

**Improving the growth, herb yield and essential oil of sage (*Salvia officinalis* L.)
by using humic acid and seaweed extract**

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Abstract:

The current experiment was established to assess the response of sage (*Salvia officinalis* L.) growth, herb yield and volatile oil production to humic acid, seaweed extract and their interactions. This experiment was done at Misurata, Libya. The tallest plants and more branch number were achieved when sage plants treated with 24 ml/L of humic acid as soil drench and 200 ppm of seaweed extract as foliar application also gave the best values for plant growth and herb yield per plant and per hectare. Increasing humic acid or seaweed extract rates gradually increased fresh and dry herb yield per plant. The highest volatile oil yield per plant and per hectare was obtained with the combination treatment of 24 ml/L of humic acid and 200 ppm of seaweed extract compared to the other combination treatments. In general, it could recommend that this treatment, produce a uniform response of sage growth and herb yield and enhancing volatile oil production under sandy soil conditions. According to the essential oil compounds, the highest values of 1,8-cineole, linalool, alpha-thujone, beta-thujone, camphor and borneol were obtained when the plants were treated with a high rate of humic acid and seaweed extract compared to the other formulations under study.

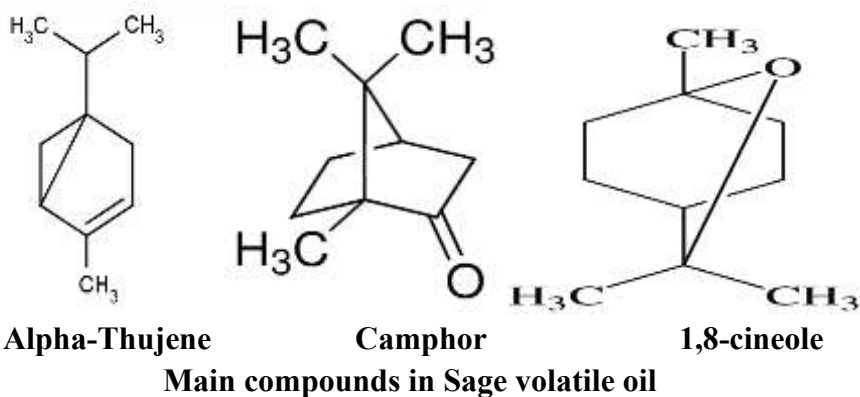
Keywords: Sage (*Salvia officinalis* L.), humic acid, seaweed extract, growth, herb yield and Essential oil compounds

Introduction

Introduction Medicinal herbs are a source of many chemical contents that are used as medicinal compounds. Products from the secondary metabolism of the plants are among the most expensive plant chemical compounds. The value of global markets for herbal medicines, such as medicinal plants and their products, has always been growing significantly. Due to the fact that most of the world market for medicinal plants is related to the output and supply of secondary metabolites derived from these plants, biologically active compounds from herbs have very high economic values (**Shabani et al. 2015; Yusupova et al. 2023; Wang et al. 2021**). The above-ground parts of sage (*Salvia officinalis* L.) from the Lamiaceae family, have several medicinal properties, as well as varied vitamins and minerals. Volatile oil from the above-ground parts of sage is one of the biologically active compositions that have various biological effects such as antiseptic, antimicrobial, antioxidant, and antiparasitic properties (**Rahmani et al. 2021**). Evaluation of the growth, yield, and secondary metabolites of the medicinal plants under water shortage conditions can be a guide for the cultivation of resistant medicinal plants in semiarid or arid climates. The medicinal herbs, unlike other horticultural crops that are damaged their yield under water deficiency conditions, may be more economically productive in such a situation by producing more phytochemicals (**Ansari et al. 2016; Caser et al. 2019; Ilkaee et al. 2011; Khalili et al. 2020, Raza et al. 2013**).

Sage (*Salvia officinalis* L.):

The genus *Salvia* belongs to the Lamiaceae family represented by 87 species, of which 44 are endemic in Turkey (**Nakipoğlu, 1993; Delamare et al., 2007**). *Salvia officinalis* L. (common sage), as the most economic species of genus *Salvia*, is a perennial woody sub-shrub with blue to purple flowers. This plant does not grow naturally in Turkey. But in recent years, due to the economic values of *Salvia* species, it has attracted the interest of producers (**Bahtiyarca et al., 2017; Sönmez and Bayram, 2017**). Common sage is widely used as the raw material of Pharmaceutical, perfumery, and the food industries (**Nadjafi et al., 2014**). Most phytochemical research of this species has focused on its volatile oil. α -Pinene (0.6-6.4%), Camphene (0.6-5.5%), 1,8-Cineole (5.3-14.6%), α -Thujone (15.2-26.6%), β -Thujone (5.2-12.9%), Camphor (16.4-20.0%), Borneol (1.8-4.9%), Bornyl acetate (2.1-2.2%), (E)- β -Caryophyllene (2.4-4.5%), α -Humulene (5.3-8.5%), Viridiflorol (4.0-8.5%) were identified as the main compounds of *S. officinalis* (**Raal et al., 2007**). The anticancer, anti-mutagenic, hypoglycemic, antioxidant, antimicrobial, antidementia, antinociceptive, and antiinflammatory activities of *S. officinalis* were reported according to various studies (**Jakovljevic et al., 2019**).



Humic acid (HA)

Organic manures contain plant promoting hormones i.e. IAA and GA, macronutrients, essential micronutrients and useful microorganisms (Sreenivasa et al., 2010). Compost is one of the most important organic manures. Zheljzkov and Worman (2004) proved that the use of compost resulted an enhance in physical and biological properties of the soil for examples: The capacity of water retention, pH, drainage, better availability of microorganism in the soil and decrease the native effect of chemical-based fertilizers and pesticides in the ecosystems

Humic acid (HA), a naturally occurring chemical in the soil as well as a byproduct of the breakdown of organic materials, has been employed successfully in the growth of a number of crops. There are several well described direct effects of HA on plant development, including improved macronutrient and micronutrient absorption and root growth (Guo Gao et al., 2015). The humic substances addition in medicinal plants has been evaluated in various studies and it has been found that these substances can enhance the production of secondary metabolites and increase the activity of bioactive substances such as flavonoids, coumarins, phenylpropanoids, total phenols, and anthocyanins. There are various methods of application, including through the soil, as a spray, or through fertigation. Regardless of the method used, the application of humic substances has been shown to significantly increase the biosynthesis of metabolism and production of biologically and pharmacologically active metabolites in medicinal plants (Pereira et al., 2019). In the last few decades, the application of organic fertilization is very important for medicinal and aromatic plants to obtain high quality and quantity of their products. Organic agriculture is based on minimizing the utilize of external inputs and avoiding applying synthetic fertilizers and pesticides (Galal and Ali, 2004). Humic acid amount of 0, 10, and 20 mg/kg of soil were used in the treatments of Basil plants. The results indicated that all humic acid dosages had a significant influence on height, number of lateral branches, and shoot dry weight when compared to the control. Humic acid treatment significantly raised the content of Fe, Mn, Zn, and Cu in plant shoots, while organic fertilizer application improved the availability of Fe, Mn, Zn, and Cu in soil. the study revealed that humic acid treatment may mitigate the negative effects of Phosphorus on the availability of micro elements, and so humic acid application is suggested for increasing plant and soil chemical conditions and enhancing basil plant development features (Jamali et al., 2015). Sardashti et al. (2012) found that the adding humic substances to the roots of these plants (*Artemisia herba-alba*) and (*Semenovia suffruticosa*) can significantly increase the percentage of essential oil composition. Sharaf-El-Deen et al. (2012) investigated the effects of humic acid application and different fertilizer types (NPK, compost, and cattle manure) on the vegetative growth, fruit yield, and essential oil quality of fennel plants. They recorded that the combined treatment of NPK (recommended dose) followed by the medium compost dose (15 m³/fed) and foliar humic acid application was the most effective. Application of humic acid, plant growth promoting rhizobacteria, and seaweed extract had positive effects on the growth parameters and essential oil content of dill (*Anethum graveolens* L.) plants, with humic acid having the greatest impact (Nasiroleslami and Safaridolatabad, 2014).

Seaweeds extract:

Seaweed extract has been shown to increase plant production and growth. Macronutrients including N, P, and K as well as Fe, Mn, Zn, and Mo as main micronutrients are found in seaweed extract. Auxins, gibberellins and cytokinins are examples of natural plant growth regulators found in seaweed extract (Crouch and Van Staden, 1993). Moreover, that seaweed extract is a fantastic source of bioactive

substances such protein, amino acids, carotenoids, vitamins, essential fatty acids, minerals, and growth regulators. Products made from seaweed, whether in powder or extract form, are a novel class of organic chemicals that are naturally occurring and help plants flourish. They promote faster germination of seeds, accelerate growth, boost agricultural yields, and raise the resistance of a number of crops (**Dhargalkar and Neelam 2005**).

Furthermore, foliar sprays of 4 ml/l seaweed extract as a high concentration considerably increased the fennel height, numbers of branches and umbels per coriander plant, plant fruit yield, feddan fruit yield, volatile oil percentage, and its production for plant and over feddan (**Ali et al., 2023**).

Abdulrahman and Sadeeq (2014) treated *Vinca roseus* plants with seaweeds extract at 0.5, 1.0 and 2.0 ml/l and revealed that plant height, branch number/ plant, leaf number/ plant, leaf area and average dry weight of plants were improved by all treatments. **Hassan (2015)** indicated that treating dill plants with seaweeds liquid extract at 0, 1, 2 and 3 ml/l led to an increase in plant height, branch number/ plant and herb dry weight/ plant. The author added that the highest values were detected by using the high concentration (3 ml/l). **Tarraf et al. (2015)** on fenugreek plants, emphasized that plant height, branch number, leaf member and plant fresh and dry weights at the stage of vegetative growth and flowering stage were augmented as a result of supplying algae extract. **Nofal et al. (2015)** obtained an augment in plant height, branch number/ plant, leaf number/ plant, total leaf area and shoot dry weight of *Calendula officinalis* due to the use of seaweeds extract at 1 ml/l under saline conditions. **Ali et al. (2017)** on anise reported that foliar spray with seaweeds extract at 1, 2 and 3 ml/l caused an increase in herb dry weight/ plant. This work aims to use safe alternatives in plant nutrition to achieve clean and pollutant-free agricultural production and determine the impact of humic acid and some seaweed extract as well as their interactions on the plant growth traits and essential oil of sage (*Salvia officinalis* L.) plants to find out the most suitable treatment for enhancing these characteristics.

Materials and Methods

Experimental site and treatments description.

This study conducted from February to September 2025 on a private farm in Misurata, Libya., to investigate the effects of a humic acid (HA), seaweed extract and their interactions on growth, herb yield characteristics and essential oil composition of sage (*Salvia officinalis* L.) plants. A separate plot design with three replicates was used in this study. Humic acid (HA) occupied four main plots (A), while the four Seaweed extract (SE) treatments (B) were distributed among subplots. Thus, 16 interactions (A × B) were achieved. Sage stems were planted on March 30 in a plot divided into 16 groups, each containing ten plants.

1- Humic acid levels at rates of 0, 8, 16, and 24 ml/L which are expressed by the following symbols (HA1, HA2, HA3 and HA4) were added to the soil in which the plants were planted three times during the study period.

2. The Seaweed extract treatments were as follows: control group (no sprayed plants), seaweed extract at concentrations of 50, 100, and 200 ppm. which are expressed by the following symbols (SA1, SA2, SA3 and SA4) The plants were foliar sprayed with these concentrations three times as follows: May 10, May 25, and June 10 for the first, second, and third sprays, respectively. The plants were foliar sprayed until the surface water runoff reached the plants. All other agricultural operations were carried out as usual during the study period.

Table 1. Some physical and chemical analysis of the experimental soil

Physical analysis										Soil texture		
Clay (%)		Silt (%)		Fine sand (%)			Coarse sand (%)			Sandy		
6.87		9.79		79.12			4.22					
Chemical analysis												
pH	E C m.mohs /Cm	Organi c mater (%)	Soluble cations (meq./ L)				Soluble anions (meq. /L)			Available (ppm)		
			Mg ⁺⁺	Ca ⁺⁺	K ⁺	Na ⁺	Cl ⁻	HC O ₃ ⁻	SO ₄ ⁻	N	P	K
8.1 1	0.84	0.74	1.3	1.4	3. 1	4.3	5.6	2.3	2.2	53. 5	75. 3	7.2

Harvest:

The plants were harvested at the end of August. Harvesting done by cutting the plants at a height of 10 cm above the soil surface. The following data recorded plant height (cm), number of branches/plants, fresh weight of herb (g), dry weight of herb (g), and essential oil content of air-dried herb according to the **British Pharmacopoeia (1963)** method.

Essential oil isolation from herb:

100 g of each replicate of all treatments were weighed, hydro-distilled (HD) for 3 hours using a Clevenger apparatus (**Clevenger, 1928**). The essential oil content was calculated as a relative percentage (volume/weight). In addition, the total essential oils were calculated as ml/100 plants using dry weight. The essential oils extracted from sage plants were collected for each treatment and dried with anhydrous sodium sulphate for chemical identification.

Sample preparation:

The sample was dissolved in dichloromethane and injected into GC.

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with VF-624ms column (30 m x 250 µm internal diameter and 1.4 µm film thickness). Analysis was carried out using Hydrogen as the carrier gas at a flow rate of 1.00 ml/min at a splitless, injection volume of 1.0 µl and the following temperature program: 40 °C for 0 min; rising at 6 °C /min to 200 °C and held for 0 min ; rising at 15 °C /min to 220 °C and held for 0 min ; rising at 30 °C /min to 250 °C and held for 2 min . The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 33-600 and solvent delay 3.50 min. The mass temperature was 230°C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Statistical analysis of the data:

The data obtained will be statistically analyzed according to the MSTATE-C model (1986) using a 5% LSD test to determine the differences between all treatments according to **Mead et al. (1993)**.

Results and discussion

Growth parameters:

The presented data in Table 2. Humic acid (HA) application significantly enhanced sage plants growth by increasing plant height and branch count compared to untreated plants. Higher natural nutrition rates consistently produced taller plants and more branches, with the highest rate (24 ml/L) yielding the best results. Plant heights reached 52.42. The highest branch counts recorded were 13.08. These findings underscore the significant impact of Humic acid on sage growth, likely due to its role in supplying essential nutrients, improving soil structure, and reducing bulk density (Benjamin et al., 2017). Moreover, these results align with earlier research on basil (El-Sayed et al., 2015), and caraway (Mahmoud et al., 2017).

Similarly, foliar application of seaweed extract (SE) significantly enhanced sage growth, as evidenced by increased plant height and branch count (Table 2). All tested seaweed extract concentrations outperformed the control, with the 200-ppm treatment producing the most pronounced improvements. Under this treatment, plant heights reached 53.75. Branch counts increased to 14.54. These results confirm the efficacy of foliar applied SE in boosting plant growth, which is consistent with findings in species such as Dill (*Anethum graveolens*, L.) Plants (Hassan 2015), *Ocimum gratissimum* (Abdou et al., 2011), *Nigella sativa* (Abd El-Khalek et al., 2023).

The interaction between Humic acid (HA) and seaweed extract (SE) significantly enhanced sweet sage's growth, as shown in Table 2 The optimal treatment was 24 ml/L of HA combined with 200 ppm SE, which produced the highest plant height and branch count. While most combined treatments outperformed the control, the control (no HA) with 50 ppm SE showed minimal improvement.

Table 2 Means plant height and number of branches per plant in sage (*Salvia officinalis* L.) plants as affected by Humic acid (HA) and seaweed extract (SE) and their interactions.

Humic acid (HA)	Seaweed extract (SE)				
	SE1	SE2	SE3	SE4	Means (B)
	Plant height (cm)				
HA1	38.00	42.33	45.00	48.00	43.33
HA2	42.33	46.00	49.00	52.67	47.50
HA3	43.67	48.33	51.33	55.00	49.58
HA4	46.00	50.67	53.67	59.33	52.42
Mean (A)	42.50	46.83	49.75	53.75	
L.S.D. at 5 %	A:3.32		B:4.31		AB:8.61
	Number of branches per plant				
HA1	7.17	8.83	11.50	12.83	10.08
HA2	8.17	10.00	12.50	14.00	11.17
HA3	8.67	11.00	13.33	15.00	12.00
HA4	9.33	12.00	14.67	16.33	13.08
Mean (A)	8.33	10.46	13.00	14.54	
L.S.D. at 5 %	For A= 1.71		For B= 1.83		For AB= 3.65

Herb fresh and dry yield per plant:

Table 3 demonstrate that both Humic acid (HA) and foliar-applied seaweed extract (SE) significantly enhanced sage herb fresh and dry weight. Increasing HA rates led to higher herb fresh and dry weight. The highest HA4 rate tested (24 ml/L) produced maximum herb fresh and dry weight 197.3 and 34.75, respectively, whereas the control (HA1) recorded the lowest values. These improvements support previous findings that Humic acid supplies essential nutrients to boost dry matter production (Ogbonna and Ubi, 2007; El-Naggar et al., 2015; Amhakhian and Isaac, 2016; Mahmoud et al., 2017) and Hassan et al (2024).

Table 3. Means herb fresh and dry weight (gm/plant) in sage (*Salvia officinalis* L.) plants as affected by Humic acid (HA) and seaweed extract (SE) and their interactions.

Humic acid (HA)	Seaweed extract (SE)				Means (B)
	SE1	SE2	SE3	SE4	
Herb fresh weight /plant (gm)					
HA1	158.3	177.3	153.7	195.3	171.2
HA2	163.3	181.0	192.3	202.3	184.8
HA3	168.3	185.0	195.0	209.0	189.3
HA4	179.3	190.0	205.3	214.7	197.3
Mean (A)	167.3	183.3	186.6	205.3	
L.S.D. at 5 %	A: 7.16		B:5.90		AB: 11.79
Herb dry weight /plant (gm)					
HA1	29.17	30.42	32.33	34.17	31.52
HA2	30.17	31.50	33.75	34.67	32.52
HA3	31.33	32.95	34.58	35.58	33.61
HA4	32.58	33.92	35.58	36.92	34.75
Mean (A)	30.81	32.20	34.06	35.33	
L.S.D. at 5 %	A:1.66		B:1.73		AB:3.45

Similarly, foliar application of seaweed extract (SE) enhanced herb fresh and dry weight compared to untreated plants. All seaweed extract concentrations increased herb fresh and dry weight, with the 200 ppm SE treatment being most effective. Under this treatment, herb fresh weights reached 205.3g, while corresponding herb dry weight were 35.33g. These results are consistent with earlier research on Ali et al & Sharma et al (2023) and Darzi et al (2012) & Hegazi et al (2015) on *Anethum graveolens*., confirming the beneficial effects of seaweed extract on herb fresh and dry weight. Table 3 reveal that the combined application of 24 ml/L of HA and 15 200 ppm SE resulted in the highest herb fresh and dry weight (g/plant), with values of 214.7and 36.92 g., respectively.

Essential oil production parameters (percentage/ ml/plant and L/hectare):

Table (4) Reveal that increasing Humic acid (HA) application rates significantly enhanced the essential oil production in sage herb. At the highest HA4 rate (24 ml/L), the essential oil % reached 1.383 and 0.484 for essential oil yield per plant (ml), while the essential oil yield per hectare (L) at 19.35 L/hectare/season. These findings support previous research on aromatic plants such as coriander (Hamza

et al., 2021; Rasouli et al., 2022), basil (El Gohary et al., 2023; Sayarer et al., 2023), and caraway (Omer et al., 2020), Sardashti et al. (2012) on (*Artemisia herbaalba*) and (Mohamed et al., 2022) on fennel.

Table 4. Means volatile oil production in sage (*Salvia officinalis* L.) plants as affected by Humic acid (HA) and seaweed extract (SE) and their interactions.

Humic acid (HA)	Seaweed extract (SE)				Means (B)
	SE1	SE2	SE3	SE4	
Volatile oil (%)					
HA1	1.137	1.207	1.263	1.320	1.232
HA2	1.200	1.247	1.310	1.363	1.280
HA3	1.213	1.263	1.323	1.383	1.296
HA4	1.443	1.300	1.357	1.433	1.383
Means (A)	1.248	1.254	1.313	1.375	
L.S.D. at 5 %	A: 0.062		B: 0.054		AB: 0.109
Volatile oil yield per plant (ml)					
HA1	0.333	0.372	0.416	0.457	0.394
HA2	0.362	0.393	0.443	0.474	0.418
HA3	0.381	0.417	0.459	0.493	0.437
HA4	0.472	0.444	0.486	0.534	0.484
Mean (A)	0.387	0.406	0.451	0.489	
L.S.D. at 5 %	A: 0.039		B: 0.034		AB: 0.068
Volatile oil yield per hectare (L)					
HA1	13.33	14.86	16.62	18.28	15.77
HA2	14.49	15.73	17.71	18.94	16.72
HA3	15.22	16.68	18.34	19.72	17.49
HA4	18.86	17.75	19.43	21.36	19.35
Mean (A)	15.48	16.26	18.03	19.58	
L.S.D. at 5 %	A: 1.54		B: 1.35		AB: 2.70

In addition, foliar application of seaweed extract (SE) significantly enhanced essential oil production. Although most seaweed extract concentrations improved the essential oil percentage relative to the control, the 200-ppm treatment was the most effective. Under 200 ppm SE4, essential oil percentages were 1.375 and 0.489 for volatile oil yield per plant (ml), with corresponding volatile oil yield per hectare (L) of 19.58 L/hectare/season. In contrast, the control treatments yielded lower percentages and yields. These results are consistent with previous findings in aromatic plants such as Ali et al & Sharma et al (2023) and Darzi et al (2012) & Hegazi et al (2015) on *Anethum graveolens*.

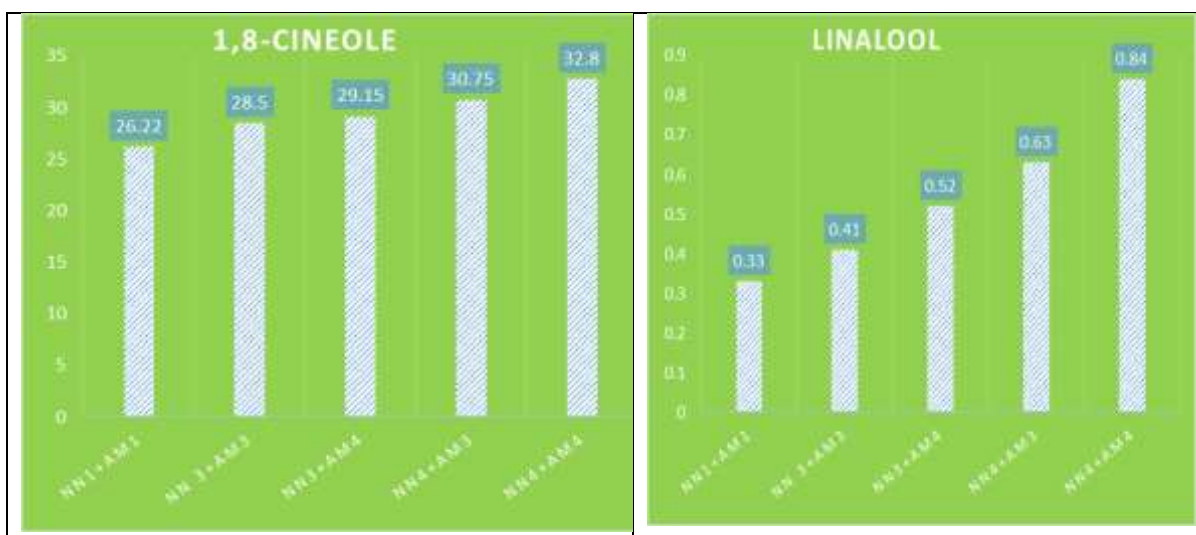
The interaction between HA and SE had a statistically significant effect on essential oil production in sage plants herb. Overall, all combined treatments boosted essential oil production compared to the control, with the exception of the treatment combining no HA1 with SE1 in some instances. Notably, the combination of 24 ml/L and 200 ppm SE was the most effective, achieving the highest essential oil production Table (4).

Effect of Humic acid (HA) and seaweed extract (SE) on essential oil compounds:

According to volatile oil compounds, HA4+SE4 combination treatment recorded the highest values of 1,8-Cineole, Linalool, α -Thujone, β -Thujone, Camphor and Borneol compared to the other combinations under study (Table 5 and Fig. 1).

Table 5. Effect of Humic acid (HA) and seaweed extract (SE) combinations on essential oil components of sage (*Salvia officinalis* L.) plants

E.O components \ Treatments	Treatments				
	HA1+SE1	HA 3+SE3	HA3+SE4	HA4+SE3	HA4+SE4
1,8-Cineole	26.22	28.50	29.15	30.75	32.80
Linalool	0.33	0.41	0.52	0.63	0.84
α -Thujone	19.05	20.51	21.40	22.70	24.33
β -Thujone	3.07	3.57	3.75	3.80	4.05
Camphor	32.11	34.25	35.75	36.22	38.74
Borneol	0.60	0.53	0.58	0.44	0.81



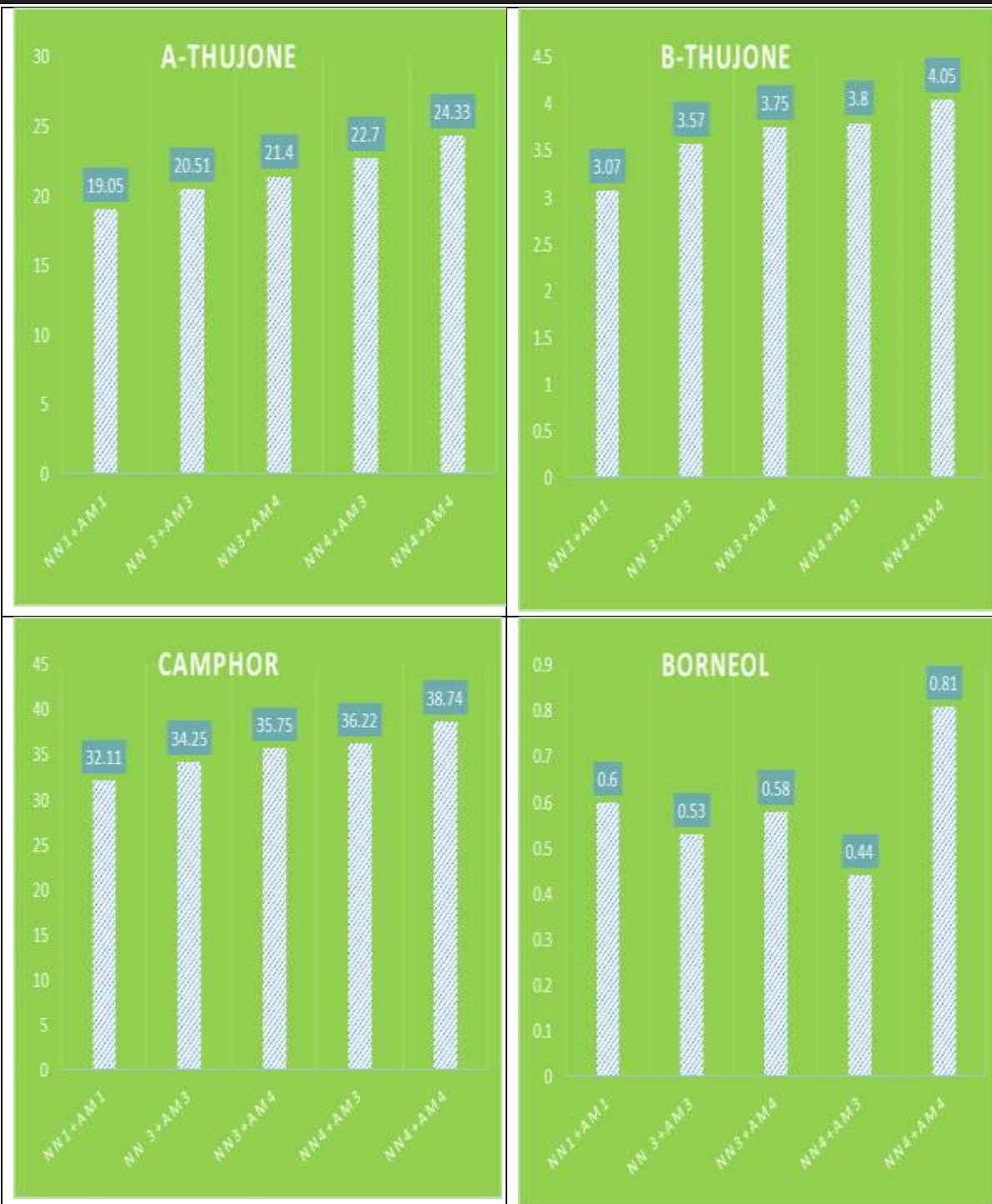


Fig. 1. Effect of Humic acid (HA) and seaweed extract (SE) combinations on essential oil components of sage (*Salvia officinalis* L.) plants.

Summary and Conclusion

The main conclusion of the study is that the Humic acid used enhanced the production of the sage (*Salvia officinalis* L.) plants. Different seaweed extract treatments significantly improved the growth, herb yield, and oil yield of the sage plants in comparison to the control. The combination between seaweed extract and humic acid had a substantial effect on growth, yield, and the oil index.

growth, yield, and the oil index. The high concentration of both agents (humic acid and seaweed extract) led to prove to be the most successful treatment .

Based on the results of this study, it is recommended that humic acid and seaweed extract led to be utilized to enhance the production of sage (*Salvia officinalis* L.) plants. It is also suggested that these two substances be employed in a safe manner to boost the production of other important medicinal and aromatic plants .

It is also proposed to include other medicinal and aromatic plants in the supply of natural and organic fertilizers in order to improve their productivity and achieve the principles of sustainable development.

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