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Growth and chemical profile of clary sage (*Salvia sclarea* L.) in response to algae and banana peel extracts

Ahmed E. El-Gohary, Heba M. Amer, Adel B. Salama, Hend E. Wahba and Khalid A. Khalid*

Abstract

Background: Clary sage (*Salvia sclarea* L.) has anti-inflammatory and anti-microbial properties; its seeds were imported from Germany for cultivation and acclimatization under the Egyptian environmental conditions as a new source of natural products. Solutions of alga and banana peel waste considered as a source of plant nutrition; it contains some bio-regulators and minerals. This research paper aimed to evaluate growth (fresh and dry weights) and chemical composition (free radical-scavenging activity, carbohydrates, flavonoids, phenols and phenolic compounds) under foliar spray of algae solution (ALS) and the extract of banana peel waste (BPW).

Results: The highest values of fresh and dry weights were detected with the treatment of 1.5 g/l (BPW) × 1.5 g/l (ALS). The maximum inhibition of free radical -cavenging activity was recorded at 1 g/l (ALS) × 0.5 g/l (BPW). 2 g/l (ALS) without BPW treatment resulted in the greatest amounts of total carbohydrates. The greatest accumulation of total flavonoids was obtained from the plants treated with 0.5 g/l (BPW) × 1 g/l (ALS). Plants exposed to 1.5 g/l (BPW) with 2 g/l (ALS) gave the greatest accumulation of total phenols. Phenolic compounds that were detected by HPLC analysis were changed due to ALS, BPW, and their interactions.

Conclusion: Different variations were observed in growth characters and various chemical constituents of Clary sage under the application of ALS and/or BPW. This research will help farmers to produce medicinal and aromatic plants by using cheap and environmentally friendly methods.

Keywords: Algae, Banana peel waste, Growth, Chemical constituents

Background

Plant nutrition is one of the most important factors that improve the growth, development, and active substances of medicinal and aromatic plants (Yassen and Khalid 2009; Khalid 2012, 2013). The ban imposed by laws and regulations on the use of agricultural chemicals leads to the search for new ways to feed medicinal and aromatic plants. Presently, a basic interest is paid to biologically active components of natural origin, including biomass, in particular, defined as plant growth bio-regulators (Ronga et al. 2019). The role of bio-regulators is to control and accelerate the physiological processes of various crops such as increase the resistance to stress and stimulate their development (Du Jardin 2015). Their applications help to reduce the amount of chemicals used in agriculture and plant protection (Radkowski and Radkowska 2013).

Alga and banana peel waste (BPW) considered as sources of some bio-regulators (Bakry et al. 2016; Ronga et al. 2019); it contains various constituents such as hormones, vitamins, amino acids, phenolic substances, and various nutrients which can change and promote growth, yield, and active ingredients of medicinal and aromatic plants (Bakry et al. 2016; Amer et al. 2019). The beneficial effects of algae and BPW on medicinal and aromatic plants were reported by Amer et al. (2019) and Danish

*Correspondence: ahmed490@gmail.com
Department of Medicinal and Aromatic Plants, National Research Centre, Dokki, Cairo 12622, Egypt

et al. (2019), and they indicated that algae or banana peel waste treatments caused significant modification in growth, yield, photosynthetic pigments, oil, flavonoids, carbohydrates, free radical-scavenging activity, and phenolic compounds.

Reconnoiter the biological activities of natural products has attracted frequently in the previous decades; these activities include antimicrobial, antiviral antioxidant or anticancer; on the other hand, audience worry have protrude versus synthetics chemicals (Alvarez-Rivera et al. 2019; Awortwe et al. 2019). Synthetics chemicals have been decided as toxic or carcinogenic (Da Cruz et al. 2019). From that prospect, the search of active constituents from medicinal plants to replace synthetic ones is one of the most attractive avenues in an endeavor to develop new drug regimen; that plants need to be investigated for detect of active components (Sadeer et al. 2019).

Clary sage (*Salvia sclarea* L.) is aromatic herb and belongs to family Lamiaceae. It is used as a flavoring factor in the food, perfumery, and cosmetic industries (Ulubelen et al. 1994; Geschel et al. 1998). The extract from aerial part has been used to treat inflammatory diseases and exert significant microbiostatic activity against *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermis*, *Candida albicans*, and *Proteus mirabilis* (Mauer and Hauser 1983; Ulubelen et al. 1997; Peana et al. 1999).

The food and pharmaceutical industries in Egypt need to increase natural resources; so, new crops have been introduced as required. Clary sage seeds were imported from Jelitto seed company, Germany, for cultivation and acclimatization under the Egyptian environmental conditions as a new source of natural products that are used in food and pharmaceutical industries. Thus, the aim of this investigation was to evaluate growth, yield, and chemical components of Clary sage in response to the application of algae solution (ALS) and the extract of BPW.

Methods

Experimental

Experiments were carried out at the Experimental farm of Faculty of Agriculture, Cairo University, Egypt, during two successive seasons, 2016/2017 and 2017/2018. Physical and chemical properties of soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) and are presented in Table 1. Clary sage seeds were introduced from Jelitto seed company, Germany. Propagation: During the last week of October in both seasons, the seeds were sown in plastic bags (23 × 18 cm) in a mixture of sand and clay soil (1:1) under a sun screen. The uniform seedlings (45 days old) were transplanted into the open field. The experimental design

Table 1 Various soil analysis

Items	Values
Sand	20.9%
Silt	33.8%
Clay	45.3%
pH	7.6
EC (dS/m)	1.8
Organic matter	1.7%
$CaCO_3$	0.8%
Total N	81.3%
Cations (mg/100 g of soil)	
P	22.7
K	24.1
Ca	78.3
Mg	19.5
Na	40.7
Anions (mg/100 g of soil)	
HCO_3	25.9
Cl	12.2
SO_4	24.1

was a complete randomized block with four replicates. The experimental area (plot) was 9 m^2 (3 m × 3 m) containing 6 lines; the distance between hills was 40 cm and 50 cm apart. Thinning for one plant per hill was made 45 days after transplanting. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into three groups: The first group was subjected to application of the extract of BPW (as foliar spray) with the rates of control (0.0), 0.5, 1.0 and 1.5 g/l, and the second and third groups were subjected to the same treatments but ALS was added at 0, 1 and 2 g/l as foliar spray. A foliar application of ALS and BPW was made twice; the first one was made after 2 weeks from thinning, and the second one was carried out after 2 weeks from the first one. Flood irrigation was used in these trials.

Preparation of the BPW extract

BPW was collected and divided into small pieces, air dried; then, dry BPW were ground with a blender. The powdered sample were soaked in ethanol 80% and allowed to stand for 24 h. The mixture was stirred occasionally by rotary Shaker. Extract was purified by filtering twice through Whatman (No. 1) filter paper. After purification, the extract was concentrated to dryness under reduced pressure at 40 C° using a Rotary Evaporator. The BPW solutions were prepared in 1000-ml volumetric flask by dissolve 0.5, 1.0 and 1.5 g (residues) in

enough distilled water to bring the total solution volume to 1000 ml.

Contents and preparation of the ALS extract

ALS extract obtained from Almottahedon for Agricultural Development Company, Cairo, Egypt. It contains oligosaccharides (3%), Alginic acid (5%), Zeatin (0.003%), Mannitol (0.001%); Natural growth regulators: Cytokines (0.001%), Indole acetic acid (IAA), Betanin (0.02%), K_2O (12%), P_2O_5 (5%), N (1%), Zn (3%), Fe (2%) and Mn (1%). The ALS solutions were prepared in 1000 ml volumetric flask by dissolve 1.0 and 2 ml (ALS extract) in enough distilled water to bring the total solution volume to 1000 ml.

Harvesting

All plants were harvested after 225 days from transplanting, and total fresh and dry plants (g /plant) were recorded as averages of both seasons.

Determination of free radical-scavenging activity

The free radical-scavenging activity was determined in leaves according to the method described by Middha and Purohit (2011).

Determination of total carbohydrates

The total carbohydrates contents were determined in leaves collected at the flowering stage of each treatment with the method of Dubois et al. (1956).

Determination of total flavonoids

The aluminum chloride colorimetric method was used to determine total flavonoids in leaves according to Pourmorad et al. (2006).

Determination of total phenols

The determination of total phenolic content of the samples was performed using the Folin–Ciocalteu reagent according to Singleton et al. (1999) with modifications. Two hundred and fifty microliters of ethanolic extract were mixed with 2.5 ml of distilled water, followed by 125 µl of 1 N Folin–Ciocalteu reagent, and stirred for 5 min. Finally, 375 µl of 20% Na_2CO_3 solution was added and kept up in dark conditions for 2 h at room temperature. Absorbance was read using an Optizen spectrophotometer (760 nm wavelength). Total phenolic contents were estimated using a gallic acid standard curve ($A_{760}=0.0196$ [gallic acid]—0.0681, $r=0.9921$), obtained using six known concentrations (8–92 µg/ml) of the compound. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry tissue (mg GAE/g DT).

Sample preparation for phenolic compounds identification

The chromatographic analysis of phenolic compounds was performed using an ethanolic extract obtained according to Villarreal-García et al. (2016). Leaf powder (1 g) of was combined with 10 ml ethanol (50, 80, and 100%, v/v) and homogenized using a Tissuemiser (Advanced Homogenizing System, VWR, Radnor, PA, USA), followed by centrifugation (11,200 × *g*, 20 min, 4 °C). The supernatant was filtered using nylon membranes (0.45 µm, VWR, Radnor, PA, USA) prior to injection into the high-performance liquid chromatography (HPLC) with diode array detection equipment.

Analytical conditions for HPLC

High-performance liquid chromatography (HPLC) instrument: Shimadzu LC-20A system (Shimadzu, Tokyo, Japan). Column: Nucleosil 120-3 C8 column; 4.6 mm (internal diameter) × 250 mm (length), 3 µm (Macherey-Nagel, Düren, Germany). Mobile phase: $A=10$ mmol/l sodium citrate buffer containing 10 mmol/l sodium L-octane sulfonate (pH 3.1); $B=$ acetonitrile; $A/B=85/15$. Flow rate: 1.0 ml/min. Column temperature: 40 °C. Detector: SPD-M20A photodiode array UV–VIS spectrophotometric detector (Shimadzu, Tokyo, Japan). Detection wavelength: 240 nm.

Identification of phenolic compounds

Reverse-phase high-performance liquid chromatography (HPLC) was used to analyze phenolic compounds present in the ethanol extract (EE) sample, using the separation module (LC-20 AT, Shimadzu Corporation, Japan) equipped with a C_{18} column (Vydac, 218 TP, 250 × 4.6 mm, 5 µm particle size, Sigma–Aldrich, St. Louis, MO, USA) and a diode array detector (Rheodyne, USA). The samples were eluted with a gradient system consisting of solvent A (2% acetic acid, v/v) and solvent B (acetonitrile/methanol, 10:15, v/v), used as the mobile phase, with a flow rate of 1 ml/min. The temperature of the column was maintained at 25 °C and the injection volume was 10 µl. The gradient system started from 90% *A* at 0 min, to 80% *A* at 10 min, 70% *A* at 15 min, 60% *A* at 25 min, 50% *A* at 30–40 min, 75% *A* at 42 min, and 90% *A* at 44 min. The peaks of the phenolic compounds were monitored at 270 nm. UV–VIS absorption spectra were recorded online from 200 to 600 nm during the HPLC analysis.

Electrospray ionization mass spectroscopic (ESI–MS) analysis of phenolic compounds in EE sample was performed using an Applied Biosystems (API2000 LC/MS/MS System, ABI, Foster city, CA). Mass spectra were achieved by electrospray ionization in both positive and negative modes. The capillaries 4500 V (negative) and

5500 V (positive) were used in this study. The electrospray probe flow was adjusted to 20 ml/min. Continuous mass spectra were obtained by scanning from 100 to 800 m/z. Identification of the phenolic compounds of the 50% EE sample from leaves was achieved by comparison with retention times of standards and their UV–VIS absorption spectra and ESI–MS spectra comparisons with literature reports or with reference standards available.

Phenolic acids were identified and quantified based on retention time and ultraviolet spectra when compared with analytical standards. Phenolic acid quantification was performed using a calibration curve for each phenolic in the range 5–250 mg/l. The concentration of phenolics was expressed as mg of each individual phenolic acid per gram of dry tissue, and then, concentrations of phenolic acids were converted from mg/g to µg/g to facilitate statistical analysis.

Statistical analysis

In this study, two factors were considered: ALS and BPW. For each treatment there were three replicates, the experimental design followed a CRBD (the CRBD was used to minimize the effects of systematic error such as the heterogeneity of soil. So the CRBD was suitable for the present experimental conditions). The averages data of both seasons were statistically analyzed using 2two-way analysis of variance (ANOVA-2) (Statsoft 2007). Significant values determined according to P values ($P<0.05$ = significant, $P<0.01$ = moderate significant and $P<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program version 10 (De-Smith 2015).

Results

Effect of ALS, BPW and their interaction on growth characters

BPW and/or ALS doses affected growth characters such as fresh and dry weights (Table 2). In general, various growth characters increased under BPW doses with or without ALS. Applying ALS with BPW doses resulted in higher values of all growth characters than BPW without ALS. The maximum values of fresh weight (1730 g/plant) and dry weight (517 g/plant) were obtained from the plants treated with 1.5 g/l (BPW) × 1.5 g/l (ALS). The

Table 2 Effect of ALS, BPW, and their interaction on the growth and chemical contents

Treatments (g/l)		FW	DW	FRSA (%)	TC (%)	TF	TP
ALS	BPW	.g/plant				.mg/g	
0.0	0.0	692.4 ± 3.2	128.4 ± 2.8	35.2 ± 3.1	12.6 ± 0.5	2.1 ± 0.1	7.7 ± 0.1
	0.5	783.0 ± 4.1	152.9 ± 3.5	47.0 ± 2.2	13.7 ± 0.3	2.4 ± 0.2	8.2 ± 0.3
	1.0	813.0 ± 3.8	163.2 ± 2.2	55.2 ± 5.7	14.3 ± 0.3	2.5 ± 0.2	8.8 ± 0.3
	1.5	859.7 ± 4.9	174.2 ± 2.7	46.6 ± 4.2	13.8 ± 0.5	2.5 ± 0.1	8.2 ± 0.3
Overall AE (0.0 g/l)		787.0 ± 6.8	154.7 ± 4.7	46.0 ± 3.8	13.6 ± 0.4	2.4 ± 0.1	8.2 ± 0.3
1.0	0.0	723.8 ± 5.4	148.2 ± 5.1	52.5 ± 4.9	20.0 ± 0.5	2.8 ± 0.2	9.3 ± 0.4
	0.5	865.0 ± 5.2	185.0 ± 5.2	74.3 ± 7.1	19.7 ± 0.6	3.2 ± 0.3	10.2 ± 0.4
	1.0	1095.0 ± 6.3	286.0 ± 3.8	65.0 ± 4.2	19.4 ± 0.3	2.9 ± 0.3	10.4 ± 0.3
	1.5	1561.7 ± 7.2	443.8 ± 3.3	59.6 ± 1.2	19.5 ± 0.5	2.8 ± 0.1	11.5 ± 0.2
Overall (1.0 g/l)		1061.4 ± 7.6	265.6 ± 4.6	62.8 ± 1.9	19.7 ± 0.5	2.9 ± 0.2	10.4 ± 0.3
2.0	0.0	805.5 ± 5.1	160.3 ± 2.7	37.2 ± 2.2	21.4 ± 0.7	2.5 ± 0.2	9.9 ± 0.4
	0.5	903.0 ± 3.2	205.0 ± 5.2	38.0 ± 3.7	20.8 ± 0.8	2.6 ± 0.2	10.5 ± 0.2
	1.0	1354.2 ± 4.1	346.5 ± 7.1	38.6 ± 6.7	18.3 ± 0.4	2.6 ± 0.1	10.5 ± 0.4
	1.5	1730.0 ± 5.8	517.0 ± 6.2	42.2 ± 3.2	19.2 ± 0.3	2.6 ± 0.1	12.8 ± 0.1
Overall (2.0 g/l)		1198.2 ± 6.2	307.2 ± 4.7	39.0 ± 4.6	19.9 ± 0.5	2.6 ± 0.2	10.9 ± 0.5
Overall BPE	0.0	740.6 ± 4.2	145.6 ± 3.9	41.6 ± 3.3	18.0 ± 0.7	2.5 ± 0.2	9.0 ± 0.4
	0.5	850.3 ± 5. 3	1801.0 ± 4.8	53.1 ± 4.2	18.1 ± 0.6	2.7 ± 0.2	9.6 ± 0.5
	1.0	1087.4 ± 6.2	265.2 ± 6.8	52.9 ± 4.8	17.3 ± 0.2	2.7 ± 0.2	9.9 ± 0.7
	1.5	1383.8 ± 5.7	378.3 ± 6.2	49.5 ± 5.1	17.5 ± 0.8	2.6 ± 0.2	10.8 ± 0.4
F ratio:							
ALS		35,947.7***	15,595.9***	555.2***	653.0***	7.2**	230.3***
BPW		49,963.6***	20,145.1***	79.8***	5.1***	0.9 ns	50.5***
ALS × BPW		8072.4***	3632.7***	32.5***	14.3***	0.4 ns	12.9***

**$P < 0.01$ (moderate significant), ***$P < 0.001$ (highly significant)

increases in fresh and dry weighs were highly significant ($P < 0.001$) for BPW, ALS and their interactions (Table 2).

Effect of ALS, BPW, and their interaction on the inhibition of free radical-scavenging activity

Applying ALS and/or BPW resulted in an increase in the inhibition of free radical-scavenging activity (Table 2). The greatest value of inhibition of free radical-scavenging activity (74.3%) produced from plants treated with 0.5 g/l (BPW) × 1 g/l (ALS). Various increases in the inhibition of free radical -scavenging activity were highly significant ($P < 0.001$) for ALS, BPW, and ALS × BPW doses.

Effect of ALS, BPW and their interaction on the total carbohydrates

The synthesis of total carbohydrates in Clary sage leaves was increased by applying different levels of BPW, ALS, and their interactions (Table 2). The highest accumulation of total carbohydrates (21.4%) resulted from the plants treated with 2 g/l (ALS) without BPW treatment. The increments in total carbohydrates were highly significant ($P < 0.001$) for ALS, BPW and ALS x BPW levels.

Effect of ALS, BPW and their interaction on the total flavonoids

BPW levels, ALS doses, and their interaction resulted in an increase of total flavonoids (Table 2). The greatest accumulation of total flavonoids (3.2 mg/g) was detected at 0.5 g/l (BPW) × 1 g/l (ALS) treatment. The increases in total flavonoids were moderately significant ($P < 0.01$) for ALS but nonsignificant for BPW treatments and the interaction between ALS and BPW.

Effect of ALS, BPW, and their interaction on the total phenols

Table 2 shows that adding ALS or BPW caused an increment in the accumulation of plant phenols. The greatest accumulation of plant phenols (12.8 mg/g) was obtained from the plants exposed to 1.5 g/l (BPW) with 2 g/l (ALS). The increases in total phenols were highly significant ($P < 0.001$) for ALS, BPW, and their interactions.

Effect of ALS, BPW, and their interaction on various phenolic compounds

As shown in Tables 3 and 4, the 13 phenolic compounds were identified and characterized by HPLC technique under ALS, BPW, and their interactions. The major constituents as detected in all treatments were gallic and chlorogenic acids which changed with ALS, BPW, and ALS + BPW levels compared with untreated control. The highest values of major compounds (gallic acid, 26.3 µg/g and chlorogenic acid 18.9 µg/g) were produced from plants treated with 1 and 0.5 g/l of BPW without ALS, respectively (Table 3). Different changes were observed in all compounds in response to various treatments (Tables 3, 4). Untreated plants resulted in the greatest values of syringic acid (3.2 µg/g) and kempferol (1.9 µg/g). 0.5, 1 and 1.5 g/l of BPW without ALS produced the maximum values of naringenin (17.5 µg/g), vanillin (1.2 µg/g) and cinnamic acid (1.3 µg/g). The treatment of 1 g/l of ALS without BPW produced the highest amounts of coumaric acid (µg/g) and taxifolin (8.9 µg/g). Plants exposed to 1 g/l (ALS) × 1 g/l (BPW) produced the maximum amounts of coffeic acid (7 µg/g) and rutin (0.8 µg/g). Plants treated with ALS at 2 g/l × BPW at 1 g/l resulted in the maximum values of Methyl gallate (2.6 µg/g) and Ferulic acid (4.3 µg/g). The changes in all phenolic components were highly significant ($P < 0.001$) for the interactions between ALS and BPW except the components of gallic and cinnamic acids, were nonsignificant (Table 3). The variations in different phenolic compounds were highly significant ($P < 0.001$) in response to BPW doses except in gallic acid and rutin, were non significant (Table 4). The differences in various phenolic compounds were highly significant ($P < 0.001$) due ALS rates except the components of gallic (nonsignificant), cinnamic acid and kempferol (significant, $P < 0.05$), and methyl gallate (moderately significant, $P < 0.01$) (Table 4).

Discussion

Obtained results from this investigation indicated that ALS and/or BPW resulted in different increases in growth characters, free radical-scavenging activity, total carbohydrates, total flavonoids and total phenols; as well as various changes were recorded in phenolic compounds. These results may be due to both ALS and BPW contain some bio stimulants and mineral those stimulate growth and the chemical content of Clary sage plants (Bakry et al. 2016; Amer et al. 2019).

Baskar et al. (2011) reported that ethanolic extract of BPW contain antioxidant substances such as phenols and flavonoids. Plant phenols were recognized as a regulator of plant physiological processes when applied exogenously to plants; the most investigated roles of phenols are associated with its interference in plant resistance response to pathogen attacks and less than optimal biotic conditions (Jalal et al. 2012). The stimulating effects of phenols on plant growth characters and chemical constituents could be attributed to phenols effects on ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, changes in the hormonal status, improvement of photosynthesis, transpiration, and stomatal conductance (Blokhina et al. 2003). The phenols increased rate of cell metabolism, prerequisite for synthesis of auxin and/or cytokinin (Gharib 2006). It was found that phenols resulted in significant increases in plant

Table 3 Effect of the interaction between ALS and BPW on the phenolic compounds

No	PC (µg/g)	ALS (g/l)												*F* ratio
		0.0				1.0				2.0				
		BPW (g/l)												
		0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
1	Gallic acid	23.7 ± 0.4	21.8 ± 0.2	26.3 ± 0.2	16.1 ± 0.1	24.0 ± 0.3	17.3 ± 0.4	19.7 ± 0.4	17.5 ± 0.3	17.4 ± 0.2	12.4 ± 0.1	15.8 ± 0.5	22.7 ± 0.5	1.3 ns
2	Chlorogenic acid	16.9 ± 0.1	18.9 ± 0.3	13.9 ± 0.1	12.4 ± 0.1	10.4 ± 0.2	13.6 ± 0.1	14.5 ± 0.1	15.4 ± 0.1	15.3 ± 0.2	12.8 ± 0.1	16.1 ± 0.2	6.9 ± 0.2	2276.0***
3	Methyl gallate	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.2	1.2 ± 0.3	1.4 ± 0.3	0.5 ± 0.1	1.0 ± 0.2	0.7 ± 0.2	0.9 ± 0.1	0.3 ± 0.1	2.6 ± 0.2	0.4 ± 0.1	27.4***
4	Coffeic acid	3.6 ± 0.4	4.1 ± 0.5	5.2 ± 0.4	2.7 ± 0.2	4.8 ± 0.5	2.0 ± 0.3	7.0 ± 0.6	3.4 ± 0.2	3.9 ± 0.5	1.5 ± 0.1	4.1 ± 0.4	2.2 ± 0.2	46.7***
5	Syringic acid	3.2 ± 0.3	2.6 ± 0.3	2.5 ± 0.4	2.1 ± 0.3	2.7 ± 0.5	2.7 ± 0.3	2.1 ± 0.1	2.9 ± 0.2	2.1 ± 0.3	2.4 ± 0.3	2.3 ± 0.3	3.9 ± 0.4	67.0***
6	Rutin	–	–	–	–	–	–	0.8	–	–	–	–	0.8 ± 0.2	48.0***
7	Coumaric acid	4.2 ± 0.4	5.6 ± 0.3	5.5 ± 0.5	5.1 ± 0.4	9.0 ± 0.7	1.8 ± 0.2	6.6 ± 0.3	4.1 ± 0.2	4.4 ± 0.5	1.9 ± 0.2	4.0 ± 0.4	4.0 ± 0.3	35.1***
8	Vanillin	–	–	1.2	–	–	–	–	–	–	–	–	–	108.0***
9	Ferulic acid	1.4 ± 0.3	1.9 ± 0.3	1.4 ± 0.4	2.0 ± 0.2	2.5 ± 0.2	1.1 ± 0.2	3.7 ± 0.6	1.0 ± 0.1	2.9 ± 0.3	0.8 ± 0.1	4.3 ± 0.5	1.0 ± 0.4	38.3***
10	Naringenin	1.0 ± 0.1	17.5 ± 0.6	3.5 ± 0.2	2.0 ± 0.2	2.5 ± 0.3	1.6 ± 0.2	2.9 ± 0.2	1.7 ± 0.2	3.7 ± 0.3	1.1 ± 0.3	–	2.8 ± 0.4	747.6***
11	Taxifolin	8.8 ± 0.2	6.2 ± 0.1	8.5 ± 0.4	2.0 ± 0.1	8.9 ± 0.4	4.7 ± 0.2	6.6 ± 0.3	9.2 ± 0.5	7.6 ± 0.4	4.0 ± 0.3	7.3 ± 0.3	9.7	107.7***
12	Cinnamic acid	1.0 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	1.3 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.9 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	1.2 ± 0.3	1.7 ns
13	Kempferol	1.9 ± 0.3	–	1.5 ± 0.2	0.8 ± 0.2	1.1 ± 0.3	–	1.3 ± 0.4	1.7 ± 0.2	1.2 ± 0.4	–	1.3 ± 0.2	2.7 ± 0.3	17.1***

***$P < 0.001$ (highly significant)

Table 4 Effect of ALS or BPW on the phenolic compounds

No	PC (µg/g)	BPW (g/l)				ALS (g/l)			*F* ratio	
		0.0	0.5	1.0	1.5	0.0	1.0	2.0	BPW	ALS
1	Gallic acid	21.7 ± 0.4	17.2 ± 0.5	20.6 ± 0.3	15.4 ± 0.2	19.5 ± 0.4	19.6 ± 0.3	17.1 ± 0.5	1.2 ns	1.1 ns
2	Chlorogenic acid	18.9 ± 0.4	16.4 ± 0.3	16.5 ± 0.4	14.9 ± 0.1	19.5 ± 0.2	16.0 ± 0.2	16.0 ± 0.3	884.8***	1454.9***
3	Methyl gallate	1.1 ± 0.1	0.6 ± 0.1	1.7 ± 0.2	0.8 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	1.1 ± 0.3	60.6***	6.5**
4	Coffeic acid	4.1 ± 0.3	2.5 ± 0.2	5.4 ± 0.4	2.8 ± 0.2	3.9 ± 0.4	4.3 ± 0.3	2.9 ± 0.2	52.2***	32.3***
5	Syringic acid	2.7 ± 0.2	2.6 ± 0.3	2.3 ± 0.3	3.0 ± 0.5	2.6 ± 0.2	2.6 ± 0.2	2.7 ± 0.4	39.0***	1.3 ns
6	Rutin	–	–	–	0.8 ± 0.2	–	–	0.8 ± 0.2	48.0***	48.0***
7	Coumaric acid	5.9 ± 0.6	3.1 ± 0.2	5.4 ± 0.3	4.4 ± 0.2	5.1 ± 0.4	5.4 ± 0.5	3.6 ± 0.3	45.2***	38.2***
8	Vanillin	–	–	1.2 ± 0.2	–	1.2	–	–	108.0***	108.0***
9	Ferulic acid	2.3 ± 0.3	1.3 ± 0.2	3.1 ± 0.4	1.3 ± 0.2	1.7 ± 0.3	2.1 ± 0.2	2.3 ± 0.4	82.5***	12.2***
10	Naringenin	2.4 ± 0.4	6.7 ± 0.6	3.2 ± 0.3	2.2 ± 0.3	6.0 ± 0.5	2.2 ± 0.6	2.5 ± 0.3	476.8***	657.7***
11	Taxifolin	8.4 ± 0.5	5.0 ± 0.3	7.5 ± 0.3	7.0 ± 0.4	6.4 ± 0.3	7.3 ± 0.5	7.2 ± 0.2	100.9***	16.3***
12	Cinnamic acid	0.8 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	1.1 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	24.7***	5.3*
13	Kempferol	1.4 ± 0.2	–	1.4 ± 0.2	1.7 ± 0.3	1.4 ± 0.2	1.4 ± 0.3	1.7 ± 0.3	89.7***	4.7*

*$P < 0.05$ (significant), **$P < 0.01$ (moderate significant), ***$P < 0.001$ (highly significant)

growth characters (plant height, fresh and dry weights), photosynthetic pigments, carbohydrates, and antioxidant enzymes activities of parsley and celery plants (Ahmed et al. 2018a, b). Flavonoids are a subgroup of secondary metabolites categorized as phenolic compounds. It protects plants against various biotic or abiotic stresses, absorbs the harmful UV radiation induced cellular damage, and influences the transport of the plant hormone (auxin) those are resulted in healthy growth of crops (Samanta et al. 2011).

The foliar spray of ALS with various rates resulted in an enhancement in growth and chemical composition; these effects may be due to the attribution to biostimulants in ALS that has important roles in cell divisions and cell enlargement which led to an increase in dry matter, growth characters, and different chemical contents (Gollan and Wright 2006). On the other hand, biostimulants against stress conditions increase the nutrient uptakes and assimilation, improve plant enzymes required for protein synthesis, and stimulate different growth characters, yield, crop productivity, and chemical composition (Panda et al. 2012). It was found that growth characters and carbohydrates of *Aechmea blanchetiana* were significantly increased under biostimulants treatments (Chu et al. 2010). ALS is a rich source of some nutrients such as N, P, K, Zn, Fe, and Mn which have various roles in physiological processes of various plant inducing the photosynthesis**,** cell divisions and cell elongation that reflect high growth characters and chemical composition (Marrez et al. 2014). Different increases in growth and carbohydrates were recorded in anise, coriander and sweet fennel plants under macro and microelements treatments (Khalid 2012). Accumulations of phenols, flavonoids, and consequently the inhibition of free radical scavenging of *Ammi visnaga* L. were significantly improved under various biostimulants (Khattab et al. 2017). The ALS contains alginic acid. However, alginic acid increases the intake of substances by application of ALS. Phytoalexins in the ALS increase plant resistance to threats from pathogens and improve plant tolerance to heat stress and also play an important role in the transportation of amino acids and nutrients as well as in the synthesis of proteins (Craigie 2011). Algal extract can increase the utilization of solar energy that reflect an increase in the chlorophyll activity and improve the crop productivity (Khan et al. 2009). The effects of blue green ALS on growth and chemical contents were confirmed by some previous investigators. ALS application resulted in various increments of fresh weight, dry weight and total carbohydrates of lupine plants (Haroun and Hussein 2003). The ALS of *Chlorella vulgaris* produced significant increases in growth and yields of maize, wheat, bean, and lettuce plants (Gonzales and Bashan 2000; Faheed and El-Fattah 2008). Some vegetables such as potato, tomato, peas, and garlic subjected to various ALS treatments (seaweed, *Spirulina platensis* and *Arthospira fusiformis*), results indicated that different ALS doses produced significant increases in growth, yield, and chemical components (Awad et al. 2006; Nour et al. 2010; Nawar and Ibraheim 2014; Shalaby and El-Ramady 2014). Growth characters and oil content of fennel and dill plants were improved due to ALS (*Spirulina platensis*) application (Abd El-Aleem et al. 2017; Toaima et al. 2017). El-Sharony et al. (2015) reported that ALS as sea wood produced significant increases in fruit yield and mineral content of mango plants. Application of ALS as mixture of *Laurencia obtusa Corallina elongate* and sea

wood resulted in significant increases of growth characters, protein and nutrient composition of maize and peanut plants (Safinaz and Ragaa 2013; Nofal et al. 2016). Cardoon plants that were cultivated under various ALS such *Spirulina platensis, Chlorella vulgaris, Amphora coffeaeformis*, and *Scenedesmus obliquus* gave higher values in morphological characters, fixed oil, flavonoids, carbohydrates, free radical-scavenging activity and phenolic compounds than those untreated control (Amer et al. 2019).

Various increments in growth characters and chemical constituents may be due to ALS and BPW and their levels, besides the effect of other conditions, including plant varieties, environmental conditions, and soil types (Piccaglia and Marottu 1993). It was found that genetic and environmental conditions produced significant changes in growth and chemical constituents of sage plants (Rowshan et al. 2010). Significant changes were recorded in chemical contents of Indian borage in response to metrological factors (Khalid and El-Gohary 2014). Different soil conditions resulted in significant variations of plant productivity (Robson 1989).

From previous studies, it turned out that there are little researches on the effect of both ALS and BPW on medicinal and aromatic plants. Therefore, this research will help farmers to produce medicinal and aromatic plants by using cheap and environmentally friendly methods because both ALS and BPW are considered as organic fertilizers.

Conclusions

In this research paper, responses of growth characters, chemical constituents of Clary sage were evaluated under the interactions between ALS and BPW. From obtained results, it may be concluded that plants treated with ALS × BPW gave higher values in growth characters and chemical constituents than those treated with BPW alone. ALS and BPW are alternative and promising organic fertilizers in order to avoid the pollution and improve the growth characters and the active substances of Clary sage plant as a natural source in food and pharmaceutical industries.

Abbreviations

ALS: Algae solution; BPW: Banana peel waste; DW: Dry weight; FRSA: Free radical-scavenging activity; FW: Fresh weight; TC: Total carbohydrates; TF: Total flavonoids; TP: Total phenols.

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Authors' contributions

AE carried out the experiments in the field. AS participated in the design of the study. HA carried out the chemical studies. HW performed the statistical analysis. KAK drafted the manuscript and participated in the sequence alignment. All authors share in every step of this work, and all of them contribute to writing the manuscript. The authors read and approved the final manuscript.

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The manuscript does not contain studies involving human participants, human data or human tissue.

Consent for publication

The authors declare that the work has consent for publication.

Competing interests

The authors declare that they have no competing interests.

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