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Enhancing growth, yield, essential oil content, and composition of holy basil (*Ocimum tenuiflorum* L.) using red algae-based bio-stimulant under acidic conditions of the Western Himalayas

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Abstract

Background The rising costs of synthetic fertilizers highlight the need for eco-friendly alternatives to enhance essential oil production in aromatic plants. This study evaluated the effects of red algae seaweed extract [*Solieria chordalis* (C. Agardh) J. Agardh] on holy basil (*Ocimum tenuiflorum* L.) in the Western Himalayas, by using five concentrations (0.0, 2.5, 5.0, 7.5, and 10.0 mL/L) and two application methods (drenching and foliar spray) in a factorial randomized block design analysing data at 5% significance level. Seaweed extract significantly improved growth and yield, with the highest essential oil content and biomass. By examining various concentrations and application techniques, the study seeks to determine the best practices for maximizing essential oil production.

Results Application of *S. chordalis* at 7.5 mL/L, increased essential oil content by 71.4% and biomass by 63.4% compared to the control. The foliar application also resulted in 50% higher essential oil content than drenching. Application of *S. chordalis* at 7.5 mL/L significantly enhanced essential oil components like eugenol, methyl eugenol, and methyl cinnamate by 61.5%, 17.6% and, 48.4%, respectively, compared to control. Based on the results of the present study, the foliar application of 7.5 mL/L seaweed extract is recommended for optimizing holy basil growth, biomass yield, essential oil content and composition.

Conclusions Based on the results of the present study, the foliar application of 7.5 mL/L seaweed extract is recommended for optimizing holy basil growth, biomass yield, essential oil content and composition. This study encourages the utilization of bio-stimulants, enhancing sustainability, and resilience in agriculture.

Keywords Tulsi, Drenching, *Solieria chordalis*, Essential oil, Seaweed, Trichome density

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Introduction

Ocimum tenuiflorum L., widely known as holy basil or tulsi, is an aromatic perennial plant (family Lamiaceae), primarily cultivated for its economically valuable essential oil (EO). The species thrives in diverse soil types and climatic conditions, that are cultivated in Brazil, China, Eastern Nepal and Indian regions like Bengal, Bihar, and the southern states [1]. In Ayurveda, holy basil is esteemed as the "Queen of Herbs" and the "Elixir of Life" for its extensive therapeutic and medicinal properties. Its EO is widely utilized in traditional and modern wellness practices for its anti-inflammatory, and antimicrobial effects, enhancing aromatherapy, skincare, and holistic health to support overall well-being [2]. The EO is extracted from the leaves and inflorescence of holy basil via steam distillation. The extraction process involves the careful harvesting of the plant's leaves and inflorescence, which are then subjected to steam distillation to release and collect the EO [3]. This EO is notable for its strong aroma and comprises a diverse array of compounds that are responsible for its medicinal and therapeutic benefits. It is notably characterized by a significant presence of eugenol, a major phenylpropanoid, with concentrations ranging from 1.94% to 60.20%. Other notable constituents include methyl eugenol (0.87% to 82.98%), methyl cinnamate (36.6% to 66.4%), β-caryophyllene (4.13% to 44.60%), and β-elemene (0.76% to 32.41%) [4, 5].

As global demand for basil EO rises, the use of inorganic fertilizers in its cultivation has emerged as a major concern. The extensive use of inorganic fertilizers, although effective for increasing crop yields, causes major issues in human health and environmental sustainability [6]. This escalating environmental concern underscores the urgent need for more sustainable agricultural practices. Replacing chemical fertilizers with bio-stimulants in agricultural practices, such as seaweed extract, shows potential as a viable and environmentally sustainable approach [7, 8]. Bio-stimulants are emerging as promising substitutes for specific chemical fertilizers due to their ability to modulate hormonal regulation, including functions associated with cytokinin, gibberellin, and auxin [9]. However, research on the bio-stimulant properties of these seaweeds remains limited, with studies often constrained to a few genera. *Solieria chordalis* (C. Agardh) J. Agardh is a species of red seaweed algae, recognized for its high concentration of sulfated galactose, which serves as the principal precursor of carrageenan [10]. Additionally, the saccharides found in seaweed extract hold the potential as elicitors, stimulating plant defence mechanisms [11]. Extensive research has consistently shown that foliar and drench applications of extract of *Ascophyllum nodosum* (L.) Le Jolis and *Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva seaweeds lead to improved growth in a variety of field crops, fruit crops, and vegetable crops [12]. Horticultural crops exhibit diverse reactions to various application techniques and concentrations of seaweed extract, potentially leading to changes in phenolic compounds, flavonoids, and antioxidant levels. Research findings have further highlighted the improvements in vegetative growth, chlorophyll levels, fruit yield, sugar content, and resistance to both foliar and soil-borne pathogens as a result of these applications [13]. However, research is scarce concerning the use of seaweed extracts in medicinal and aromatic crops. Specifically, meagre results are reported for the extract of *S. chordalis*. Nevertheless, there has been a notable increase in the utilization of seaweed extracts in organic and sustainable agricultural methods, driven by their perceived efficacy [14]. Numerous research investigations have determined that seaweed extract contains a broad spectrum of minor and major elements, qualifying it as a potential partial substitute for traditional fertilizers [15]. Similarly, several studies have shown that organic amendments and bio-inoculants can enhance EO production in various medicinal and aromatic plants (MAPs) such as *Dracocephalum moldavica* L. [16], *D. kotschyi* Boiss. [17], and *O. basilicum* L. [18]. This study aimed to assess the impact of different levels of *S. chordalis* extract on the morphological and physiological traits of holy basil plants. Specifically, we examined the alterations in EO content and composition after the application of seaweed extract.

Materials and methods

Experimental site

The study was conducted at the CSIR-Institute of Himalayan Bio-resource Technology, Palampur (altitude 1404 m amsl, 32°11′35"N latitude, 76°56′49" E longitude), Himachal Pradesh, India in 2022. Mean monthly data during crop growing season was obtained from agro-meteorological advisory and crop weather outlook (Fig. 1). The mean maximum temperature was 24.9 °C, ranging from 17.5 °C to 29.8 °C, while the mean minimum temperature was 15.4 °C, ranging from 6.8 °C to 20.0 °C, respectively. The mean relative humidity during the crop season was 64.3% with 7.15 h bright sunshine hours. A total rainfall of 441.63 mm was received during the crop-growing season. The experimental soil had a clay loam texture, with an acidic pH of 5.4 and non-saline with an electrical conductivity of 0.3 m mhos/cm. The organic carbon content was low (0.8%), available nitrogen was medium (293.6 kg/ha), available phosphorus was low (9.3 kg/ha) and available potassium was high (870.9 kg/ha).

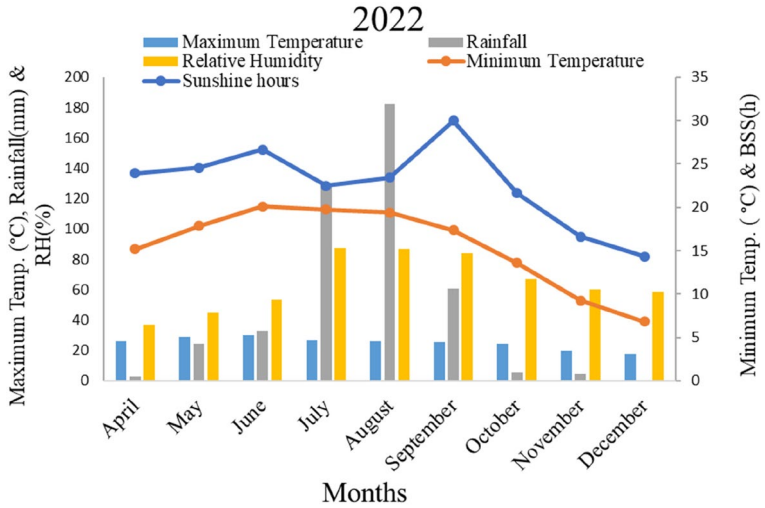

Fig. 1 Monthly mean maximum and minimum temperature (°C), bright sunshine (BSS) hours (h), rainfall (mm), and relative humidity (RH %) during the cropping season of 2022 at Palampur, India

Experimental details

The experiment was designed using a factorial randomized block design (FRBD) with ten treatment combinations and three replications. It was conducted on plots, each measuring (5 m × 2.5 m) 12.5 m^2 in size. Five different concentrations of *S. chordalis* (0.0, 2.5, 5.0, 7.5 and 10.0 mL/L) were applied through foliar spray and drenching. Seaweed extract was applied two times to the crop at 30 and 60 days after transplanting (DAT). The extract of *S. chordalis* was prepared by squeezing fresh seaweed harvested from the Tamil Nadu coast, following which it was filtered using muslin cloth and preserved for further use as a bio stimulant. The bioactive compounds in *Solieria chordalis*, including IAA, Zeatin, GA3, phenolics, flavonoids, and essential minerals, and its composition is given in (Table 1). Standard agronomic practices were followed to grow the crop. Five plants were chosen from each plot for recording observations. Plant height, plant spread north–south (NS) and east–west (EW), number of branches and Chlorophyll Content (CCI) were recorded in the standing crop. CCI was measured using a Chlorophyll Content Meter (Model CCM200 Plus GPS, Forestry Suppliers, Inc., Jackson, MS, USA. After 120 days of transplanting, the plants were uprooted and roots were carefully washed to remove soil. The biomass was chopped and hydro-distilled in the Clevenger apparatus for four hours. Anhydrous sodium sulphate (Merck) was used to remove moisture from EO and the oil was thereafter stored in dark glass bottles at 4 °C. The EO content and composition were evaluated in three replicates. The EO content was calculated as a percentage of fresh weight (v/w).

Table 1 Characteristics of *S. chordalis* lyophilized seaweed extract (3.92 g 100 mL^{-1}) values represented are mean ± standard deviation of three replicates

Parameters	Unit	Values
Micronutrients		
Boron	mg kg^{-1}	46.0 ± 8.20
Iron	mg kg^{-1}	726.1 ± 168.0
Manganese	mg kg^{-1}	61.4 ± 3.7
Zinc	mg kg^{-1}	32.2 ± 6.2
Copper	mg kg^{-1}	5.25 ± 0.50
Molybdenum	mg kg^{-1}	0.805 ± 0.25
Cobalt	mg kg^{-1}	1.39 ± 0.10
Macronutrients		
Nitrogen	g 100 g^{-1}	0.739 ± 0.15
Phosphorus	g 100 g^{-1}	1.38 ± 0.05
Potassium	g 100 g^{-1}	33.10 ± 0.0
Sodium	g 100 g^{-1-}	23.6 ± 3.52
Calcium	g 100 g^{-1}	0.05 ± 0.00
Magnesium	g 100 g^{-1}	1.04 ± 0.03
Hormones and bio-actives		
Total Phenolics	mg GAE (100 g)$^{-1}$	136.7 ± 26.2
Total flavonoids	mg QE (100 g)$^{-1}$	60.2 ± 3.5
Indole acetic acid*	mg L^{-1}	45.9 ± 0.0
Zeatin*	mg L^{-1}	19.50 ± 0.0
Gibberellic acid (GA_3)*	mg L^{-1}	12.50 ± 0.0

NB: *GAE* gallic acid equivalent, *QE* quercetin equivalent, *Values of IAA, GA and Zeatin pertains to single technical replicate of unlyophilized liquid extract

Glandular trichome characteristics

To observe the morphological characteristics of the leaf glandular trichomes, the surface topography of the leaf sample was examined at the full boom stage of the

crop by Scanning Electron Microscope (SEM S-3400 N, Hitachi, Japan). Fresh leaf samples were mounted on aluminium stubs using double-sided carbon tape and then coated with a thin layer of gold with the help of a sputter-coater at a vacuum of 10 Pa for 10 s to provide electrical conductivity. The sample stub was further loaded into the specimen holder and connected to the specimen exchange chamber in SEM. The images were captured at the desired magnification at an accelerating filament voltage of 30 kV. At least three leaflets per treatment and one leaflet per plant were used to observe the distribution and density of distinctive structures, averaging the density on 1 mm^2 areas on the micrograph obtained for each leaf.

Essential oil isolation and identification

The essential oil components were analyzed using GC and GC–MS techniques. The GC analysis was performed with a Shimadzu GC-2010 system equipped with a flame ionization detector (FID) and a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). For the GC–MS analysis, a Shimadzu GC–MS QP 2010 system equipped with an Auto-injector (AOC-5000) and a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) from SGE International, Ringwood, Australia, was used. The essential oil (10.0 mL) was dissolved in 2 mL of dichloromethane and injected into the gas chromatograph in split mode, with 2 mL per injection. Nitrogen gas was used as the carrier, flowing at 1.5 mL/min. The temperature was initially set to 70 °C for 3 min, followed by a ramp of 4 °C/min for 5 min. The injector and detector temperatures were set at 280 °C and 300 °C, respectively. The mass spectrometer operated at 70 eV ionization energy, with a reading range of 50–500 m/z and was set at 1 per scan. The chromatographic peaks were analysed to identify and quantify the volatile components, which were then arranged according to their elution order. Retention indices (RI) were determined using a homologous series of n-alkanes (C_8-C_{24}), and the percentage of peak area in the chromatogram was utilized for component identification [19, 20].

$$\text{Essential oil concentration (\%)} = \frac{\text{Amount of essential oil recovered (mL)}}{\text{Amount of crop biomass distilled (g)}} \times 100$$

Statistical analysis

Statistical analysis was performed using SYSTAT-12 software (Chicago, IL, USA) and standard analysis of variance (ANOVA). To compare treatment means, the least significant difference (LSD) was applied at the 5% probability level. Multivariate principal component analysis was carried out using PAST 3.0 (Paleontological Statistics Software Package for Education and Data Analysis Version 3.0) to assess the impact of treatments on essential oil components [21].

Results

Growth parameters

The application of *S. chordalis* extract significantly increased the number of branches per plant, plant spread (NS and EW), and CCI, as compared to the control. However, its effect on plant height was not significant (Table 2). The highest number of branches per plant, plant spread and CCI at 60, 90, and 120 DAT were recorded with the application of 7.5 mL/L. We observed a significant increase in the number of branches, plant spread and CCI with an increase in the concentration of seaweed up to 7.5 mL/L, however, a further increase in the concentration of seaweed 10.0 mL/L caused a significant decline in these growth parameters, although not inferior to the control in any case. The CCI increased from 60 to 120 DAT. Furthermore, the number of branches per plant, plant spread (NS and EW), and CCI were found to be significantly higher with the foliar application compared to the drenching. The interaction between application method and concentration was not significant for all the parameters.

Yield attributes and biomass yield

The effect of seaweed extract application on the aboveground biomass yield of holy basil was significant (Table 3). The application of 7.5 mL/L recorded maximum herbage yield (leaf + inflorescence) and was significantly superior to the rest of the treatments. A higher concentration of seaweed extract *i.e.*, 10.0 mL/L declined the herbage yield by 55% as compared to the 7.5 mL/L. Foliar application of seaweed extract enhanced the fresh and dry yield of herbage significantly over the drenching method. The interaction between seaweed extract dose and method of application was found to be significant. However, no significant effect of seaweed application was observed on the root biomass (fresh and dry).

Dry matter partitioning

Seaweed extract application showed significant effect on dry matter partitioning in different plant parts of *O. tenuiflorum* (Table 3). *S. chordalis* at 7.5 mL/L recorded significantly higher fresh and dry biomass of stems, leaves, and inflorescences than other treatments. Regarding application methods, foliar application significantly

Table 2 Effect of *S. chordalis* on growth parameters of *O. tenuiflorum*

Treatment		Plant height (cm) DAT			Primary branches DAT			Plant spread EW(cm) DAT			Plant spread NS (cm) DAT			CCI DAT		
		60	90	120	60	90	120	60	90	120	60	90	120	60	90	120
Sole Effect																
Application method	Drench	10.4	17.2	51.4	5.8^{b}	8.3^{b}	12.2^{b}	13.7^{b}	21.2^{b}	52.6^{b}	13.2^{a}	28.8^{b}	68.6^{b}	10.6^{b}	22.4^{b}	26.3^{b}
	Foliar	10.9	17.3	52.3	6.6^{a}	9.3^{a}	13.7^{a}	14.2^{a}	22.1^{a}	54.1^{a}	14.2^{b}	29.5^{a}	69.5^{a}	11.5^{a}	24.7^{a}	28.0^{a}
	SEm ±	0.252	0.07	0.32	0.20	0.21	0.226	0.171	0.12	0.144	0.103	0.134	0.21	0.076	0.137	0.242
	LSD (*P* = *0.05*)	NS	NS	NS	0.61	0.62	0.677	0.51	0.38	0.431	0.307	0.4	0.628	0.227	0.411	0.723
Concentration	Control	10.3	17.5	51.3	4.6^{b}	6.6^{c}	11.0^{c}	11.1^{d}	19.1^{d}	50.2^{d}	11.3^{b}	26.1^{d}	63.0^{d}	9.1^{d}	16.9^{e}	20.3^{c}
	2.5 mL/L	10.3	17.1	52.6	5.8^{b}	7.8^{bc}	11.8^{bc}	13.8^{bc}	21.7^{c}	52.0^{c}	13.8^{ab}	28.0^{c}	70.8^{c}	10.6^{c}	21.0^{c}	25.0^{b}
	5.0 mL/L	11.2	17.3	51.2	6.1^{b}	9.0^{bc}	13.5^{bc}	15.2^{bc}	23.3^{b}	55.5^{b}	15.3^{a}	31.7^{b}	72.7^{b}	11.7^{b}	24.0^{b}	26.9^{b}
	7.5 mL/L	11.0	17.1	51.6	9.3^{a}	12.5^{a}	16.7^{a}	16.7^{a}	25.4^{a}	58.8^{a}	16.9^{a}	34.0^{a}	75.8^{a}	14.7^{a}	37.0^{a}	42.5^{a}
	10.0 mL/L	10.5	17.2	52.6	5.0^{b}	8.1^{bc}	12.0^{bc}	13.3^{c}	19.1^{d}	50.3^{d}	11.1^{d}	26.1^{d}	63.0^{d}	9.11^{d}	18.9^{d}	20.9^{c}
	SEm ±	0.398	0.12	0.51	0.32	0.33	0.357	0.271	0.20	0.228	0.162	0.211	0.332	0.12	0.217	0.382
	LSD (*P* = *0.05*)	NS	NS	NS	0.973	0.99	1.07	0.81	0.60	0.682	0.486	0.633	0.99	0.36	0.651	1.143
Interaction Effect																
	LSD (*P* = *0.05*)	NS	NS	NS	NS	NS	NS	NS	0.852	0.964	0.68	NS	NS	0.509	0.92	1.617

SEm standard error of mean, *LSD* least significant difference, *NS* not significant, *DAT* Days after transplanting. Means within each column with similar letter are not significantly different at the 5% probability level

Table 3 Effect of *S. chordalis* on fresh and dry biomass (gram/plant) of different plant parts of *O. tenuiflorum*

Treatment		Stem		Leaf		Inflorescence		Root	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Sole Effect									
Application method	Drench	82.0^{b}	39.7^{b}	26.4^{b}	13.5	21.7^{b}	10.8^{ab}	16.5	8.1
	Foliar	87.9^{a}	43.5^{a}	28.9^{a}	14.0	23.3^{a}	11.5^{a}	16.4	8.0
	SEm ±	0.634	0.387	0.441	0.178	0.321	0.167	0.308	0.127
	LSD (*P* = *0.05*)	1.898	1.158	1.32	NS	0.96	0.5	NS	NS
Concentration	Control	61.5^{d}	29.6^{d}	18.7^{d}	8.5^{d}	10.2^{e}	4.9^{e}	16.1	7.8
	2.5 mL/L	79.8^{c}	37.2^{c}	27.0^{c}	14.1^{c}	13.4^{d}	6.7^{d}	16.5	8.2
	5.0 mL/L	97.1^{b}	48.6^{b}	32.9^{b}	16.3^{b}	33.6^{b}	16.8^{b}	16.6	8.2
	7.5 mL/L	123.4^{a}	61.4^{a}	41.0^{a}	20.6^{a}	38.1^{a}	18.9^{a}	16.3	8.0
	10.0 mL/L	63.2^{d}	31.1^{d}	18.8^{d}	9.35^{d}	17.1^{c}	8.4^{c}	16.5	8.1
	SEm ±	1.002	0.61	0.697	0.281	0.50	0.26	0.487	0.201
	LSD (*P* = *0.05*)	3.001	1.83	2.087	0.84	1.51	0.79	NS	NS
Interaction Effect									
	LSD (*P* = *0.05*)	4.24	2.58	2.952	1.189	2.147	1.119	NS	NS

SEm standard error of mean, *LSD* least significant difference, *NS* not significant. Means within each column with similar letter are not significantly different at the 5% probability level

increased the weights of stems, leaves, and inflorescences compared to drenching. However, there was no effect of seaweed concentration or application method on fresh and dry root weights.

Glandular trichrome characteristics

Electron-microscopic observations of the adaxial surface of leaves showed a significant change in the glandular trichrome density of holy basil leaves due to the seaweed extract application (Fig. 2). The glandular trichomes were denser in the treatment receiving the foliar application of seaweed extract at the concentration of 7.5 mL/L, as compared to the control. There was a noticeable reduction in the glandular trichrome density when the dose of seaweed extract was increased from 7.5 mL/L to 10.0 mL/L, regardless of the method of application.

Essential oil content

The effect of different concentrations of seaweed extract and its application method on the EO content

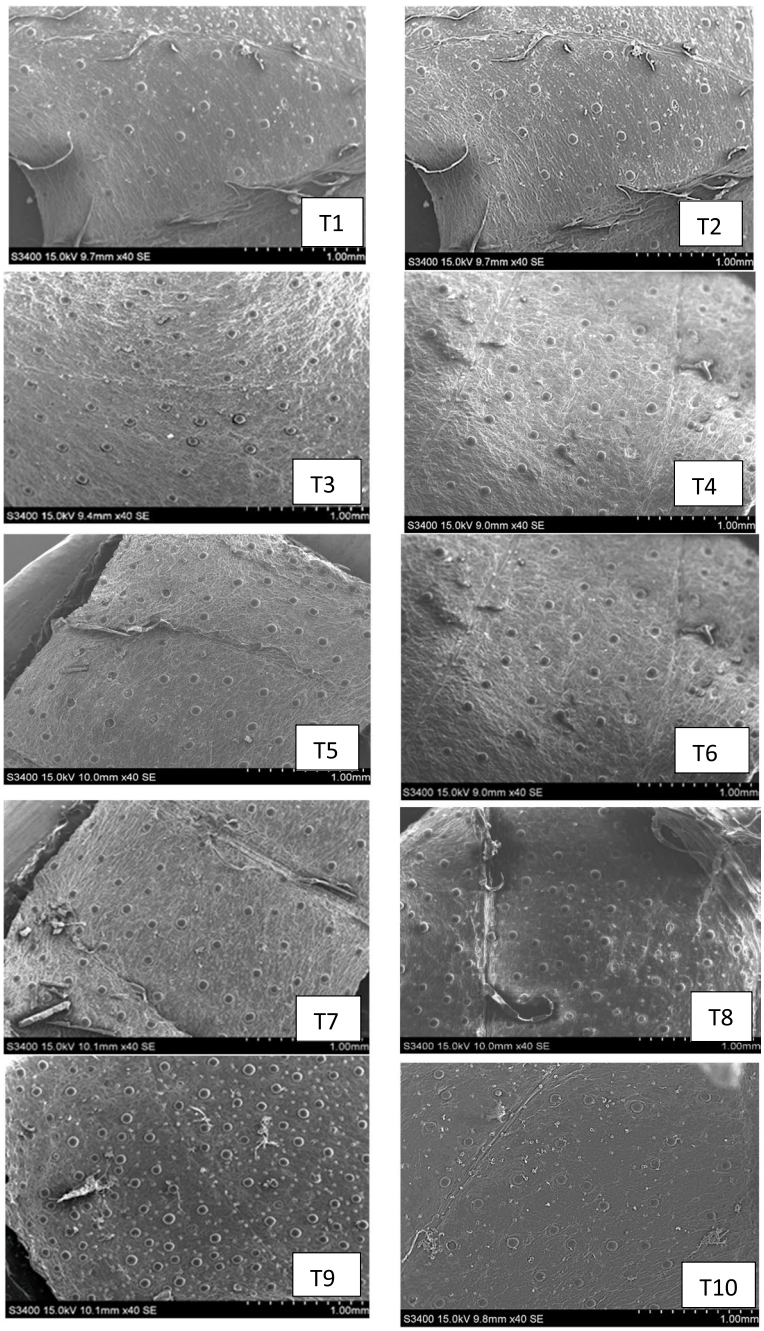

Fig. 2 Effect of *S. chordalis* on density of glandular trichomes of *O. tenuiflorum*. T1: Drenching with distilled water, T2: Drenching SC @ 2.5 mL/L, T3: Drenching SC @ 5.0 mL/L, T4: Drenching SC @7.5 mL/L, T5: Drenching SC @ 10.0 mL/L, T6: Foliar application with distilled water, T7: Foliar application SC @ 2.5 mL/L, T8: Foliar application SC @ 5 mL/L, T9: Foliar application SC @ 7.5 mL/L, T10: Foliar application SC @ 10.0 mL/L

was significant (Table 4). Significantly higher EO content was recorded with the application of 7.5 mL/L compared to other treatments. This treatment recorded five times higher EO content than the control. Seaweed extract at 2.5, 5.0, and 10.0 mL/L remained statistically at par with each other. In the case of the application method, the essential oil content was 50% higher with the foliar application as compared to drenching. However, the interaction between seaweed extract concentration and the method of application was not significant.

Table 4 Effect of *S. chordalis* on essential oil content (%) and composition (area %) of *O. tenuiflorum*

Treatment		Oil Content (v/w%)	Camphene	Limonene	Eugenol	Methyl Cinnamate	β- elemene	Methyl Eugenol	α-Humulene	β—Selinene	β- Cadinene	Elemol
RI (exp.)			960	1038	1360	1362	1387	1470	1494	1509	1528	1559
RI (lit.)			946	1024	1345	1376	1391	1452	1484	1522	1546	1548
Sole Effect												
Application method	Drenching	0.19^{b}	0.99^{a}	0.66^{a}	26.50	20.32	1.36^{b}	23.48^{b}	1.95	3.22^{b}	2.40^{b}	3.47^{b}
	Foliar	0.26^{a}	0.60^{b}	0.62^{b}	27.98	20.27	1.40^{a}	24.63^{a}	1.22	3.68^{a}	3.95^{a}	3.76^{a}
	SEm ±	0.015	0.006	0.005	1.37	0.181	0.005	0.24	0.452	0.049	0.025	0.021
	LSD (*P = 0.05*)	0.046	0.019	0.015	NS	NS	0.016	0.73	NS	0.148	0.076	0.063
Concentration	Control	0.07^{c}	0.63^{d}	0.43^{d}	18.50^{b}	13.30^{d}	1.70^{a}	22.53^{c}	1.28	1.43^{d}	5.24^{a}	1.03^{e}
	2.5 mL/L	0.18^{b}	0.70^{c}	0.83^{a}	24.56^{b}	18.40^{c}	1.44^{c}	22.55^{c}	0.97	2.05^{c}	3.37^{b}	2.98^{d}
	5.0 mL/L	0.23^{b}	0.75^{b}	0.58^{b}	24.88^{b}	22.18^{b}	1.48^{b}	23.01^{bc}	0.82	2.60^{b}	1.63^{d}	3.45^{c}
	7.5 mL/L	0.40^{a}	1.25^{a}	0.56^{bc}	48.08^{a}	25.76^{a}	0.85^{d}	27.36^{a}	2.98	9.35^{a}	2.21^{c}	6.20^{a}
	10.0 mL/L	0.24^{b}	0.69^{c}	0.83^{a}	20.18^{b}	21.80^{b}	1.44^{c}	24.81^{b}	1.89	1.81^{C}	3.54^{b}	4.43^{b}
	SEm ±	0.024	0.01	0.008	2.165	0.286	0.009	0.388	0.715	0.078	0.04	0.033
	LSD (*P = 0.05*)	0.073	0.03	0.024	6.484	0.856	0.026	1.162	NS	0.234	0.121	0.1
Interaction Effect												
	LSD (*P = 0.05*)	NS	0.043	0.034	9.169	NS	0.036	NS	NS	NS	0.171	0.141

*SEm (±)*Standard Error of Mean, *LSD* Least Significant Difference, *NS* Non-significant, *RI* Retention Index. Means within each column with similar letter are not significantly different at the 5% probability level

EO composition

The GC and GC–MS analysis of holy basil EO revealed that eleven constituents accounted for 97.38% of the total EO percentage (Table 4), a representative chromatogram is given in (Fig. 3). Phenyl propanoids, viz., eugenol and methyl eugenol predominated the essential oil composition, followed by esters (methyl cinnamate) and sesquiterpenes (β-elemene). The range of these identified chemical constituents was, eugenol (18.5–48.0%), methyl eugenol (22.5–27.3%), methyl cinnamate (13.3–25.7%), β-elemene (0.85–1.70%), β-selinene (1.43–9.35%), β-cadinene (1.63–5.24%), α-humulene (0.82–2.98%), limonene (0.43–0.83%), elemol (0.58–1.19%) and camphene (0.63–1.25%).

These essential oil constituents were significantly influenced by the application of seaweed extract. Camphene was highest in the treatment 7.5 mL/L seaweed extract dose (1.25%), followed by 5.0 mL/L (0.98%). On the other hand, limonene was maximum in treatments 10.0 mL/L and 2.5 mL/L seaweed extract (0.83%) and minimum in control (0.43%). β-elemene and β-cadinene were found to be maximum in control. However, eugenol, methyl cinnamate, methyl eugenol, β-selinene and elemol were significantly highest in 7.5 mL/L seaweed extraction treatment, with a respective increase of 61.5, 48.2, 17.5, 84.7 and 83.4% over the control. Seaweed extract doses and application method had no significant effect on α-humulene, however, its numerically highest value was recorded with the foliar application of 7.5 mL/L seaweed extract. A marked increase in the β-elemene, methyl eugenol, β-selinene, β-cadinene and elemol content of the holy basil EO was recorded with the foliar application of seaweed extract while drenching enhanced the camphene, limonene contents. However, no significant change in the eugenol, methyl cinnamate and α-humulene content was recorded due to different seaweed extract application methods.

Principal component analysis (PCA)

The identified components of *O. tenuiflorum* EO were subjected to principal component analysis (PCA) to analyse variability in the various treatment combinations (Fig. 4). The calculated variance of PC-1 and PC-2 were 88.6% and 11.5%, respectively, which accounted for 97.1% of the total variance. The PC-1 and PC-2 separated eugenol and EO content from other constituents and were positioned in the positive end of both PC-1 and PC-2. In contrast, methyl cinnamate and methyl eugenol were positioned in the negative end of PC-1, and camphene, methyl cinnamate, methyl eugenol, and essential oil content were in the positive end of PC-2. The scree plot and loading plot have been illustrated in (Fig. 4), with PC-1 and PC-2 being the most informative with Eigen values above one. Principal Component Analysis (PCA) separated the treatments into three distinguishable clusters, where cluster I comprised of T7 and T5, exhibited a higher content of camphene, eugenol, methyl eugenol, and methyl cinnamate. Cluster II consists of T2, T3, T6, and T9 exhibiting a lower content of methyl eugenol and EO content. Cluster III consists of T1 and T10 with lower content of camphene, eugenol, and methyl eugenol.

Discussion

Extensive research has demonstrated that varying concentrations and application methods of *S. chordalis* can potentially enhance crop yield and quality. Seaweed extract enhances plant growth and development by maintaining hormonal balance, activating nutrient transporter genes, promoting photosynthesis, and enhancing stress tolerance through antioxidant induction and reduced lipid peroxidation, including the modulation of reactive oxygen species (ROS) levels [22]. The spraying of seaweed extract at 30 and 60 days after transplanting recorded the highest crop yield [23]. In the present study, we observed a significant improvement in the glandular trichome

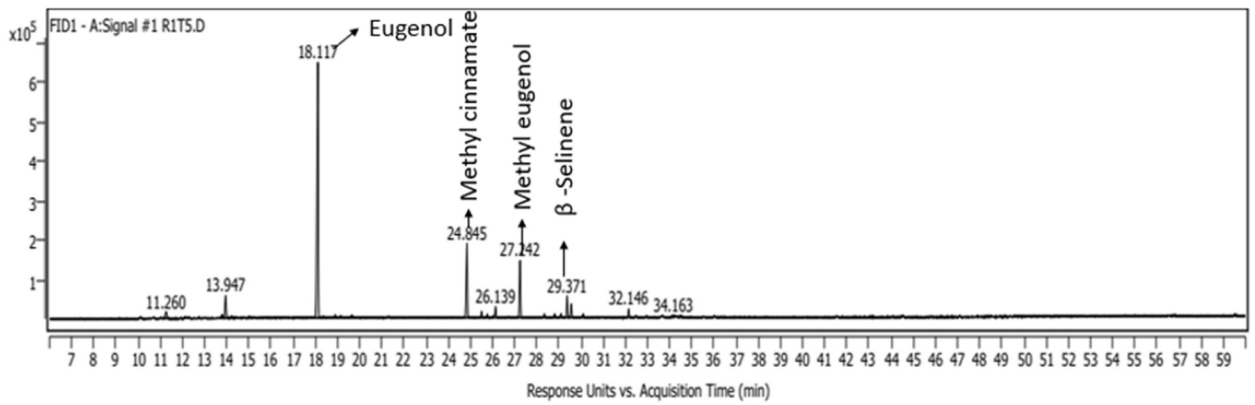

Fig. 3 Representative chromatogram of *O. tenuiflorum*

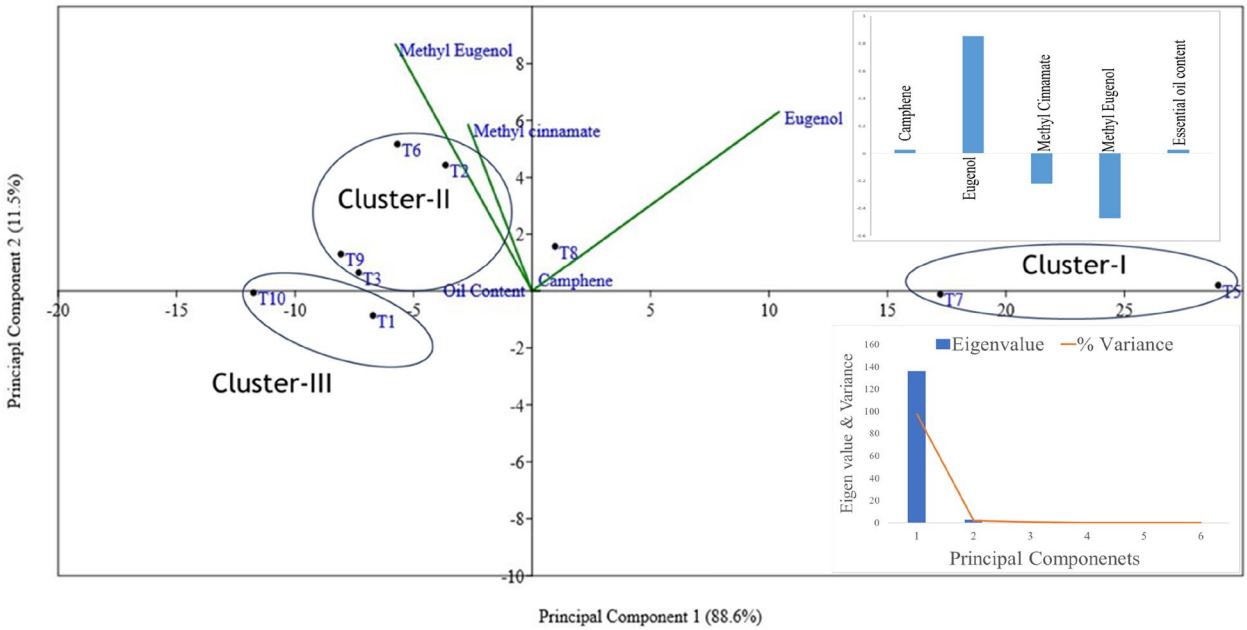

Fig. 4 Bi-plot of principal components based on mean value of composition of *O. tenuiflorum*. PCA explains 97.1% of the data variation. T1: Drenching with distilled water, T2: Drenching SC @ 2.5 mL/L, T3: Drenching SC @ 5.0 mL/L, T4: Drenching SC @7.5 mL/L, T5: Drenching SC @ 10.0 mL/L, T6: Foliar application with distilled water, T7: Foliar application SC @ 2.5 mL/L, T8: Foliar application SC @ 5.0 mL/L, T9: Foliar application SC @ 7.5 mL/L, T10: Foliar application SC @ 10.0 mL/L

density of the plant [24]. Seaweed extract is highly rich in phyto-hormones such as auxins, cytokinins, gibberellins, and abscisic acid which play an important role in regulating plant growth, development, and responses to environmental stresses [25]. The enhancement effect of the application of an appropriate amount of *S. chordalis* extract on essential oil content can be attributed to the presence of essential nutrients, and growth regulators such as auxins which are responsible for the increased biosynthesis of secondary metabolites, vitamins and hormones leading to higher production of essential oil [7, 26]. The foliar application of seaweed was more effective than drenching to improve the biomass yield and essential oil content of holy basil. This could be attributed to the direct and fast absorption of seaweed extract by plant tissues and also, the losses were less with the foliar application [23, 27].

Examination of the essential oil composition of *O. tenuiflorum* revealed that among all the constituents, eugenol, methyl eugenol, and methyl cinnamate emerged as the primary chemical compounds within the essential oil. Plants inoculated with bio-stimulants may exhibit enhanced primary metabolite production through boosted photosynthesis and other metabolic activities, thereby potentially augmenting the accumulation of secondary metabolites [28, 29]. Various researchers have reported that foliar application of *A. nodosum* seaweed extract improved the growth and production in different field crops [30], fruit crops and vegetable crops [15, 31]. The stimulatory effects of seaweed extract on the biomass yield, EO content, and composition have been reported in *Coriandrum sativum* L. [32], and *Hyssopus officinalis* L. [33]. Seaweed extracts may primarily affect the pathways related to growth, development, and stress response rather than those directly involved in the synthesis of chemical constituents, which could be the reason for no significant change in the percentage of eugenol, methyl cinnamate, α-humulene in essential oil with different seaweed application methods [34]. These findings indicate that secondary metabolism, which includes the production of these metabolites, is influenced by the application of seaweed extracts. Such secondary metabolites are known to enhance the plant's ability to adapt and survive under various environmental conditions.

Conclusions

The integration of *S. chordalis* into agricultural practices significantly enhances the growth and quality of aromatic plants, improving productivity and essential oil content. Our findings demonstrate that foliar application of *S. chordalis* at 7.5 mL/L on *O. tenuiflorum*, applied twice at 30 and 60 days after transplanting, led to increased essential oil content, enhanced by better nutrient uptake, plant tissue interaction, and rapid absorption rates. The

composition of essential oil was particularly enriched in compounds such as eugenol, methyl cinnamate, and methyl eugenol. However, higher concentrations (10.0 mL/L) had negative effects on crop growth and essential oil content. The mechanisms underlying these effects are not yet fully understood, emphasizing the need for further research into the action modes of bio-stimulants.

Moreover, *S. chordalis* is cost-effective when used in small amounts, offering a sustainable alternative to synthetic fertilizers. Its bioactive compounds enhance plant growth, stress tolerance, and soil health without high processing costs. For farmers with local access to *S. chordalis*, its use reduces overall expenses, making it an affordable and eco-friendly option. Long-term benefits to soil and plant health may result in further cost savings, improving its economic viability.

Future research should aim to optimize application techniques, clarify the underlying mechanisms, and explore the broader use of bio-stimulants across various agricultural systems. Ultimately, *S. chordalis* and other seaweed extracts represent a promising and emerging area of research in agriculture, with significant potential to enhance crop productivity, quality, and sustainability. These bio-stimulants offer a cost-effective and environmentally sustainable alternative to conventional synthetic fertilizers and inputs.

Abbreviations

EO	Essential Oil
DAT	Days After Transplanting
CCI	Chlorophyll Content Index
FRBD	Factorial Randomized Block Design
SEM	Scanning Electron Microscope
GC	Gas Chromatography
GC–MS	Gas Chromatography-Mass Spectrometry
PCA	Principal Component Analysis
ROS	Reactive Oxygen Species
AOC	Auto-injector (part of the GC–MS system)
RI	Retention Index
LSD	Least Significant Difference
mgL^{-1}	Milligrams per liter (unit of concentration)
mL/L	Millilitres per liter (unit of concentration)
DAS	Days After Sowing (sometimes used interchangeably with DAT)
g	Grams (unit of mass)
kg/ha	Kilograms per hectare (unit of biomass)
°C	Degrees Celsius (temperature unit)
mhos/cm	Reciprocal ohm (a unit of electrical conductivity)
T	Treatment (often used in experimental contexts, e.g., T1, T2)

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Authors' contributions

Saizal Jamwal- Experimental execution, formal analysis, data observation, data processing, oil analysis, identification of compounds, data curation, data compilation, data presentation, statistical analysis, literature search, and manuscript writing. Avnesh Kumari- Conducted SEM (Scanning Electron Microscopy) imaging of the samples, including sample preparation. Veeraprakasam Veeragurunathan- Preparation of Seaweed extract, characterization. Kamalesh Prasad- Hormone analysis. Arup Ghosh- Design, analysis, and reviewing the manuscript. Rakesh Kumar—Develop the idea, design the experiment, and supervise the investigation, data curation, validation, data processing, and paper editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study did not require ethics approval as it involved no human participants or sensitive data.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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