yeast and humic acid alone and in a mixture increased the relative water content of basil by (3, 5, and 6%), respectively, under normal conditions. Yet, under saline conditions, the percentage increase in relative water content of the plants was (25, 40, and 53%) as a result of applying dry yeast solution, humic acid, and a mixture of both, respectively, compared with the control plants (Figure 4B).

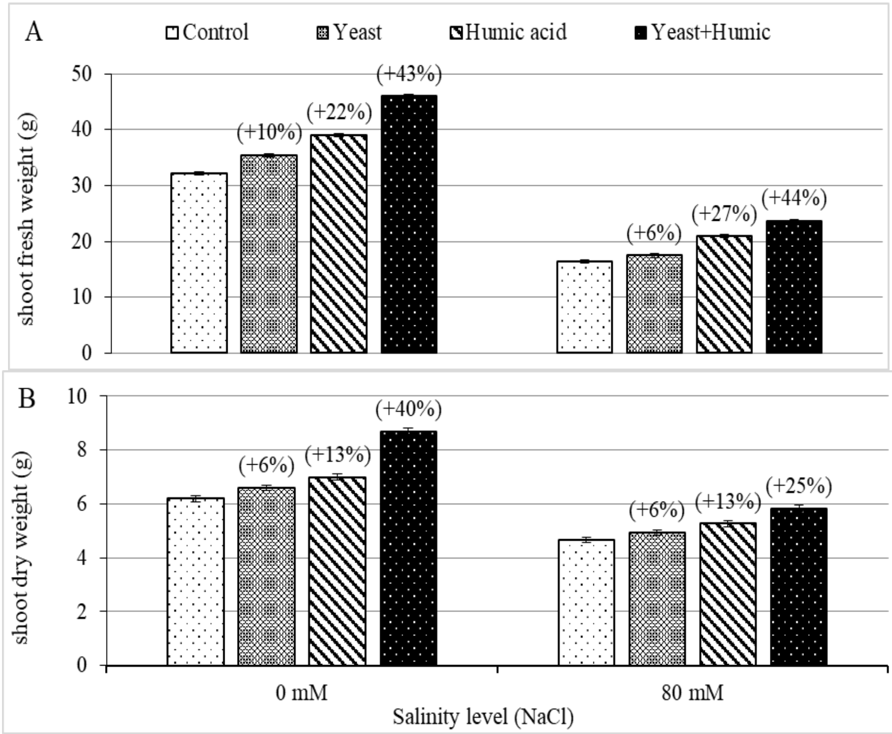

Figure: (3). The effects of (DY), (HA), and (DY+HA) on (A) shoot fresh weight (g) and (B) shoot dry weight (g) of basil plants grown under saline and non-saline environments. Values in parentheses indicate percentage increase from the control.

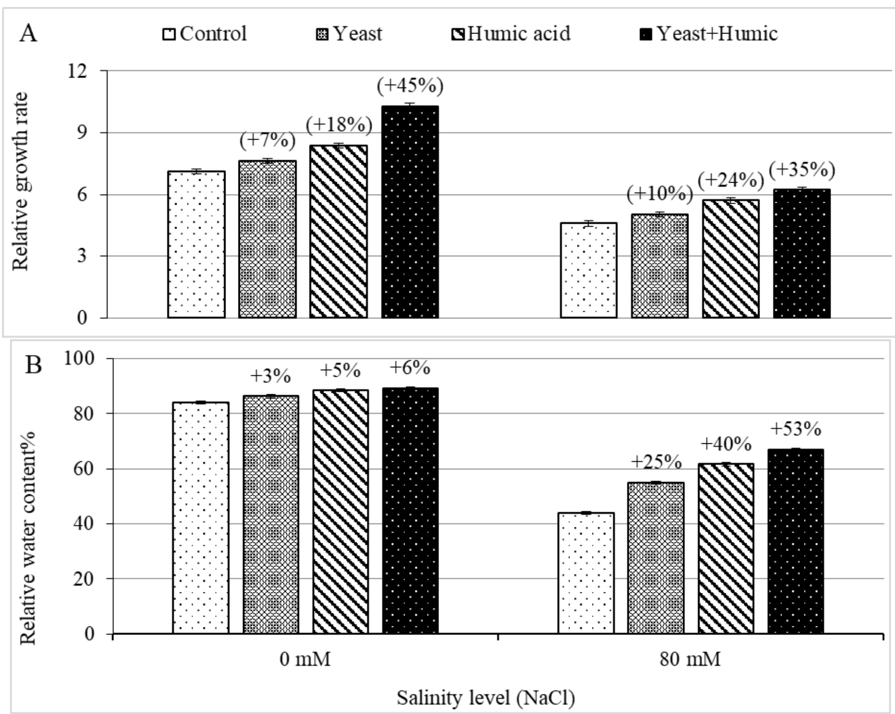

Figure: (4). The effects of (DY), (HA), and (DY+HA) on (A) relative growth rate and (B) relative water content (%) of basil plants grown under saline and non-saline environments. Values in parentheses indicate percentage increase from the control.

DISCUSSION

The results showed that salinity stress significantly decreased growth traits (Tables 1 and 2). Salinity leads to a decrease in soil water potential, making water absorption more difficult and consequently reducing cell turgor pressure and weakening cell elongation (Ehtaiwesh, 2016). Salinity also causes mineral imbalance, as sodium and chloride compete with essential elements such as potassium, calcium, and magnesium, thus reducing the absorption of elements necessary for proper growth (and Tester, 2008; Jadczak et al., 2021). This results in weakened cell wall structure, decreased photosynthetic efficiency, and reduced overall vegetative growth (Heidari, 2012). The application of humic acid reduced the negative consequences of salt stress and significantly improved basil growth under both conditions. Humic acid improves soil properties, increases water retention, and reduces nutrient leaching, thus providing a better environment for root growth. This leads to more efficient absorption of water and nutrients, resulting in increased vegetative growth (Yang et al., 2021). It also increases nutrient availability by forming chelated complexes with micronutrients. It balances the effect of sodium in saline soils, thereby improving mineral nutrition and increasing vegetative growth and biomass (Yang et al., 2021). Humic acid increases chlorophyll content, activates enzymes involved in photosynthesis, and improves intracellular water balance, thereby increasing leaf area and the relative growth rate of the plant (Yıldız et al., 2022; Ehtaiwesh, 2025). The application of yeast reduced the adverse effect of salinity and significantly improved basil growth in both conditions. Because yeast contains compounds similar to auxins, cytokinins, and gibberellins, it stimulates cell division and elongation (Alzweek and Salem, 2024). Yeast is also rich in vitamins, amino acids, proteins, and sugars, thus activating metabolic processes and increasing the synthesis of proteins and enzymes, and improving vegetative growth (Nimsi et al., 2023). Yeast also enhances photosynthesis by increasing chlorophyll content, improving chloroplast efficiency, and increasing leaf area and number, leading to higher photosynthetic efficiency and providing sufficient energy for rapid growth. Furthermore, yeast stimulates the root system, increasing lateral root growth and improving water and nutrient absorption, thus supporting growth (Ehtaiwesh and Abuiflayjah, 2024; Yousif et al., 2024).

Yeast increases plant resistance to salt stress by stimulating the accumulation of osmotic compounds (such as proline) and reducing oxidative damage through activation of antioxidant systems, thus mitigating the negative effects of stress (Ehtaiwesh, 2023). It is worth noting that both separate and combined application of yeast solution and humic significantly improved the vegetative growth of basil under saline and non-saline conditions. In particular, the synergistic use of yeast and humic reduced stress-induced damage. The improvement in plant growth when spraying with a mixture of yeast and humic is attributed to their synergistic effect. Yeast acts as a natural source of growth regulators and vital elements, while humic acid improves nutrient absorption and reduces the harmful effects of salinity, thus enhancing physiological processes and improving growth and productivity under stress conditions (Ibrahim et al., 2022; Yousif et al., 2024). The improvement in the physiological indicators of the plant treated with the yeast and humic mixture is attributed to their synergistic effect, which led to an increase in relative water content as a result of improved water uptake and maintenance of cell turgor pressure, in addition to increased proline accumulation, which plays an important role in osmotic adaptation and protection of cell membranes under stress conditions (Saidimoradi et al., 2019; Silva et al., 2025). The treatment also contributed to activating the antioxidant system, which reduced the harmful effects of oxidative stress and maintained cell integrity, which had a positive impact on plant growth and physiological efficiency under stress and non-stress conditions (Abu-Ria et al., 2023; Nimsi et al., 2023; Dmytruk et al., 2025). The results presented here provide a comprehensive assessment of the effects of both dry yeast solution and humic acid on the growth and development of basil plants under normal and salt stress conditions.

CONCLUSION

The results showed a significant difference in the response of basil plants to salinity stress when the plants were treated with both humic acid and yeast, as demonstrated by the growth and productivity characteristics under salinity conditions. Foliar spraying with humic acid and/or yeast solution enhanced basil plant growth and achieved higher yields. Therefore, these findings suggest the use of humic acid and yeast to mitigate the effects of salinity, with further research to be conducted on other plants and under different environmental stresses such as heat, drought and other abiotic stresses. Future studies should also evaluate the effectiveness of humic acid and yeast under high salinity and poor-quality water conditions, and assess their effect on different basil varieties, including essential oils and aroma-related characteristics.

ACKNOWLEDGEMENT

The author acknowledges the Department of Botany, Faculty of Science, University of Zawia for the research support.

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