Effect of algae and yeast on the production of essential oil and some active constituents in rosemary

Rasha Fouad[a], Hend Fouad[a], Eman E. Aziz[a], Osama A. Nofal[b], Abdelhalim I. Rezk[c], Adel B. El-Nasharty[c], Elsayed A. Omer[a]

[a]Medicinal and Aromatic Plants Research Department, National Research Centre, 33 El-Bohouth St., P.O. 12622, Dokki, Giza, Egypt, [b]Plant Nutrition Department, National Research Centre, 33 El-Bohouth St., P.O. 12622, Dokki, Giza, Egypt, [c]Fertilization Technology Department, National Research Centre, 33 El-Bohouth St., P.O. 12622, Dokki, Giza, Egypt

Correspondence to Rasha Fouad, PhD, Department of Medicinal and Aromatic Plants, Institute of Pharmaceutical and Drug Industries, National Research Centre, 33 El-Bohouth Street, PO Box 12622, Dokki, Giza, Egypt. Tel: +20 100 355 0340; fax: +20 3337 0931; e-mail: frasha42@yahoo.com

Received: 29 January 2023
Revised: 25 February 2023
Accepted: 26 February 2023
Published: 28 September 2023

Egyptian Pharmaceutical Journal 2023, 22:424–431

Background
Rosemary is an important medicinal plant and one of the main aromatic spices in the world. Nowadays, it is very important to use natural substances such as algae and yeast in the green agriculture to increase quantity and quality of crops, in addition to preserving environment from the harms of using chemicals in the agriculture.
Objective
The study aimed to investigate growth, yield, and active constituents of rosemary under foliar spraying of different concentrations of both algae and yeast extracts.
Materials and methods
The experiment was performed during the two successive seasons 2019 and 2020 in completely randomized blocks design and consisted of seven treatments: two biostimulants with three levels of each factor, in addition to the control (tap water). Algae extract was sprayed with concentrations of 0.5, 1, and 2 g/l, whereas yeast concentrations were 5, 10, and 20 g/l. The growth parameters, total phenolics, antioxidant activity, essential oil percentage, yield, and its main constituents were studied.
Results and conclusion
The main components of essential oil were found to be endo-borneol followed by (+)-2-bornanone. The growth, yield, total phenolics, antioxidant activity, essential oil, and the main components of rosemary increased with all used concentrations of algae and yeast extracts compared with control. These increments reached their maximum with application of algae at 1 and 2 g/l and yeast extract at 10 and 20 g/l. In general, spraying yeast extract resulted in the highest average of growth, yield, and chemical constituents of rosemary, and the best parameters were obtained by spraying yeast at 20 g/l. It is recommended to spray rosemary with yeast extract at a dose of 20 g/l to obtain the best plant herbal yield, essential oil, total phenolic content, and antioxidant activity.

Keywords:
algae, antioxidant activity, essential oil, gas chromatography–mass spectrometry, *Rosmarinus officinalis*, total phenolics, yeast

Egypt Pharmaceut J 22:424–431

1687-4315

Introduction

Rosemary (*Rosmarinus officinalis* L) is one of the most important aromatic and medicinal plants in the world. It is grown in a variety of climates, native to Asia, Africa, and Europe, mainly in the regions around the Mediterranean Sea [1]. It is used as a natural preservative in the food industry, as a spice in cooking and as an ornamental plant [2,3]. *R. officinalis* has strong antidiabetic, anti-inflammatory, hepatoprotective, diuretic, antithrombotic, antimicrobial, and antioxidant activities [4]. It also has an inhibitory effect on the growth of breast, liver, leukemia, lung, and prostate cancer cells [5].

Enhancing crop productivity and yield quality while reducing dangers to the environment are the main goals of improving crop cultivation technology. Natural biostimulators are widely employed in the current agricultural production to increase crop yield and quality, while ensuring environmental and human safety [6]. Such substances are usually a rich source of phytohormones (e.g. auxins, gibberellins, cytokinins, and abscisic acid) and phenolic compounds [7]. Several biostimulants have shown promising results for boosting yield under normal conditions or preserving it under unfavorable conditions [8]. The results of Pereira *et al.* [9] highlighted the importance of using biostimulants in arid conditions to increase yield and nutrient content of the plant.

DOI: 10.4103/epj.epj_17_23

The plant biostimulants through different mechanisms of action are sustainable management practice for production of medicinal plants, increasing biomass production and enhancing secondary metabolites synthesis [10]. Biostimulants can effectively improve plant metabolic processes, leading to increased essential oil production in medicinal plants [11].

Algae as a biofertilizer improve the physiological performance of the plant. They are considered as an important group of microorganisms capable of fixing atmospheric nitrogen. In addition, algae extract naturally contains auxin, cytokinins, and gibberellic acid [12]. Application of algae extract resulted in noticeable higher increases in herb yield, nutrient content and their uptake, carbonic anhydrase, and essential oil production of *Mentha pulegium* plant [13].

Yeast is one of the richest sources of high-quality protein, namely, the essential amino acids like lysine and tryptophan; contains essential minerals and trace elements, namely calcium, cobalt, and iron; and is the best source of B-complex vitamins. The extract is a valuable source of bio-constituents, especially cytokinins [14].

The aim of this study was to investigate the effect of spraying of algae and yeast extracts on rosemary plants to improve plant growth and yield, essential oil yield, and the main components and providing growers with useful information about the response of rosemary plants to these biostimulators.

Materials and methods

Two field experiments were carried out at the Agricultural Experimental Station of National Research Center in Nubaria District, west of the Nile Delta of Egypt (its location is latitude 30° 30' 1.4' N, and longitude 30° 19' 10.9' E, Egypt), using a drip irrigation system during the two successive seasons of 2019 and 2020. This study was designed to investigate the effect of foliar application of two biostimulators (algae and yeast extracts) on some growth parameters, essential oil production and its constituents, as well as nutrients content and uptake of rosemary plants.

Representative soil samples were taken from one layer (0–30 cm) before cultivation for physical and chemical analyses according to Chapman and Pratt [15]. The soil was loamy sand with 1.1 ds/m EC, 8.1 pH, 0.5 OM, 6.0% $CaCO_3$,180 ppm nitrogen, 19 ppm phosphorous, 116 ppm potassium, 280 ppm calcium, 170 ppm magnesium, 0.3 ppm iron, 4.3 ppm manganese, 1.1 ppm zinc, and 1.4 ppm cupper.

Seedlings of *R. officinalis* were obtained from Horticulture Research Institute, Agricultural Research Center, Egypt. The seedlings were transplanted on March 5, 2019 and March 3, 2020, respectively, into the experimental field at 30 cm between drippers with 75 cm between lines.

This study was performed as a completely randomized blocks design with three replications. The treatments were foliar applications of algae and yeast extracts, in addition to control treatment; algae extract was sprayed at the rate of 0.5, 1.0, and 2.0 g/l and yeast extract (concentrated molas solution which is a secondary byproduct from yeast production) at the rate of 5, 10, and 20 g/l. The foliar application was applied four times during growth stages and the two cultivation seasons (2019 and 2020) of rosemary on shoot of rosemary. The first spray was applied at the end of March, April, June, and September.

The formulations of biostimulators were as follows: algae extract (3.2 mg/g indole butyric acid, 13.7 mg/g indole acetic acid, 15.89% total amino acids, 13.3% N, 2.22% P, 2.13% K, 0.44% Ca, 0.22% Mg, 0.01% Na, 19.3 ppm Fe, 6.8 ppm Mn, 4.5 ppm Zn, and 1.8 ppm Cu) and yeast extract (762.6 mg/l CYT, 195 mg/l Gib, 20% total amino acids, 7% free amino acids, 34.66% organic carbon, 59.75% organic matter, 7.23 pH, 4.62% total-N, 0.2% P_2O_5, 9.8% K_2O, 0.0% S, 0.87% Ca, 0.16% Mg, 5.3 mg/l Mo, 71.0 mg/l Fe, 11.3 mg/l Mn, 483.9 mg/l Zn, and 5.3 mg/l Cu).

The normal agricultural practices for rosemary cultivation were carried out as recommended. The plants were harvested on October 22, of the two seasons by cutting the aerial parts of each plant at 10 cm above the soil surface. Plant height (cm), fresh and dry weights of herb (g/plant and ton/ha), as well as essential oil percentage (%) and yield (ml/plant and l/ha) were recorded. Macronutrient and micronutrient contents of the herb were determined according to Chapman and Pratt [15].

Essential oil percentages of fresh herb were determined by hydro-distillation using the Clevenger-type apparatus according to the Egyptian Pharmacopoeia [16] and Omer *et al.* [17], and the essential oil yield (l/ha) was calculated. The resulted essential oil was separately dried over anhydrous sodium sulfate and was kept in the deep freezer till used for chemical analyses. To identify the main constituents and to determine their relative percentages, the essential oils were separately subjected to gas chromatography–mass spectrometry analysis using gas chromatography–mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center, following the conditions mentioned by Ibrahim *et al.* [18]. Preparation of plant extract to determine total phenolics (mg/g dry herb) and antioxidant activity (%) was done as follows: 0.1 g of the crushed dry herb was weighed into Eppendorf tubes then was mixed with 1.8 ml of 70% methanol and stored at room temperature, and after 48 h, the samples were centrifuged for 15 min at 10000 rpm.

Total phenolics (mg/g dry herb) was determined in the dried herb according to Singleton *et al.* [19]. Antioxidant activity of dried herb (%) was determined depending on the ability of the extract to scavenge DPPH free radicals according to the standard method by Tekao *et al.* [20] and the suitable modifications of Kumarasamy *et al.* [21].

The recorded data were analyzed as completely randomized blocks design by analysis of variance using the General Linear Models procedure of CoStat [22]. Least significant difference test was applied at 0.05 probability level to compare the means of the treatments.

Results and discussions

Plant growth parameters and yield

Evaluation of the effects of biostimulators (algae and yeast extracts) on growth traits of rosemary (*R. officinalis* L) showed that all foliar application treatments were significant on all the studied parameters (Table 1). Application of algae at the concentrations of 1 and 2 g/l and yeast at 10 and 20 g/l increased significantly plant height, herb fresh, and dry weights (g/plant and ton/ha) of rosemary plants in both seasons, except plant height of plants sprayed with 1 g/l of algae extract in the second season. No significant differences were observed between the medium and the high concentrations of algae.

At the low concentrations of algae (0.5 g/l) and yeast (5 g/l), there was no significant increase in plant height, herb fresh, and dry weights (g/plant and ton/ha) of rosemary plants as compared with control plants during the two seasons, except in plant height in the first season for both extracts, fresh weight and yield (g/plant and ton/ha) when sprayed with yeast in both seasons, and dry weight (g/plant and ton/ha) when sprayed with yeast in the second season. The highest values were recorded with yeast at 20 g/l during the first and the second seasons.

It is clearly observed that the mean values of all recorded growth parameters and yield resulted from application of either yeast or algae treatments were significant compared with the values of control treatment, except plant height mean values of plants sprayed with algae extract in the second season, and the means of yeast were greatly outperformed than the means of algae in both seasons (Table 1).

The maximum values of plant height (58.3 and 60.7 cm), fresh weight (595.4 and 639.9 g/plant; 26.46 and 28.4 ton/ha), and dry weight (209.9 and 236.6 g/plant; 9.3 and 10.5 ton/ha) in the first and second seasons, respectively, were obtained by foliar application of yeast extract at the rate of 20 g/l. However, foliar application by algae extract at spray rate of 2 g/l resulted in lower values of plant height (57.3 and 57.7 cm), fresh weight (413.8 and 463.6 g/plant which equal to 18.39 and 20.6 ton/ha), and dry weight (152.6 and 159.5 g/plant which equal to 6.8 and

Table 1 Effect of algae and yeast on vegetative growth and yield of *Rosmarinus officinalis* plants during 2019 and 2020 seasons

Treatment	Plant height (cm)		Fresh weight (g/plant)		Dry weight (g/plant)		Fresh yield (ton/ha)		Dry yield (ton/ha)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	50.7	53.7	318.8	357.6	113.3	120.2	14.17	15.9	5.0	5.3
Algae (0.5 g/l)	53.7	54.3	360.5	384.6	121.5	135.1	16.02	17.1	5.4	6.0
Algae (1 g/l)	56.3	55.3	408.8	436.7	148.5	151.8	18.17	19.4	6.6	6.8
Algae (2 g/l)	57.3	57.7	413.8	463.6	152.6	159.5	18.39	20.6	6.8	7.1
Mean of algae	55.7	55.8	394.4	428.3	140.9	148.8	17.5	19.0	6.3	6.6
Yeast (5 g/l)	54.3	55.0	380.4	420.9	129.1	144.7	16.91	18.7	5.7	6.4
Yeast (10 g/l)	57.0	58.3	480.7	569.6	179.8	209.0	21.36	25.3	8.0	9.3
Yeast (20 g/l)	58.3	60.7	595.4	639.9	209.9	236.6	26.46	28.4	9.3	10.5
Mean of yeast	56.6	58.0	485.5	543.5	172.9	196.8	21.6	24.2	7.7	8.7
L.S.D. at 5%	2.26	3.18	51.10	42.17	29.25	22.61	2.27	1.87	1.30	1.01

7.1 ton/ha) in the first and second seasons, respectively. The least amounts of traits were observed in control treatment.

Algae extracts have positive effects on several plants, as they naturally contain gibberellic acid, cytokinins, and auxin which promote cell division and cell enlargement and enhance nutrient uptake, which leads to an increase in shoot growth, dry matter, and several morphological characteristics [23,24].

This result of algae agreed with those of Elansary *et al.* [25] and Aziz *et al.* [13] on *M. pulegium*, who concluded that the foliar application of algae extract led to significant increment in herb yield.

The improvement of rosemary growth and yield as a result of yeast extract may be attributed to its content of numerous micro- and macronutrients, growth regulators, proteins, amino acids, and vitamins that enhance dry matter production [26]. Additionally, the increment may be a result of the numerous functions that amino acids play in the protein structure of several plant enzymes that are necessary for vegetative growth [27]. It is also a natural source of cytokinins, which promote cell proliferation and differentiation and also regulate shoot and root morphogenesis, chloroplast maturation, protein, and nucleic acid synthesis [28]. Yeast extract contains high amounts of tryptophan (a precursor to indole acetic acid) that promotes cell division and elongation [29]. Moreover, yeast extract has a bioregulator role in plants, affecting photosynthesis and photorespiration balance and delaying the leaf senescence by reducing the chlorophyll degradation and enhancing protein and RNA synthesis [30,31].

Yeast results are in agreement with those of Putalun *et al.* [32] on *Artemisia annua* and Tarek Elsayed and El Sayed [33] on rosemary.

Generally, the stimulatory effect of biostimulants on growth parameters may be attributed to promote cell growth, division, differentiation, and enlargement as well as protein and nucleic acid metabolism and a change in membrane potentials [34–37].

These results concerning the effect of algae and yeast extracts as biostimulants on vegetative growth of rosemary plants are in good agreement with the results obtained by Abd El-Wahed and Gamal El-Din [38] on chamomile, Balbaa *et al.* [39] on *Tagetes*, Eskandari and Eskandari [40] on *Satureja khuzestanica*, Metwally *et al.* [41] on *Lathyrus odoratus*, and Naeem *et al.* [42] on *Mentha arvensis*.

Essential oil content, total phenolics, and antioxidant activity

Application of algae or yeast with all different concentrations increased essential oil percentage and yield (ml/plant and l/ha), total phenolics (mg/g), and antioxidant activity (%) in both seasons compared with the control, except antioxidant activity (%) in the second season (Table 2).

Increasing concentration of algae and yeast extract led to an increase in essential oil, total phenolics, and antioxidant activity of rosemary. When plants were sprayed with algae, the highest essential oil percent (%) and yield (ml/plant and l/ha), total phenolics (mg/g), and antioxidant activity (%) were recorded with the high concentration (2 g/l) of algae, but there were no significant differences from spraying with 1 and 2 g/l of

Table 2 Effect of algae and yeast on essential oil, total phenolics and antioxidant activity of *Rosmarinus officinalis* plant during 2019 and 2020 seasons

	Oil percentage (%)		Oil yield (ml/plant)		Oil yield (l/ha)		Total phenolics (mg/g)		Antioxidant activity (%)	
Treatment	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	0.20	0.18	0.64	0.64	28.27	28.39	50.7	48.8	82.1	82.4
Algae (0.5 g/l)	0.23	0.22	0.84	0.84	37.39	37.48	50.7	49.0	82.6	82.5
Algae (1 g/l)	0.24	0.22	1.00	0.96	44.32	42.72	51.9	49.9	84.5	83.7
Algae (2 g/l)	0.27	0.22	1.10	1.04	49.04	46.21	55.3	50.0	86.5	85.5
Mean of algae	0.25	0.22	0.98	0.95	43.6	42.1	52.6	49.6	84.5	83.9
Yeast (5 g/l)	0.24	0.21	0.91	0.89	40.32	39.57	51.3	49.8	83.3	82.6
Yeast (10 g/l)	0.30	0.24	1.44	1.34	64.09	59.58	52.2	54.2	85.4	85.1
Yeast (20 g/l)	0.30	0.25	1.79	1.61	79.39	71.66	57.6	54.9	88.0	87.4
Mean of yeast	0.28	0.23	1.38	1.28	61.3	56.9	53.7	53.0	85.6	85.0
L.S.D. at 5%	0.041	0.021	0.184	0.124	8.172	5.521	3.54	3.58	2.07	ns

algae in the two seasons. On the contrary, application of yeast at the highest level of 20 g/l gave the highest significant increase of essential oil yield (1.79 and 1.61 ml/plant and 79.4 and 71.8 l/ha) and total phenolics (57.6 and 54.9 mg/g) in the first and second seasons, respectively and antioxidant activity (88.0%) in the first season.

Generally, the mean values of essential oil, total phenolic, and antioxidant activity resulted from application of either yeast or algae treatments were significant compared with the values of control treatment, and the means of yeast were greatly outperfomed than the means of algae in both seasons (Table 2).

The improved effect of algae on secondary metabolites (essential oil and total phenolics) and antioxidant activity may be attributed to the presence of auxin in algae extract that plays a basic role in cell division and cell enlargement, which led to an increase in various morphological characters and numerous chemical components [23]. Algae foliar application increases in plants the antioxidant enzyme activities such as superoxide dismutase, glutathione reductase, and ascorbate peroxidase as well as antioxidant metabolites such as α-tocopherol, ascorbic acid, and β-carotene [43].

Algae results are in accordance with Elansary *et al.* [25], who mentioned that seaweed extract has an important role in promoting active constituents of medicinal plants, and Tawfeeq *et al.* [44], who found that seaweed extract boosted essential oil yield in rosemary (*R. officinalis*).

Different elicitors such as yeast extract activate the plant's defense response, causing consecutive cellular and molecular events as well as activating biosynthetic genes involved in the production of secondary metabolites [45]. Moreover, Abraham *et al.* [46] reported that the yeast extract stimulated the production of endogenous hormones, which resulted in the accumulation of secondary metabolites such as flavonoids, phenolic, glycosides, and total soluble sugars.

The mentioned results of yeast extract agreed with those of van der Heijden *et al.* [47] on *Tabernaemontana divaricata*, Yoon *et al.* [48] on *Scutellaria baicalensis*, Farjaminezhad and Garoosi [49] on *Azadirachta indica*, and Putalun *et al.* [32] on *A. annua*, who stated that yeast extract promotes growth and secondary metabolites.

Essential oil composition

Approximately 23 compounds (22 compounds were identified and one compound was unidentified) were ranged between 98.39 and 100% of the separated compounds in the essential oil of all treatments during 2020 season (Table 3). The essential oil composition of rosemary was characterized by high percentages of oxygenated compounds (82.99–95.59%), whereas nonoxygenated compounds ranged from 3.35 to 17.01%. Monoterpenes ranged between 96.25 and 98.75%, whereas sesquiterpene percentage ranged between 1.25 and 2.45%. The major constituents were found to be endo-borneol (27.82–43.36%) followed by (+)-2-bornanone (14.89–19.33%) and bornyl acetate (6.56–11.44%). The application of algae and yeast at different concentrations increased the major constituent of endo-borneol, and the highest relative concentration (43.36%) was obtained from the high level (20 g/l) of yeast, and this effect was accompanied with a decrease in the relative concentration of (+)-2-bornanone (14.89%), bornyl acetate and eucalyptol. Moreover, the high level of yeast (20 g/l) led to the maximum content of oxygenated compounds (95.59%). The highest relative content of (+)-2-bornanone (19.33%) was recorded with algae at the highest concentration (2 g/l). The relative percent of bornyl acetate (10.80 and 11.44%) increased with algae at 1 and 2 g/l, respectively.

Foliar application of seaweed extract improved the essential oil composition in rosemary (*R. officinalis*), as it increased significantly percentage of monoterpenes (α-thujene, β-pinene, α-terpinene, α-phellandrene, 3-methylenecycloheptene and E-isocitral) and sesquiterpenes (Italicene and α-bisabolol) than control [44].

These results agreed with Tawfeeq *et al.* [44] on rosemary (*R. officinalis*)

Mineral contents

Foliar applications of the two biostimulants positively affected the rosemary nutrient content, as presented in Tables 4 and 5. The application of algae and yeast extracts at all rates significantly increased macro- and micronutrient content compared with the control treatment and also increased with increasing the rates of applications of both biostimulants in the two seasons. The data revealed that the highest level of algae (2 g/l) recorded the maximum concentrations of N, P, Ca, Mg, Fe, Mn, Zn, and Cu (Tables 4 and 5). However, 20 g/l of yeast gave the highest concentration of K.

Table 3 Effect of algae and yeast on essential oil constituents of *Rosmarinus officinalis* plant during 2020 season

			Area %						
R.T.	Compounds	KI	Control	Algae 0.5 g/l	Algae 1 g/l	Algae 2 g/l	Yeast 5 g/l	Yeast 10 g/l	Yeast 20 g/l
3.70	α-Pinene	922	5.97	Traces	1.41	1.59	Traces	1.56	Traces
4.07	Camphene	941	1.57	Traces	Traces	Traces	Traces	Traces	Traces
4.71	β-Pinene	971	1.31	Traces	Traces	Traces	Traces	Traces	Traces
6.06	p-Cymene	1024	1.51	Traces	Traces	Traces	Traces	Traces	Traces
6.12	D-Limonene	1026	3.01	Traces	0.67	0.77	Traces	1.21	Traces
6.23	Eucalyptol	1030	5.95	1.66	4.22	4.03	3.36	4.30	0.46
8.57	Linalool	1101	4.84	4.28	4.96	5.36	6.05	5.26	4.93
9.46	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl	1128	2.39	1.76	1.69	2.13	2.14	2.76	1.16
10.35	Verbenol	1152	Traces	0.74	0.58	–	0.61	0.49	0.63
10.52	(+)-2-Bornanone	1157	17.42	16.14	18.43	19.33	17.62	18.34	14.89
11.00	Trans-3-Pinanone	1169	–	0.65	0.77	0.95	0.97	0.97	0.71
11.48	Endo-Borneol	1180	27.82	38.99	31.92	35.24	34.55	32.28	43.36
11.66	3-Pinanone, cis	1184	3.38	3.01	3.69	3.28	3.04	3.55	2.38
11.77	Terpinen-4-ol	1187	1.17	1.18	1.16	1.26	1.45	1.48	1.46
12.41	Myrtenol	1202	0.86	1.09	–	1.16	–	–	1.23
12.48	α-Terpineol	1204	2.21	2.45	3.27	2.51	4.21	3.85	3.28
12.75	Iso-borneol	1211	3.57	4.63	4.18	3.84	4.79	4.78	5.22
13.01	l-Verbenone	1218	3.85	3.66	3.64	3.77	4.54	5.47	4.18
14.33	p-Menth-2-en-7-ol, cis	1252	3.64	5.21	4.51	4.46	4.89	4.75	5.92
16.02	Bornyl acetate	1291	8.28	10.80	11.44	7.91	8.10	6.56	6.94
19.78	Not Idendified	1382	–	1.04	1.02	0.74	0.79	0.69	0.72
21.53	Caryophyllene	1426	1.25	1.38	1.54	1.03	1.28	1.06	1.38
23.11	Humulene	1465	–	0.88	0.91	0.66	0.79	0.64	0.81
Monoterpenes			98.75	96.25	96.54	97.59	96.32	97.61	96.75
Sesquiterpenes			1.25	2.26	2.45	1.69	2.07	1.7	2.19
Total of nonoxygenated compounds			17.01	4.02	6.22	6.18	4.21	7.23	3.35
Total of oxygenated compounds			82.99	94.49	92.77	93.10	94.18	92.08	95.59
Total of identified compounds			100	98.51	98.99	99.28	98.39	99.31	98.94

Table 4 Effect of algae and yeast on macro- and micronutrients of *Rosmarinus officinalis* herb during 2019 season

	%						ppm			
Treatment	N	P	K	Ca	Na	Mg	Fe	Mn	Zn	Cu
Control	2.95	0.25	1.07	1.87	0.30	0.15	207.3	24.0	44.3	6.33
Algae (0.5 g/l)	3.48	0.31	1.27	2.07	0.34	0.23	292.3	30.7	55.3	11.7
Algae (1 g/l)	3.73	0.33	1.33	2.37	0.36	0.21	365.7	36.0	59.0	14.7
Algae (2 g/l)	3.90	0.37	1.53	2.47	0.40	0.23	388.3	39.3	65.3	17.3
Mean of algae	3.70	0.34	1.38	2.30	0.37	0.22	348.8	35.3	59.9	14.6
Yeast (5 g/l)	3.18	0.28	1.57	1.97	0.34	0.17	229.3	25.7	47.3	8.0
Yeast (10 g/l)	3.20	0.31	1.80	2.03	0.35	0.18	246.3	27.3	48.3	10.0
Yeast (20 g/l)	3.19	0.32	1.97	2.17	0.36	0.19	264.0	28.0	49.3	10.0
Mean of yeast	3.19	0.30	1.78	2.06	0.35	0.18	246.5	27.0	48.3	9.3
L.S.D. at 0.05%	0.07	0.02	0.12	0.11	0.02	0.03	10.2	1.51	2.39	1.74

Foliar application of algae extract at all rates was more effective than yeast extract in increasing rosemary content of all macronutrients and micronutrients except for potassium, which reached the highest content of 2.2% by yeast extract at 20 g/l compared with 1.6% K that resulted from 2 g/l algae extract (as means of the two seasons). In other words, the mean values of the determined elements that resulted from application of different treatments either for algae or yeast indicated significant differences compared with control and the means of algae were greatly outperformed than the means of yeast, except in K mean values.

This may be attributed to the higher content of all nutrients in algae extract compared with yeast extract

Table 5 Effect of algae and yeast on macro- and micronutrients of *Rosmarinus officinalis* herb during 2020 season

	%						ppm			
Treatment	N	P	K	Ca	Na	Mg	Fe	Mn	Zn	Cu
Control	1.58	0.36	1.13	3.27	0.26	0.23	102.7	25.3	26.3	12.0
Algae (0.5 g/l)	2.08	0.41	1.40	3.57	0.30	0.28	169.3	58.7	34.7	17.3
Algae (1 g/l)	2.31	0.43	1.47	3.73	0.32	0.38	183.0	73.3	40.7	20.7
Algae (2 g/l)	2.36	0.47	1.67	4.13	0.31	0.49	201.0	82.7	50.0	25.0
Mean of algae	2.25	0.44	1.51	3.81	0.31	0.38	184.4	71.6	41.8	21.0
Yeast (5 g/l)	1.77	0.39	1.50	3.90	0.28	0.25	151.0	33.7	28.7	14.3
Yeast (10 g/l)	1.80	0.40	2.00	3.77	0.27	0.26	168.7	37.0	32.0	13.3
Yeast (20 g/l)	1.79	0.42	2.47	3.80	0.30	0.27	177.7	37.7	33.0	13.7
Mean of yeast	1.79	0.40	1.99	3.82	0.28	0.26	165.8	36.1	31.2	13.8
L.S.D. at 0.05%	0.09	0.016	0.17	0.18	0.01	0.03	6.16	3.67	3.68	1.11

except potassium, which is only 2.13% in algae extract, whereas its content in yeast extract is 9% (as mentioned in materials and methods section).

These findings agreed with those of Aziz *et al.* [13], who found that application of algae increased nutrient contents and their uptake in *M. pulegium*.

Similarly, Nia *et al.* [50] reported that the commercial formulation of biostimulators with basis of bioactive amino acid compound accompanied by macronutrients (N, P, and K) and micronutrients (Fe, Mn, Zn, and Cu) had positive effects on different growth parameters of rosemary plants (*R. officinalis* L.). Moreover, Starck [51] and Nia *et al.* [50] stated that biostimulators as biological substances stimulate metabolic processes to raise plant yield, and these compounds, which include the basis of amino acid, enhance both quantitative and qualitative plant growth.

Accordingly, the nutrient content and growth parameters of rosemary plants were significantly affected positively owing to foliar application by both biostimulators, but the yeast extracts gave the highest effect.

Conclusion

The growth, yield, essential oil and its main components, total phenolics, and antioxidant activity of rosemary increased with all used concentrations of algae and yeast extracts compared with control. These increments reached their maximum with application of algae at 1 and 2 g/l and yeast extract at 10 and 20 g/l. In general, spraying with yeast extract resulted in the highest average of growth yield and chemical constituents of rosemary, and the best parameters were obtained by spraying yeast at 20 g/l. It is recommended to spray rosemary with yeast extract at a dose of 20 g/l for the best herb yield, essential oil, total phenolic content, and antioxidant activity.

Acknowledgements

This work was financially supported from the Project No. 12050101 which was funded from National Research Centre, Egypt, so all authors acknowledge National Research Centre, Egypt for the facilities provided during this work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, *et al.* Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from sardinia and corsica. Flavour Fragr J 2002; 17:15–19.

2 Gharib F, Ghazi S, Aly H, EL-Araby M, Mousstafa S. Effect of soil type and water content on rosemary growth and essential oil yield. Int J Sci Eng Res 2016; 7:183–189.

3 González-Trujano ME, Peña EI, Martínez AL, Moreno J, Guevara-Fefer P, Déciga-Campos M, *et al.* Evaluation of the antinociceptive effect of *Rosmarinus officinalis* L. using three different experimental models in rodents. J Ethnopharmacol 2007; 111:476–482.

4 Altinier G, Sosa S, Aquino RP, Mencherini T, Loggia RD, Tubaro A. Characterization of topical antiinflammatory compounds in *Rosmarinus officinalis* L. J Agric Food Chem 2007; 55:1718–1723.

5 Johnson JJ. Carnosol: a promising anti-cancer and anti-inflammatory agent. Cancer Lett 2011; 305:1–7.

6 Dhargalkar VK, Pereira N. Seaweed: promising plant of the millennium. Sci Cult 2005; 71:60–66.

7 Van Oosten MJ, Pepe O, De Pascale S, Silletti S, Maggio A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chem Biol Technol Agric 2017; 4:5.

8 Rouphael Y, Colla G. Editorial: Biostimulants in Agriculture. Front Plant Sci 2020; 11:40.

9 Pereira C, Dias MI, Petropoulos SA, Plexida S, Chrysargyris A, Tzortzakis N, *et al.* The effects of biostimulants, biofertilizers and water-stress on nutritional value and chemical composition of two spinach genotypes (*Spinacia oleracea* L.). Molecules 2019; 24:4494.

10 Rafiee H, Naghdi Badi H, Mehrafarin A, Qaderi A, Zarinpanjeh N, Sekara A, *et al.* Application of plant biostimulants as new approach to improve the biological responses of medicinal plants – a critical review. J Med Plants 2016; 15:6–39.

11 Truzzi E, Benvenuti S, Bertelli D, Francia E, Ronga D. Effects of biostimulants on the chemical composition of essential oil and hydrosol of lavandin (*Lavandula* x *intermedia Emeric* ex *Loisel*.) cultivated in Tuscan-Emilian Apennines. Molecules 2021; 26:1–15.

12 Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 2014; 26:1–20.

13 Aziz EE, Rezk AI, Omer EA, Nofal OA, Salama ZA, Fouad H, *et al.* Chemical composition of *Mentha pulegium* L. (Pennyroyal) plant as influenced by foliar application of different sources of zinc. Egypt Pharm J 2019; 18:53–59.

14 Amer SSA. Growth, green pods yield and seeds yield of common bean (*Phaseolus vulgaris* L) as affected by active dry yeast, salicylic acid and their interaction. J Agric Sci Mansoura Univ 2004; 29:1407–1422.

15 Chapman HD, Pratt PF. Methods of analysis for soils, plants and waters. Oakland, USA: Univ California, Div Agric Sci; 1978.

16 Egyptian Pharmacopoeia. General organization for governmental printing office. Cairo, Egyp: Ministry of Health; 1984 t; 31–33.

17 Omer EA, Aziz EE, Fouad R, Fouad H. Qualitative and quantitative properties of essential oil of *Mentha Pulegium* L. and *Mentha Suaveolens* Ehrh. affected by harvest date. Egypt J Chem 2022; 65:709–714.

18 Ibrahim FM, Fouad R, EL-Hallouty S, Hendawy SF, Omer EA, Mohammed RS. Egyptian *Myrtus communis* L. essential oil potential role as in vitro antioxidant, cytotoxic and α-amylase inhibitor. Egypt J Chem 2021, 84:3005–3017.

19 Singleton VL, Orthofer R, Lamuela-Raventos M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299:152–178.

20 Tekao T, Watanabe N, Yagi I, Sakata K. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 1994; 58:1780–1783.

21 Kumarasamy Y, Byres M, Cox PJ, Jasapars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother Res 2007; 21:615–621.

22 Snedecor GW, Cochran WG. Statistical methods. Ames, Iowa, USA: Iowa State University Press 1967; 593.

23 Gollan JR, Wright J. Limited grazing pressure by native herbivores on the invasive seaweed *Caulerpa taxifolia* in a temperate Australian estuary. Mar Freshw Res 2006; 57:685–694.

24 Nofal OA, Hellal FA, El-Sayed SAA, Bakry AB. Response of peanut and maize crops to foliar application of algae extracts under sandy soil condition. Res J Pharm Biol Chem Sci 2016; 7:151–157.

25 Elansary HO, Skalicka-Wozniak K, King IW. Enhancing stress growth traits as well as phytochemical and antioxidant contents of *Spiraea* and *Pittosporum* under seaweed extract treatments. Plant Physiol Biochem 2016; 105:310–320.

26 Sarhan AMZ, Habib AM, Mahmoud AN, Noor El-Deen TM. Effect of nano, bio, chemical fertilization and leaves extract of moringa plant on flowering and chemical constituents of gladiolus plant. Egypt J Chem 2002; 65:221–230.

27 Shafeek MR, Helmy YI, Shalaby MA, Omer NM. Response of onion plants to foliar application of sources and levels of some amino acids under sandy soil conditions. J Appl Sci Res 2012; 8:5521–5527.

28 Dawood MG, El-Lethy SR, Sadak MS. Role of methanol and yeast in improving growth, yield, nutritive value and antioxidants of Soybean. World Appl Sci J 2013; 26:6–14.

29 Wareing PE, Phillips IDG. The control of growth and differentiation in plants. Oxford, New York: Pergamon Press Ltd; 1973.

30 Olaiya CO. Presowing bioregulator seed treatments increase the seedling growth and yield of tomato (*Lycopersicon esculentum*). J Plant Growth Regul 2010; 29:349–356.

31 Alavi Mehryan SM, Zare N, Masumiasl A, Sheikhzadeh P, Asghari R. Effect of salicylic acid and yeast extract elicitors on the expression of HMGR and GPPS genes involved in biosynthesis of terpenes in medicinal plant *Ferulago angulata* under cell suspension culture condition. Plant Genet Resour 2020; 7:63–76.

32 Putalun W, Luealon W, De-Eknamkul W, Tanaka H, Shoyama Y. Improvement of artemisinin production by chitosan in hairy root cultures of *Artemisia annua* L. Biotechnol Lett 2007; 29:1143–1146.

33 Tarek Elsayed SA, El Sayed SA. Evaluating biotic elicitation with phenylalanine and/or yeast for rosemary (*Rosmarinus officinals* L.) sustainable improvement under traditional and organic agriculture. Agric Sci 2021; 12:273–292.

34 Clouse SD, Sasse JM. Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 1998; 491:427–451.

35 Khripach V, Zhabinskii V, Groot A. Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century. Ann Bot 2002; 86:441–447.

36 Müssig C. Brassinosteroid-promoted growth. Plant Biol 2005; 7:110–117.

37 Prins C, Vieira JC, Freitas SP. Growth regulators and essential oil production. Braz J Plant Physiol 2010; 22:91–102.

38 Abd El-Wahed MS, Gamal El-Din KM. Stimulation of growth, flowering, biochemical constituents and essential oil of chamomile plant (*Chamomilla recutita* L.) with spermidine and stigmasterol application. Bulg J Plant Physiol 2004; 30:89–102.

39 Balbaa LO, Abd El Aziz N, Youssef AA. Physiological effects of stigmasterol and nicotinamide on growth, flowering, oil yield and some chemical compositions of *Tagetes erecta* L. Plant. J Appl Sci Res 2008; 3:1936–1942.

40 Eskandari M, Eskandari A. Effects of brassinolide on growth, photosynthesis and essential oil content of *Satureja Khuzestanica*. Inte J Plant Physiol Biochem 2013; 5:36–41.

41 Metwally S, Ibrahim G, Abo-Leila BH, Hussein AM. Effect of gibberellic acid and progesterone on growth, vase life and volatile component of *Lathyrus odoratus* plant. Res J Pharm Biol Chem Sci 2016; 7:2307–2317.

42 Naeem M, Tariq A, Idrees M, Singh MA, Masroor M, Khan M, *et al.* Modulation of physiological activities, active constituents and essential oil production of *Mentha arvensis* L. by application of depolymerised carrageenan, triacontanol and 28- homobrassinolide. J Essent Oil Res 2017; 29:179–188.

43 Allen VG, Pond KR, Saker KE, Fontenot JP, Bagley CP, Ivy RL, *et al.* Tasco: influence of a brown seaweed on antioxidants in forages and livestock – a review. J Anim Sci 2021; 79:21–31.

44 Tawfeeq A, Culham A, Davis F, Reeves M. Does fertilizer type and method of application cause significant differences in essential oil yield and composition in rosemary (*Rosmarinus officinalis* L.). Ind Crops Prod 2016; 88:17–22.

45 Jiao J, Gai Q-Y., Wang W, Luo M, Zu Y-G, Fu Y-J, Ma W. Enhanced astragaloside production and transcriptional responses of biosynthetic genes in *Astragalus membranaceus* hairy root cultures by elicitation with methyl jasmonate. Biochem Eng J 2016; 105:339–346.

46 Abraham F, Bhatt A, Keng CL, Indrayanto G, Sulaiman SF. Effect of yeast extract and chitosan on shoot proliferation, morphology and antioxidant activity of *Curcuma mangga in vitro* Plantlets. Afr J Biotechnol 2011; 10:7787–7795.

47 van der Heijden R, Threlfall DR, Verpoorte R, Whitehead IM. Regulation and enzymology of pentacyclic triterpenoid phytoalexin biosynthesis in cell suspension cultures of *Tabernaemontana divaricata*. Phytochemistry 1989; 28:2981–2988.

48 Yoon HJ, Kim HK, Ma C-J., Huh H. Induced accumulation of triterpenoids in *Scutellaria baicalensis* suspension cultures using a yeast elicitor. Biotechnol Lett 2000; 22:1071–1075.

49 Farjaminezhad R, Garoosi G. Improvement and prediction of secondary metabolites production under yeast extract elicitation of *Azadirachta indica* cell suspension culture using response surface methodology. AMB Express 2021; 11:43.

50 Nia F, Mehrafarin A, Hadavi E, Badi N. The influences of bio-stimulators compounds on growth traits and essential oil content of rosemary (*Rosmarinus officinalis* L.). J Med Plants 2014; 13:51–61.

51 Starck Z. Growing assistant: Application of growth regulators and biostimulators in modern plant cultivation (in Polish). Rolnik Dzierawca 2005; 2:74–76.