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Effect of humic acid and seaweed extract on growth, yield and essential oil of rosemary (*Rosmarinus officinalis L.*)

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Abstract

Objective

Rosemary (*Rosmarinus officinalis* L.) is a significant perennial aromatic and medicinal plant. It has economic, industrial, and medicinal value. This plant is widely used in the pharmaceutical, food, and cosmetic industries. The study was conducted to evaluate the impact of humic acid and seaweed extract on growth, yield, essential oil and active compound content of rosemary.

Materials and methods

The field trial was implemented during the crop season 2024-2025 at the research station of College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad. The trial implemented as factorial arrangement according to a randomized complete block design (RCBD) with three replications. Factor A: Humic acid (0, 1 and 2 g/L). The second factor assessed was the concentration of seaweed extract (0, 1 and 2 mg/L).

Results

The addition of humic acid significantly affected most of the measured traits, as the treatment of 2 g/L achieved the highest average in the number of branches, reaching 6.696 branch/plant, root length 28.06 cm, the percentage of volatile oil in the leaves 4.703 %, the plant yield of essential oil 297.3 mg, and the leaf content of the active compound 765.9 mg Cineole. Spraying with seaweed extract at a concentration of 2 mg/L significantly outperformed by achieving the highest significant average in most of the measured traits. As the treatment of 2 mg/L achieved the highest average in the number of branches, reaching 6.784 branch/plant, root length 27.13 cm, the percentage of volatile oil in the leaves 4.455 %, the plant yield of essential oil 289.8 mg, and the leaf content of the active compound 773.3 mg Cineole. The interaction effect between the two study factors was significant in most of the studied traits.

Conclusion

The results of this study showed that the combined application of humic acid and seaweed extract (especially at a concentration of 2 g/L humic acid and 2 mg/L seaweed extract) can significantly

improve the vegetative growth, biomass production, essential oil percentage and 1,8-cineole content of rosemary plants. Therefore, it can be concluded that the use of natural biostimulants can increase the productivity of rosemary and support sustainable agricultural practices.
Keywords: 1,8-cineole, Essential Oil, Humic substances, *Rosmarinus officinalis* L., seaweed extract

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Introduction

Rosemary (*Salvia rosmarinus Spenn*., formerly *Rosmarinus officinalis L.*) is a significant perennial aromatic and medicinal plant. It belongs to the family Lamiaceae. This plant has economic, industrial, and medicinal value. It is widely used in the pharmaceutical, food, and cosmetic industries. This is due to its essential oil, which is rich in biologically active compounds such as cineole, camphor, alpha-pinene, and borneol. These compounds have antioxidant, antimicrobial, and anti-inflammatory properties. Therefore, the demand for rosemary cultivation and essential oil production has increased (Nieto et al., 2018). Environmental risks associated with chemical fertilizers have increased. These risks include soil degradation, pollution, and negative effects on ecological balance. Therefore, sustainable agriculture has become necessary to improve soil health and plant productivity (Tilman et al., 2002). In this context, humic substances and biostimulants have emerged as environmentally friendly natural alternatives. They provide support for plant growth and soil health at the same time (Calvo et al., 2014). In general, humic acid is the most important natural humic substance in soil organic matter. It enhances soil structure and makes nutrients more available. It also stimulates root growth and plant metabolic activity. Studies have shown the addition of humic acid leads to the enhancement of vegetative growth, increased accumulation of secondary metabolites in medicinal and aromatic plants, including rosemary. In addition, it improves plant height, branch number, and biomass. It also increases essential oil content and active compounds (Khaled & Fawy, 2011). Seaweed extracts, such as Acadian extract, are obtained from brown algae such as Ascophyllum nodosum. These extracts are rich in macro- and micronutrients, amino acids, polysaccharides, and natural plant growth regulators (Craigie, 2011). Foliar spraying with Acadian extract has been shown to improve photosynthetic activity. Seaweed extracts stimulate cell division, improve tolerance to biotic and abiotic stresses, and enhance the accumulation of secondary metabolites. It also increases the essential oil content in aromatic plants, including rosemary, basil, and thyme.

Although many studies have examined the effect of humic acid or seaweed extract separately, recent studies have indicated that the use of innovative techniques for intervention in the growth of rosemary plant can improve vegetative growth, biomass, and content of active compounds in essential oil. This confirms the importance of searching for effective and environmentally friendly methods to improve the productivity of medicinal and aromatic plants (Al-Khazraji et al., 2025). Moreover, phytobiotics and medicinal plants have gained considerable attention in recent years due to their potential as natural alternatives to synthetic additives in nutrition (Amirteymoori et al., 2021). These natural products are rich in bioactive compounds such as essential oils, alkaloids, flavonoids, and phenolic acids, which contribute to their antimicrobial, antioxidant, and anti-inflammatory properties (Mohammadabadi et al., 2025a). Consequently, phytobiotics play a crucial role in improving health, performance, and product quality (Safaei et al., 2025). The use of phytobiotics and medicinal plants as natural antimicrobial growth promoters in place of antibiotics in feed offers numerous advantages (Khezri et al., 2025). These benefits include improved zootechnical efficiency parameters, suppression of specific diseases (Mohammadabadi et al., 2025b), antimicrobial and antioxidant activities, hypocholesterolemic effects, enhancement of digestive enzymes, and improved function (Roudbar et al., 2015). Moreover, phytobiotics have been shown to modulate microbiota, which enhances nutrient absorption and supports overall immune function (Vahabzadeh et al., 2021). Studies have demonstrated that incorporating these plants into the diets can increase feed consumption, improve feed conversion ratios, and enhance yield (Vahabzadeh et al., 2020). Furthermore, phytobiotics are associated with reducing stress-related impacts in animals, improving meat quality, and decreasing the environmental impact of animal production systems by optimizing nutrient utilization (Alhasoon et al., 2026; Mohammadabadi et al., 2025c). Given the growing global concerns regarding antibiotic resistance and the demand for safer and healthier products, the incorporation of phytobiotics and medicinal plants into feed presents a promising and sustainable alternative strategy (Mohammadabadi et al., 2022). Therefore, this study was conducted to evaluate the individual and combined effects of humic acid and seaweed extract on vegetative growth, biomass yield, essential oil percentage, and 1,8-cineole content of rosemary plants under field conditions.

Materials and methods

A field trial was conducted during crop season of 2024-2025 at the research fields belonging to the College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, in loamy soil (silty clay loam). The experiment aimed to assess the impact on growth traits, aromatic yield, and some active compounds of rosemary plant through the incorporation of humic acid and application of seaweed extract. The experiment field was subjected to soil service operations in the experimental field (plow, smooth and leveling. Next, the field was subdivided into 27 experimental units. Each experimental unit had an area of 1 m^2. Nitrogenous and phosphate fertilizers were applied in accordance with the recommended guidelines for soil improvement. Uniform rosemary seedlings (6 months old) were selected and transplanted to the field on 20 March 2025. They were sown at 50 cm spaced between rows and also 25 cm between one plant and another. This was done to ensure suitable spacing and proper provision of nutrients. The experiment was designed using a randomized complete block design (RCBD) with three replicates for statistical accuracy. The experiment included two factors. The first factor (H) included three concentrations of humic acid (0, 1 and 2 g/L). The second factor was seaweed extract spray, represented by (SW), at three concentrations (0, 1 and 2 mg/L). The aqueous solutions of seaweed extract and humic acid were prepared by dissolving the required weights, each separately, at the rate of 1 g per liter of water. The plants were sprayed with seaweed extract (Acadian) twice. The initial spray was performed 30 days post-transplanting. One month after the first spray, a second spray was used. We conducted spraying in the early morning by applying a 200 L hand sprayer until fully wetting on vegetative growth. The irrigation process was applied with the addition of Humic acid.

Studied traits: The following traits were measured at the end of June 2025 as follows:

Number of branches per plant: The number of main branches was counted for five plants taken randomly from each experimental unit.

Total leaf yield (kg/ha): It was calculated using the following equation:

$$\text{Leaf yield (kg/ha)} = (\text{Plant leaf yield (g/plant)} \times \text{density (plant/ha)}) / 10^3$$

Leaf chlorophyll content (mg/g fresh weight): It was measured according to the method described by Mackinney (1941).

Root length (cm): It was measured from the point of its connection with the crown to the farthest point by using a measuring tape. The longest main roots of five plants taken randomly from each experimental unit were measured.

Fresh weight of leaves (g): Five plants were taken from each experimental unit. The leaves were separated from the branches. Then they were washed from soil particles. A sensitive balance recorded their fresh weight. After that, their average was calculated.

Dry weight of leaves (g): After recording the fresh weight of the leaves, the leaves were separated manually from the branches for five plants from each experimental unit. Then they were air-dried at room temperature. This was done by placing them in perforated paper bags with turning from time to time until they dried completely and the weight became constant. Then their weight was recorded and their average was calculated.

Percentage of volatile oil in the leaves (%): The percentage of volatile oil was determined by utilizing the following equation (Guenther, 2008):

$$\text{volatile oil (\%)} = \frac{\text{volatile oil weight (g)}}{\text{Sample weight (g)}} x\ 100$$

Plant yield of essential oil (mg): Essential oil was extracted from dried leaf samples by hydro-distillation using a Clevenger-type apparatus for 3 h according to the British Pharmacopoeia method. Plant yield of essential oil was calculated according to the following equation:

Plant yield of essential oil (mg) = percentage of volatile oil × total leaf yield per plant (kg)

Then the averages were calculated.

Estimation and diagnosis of Cineole by using HPLC: The separation of 1,8-cineole was performed using an HPLC device under the conditions indicated in a Tab. 1. 1,8-cineole was qualitatively identified by comparing the retention time of the crude sample with that of standard compound (Nahar et al., 2022). Thereafter, the concentration of 1,8-cineole was determined applying the equation below:

$$\text{Concentration of active compounds} = (Ac / As) \times C \times D$$

Where, Ac = area of the active compound in the crude sample, As = area of the active compound in the standard compound, C = concentration of the standard compound, and D = number of dilution times.

Table 1. Separation conditions

Separation column	C-18 DB Colum (50 x 2.0)
Mobile phase	Phosphoric acid (0.1%) + methanol 88: 12 (V : V)
Flow rate	1.2 mL/min
Sample concentration	25 µg/mL
Detector	UV at 285 nm wavelength and 20°C

Statistical analysis: After the findings collection and tabulation, statistical analysis was performed on the data using Genstat software, according to the experimental design used. The least significant difference (LSD) at the 0.05 probability level was used to compare the average treatments (Steel et al., 1997).

Results and discussion

The results revealed that humic acid significantly affected branch number (Table 2). The dose of 2 g/L achieved the highest average of 6.696 branch/plant. The concentration of 1 g/L gave 6.251 branch/plant. The control treatment, without humic acid addition, gave the lowest mean, which reached 5.801 branch/plant. This might result from the function of humic acid in enhancing photosynthesis efficacy. It may also increase nutrient accumulation. These nutrients may increase cytokinin activity that counter the effect of auxins. As a result, apical dominance decreases. At the same time, the vascular connection region between lateral buds and the stem becomes more differentiated. This leads to the growth of a greater number of main vegetative branches (Moore, 1982). This result agrees with Farruggia et al. (2024) and Al-Fraihat et al. (2023). It was stated that the application of humic acid results in a significant enhancement in the number of rosemary branches. The results in Table 2 also indicated that seaweed extract significantly affected branch number. Spraying at 2 mg/L achieved the highest average, which reached 6.784 branch/plant. The concentration of 1 mg/L gave 6.158 branch/plant. Spraying with distilled water (SW0) gave the lowest mean, which reached 5.806 branch/plant. The increase in branch number may be due to the nutrients present in seaweed extract. The impact of these nutrients extends to photosynthesis, respiration, and cellular metabolism, and contributes to the synthesis of nucleic acids vital for cell division. In addition, they contribute to the formation of enzymes, proteins, and hormones. These compounds promote branch formation in the plant (Dobermann et al., 2022; Hernandez et al., 2024). This result agrees with Farruggia et al. (2024) and Al-Khamas & Al-Rubaie (2023). They additionally documented a significant rise in the number of rosemary branches after foliar application of seaweed extracts. The statistical significance of the interaction between the two study factors on branch number is evident in Table 2. The combination H2 × SW2 gave the highest value, which reached 7.280 branch/plant. In contrast, the combination H0 × SW0 gave the lowest value, which reached 5.143 branch/plant. The results in Table 3 showed that humic acid significantly affected leaf yield. The concentration of 2 mg/L achieved the highest average, which reached 107.1 kg/ha. The concentration of 1 mg/L gave 105.7 kg/ha. The control treatment, without humic acid addition, gave the lowest mean. It reached 5.801 kg/ha. The elevation in leaf harvest could possibly stem from the effectiveness of humic acid in enhancing nutrient uptake. It may also increase nutrient availability in the soil. In addition, it stimulates physiological processes such as cell division and photosynthesis. This leads to increased leaf formation (Chen and Aviad, 1990), that agrees with results reported by Al-Fraihat et al. (2023).

Table 2. Effect of humic acid addition, spraying with seaweed extract, and their interaction on number of branches (branch/plant)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW_0	SW_1	SW_2	
H_0	5.143	5.990	6.270	5.801
H_1	5.890	6.060	6.803	6.251
H_2	6.383	6.423	7.280	6.696
Average	5.806	6.158	6.784	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.111	0.111	0.193	

The results in Table 3 also showed that seaweed extract significantly affected leaf yield. Spraying at 2 mg/L achieved the highest average of 107.3 kg/ha. The concentration of 1 mg/L gave 105.8 kg/ha. The control treatment, without seaweed extract addition, gave the lowest mean, which reached 104.4 kg/ha. This could be a result of the function of seaweed extracts in stimulating vegetative growth. This increases the number of leaves and branches. It also helps create an efficient source for light interception. As a result, photosynthesis increases. This leads to a greater accumulation of manufactured nutrients. These effects are reflected positively in increasing leaf yield (Doğan & Yazar, 2025). These results are consistent with the results of Al-Khamas & Al-Rubaie (2023). The interaction between the two study factors demonstrated significance for leaf yield, as indicated in Table 3. The combination H2 × SW2 gave the highest interaction value, which reached 108.3 kg/ha. In contrast, the combination H0 × SW0 gave the lowest interaction value, which reached 102.5 kg/ha.

Table 3. Effect of humic acid addition, spraying with seaweed extract, and their interaction on total leaf yield (kg/ha)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	102.5	105.1	106.6	104.7
H1	104.5	105.7	107.0	105.7
H2	106.3	106.6	108.3	107.1
Average	104.4	105.8	107.3	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.59	0.59	1.02	

The results in Table 4 showed that humic acid significantly affected leaf chlorophyll content. The concentration of 2 g/L gave the highest mean, which reached 1.691 mg/g fresh weight. The concentration of 1 g/L gave 1.565 mg/g fresh weight. The control treatment, without humic acid addition, gave the lowest mean, which reached 1.531 mg/g fresh weight. This increase may be due to the effectiveness of humic acid in activating the series of vital processes inside the plant. It may also increase the permeability of cell membranes. This helps increase the movement of elements, including nitrogen. Nitrogen enters into the composition of the four porphyrin groups that are involved in chlorophyll formation inside the plant (White & Brown, 2010; Asadu et al., 2024). Al-Fraihat et al. (2023) repoted the same results on effect of humic acid on leaf chlorophyll content. The results also proved that leaf chlorophyll content was significantly affected by seaweed extract (Table 4). The mean for this treatment, 2 mg/L, was the highest (1.701 mg/g fresh weight). 1 mg/L yielded 1.562 mg/g FW. The lowest mean, 1.524 mg/g of fresh weight was obtained from the control treatment (without addition of seaweed extract). Perhaps it is because spraying seaweed extract on the plant is crucial. It plays an effective role in increasing the number of leaves. This is an expansive training enhances the photosynthetic material index of plant pigments and biochemical growth parameters. Chlorophyll is one of the main photosynthetic pigments. In addition, seaweed extract contains compounds similar to cytokinins. These compounds stimulate chloroplast formation and delay leaf senescence. This leads to increased chlorophyll accumulation and higher photosynthetic efficiency in the plant (Barbosa et al., 2024). These results were consistent with the results of Danteswari et al. (2025), Farruggia et al. (2024), and Al-Khamas & Al-Rubaie (2023). These researchers indicated an increase in leaf chlorophyll content when seaweed extract was sprayed on rosemary plants. Significant interaction of the two studied factors for leaf chlorophyll content showed in mg/g fresh weight. Among the combinations, H2 × SW 2 reached maximum interaction value of 1.833 mg/g fresh weight. In contrast, the H0 × SW1 had the least interaction value. It reached 102.5 mg/g fresh weight. The root length was significantly influenced by humic acid (Table 5). The highest mean root length (28.03 cm) was recorded at 2 mg/L. The least average achieved in the case of control treatment, which did not receive humic acid, and it achieved 22.05 cm. It could be because humic acid affects plant growth in general. This led to greater availability of nutrients. As a result, root cell elongation and cell division were stimulated. This improved the ability of roots to absorb nutrients (Chen and Aviad, 1990; Dobermann et al., 2022; Hernandez et al., 2024; Canellas and Olivares, 2014).

Table 4. Effect of humic acid addition, spraying with seaweed extract, and their interaction on leaf chlorophyll content (mg/g fresh weight)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	1.584	1.437	1.572	1.531
H1	1.44•	1.555	1.699	1.565
H2	1.547	1.694	1.833	1.691
Average	1.524	1.562	1.701	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.094	0.094	0.163	

This result agrees with that reported by Saber et al. (2019) on thyme plants. The results also showed that seaweed extract significantly affected root length. Spraying at 2 mg/L achieved highest average, which reached 27.13 cm. The dose of 1 mg/L gave 24.99 cm. The control treatment, without seaweed extract addition, gave the lowest mean, which reached 23.06 cm. The increase could be attributed to the function of seaweed extracts in enhancing root elongation. They contain natural plant hormones such as auxins, cytokinins, and gibberellins. These hormones promote root elongation and stimulate cell division. They also increase the availability of minerals required for cell growth. This improves root efficiency in absorbing nutrients from the soil. In this way, the plant can meet its nutritional requirements. This is reflected positively in plant growth.

Seaweed extracts also increase the activity of beneficial enzymes in the soil. They support beneficial microorganisms as well. This creates an ideal root environment for longer growth. In addition, they enter into the structure and formation of important compounds in the plant, such as DNA, RNA, and proteins. They also participate in all vital processes necessary for the plant. These include respiration and photosynthesis. Therefore, cell division and elongation increase (Craigie, 2011). These results confirm the results of Al-Khamas & Al-Rubaie (2023), who indicated that spraying rosemary plants with seaweed extract increased root length. The interaction between the two study factors did not significantly affect mean root length, (Table 5). This indicates the independent behavior of each factor in influencing this trait. The amount of fresh leaves weights was significantly affected by humic acid (Table 6). Overall, the mean for dose 2 mg/L was the highest achieved 107.2 g, followed by dose of 1 mg/L with an average of 104.2 g and control treatment (without humic acid addition) with a mean of 101.3 g; therefore, it is indicated that the increase in plant fresh weight may be related to the contribution of humic acid to improving plant growth. It acts effectively in enhancing plant growth and availability of nutrients. In addition, it enhances the permeability of cell membrane. So, it helps plant to absorb water and nutrients more efficiently. This helps the movement and translocation of water and minerals within the plant. It also reactivates plant enzymes. These are among the important functions performed by humic acid (Dobermann et al., 2022; Hernandez et al., 2024; Shamsa, 2022; Tang, 2025). This result agrees with Farruggia et al. (2024). The findings additionally indicated that applying seaweed extract to plants at a dose of 2 mg/L resulted in the highest average fresh weight of leaves (106.2 g).

Table 5. Effect of humic acid addition, spraying with seaweed extract, and their interaction on root length (cm)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	19.89	21.87	24.4	22.05
H1	23.13	25.46	26.62	25.07
H2	26.16	27.64	30.38	28.06
Average	23.06	24.99	27.13	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.564	0.564	NS	

When applying a concentration of 1 mg/L, the fresh weight of leaves reached 104.2 g per plant. The control treatment, without seaweed extract addition, gave the lowest mean, which reached 102.3 g. The possible explanation for this could be the contribution of seaweed extracts due to its presence of natural plant hormones, amino acids and vitamins. These compounds activate the plant's physiology, including photosynthesis, nutrient uptake and cell elongation. This was reflected positively in increasing the fresh weight of the plant (Ali et al., 2021). This result agrees with Farruggia et al. (2024). The interaction between the two study factors did not significantly affect plant fresh weight, as shown in Table 6. This indicates the independent behavior of each factor in influencing this trait.

Table 6. Effect of humic acid addition, spraying with seaweed extract, and their interaction on fresh weight of the plant (g)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	99.5	101.1	103.2	101.3
H1	102.1	104.1	106.3	104.2
H2	105.3	107.5	108.9	107.2
Average	102.3	104.2	106.2	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.42	0.42	NS	

The results in Table 7 showed that humic acid significantly affected the dry weight of leaves. The dose of 2 g/L achieved highest average of 34.23 g. The dose of 1 g/L achieved 32.14 g. The control treatment, without humic acid addition, gave the lowest mean, which reached 30.00 g. The elevation in the average of the characteristic could be attributed to the rapid assimilation of humic acid. In turn, this increases the activity of metabolic processes in plant cells. It also stimulates growth and cell division. As a result, leaf area increases. This affects photosynthesis positively. Therefore, plant dry weight increases (Dobermann et al., 2022; Hernandez et al., 2024). This result agrees with Farruggia et al. (2024). The outcomes presented in Table 7 revealed that applying seaweed extract to plants at a concentration of 2 mg/L resulted in the highest average dry weight of leaves, which measured 34.06 g. The concentration of 1 mg/L gave 32.09 g plant-1. The control treatment, without seaweed extract addition, gave the lowest mean, which reached 30.22 g. The plants treated with seaweed extract's superiority may arise from its significant nutrient composition. These nutrients contribute to the biosynthetic processes in the plant. They also play a role in activating photosynthesis and carbohydrate synthesis in the leaves. In addition, seaweed extract contains a high percentage of growth regulators. These stimulate vegetative growth. This was reflected positively in plant dry weight (Ali et al., 2021). This result agrees with Farruggia et al. (2024). The interaction between the two study factors did not significantly affect leaf dry weight, as shown in Table 7. This indicates the independent behavior of each factor in influencing this trait.

Table 7. Effect of humic acid addition, spraying with seaweed extract, and their interaction on dry weight of the plant (g)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	28.44	30.01	31.54	30.00
H1	29.86	32.29	34.26	32.14
H2	32.34	33.96	36.38	34.23
Average	30.22	32.09	34.06	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.39	0.39	NS	

The results in Table 8 showed that humic acid significantly affected the percentage of volatile oil in the leaves. The dose of 2 g/L achieved highest average, which reached 4.703%. The dose of 1 g/L gave 3.896%. The treatment, without humic acid addition (control), gave the lowest mean, which reached 3.297%. The increase in volatile oil percentage of rosemary with the addition of humic acid can be related to its nature as a humic substance. They are composed of carbon, hydrogen, oxygen and nitrogen in varying amounts. This yields compounds with variations in molecular weight. They act as plant nutrients when they added to the plant. This is seen in improved growth via their impacts on respiration and photosynthesis. Humic acid may stimulate endogenous phytohormone activity and nutrient uptake, thereby promoting vegetative growth. More secondary metabolites are generated as a result of this. This positive role of humic substances in plant growth is reflected in the increase in volatile oil percentage (Masidur Alam et al., 2012). This result agrees with Al-Fraihat et al. (2023) and Farruggia et al. (2024). Spraying the seaweed extract at 2 mg/L resulted in the highest average content of leaf volatile oil, which reached 4.455% (Table 8). for the dose of 1 mg/L gave 4.134%. The treatment without the addition of seaweed extract (control) achieved the least average of 3.308%. This rise could be attributed to the seaweed extract's function in providing the plant with its nutritional needs. It also improves the efficiency of vital processes, especially respiration and photosynthesis. As a result, the synthesis and accumulation of carbohydrates increase. This leads to increased production of secondary compounds, including volatile oils, and thus increases volatile oil yield. In addition, the increase in carbohydrate production as a result of efficient respiration and photosynthesis leads to increased production of terpene compounds. This occurs through the involvement of carbohydrates in the production of isoprene units (C_5H_8), which are the main component in the structure and formation of oils (Özyazici, 2021). This result agrees with that reported by Al-Khamas & Al-Rubaie (2023) and Farruggia et al. (2024). The interaction of both study factors was shown to be significant with respect to volatile oil in leaves (%) (Table 8). Among all combinations, the highest interaction value was reached at H2 × SW2 (5.152%). Conversely, the combination H0 × SW0 (the interaction value of which achieved 2.392%) provided the lowest possible interaction value.

Table 8. Effect of humic acid addition, spraying with seaweed extract, and their interaction on the percentage of volatile oil in the leaves (%)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	2.392	3.583	3.916	3.297
H1	3.485	3.907	4.296	3.896
H2	4.046	4.91	5.152	4.703
Average	3.308	4.134	4.455	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	0.066	0.066	0.114	

The results in Table 9 showed that humic acid significantly affected plant essential oil yield. The dose of 2 g/L achieved highest average, which reached 297.3 mg. The dose of 1 g/L gave 247.2 mg. The control treatment, without humic acid addition, gave the lowest mean, which reached 157.3 mg. The increase could possibly be attributed to the function of humic acid as a physiological stimulant. It enhances the absorption of essential nutrients by the plant. It also stimulates root growth and the activity of enzymes associated with the pathways of volatile oil compound synthesis. In addition, it improves photosynthesis efficiency and carbon transport to the leaves. This provides more raw materials for the synthesis of essential oils and oil glands. As a result, essential oil yield in the plant increases. This result agrees with Al-Fraihat et al. (2023). Spraying the seaweed extract at 2 mg/L resulted in the highest average content of essential oil yield, which reached 4.455% (Table 9). The dose of 1 mg/L gave 226.1 mg. The control treatment, without seaweed extract addition, gave the lowest mean, which reached 185.9 mg. The reason for this increase may be due to the concentration and efficiency of the extract. This leads to increased protein formation activity and the availability of NADH (Sharma et al., 2026). Volatile oils are biosynthesized and stored in different sites within plant parts. These include trichomes, trichome bases, and secretory cells. These structures are also affected by auxin as a growth hormone. This is reflected positively in plant essential oil yield (Prins et al., 2010). This result agrees with El-Nagar et al. (2025) and Al-Khamas & Al-Rubaie (2023). They indicated that essential oil yield increased with increasing spray levels of seaweed extracts in rosemary plants. The interaction of both study factors was shown to be significant with respect to essential oil yield (Table 9). The combination H2 × SW2 gave the highest interaction value, which reached 372.9 mg. In contrast, the combination H0 × SW0 gave the lowest interaction value, which reached 135.8 mg.

Table 9. Effect of humic acid addition, spraying with seaweed extract, and their interaction on plant essential oil yield (mg)

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	135.8	143.8	192.4	157.3
H1	186.1	251.2	304.2	247.2
H2	235.8	283.3	372.9	297.3
Average	185.9	226.1	289.8	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	3.12	3.12	5.40	

The results showed that humic acid significantly affected Cineole content (Table 10). The dose of 2 g/L achieved highest average of 765.9 mg. The concentration of 1 g/L gave 639.5 mg. The control treatment, without humic acid addition, gave the lowest mean, which reached 565.9 mg. The addition of humic acid may increase the content of active compounds in the essential oil, such as Cineole, because of its direct effect on plant physiological processes. Humic acid acts as a stimulant for the activity of enzymes involved in the synthesis of secondary metabolites. It also enhances photosynthesis and carbon transport to vegetative tissues. This provides more raw materials for the synthesis of phenolic compounds and terpenes. These compounds are responsible for the properties and quality of the essential oil. Additionally, humic acid enhances the assimilation of nutrients like nitrogen and phosphorus. These nutrients play an important role in activating secondary metabolic pathways. This leads to an increase in the amount and percentage of active compounds such as Cineole in the leaves, stems, and glandular hairs of the plant. This result agrees with that reported by Farruggia et al. (2024). Spraying plants with seaweed extract at a dose of 2 mg/L produced the largest mean for Cineole content, which equaled 773.3 mg fresh weight. This corresponded to a dose of 1 mg/L achieved 667.4 mg. The control treatment, without seaweed extract addition, gave the lowest average of 530.5 mg. The increase in active compounds after spraying rosemary plants with seaweed extract may be due to its content of nutrients. This is reflected positively in increasing active compounds. It can even enhance the glandular hairs, particularly the peltate type and secretory structures that are on the leaf surface. This interface consists of Phenylpropenes and Monoterpenes that increase the generation of active compounds such as Cineole within cell tissues. Moreover, seaweed extract contains hormonal components that play a role in biological metabolism inside the plant cell (Sangwan et al., 2001). This result agrees with that reported by Al-Khamas & Al-Rubaie (2023) and Hassan et al. (2024). The interaction of both study factors was shown to be significant with respect to Cineole content. The combination H2 × SW2 gave the highest interaction value, which reached 877.3 mg. In contrast, the combination H0 × SW0 gave the lowest interaction value, which reached 446.7 mg (Table 10).

Table 10. Effect of humic acid addition, spraying with seaweed extract, and their interaction on leaf content of the active compound Cineole

Humic Acid (g/L)	Sea weeds extract (mg/L)			Average
	SW0	SW1	SW2	
H0	446.7	584.8	666.1	565.9
H1	509.8	632.2	776.5	639.5
H2	635.0	785.4	877.3	765.9
Average	530.5	667.4	773.3	
LSD 0.05	Humic Acid	Sea weeds	Interaction	
	6.41	6.41	11.11	

Conclusions: The results of this study showed that vegetative growth, biomass accumulation, essential oil percentage and 1,8-cineole content in rosemary plant increased significantly with the use of humic acid and seaweed extract. The most favorable results for most of the measured traits were the combined treatment of 2 g/L humic acid and 2 mg/L seaweed extract. These improvements could probably be related to increased nutrient uptake, stimulation of physiological processes and increased biosynthesis of secondary metabolites. Therefore, the combined use of humic acid and seaweed extract can be recommended as an environmentally friendly strategy to improve rosemary productivity and essential oil quality under field conditions.

Author contributions

F. N. A. contributed to the study methodology and experimental design and performed data collection, G.A.A. assisted with data analysis and contributed to writing the initial draft and statistical analysis, and S. M. S. drafted the original manuscript and reviewed, F. N. A. discussed, and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical Considerations

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

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اثر اسید هیومیک و عصاره جلبک دریایی بر رشد، عملکرد و اسانس رزماری (*Rosmarinus officinalis L.*)

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چکیده

هدف: رزماری (*Rosmarinus officinalis L.*) یک گیاه معطر و دارویی چندساله مهم است که دارای ارزش اقتصادی، صنعتی و دارویی می‌باشد و به‌طور گسترده در صنایع داروسازی، غذایی و آرایشی استفاده می‌شود. هدف این مطالعه ارزیابی تأثیر اسید هیومیک و عصاره جلبک دریایی بر رشد، عملکرد، میزان اسانس و محتوای ترکیبات فعال رزماری بود.

مواد و روش‌ها: آزمایش مزرعه‌ای در فصل زراعی ۲۰۲۴-۲۰۲۵ در ایستگاه تحقیقاتی دانشکده آموزش علوم پایه، ابن‌الهیثم، دانشگاه بغداد انجام شد. طرح آزمایشی به‌صورت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی (RCBD) با سه تکرار اجرا گردید. عامل اول شامل اسید هیومیک در سه سطح (۰، ۱ و ۲ گرم در لیتر) و عامل دوم شامل عصاره جلبک دریایی در سه سطح (۰، ۱ و ۲ میلی‌گرم در لیتر) بود.

نتایج: افزودن اسید هیومیک تأثیر معنی‌داری بر بیشتر صفات مورد بررسی داشت؛ به‌طوری‌که تیمار ۲ گرم در لیتر بیشترین مقدار را در تعداد شاخه‌ها (۶/۶۹۶ شاخه در بوته)، طول ریشه (۲۸/۰۶ سانتی‌متر)، درصد اسانس در برگ‌ها (۴/۷۰۳٪)، عملکرد اسانس در هر گیاه (۲۹۷/۳ میلی‌گرم) و محتوای ترکیب فعال ۱،۸-سینئول (۷۶۵/۹ میلی‌گرم) نشان داد. محلول‌پاشی عصاره جلبک دریایی در غلظت ۲ میلی‌گرم در لیتر نیز بیشترین تأثیر مثبت را بر اغلب صفات داشت؛ از جمله تعداد شاخه‌ها (۶/۷۸۴ شاخه در بوته)، طول

ریشه (۲۷/۱۳ سانتی‌متر)، درصد اسانس (۴/۴۵۵٪)، عملکرد اسانس (۲۸۹/۸ میلی‌گرم) و محتوای ۱،۸–سینئول (۱۷۳/۳ میلی‌گرم). اثر متقابل بین دو عامل نیز در بیشتر صفات معنی‌دار بود.

نتیجه‌گیری: نتایج نشان داد که کاربرد همزمان اسید هیومیک و عصاره جلبک دریایی، به‌ویژه در غلظت ۲ گرم در لیتر اسید هیومیک و ۲ میلی گرم در لیتر عصاره جلبک دریایی، می‌تواند به‌طور قابل توجهی رشد رویشی، تولید زیست‌توده، درصد اسانس و محتوای ۱،۸–سینئول در رزماری را افزایش دهد. بنابراین استفاده از بیواستیمولانت‌های طبیعی می‌تواند بهره‌وری رزماری را افزایش داده و از کشاورزی پایدار حمایت کند.

کلمات کلیدی: ۱،۸–سینئول، اسانس، مواد هیومیکی، عصاره جلبک دریایی، *Rosmarinus officinalis L.*

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