



The Alteration in Physiological Status, Growth and Essential Oil Profile of French Marigold (*Tagetes patula* L.) Owing to Seaweed Extract and Salicylic Acid Application

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Abstract

Purpose Medicinal plants show a relevant importance in human life and health. The use of natural substances in crop management is not only significant for the plant healthy growth and yield, but also for obtaining safe products. The study aimed to examine the effect of seaweed extract (SW) as a natural source of nutrition and salicylic acid (SA) on plant health, physiology and essential oil profile of French marigold (*Tagetes patula* L.).

Methods Three levels of salicylic acid, SA50, SA100 and SA200, (50, 100, and 200 mg L⁻¹) and three levels of seaweed extract, SW2, SW4 and SW6 (2.0, 4.0, and 6.0 ml L⁻¹), in comparison with control treatment (tap water) were applied as foliar sprayings. The seven treatments were arranged in randomized complete block design and were replicated thrice in two growing seasons (2020 and 2021).

Results Findings pointed out that SA200 increased plant height, branches number plant⁻¹, stem diameter, leaf area, leaves number plant⁻¹, plant fresh weight, and plant dry weight by 32.9, 112.2, 59.2, 34.4, 44.3, 33.0 and 56.9% (averages of the two seasons), respectively, compared to the control treatment (tap water). SW6 increased carotenoids content by 34.8 and 46.4% in 2020 and 2021 seasons, respectively. Salicylic acid at the rate of 200 mg L⁻¹ along seaweed extract at 6 ml L⁻¹ gave the highest values of total sugars and free amino acids. SA200 alone recorded the maximal value of peroxidase activity surpassing the other treatments. SW6 possessed the greatest essential oil content in both seasons, statistically equaling SA200 in the first season. Huge variations in essential oil profile were recorded under the tested trial treatments.

Conclusion It could be concluded that salicylic acid and seaweed extract foliar application unveiled their involvement in diverse physiological and developmental responses; pigment formation, enzyme activities, flower induction, nutrient uptake, essential oil metabolism, and overall plant growth and development. It is recommended to use the highest concentrations of the tested substances (SA at 200 mg L⁻¹ or SW at 6.0 ml L⁻¹) for achieving distinctive improvements in physiological responses of French marigold plant.

Keywords Antioxidants · Marigold Essential oil · Natural Nutritious · Ortho-Hydroxybenzoic acid

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1 Introduction

Recently, the ornamental and medicinal plants industry is one of the most agricultural production sectors (Vita et al. 2015; Barna et al. 2021). Therefore, cultivation of these plant species while following appropriate and effective practices has received great attention recently (Ali et al. 2024a; Lasheen et al. 2024). One of these plants is *Tagetes patula* L. that belongs to the family Asteraceae. *Tagetes patula* L. is called French marigold and it is an important annual flower crop used for ornamental and commercial purposes (Ilbi et al. 2020). The main components of French marigold

are phenols, essential oil, vitamins (carotenoids), and flavonoids (Kurkina et al. 2021). French marigold was used to treat various diseases, for example, cough, colic, constipation, diarrhea, rheumatism, and eye problems. Nowadays, marigolds are used as an antimicrobial, antiseptic, hepatoprotective, blood purifying, and diuretic agent (Liu et al. 2020).

Plant growth regulators play a crucial role within the life cycle of plants and these are often produced naturally by leaves factories of the plant or synthetically by chemical laboratories (Saady et al. 2021a; El-Bially et al. 2022a; El-Metwally et al. 2022a). The exogenous supply of growth regulators showed good potential to improve crop physiological status (Hadid et al. 2023; Ramadan et al. 2023; Saady et al. 2023; Doklega et al. 2024) while enhancing growth and productivity (El-Bially et al. 2018; El-Metwally et al. 2021; Rizk et al. 2023). One of the most important external applications of the industrial plant growth regulators is ortho-hydroxybenzoic acid named salicylic acid (SA), which is inexpensive and functions as a plant natural hormone, derivatized from phenols (Kulak et al. 2021).

SA had a mainly important role in multiple functions in physiological and biochemical processes of plant metabolism including modulating growth by improving morphological characteristics, photosynthesis, cellular metabolism, protein synthesis, enzymatic activities, stomatal closure, ion uptake, avoiding leaf senescence, transpiration, cell elongation, cellular division, cell differentiation, and gas exchange (Shadmehri and Khatiby 2020). Moreover, it can effectively alleviate the harmful effects caused by abiotic stresses by enhancing the defense system of the plant, which leads to an increase in enzymatic and non-enzymatic antioxidants (Zulfikar et al. 2021). SA foliar application enhanced growth, root, and flowering parameters, photosynthesis, transformation, and storage carbohydrates, additionally improved mineral nutrients percentages of leaves, thus it had a positive significant effect on gazania (*Gazania linearis*) plant (Saeed 2020). SA functions as an endogenous regulator on flowering and together with plant regulators, like gibberellin, induced flowering (Sewedan et al. 2018). SA application led to a rise production of additional petals and enhanced the biosynthesis of volatile oil (Elsadek 2018).

Under traditional agriculture conditions, as in most developing countries, synthetic chemicals are widely used in crop production despite their harmful impacts on environment and human health (Saady et al. 2020, 2021b; Abou El-Enin et al. 2023; Shaaban et al. 2023). Therefore, many organic alternatives have shown immense potential for use as safe agricultural inputs in plant production to increase plant quality while preserving and sustaining environmental resources (Abd-Elrahman et al. 2022; Dookie et al. 2021; El-Metwally et al. 2022b; Elgala et al. 2022; Makhoulf

et al. 2022). Since seaweed extract has elevated levels of macro and micronutrients and many vitamins and plant hormones that promote plant growth and flowering, enhancing the tolerance to various stresses, significant increases in growth and reproductive parameters in several crop types were reported (Shukla et al. 2019). Also, noticed significant increases in plant height and leaves number of Chinese carnation and gazania due to spraying of seaweed extract at different concentrations (Al-Hamzawi 2019). However, little knowledge related to the response of French marigold plants toward seaweed application is available. In this connection, the current research hypothesized that diversified concentrations of salicylic acid and seaweed extract could have varied influences on French marigold performance. Therefore, this work aimed to assess the alternations in physiology, growth, nutritional status and essential oil constituents owing to exogenous supply of salicylic acid and seaweed extract, at different concentrations, as efficient eco-friendly alternatives for *Tagetes patula* production.

2 Materials and Methods

2.1 Experimental Location

Along two successive seasons of 2020 and 2021, greenhouse experiments were conducted at Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt (30° 01' 02.7" N, 31° 12' 32.0" E). The study area has typical Mediterranean climatic conditions with no rainfall and hot-dry in summer. The averages of air temperatures, humidity and solar radiation during the plant growth period (March-June) were approximately 25.0 °C, 52.4% and 28.7 MJ m⁻² day⁻¹, respectively. The used soil media was formed by mixing clay loam and loamy sand (1:1 v/v) having the physicochemical properties shown in Table 1. Soil analysis was performed according to Piper (1950).

2.2 Trial Design and Treatments Application

On the first of March in 2020 and 2021, 3–5 seeds of French marigold were sown in plastic pots (30-cm in diameter), filled with the previously prepared mixture of clay and sand (1:1 v/v). At 4–6 leaf old, 35 days after sowing (DAS), seedlings were thinned to secure one plant per pot. Seven treatments were arranged in a randomized complete block design with three replicates. In addition to the control treatment (tap water), three levels of salicylic acid (50, 100, and 200 mg L⁻¹) and three levels of seaweed extract (2.0, 4.0, and 6.0 ml L⁻¹) were applied as foliar sprayings. The spray solutions of both salicylic acid and seaweed extract were sprayed separately three times, 50, 65 and 80 DAS, using

Table 1 Initial physicochemical properties of loamy sand and clay loam used in soil media preparation

Property	Unit	Value	
Texture	-	Loamy sand	Clay loam
Cation exchange capacity	meq 100 g ⁻¹	15.3 ± 0.12	31.4 ± 1.00
Field capacity	%	26.0 ± 0.15	47.3 ± 0.18
pH (Extract 1:2.5 H ₂ O)	-	7.74 ± 0.05	8.10 ± 0.04
EC 20 °C	dSm ⁻¹	0.31 ± 0.01	0.29 ± 0.02
Organic matter	%	1.5 ± 0.02	2.4 ± 0.06
Nitrogen	mg kg ⁻¹	60.0 ± 1.11	538.4 ± 3.20
Phosphorus	mg kg ⁻¹	43.7 ± 0.95	22.0 ± 0.21
Potassium	mg kg ⁻¹	1.20 ± 0.02	278 ± 0.81
Calcium	mg kg ⁻¹	32.4 ± 1.01	5.0 ± 0.02
Magnesium	mg kg ⁻¹	6.8 ± 0.01	3.5 ± 0.24
Sodium	mg kg ⁻¹	8.0 ± 0.03	38.8 ± 1.30
Zinc	mg kg ⁻¹	8.3 ± 0.02	39.0 ± 1.05
Manganese	mg kg ⁻¹	19.9 ± 0.09	7.0 ± 0.04
Iron	mg kg ⁻¹	68.9 ± 1.32	9.2 ± 0.05
Copper	mg kg ⁻¹	2.50 ± 0.09	5.0 ± 0.06

Table 2 Composition of seaweed extract

Constituent	Unit	Value
Moisture	%	0.06
Protein	%	6–8
Alginic acid	%	10–20
Mannitol	%	4–7
Carbohydrate	%	35–50
Organic matter	%	45–60
Inorganic matter	%	45–60
Indole acetic acid	%	0.0003
Cytokinin	%	0.0002
Abscissic acid	%	0.0001
Nitrogen	%	1.0–1.5
Phosphorus	%	0.02–0.09
Sulfur	%	3–9
Potassium	%	1.0–1.2
Calcium	%	0.2–1.5
Magnesium	%	0.5–0.9
Zinc	mg L ⁻¹	10–100
Manganese	mg L ⁻¹	5–12
Iron	mg L ⁻¹	50–200
Boron	mg L ⁻¹	20–100
Copper	mg L ⁻¹	1.0–6.0
Molybdenum	mg L ⁻¹	1–5

manual back-pack knapsack sprayer fitted with a flat-fan nozzle. Seaweeds extract was obtained from Algeser product from Shoura Chemicals Company, Cairo, Egypt. The constituents of seaweed extract were determined (Bonner 1994) and illustrated in Table 2.

2.3 Assessments

2.3.1 Vegetative and Flowering Traits

At the end of June in 2020 and 2021, the experiment was completed and data were recorded on the different vegetative growth characteristics (plant height, branches number plant⁻¹, stem diameter, leaf area, leaves number plant⁻¹, fresh and dry weights of shoots (stems + leaves). Also, data were recorded on the flowering including the number, fresh weight and dry weight of flowers plant⁻¹.

2.3.2 Plant Pigments

Based on the Eqs. 1–4, described by Moran (1982) and Wellburn (1994) chlorophylls and carotenoids were estimated. The leaf samples of 100 mg fresh weight were ground using a mortar and extracted with 85% methanol. Then, the extracts were centrifuged for 10 min at 8000 rpm. The pigment quantification was performed by spectrophotometry, with wavelengths at 665, 646 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. After extraction, the absorbance readings were performed in a digital spectrophotometer (Edutec EEQ 9023).

$$\text{Chlorophyll a concentration (mg g FW}^{-1}) = 12.64 * A_{665} - 2.99 * A_{646} \quad (1)$$

$$\text{Chlorophyll b concentration (mg g FW}^{-1}) = 23.26 * A_{646} - 5.6 * A_{665} \quad (2)$$

$$\text{Total Chlorophyll concentration (mg g FW}^{-1}) = 7.04 * A_{665} + 20.27 * A_{646} \quad (3)$$

$$\begin{aligned} \text{Carotenoids concentration (mg L}^{-1}) \\ = \frac{1000 * A_{470} - 0.89 * Chla - 52.02 * Chlb}{245} \end{aligned} \quad (4)$$

2.3.3 Biochemical Constituents

Total sugars were determined by using the method of phenol sulphuric acid reagent as described by Dubois et al. (1956). Total free amino acids analysis was determined by spectrophotometer at 650 nm, using ninhydrine reagent (Singleton et al. 1999).

2.3.4 Antioxidant Activity

The activity of antioxidant enzymes was determined in leaves samples. Peroxidase (POX; EC 1.11.1.7), according to Hammerschmidt et al. (1982) using a spectrophotometer at 470 nm and Glutathione-s-transferase (EC 2.5.1.13) determined by the method of Habig and Jakoby (1981) at 340 nm. Furthermore, Vitamin C was extracted and

estimated according to the method mentioned by Obouayeba et al. (2014).

2.3.5 Leaf Macronutrient Content

Nitrogen content (N) was determined using the micro-Kjeldahl method, according to Pregl (1945). Phosphorus (P) content was estimated using the method described by King (1951). Potassium (K) content was assessed according to the methods described by Cottenie et al. (1982).

2.3.6 Essential Oil Content and Composition

In the fresh herb, essential oil percentage was extracted for 2 h by water distillation of 20 g of aerial parts of plant (Guenther 1961). The essential oil extracted was dehydrated with anhydrous sodium sulphate. Furthermore, the GC-MS analysis of the essential oil of the different treatments was carried out using gas chromatography/mass spectrometry instrument stands in the Research Institute of Medicinal & Aromatic plants (RIMAP) with the following specifications. Instrument: a traces GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a Tr-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min and a split ratio of 1:10 using the following temperature program: 60 °C for 1 min; rising at 4 °C/min to 160 °C and held for 6 min; rising at 6 °C/min

to 210 °C and held for 1 min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 0.1 µL of the mixtures were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450.

2.4 Statistical Analysis

The measured data were statistically analyzed based on the analysis of variance technique (ANOVA) for randomized complete blocks design in triplicates by Genstat computer software package (VSN International Ltd., Oxford, UK). For post-ANOVA-mean separation, Duncan's multiple range test ($p \leq 0.05$) was used. Treatments of SA and SW were considered fixed variables, while replications and seasons were regarded as random factors. Pearson's correlation analysis expressed in heat map was performed to quantify relationships among observed parameters.

3 Results

3.1 Vegetative and Flowering Traits

The treatments of salicylic acid and seaweed extract showed significant ($p \leq 0.05$) variation in vegetative and flowering parameters (Table 3). SA200 was the efficient treatment for enhancing all growth traits in both seasons of 2020 and 2021. As averages of the two seasons, SA200 increased plant height, branches number plant^{-1} , stem diameter, leaf

Table 3 Effect of salicylic acid and seaweed extracts foliar application on vegetative traits of *Tagetes patula* L. in 2020 and 2021 seasons

Variable	Plant height (cm)	Branches number plant^{-1}	Stem diameter (cm)	Leaf area (cm^2)	Leaves number plant^{-1}	Plant fresh weight (g)	Plant dry weight (g)
Season of 2020							
Control, tap water	39.75 c	6.00 d	5.21e	4.65 f	73.00 e	66.91 d	18.83 c
SW2	41.50 bc	8.33 cd	6.00 d	4.87 e	84.67 d	71.66 cd	20.89 c
SW4	46.56 ab	10.00 bc	7.02 bc	5.08 d	86.33 d	76.13 bc	24.29 b
SW6	51.50 a	12.67 ab	7.08 b	5.94 b	98.33 c	85.41 a	27.27 a
SA50	43.75 bc	10.33 bc	6.55 c	5.20 d	94.67 c	69.85 d	21.50 c
SA100	47.75 ab	12.67 ab	7.13 b	5.61 c	108.0 b	78.86 b	25.49 ab
SA200	52.00 a	14.00 a	7.77 a	6.22 a	115.7 a	88.53 a	27.60 a
Season of 2021							
Control, tap water	36.83 b	7.33 e	4.79 f	4.50 d	82.00 d	73.93 e	19.35 d
SW2	39.63 b	9.42 d	5.87 e	4.65 d	87.92 cd	78.86 de	22.00 cd
SW4	46.62 a	11.33 c	7.07 cd	5.43 bc	92.25 bc	82.81 cd	24.71 bc
SW6	50.32 a	14.00 a	7.43 bc	6.08 a	101.3 ab	94.39 ab	30.21 a
SA50	38.54 b	9.92 d	6.53 d	5.27 c	97.08 b	76.31 e	22.54 cd
SA100	45.88 a	12.75 b	7.65 ab	5.81 ab	99.92 ab	88.42 bc	29.44 ab
SA200	49.71 a	14.00 a	8.11 a	6.08 a	106.7 a	98.82 a	32.36 a

SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L^{-1} , respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L^{-1} , respectively. Means values in each column followed by different lower-case letters are significantly different based on the Duncan test ($p \leq 0.05$)

Table 4 Effect of salicylic acid and seaweed extracts foliar application on flowering traits of *Tagetes patula* L. in 2020 and 2021 seasons

Variable	Flowers number plant ⁻¹	Flower fresh weight plant ⁻¹ (g)	Flower dry weight plant ⁻¹ (g)
Season of 2020			
Control, tap water	7.00 f	2.96 f	0.317 e
SW2	8.50 ef	3.23 ef	0.343 de
SW4	11.17 cd	3.78 cd	0.397 cd
SW6	13.50 b	4.24 b	0.470 ab
SA50	9.67 de	3.45 de	0.403 c
SA100	12.50 bc	4.08 bc	0.450 bc
SA200	15.83 a	4.58 a	0.523 a
Season of 2021			
Control, tap water	7.42 d	3.03 e	0.327 f
SW2	8.50 cd	3.30 de	0.367 ef
SW4	10.17 bc	3.83 bc	0.433 cd
SW6	12.17 b	4.21 ab	0.493 b
SA50	8.25 cd	3.58 cd	0.413 de
SA100	11.25 b	4.25 ab	0.483 bc
SA200	14.58 a	4.64 a	0.573 a

SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L⁻¹, respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L⁻¹, respectively. Means values in each column followed by different lower-case letters are significantly different based on the Duncan test ($p \leq 0.05$)

area, leaves number plant⁻¹, plant fresh weight, and plant dry weight by 32.9, 112.2, 59.2, 34.4, 44.3, 33.0 and 56.9%, respectively, compared to the control treatment (tap water). However, the improvements in plant height (with SW4, SW6 and SA100), branches number plant⁻¹ (with SW6 and SA100), plant fresh weight (with SW6), and plant dry weight (with SW6 and SA100) were as similar as that of SA200 in the first season. In the second season, the values of plant height (with SW4, SW6 and SA100), branches number plant⁻¹ (with SW6), and stem diameter (with SA100) as well as leaf area, leaves number plant⁻¹ and plant dry weight (with SW6 and SA100) significantly equaled ($p \geq 0.05$) the corresponding values of SA200 treatment.

For flowering traits, spraying of SA200 was the most effective practice for enhancing flowers number plant⁻¹, flower fresh weight plant⁻¹ and flower dry weight plant⁻¹ in both growing seasons, surpassing the other treatments, except SW6 for flower dry weight plant⁻¹ in the first season and SW6 and SA100 for flower fresh weight plant⁻¹ in the second season (Table 4). In this context, SA200 increased flowers number plant⁻¹ by 2.26 and 1.96 times, flower fresh weight plant⁻¹ by 1.54 and 1.53 times and flower dry weight plant⁻¹ by 1.64 and 1.75 times, in the first and second seasons, respectively, compared to the control treatment.

Table 5 Effect of salicylic acid and seaweed extracts foliar application on plant pigments of *Tagetes patula* L. in 2020 and 2021 seasons

Variable	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Total Chlorophyll (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)
Season of 2020				
Control, tap water	1.15 f	0.48 c	1.63 d	0.23 c
SW2	1.39 de	0.45 d	1.84 c	0.26 bc
SW4	1.48 cd	0.74 b	2.22 b	0.28 ab
SW6	1.78 bc	0.36 e	2.14 bc	0.31 a
SA50	1.33 ef	0.40 de	1.73 cd	0.26 bc
SA100	1.69 b	0.35 e	2.04 bc	0.20 d
SA200	1.80 a	0.87 a	2.67 a	0.27 ab
Season of 2021				
Control, tap water	1.01 f	0.30 c	1.31 d	0.28 c
SW2	1.19 de	0.32 d	1.51 c	0.31 bc
SW4	1.26 cd	0.44 b	1.70 b	0.33 ab
SW6	1.55 bc	0.78 e	2.33 bc	0.41 a
SA50	1.20 ef	0.62 de	1.82 cd	0.21 bc
SA100	1.58 b	0.71 e	2.29 bc	0.26 d
SA200	1.88 a	0.86 a	2.74 a	0.28 c

SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L⁻¹, respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L⁻¹, respectively. Means values in each column followed by different lower-case letters are significantly different based on the Duncan test ($p \leq 0.05$)

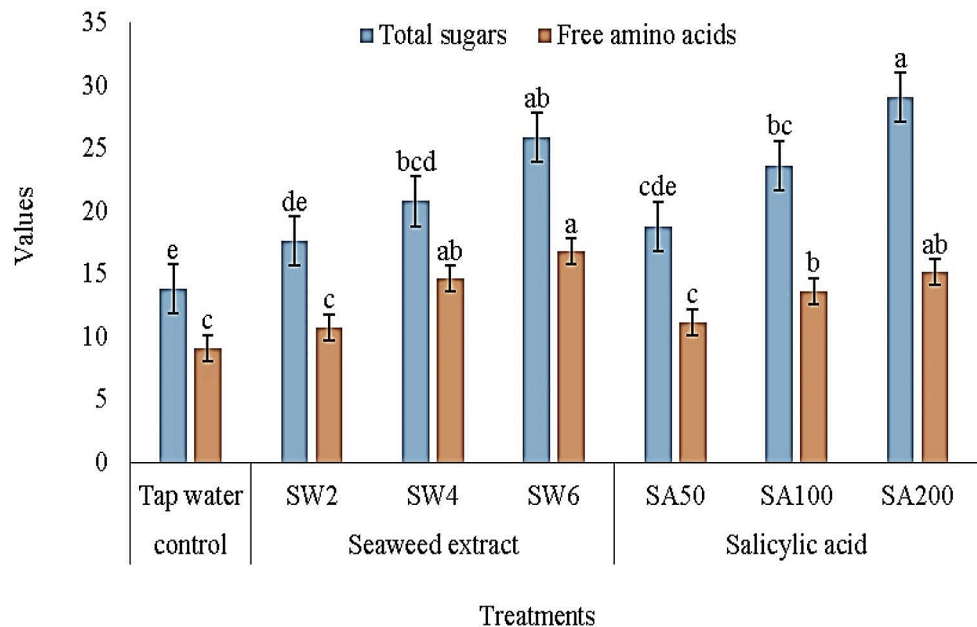
3.2 Plant Pigments

The pigment values of leaves increased significantly ($p \leq 0.05$) after spraying salicylic acid or seaweed extract when compared to control in both seasons (Table 5). Chlorophylls content in plants treated with SA at 200 mg L⁻¹ were significantly higher than that treated with seaweed and control, but there was higher significant ($p \leq 0.05$) increase in carotenoids content when plants were treated with seaweed extract at 6 ml L⁻¹. Result revealed also that the concentration of chlorophyll a, chlorophyll b, and total chlorophyll in the leaves increased with increasing the concentration of salicylic acid and seaweed. Increases were 56.5 and 86.1% in chlorophyll a, 81.2 and 186.7% in chlorophyll b and 63.8 and 109.1% total chlorophyll, owing to application of SA200 compared to tap water, in the first and second seasons, respectively. While, SW4 and SW6 increased carotenoids content by 21.7 and 34.8% in 2020 season and 17.8 and 46.4% in 2021 season, respectively, compared to the control. Moreover, in the first season SA200 treatment possessed 17.4% increase in carotenoids content.

3.3 Biochemical Constituents

Total sugar and free amino acids increased gradually ($p \leq 0.05$) with increase the concentrations of salicylic acid and seaweed extract (Fig. 1). Salicylic (200 mg L⁻¹) and

Fig. 1 Effect of salicylic acid (mg L^{-1}) and seaweed extract (ml L^{-1}) foliar application on total sugar and free amino acid contents (mg g^{-1} FW) in *Tagetes patula* L. leaves. SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L^{-1} , respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L^{-1} , respectively. Means values in each bar followed by different letters are significantly different based on the Duncan test ($p \leq 0.05$)



seaweed extract (6 ml L^{-1}) gave the highest values of total sugars and free amino acids. In this situation, SA200 and SW6 gave rise to about 2.09- and 1.86-fold increments in total sugars and 1.66- and 1.84-fold increments in free amino acids, respectively, compared to the control (tap water).

3.4 Antioxidant Activity

As depicted in Fig. 2, peroxidase activity and vitamin C remarkably ($p \leq 0.05$) influenced by salicylic acid and seaweed extract treatments, while glutathione-s-transferase was not modified. SA200 alone recorded the maximal value of peroxidase surpassing the other treatments, recording increase of 28.8% compared to the control. Except SA50 treatment, all other salicylic acid and seaweed extract treatments showed statistically similar values of vitamin C greater than the control treatment.

3.5 Leaf Macronutrient Content

As shown in Table 6, macronutrients content of *Tagetes patula* L. leaves significantly ($p \leq 0.05$) responded to foliar applications of SA or SW. SA200 showed the maximum increases in nitrogen, phosphorus and potassium content in both growing seasons, surpassing the other treatments, except SW6 for potassium content in the first season and SW6 or SA100 for phosphorus content in the second season. Leaves of *Tagetes patula* L. had increased values of N, P and K by about 2.15 and 1.94 folds, 1.79 and 1.74 folds as well as 1.52 and 2.51 folds in 2020 and 2021 seasons, respectively, due to SA200.

3.6 Essential Oil Content and Composition

Figure 3 illustrates the significant ($p \leq 0.05$) impact of salicylic acid and seaweed extract treatments on leaf essential oil content of *Tagetes patula* L. in 2020 and 2021 growing seasons. It is interesting to observe that SW6 possessed the greatest essential oil content in both seasons, statistically equaling ($p \geq 0.05$) SA200 in the first season. Comparing with the control treatment (tap water), increases in essential oil content reached 1.26 and 1.27 times due to SW6 in first and second seasons, respectively, and 1.25 times due to SA200 in the first season.

As for essential oil composition defined using GC-MS, there were variations in essential oil profile due to application of salicylic acid and seaweed extract (Supplementary Table 1). Both of SA200 and SW6 showed increments in majority of essential oil components detected with notable presence of identified components when compared to other treatments and approximately 1.22-fold increment when compared to control. SA200 increased D-Limonene, cis-Ocimene, α -terpinolene, Linalool, (-)- Carvyl Acetate, 1,3,8-p-Menthatriene, cis-p-Mentha-2,8-dien-1-ol and Piperitone. Nevertheless, SA200 showed a notable reduction in one of the major components of essential oil which is Caryophyllene. SW6 showed notable increase in some component such as α -terpinolene, p-Cymen-8-ol, dihydroedulan II and Neophytadiene.

3.7 Correlation Coefficient

The associations relationship expressed in correlation coefficients between the studied traits of *Tagetes patula* L. are

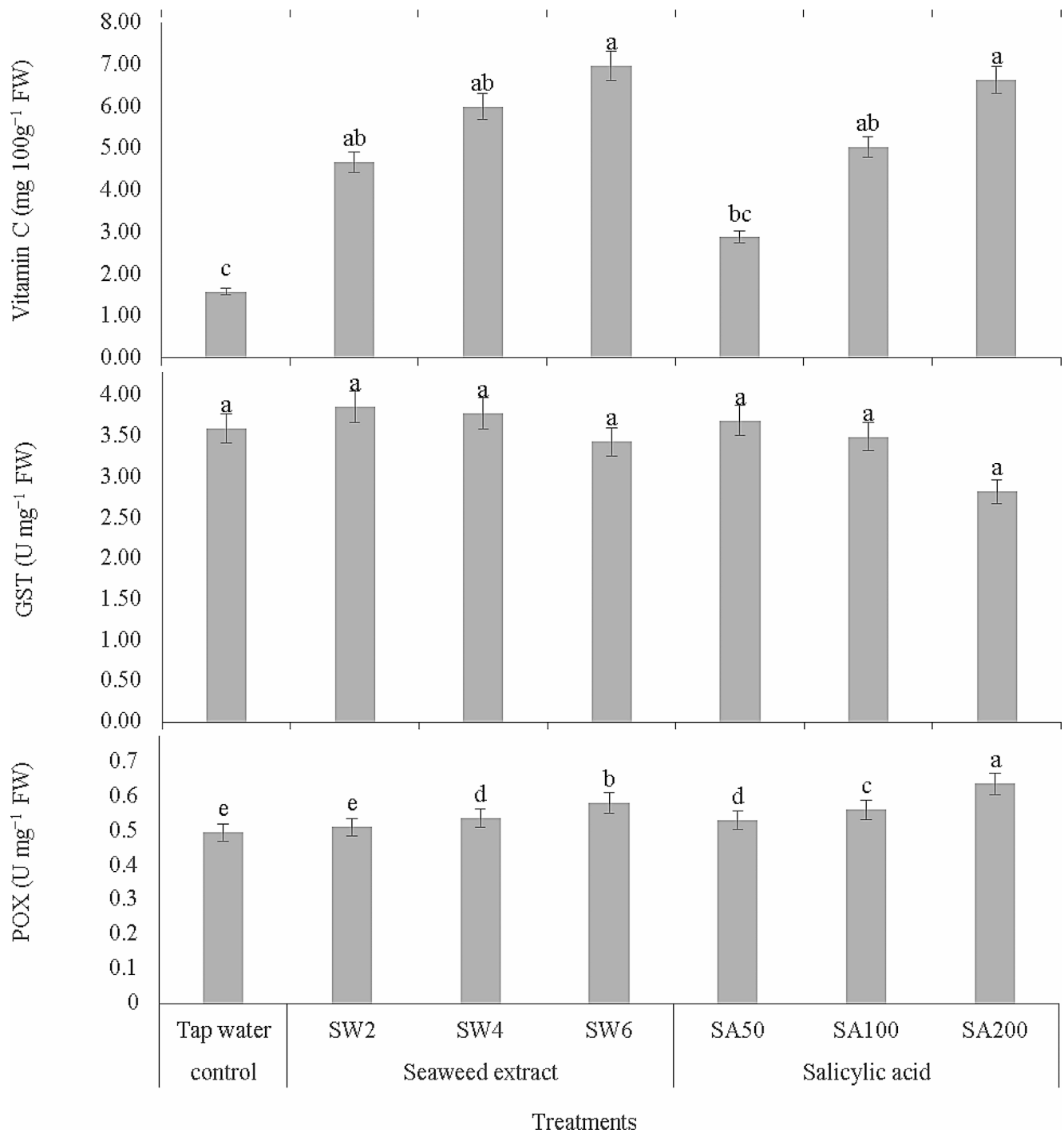


Fig. 2 Effect of salicylic acid (mg L⁻¹) and seaweed extract (ml L⁻¹) foliar application on glutathione-s-transferase (GST) and peroxidase (POX) in *Tagetes patula* L. leaves. SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L⁻¹, respectively; SA50, SA100

and SA 200: salicylic acid at 50, 100 and 200 mg L⁻¹, respectively. Means values in each bar followed by different letters are significantly different based on the Duncan test ($p \leq 0.05$)

presented in heat map (Fig. 4). Significant and positive associations were obtained between chlorophyll b and each of branches number plant⁻¹, leaf area, plant fresh weight, flower number plant⁻¹ and chlorophyll a as well as carotenoids and each of potassium, vitamin C and essential oil.

While, glutathione-s-transferase showed significant and negative correlations with plant height, branches number plant⁻¹, and stem diameter. Furthermore, highly significant and negative correlations were observed with glutathione-s-transferase and each of leaf area, leaves number plant⁻¹,

Table 6 Effect of salicylic acid and seaweed extracts foliar application on leaf macronutrients content of *Tagetes patula* L. in 2020 and 2021 seasons

Variable	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Season of 2020			
Control, tap water	1.96 f	1.13 f	3.42 e
SW2	2.11 ef	1.49 ef	3.66 de
SW4	2.90 cd	1.51 cd	4.73 cd
SW6	3.94 b	1.67 b	5.06 ab
SA50	2.23 de	1.47 de	3.43 c
SA100	3.80 bc	1.65 bc	4.87 bc
SA200	4.22 a	2.03 a	5.21 a
Season of 2021			
Control, tap water	2.08 d	1.02 e	2.44 f
SW2	2.83 cd	1.41 de	2.45 ef
SW4	3.01 bc	1.47 bc	3.29 cd
SW6	3.29 b	1.62 ab	4.92 b
SA50	2.93 cd	1.29 cd	2.62 de
SA100	3.01 b	1.55 ab	5.68 bc
SA200	4.05 a	1.78 a	6.13 a

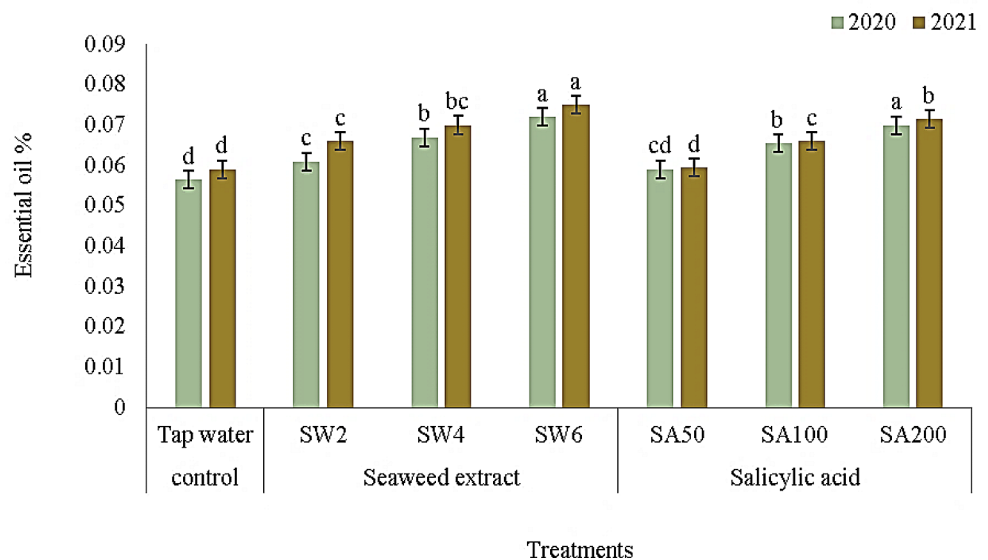
SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L⁻¹, respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L⁻¹, respectively. Means values in each column followed by different lower-case letters are significantly different based on the Duncan test ($p \leq 0.05$)

plant fresh weight, plant dry weight, flower number plant⁻¹, flower fresh weight plant⁻¹, flower dry weight plant⁻¹, and chlorophyll a. On the contrary, there were no noticeable correlation between chlorophyll b and each of plant height and leaves number plant⁻¹ as well as carotenoids and each of plant height, branches number plant⁻¹, stem diameter, leaf area, leaves number plant⁻¹, plant fresh weight, plant dry weight, flower number plant⁻¹, flower fresh weight plant⁻¹, flower dry weight plant⁻¹, and chlorophyll a. On the other hand, the other remained possible correlations between

Tagetes patula L. studied traits were positive and highly significant.

4 Discussion

Obviously, French marigold (*Tagetes erecta* L.) physiology, growth and essential oil were distinctively affected by exogenous supply of salicylic acid and seaweed extract. Generally, findings of the current work exhibited that application of salicylic acid at 200 mg L⁻¹ and seaweed extract at 6 ml L⁻¹ achieved promising enhancements in plant growth, antioxidant activity, sugar and amino acid contents as well as the nutritional status, and final economical yield. Herein, SA had the potency to enhance the activity of photosynthesis and enzymatic antioxidant (Abdelaal 2015), while stimulates the metabolisms of proline (Khan et al. 2014). Also, plant growth was improved by exogenous supply of SA (Abbaszadeh et al. 2020), since it serves as protectant against lipid peroxidation (Kang et al. 2013). Therefore, the improvements in French marigold growth with application of SA could be ascribed to the important action of SA in maintaining the photosynthetic apparatus and activity. SA, as a plant hormone (Davies 2010) can stimulate, plant pigments, growth, development and flowering, in addition to photosynthesis (Arif et al. 2020; El-Bially et al. 2022b). Furthermore, growth and flowering and antioxidant system were improved as well as photosynthesis and enzymatic scavenging activity of free radicals was up-regulated owing to SA application (Arif et al. 2020; Zulfikar et al. 2021). The enhancement in photosynthetic pigments with SA supply could be attributed to its potential to quench the reactive oxygen species (ROS) that naturally produced during photosynthesis process and caused several injuries to chlorophyll molecules and oxidative stress to plant tissues (El-Bially et

Fig. 3 Effect of salicylic acid and seaweed extracts foliar application on essential oil content of *Tagetes patula* L. leaves in 2020 and 2021 seasons. SW2, SW4 and SW6: seaweed extract concentrations of 2, 4 and 6 ml L⁻¹, respectively; SA50, SA100 and SA 200: salicylic acid at 50, 100 and 200 mg L⁻¹, respectively. Means values in each bar followed by different letters are significantly different based on the Duncan test ($p \leq 0.05$)

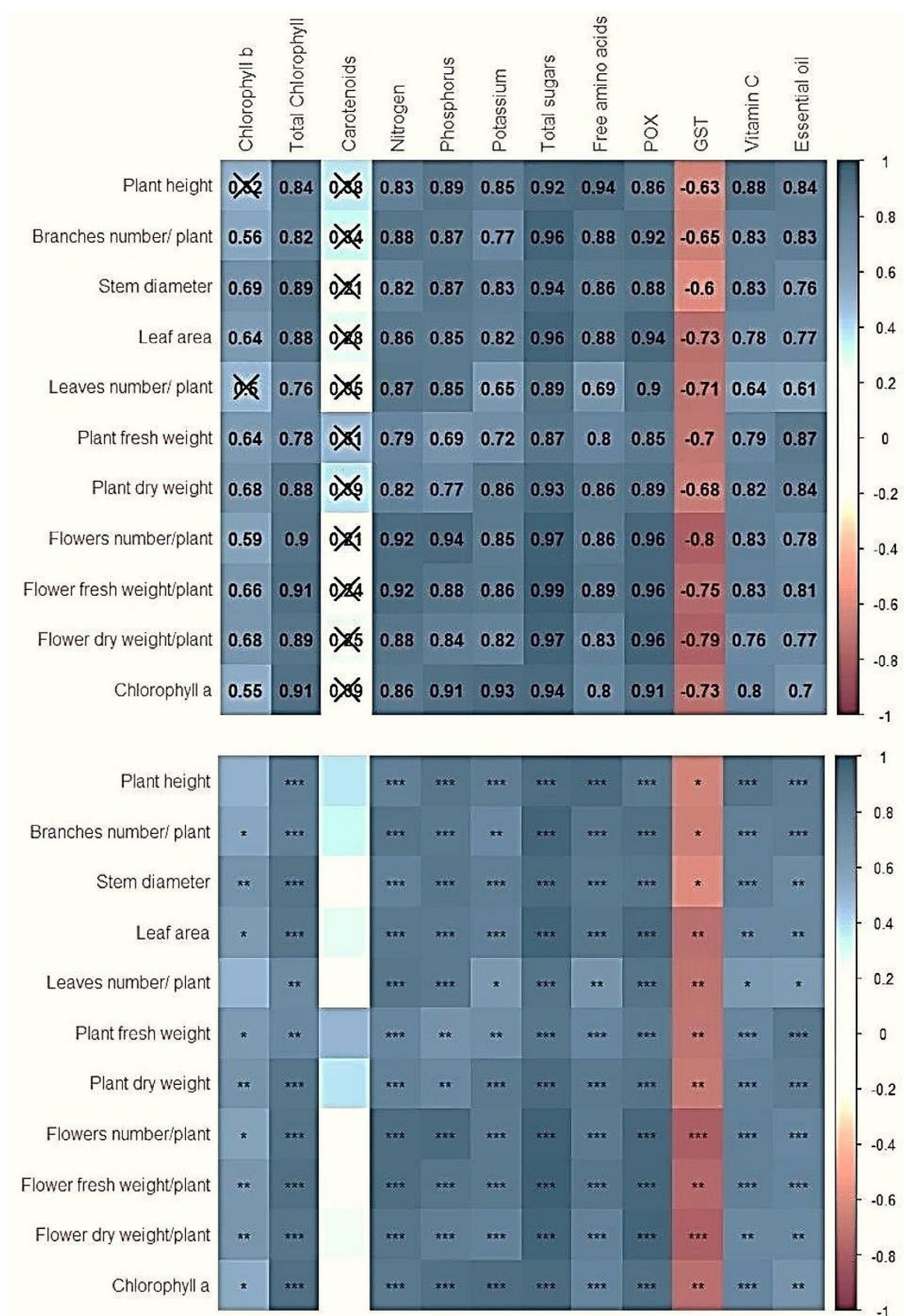


Fig. 4 Heat map shows the analysis of correlation coefficient between different *Tagetes patula* L. traits. POX: peroxidase, GST: glutathione-s-transferase, *, ** and ***: imply that the correlation is significant at 0.05, 0.01 and 0.001 level of significance, respectively

al. 2022b; Ali et al. 2024b). Wen et al. (2005) proved that SA stimulated the stress tolerance via induction and activation of phenylalanine ammonia lyase (PAL) gene expression. The action of PAL to reduce oxidative damage through the antioxidant pathway involves high accumulation of phenolics and flavonoids, resulting in better photosynthesis and low membrane damage (Li et al. 2020). It has been documented that phenolics and flavonoids and antioxidant enzymes are effective scavengers of ROS in plants (Dias et al. 2021). Thus, PAL and other enzymatic activities are often used as enzymes of the antioxidant system to evaluate the roles of genes in stresses (Zhang et al. 2019). Exogenous SA treatment induced PAL gene expression in *Salvia officinalis* and *Salvia virgata* (Ejtahed et al. 2015) and *Salvia miltiorrhiza* (Li et al. 2016). Salicylic acid-induced cytosolic acidification increases the accumulation of phenolic acids in *Salvia miltiorrhiza* cells and secondary metabolic components (Li et al. 2016). Moreover, SA treatment increased the activities of the enzymes of phenylpropanoid pathway in *Citrus sinensis* leading to the accumulation of phenolic acids in the fruits while enhanced the level of lignin (Zhou et al. 2018). The increase in the secondary metabolites was mediated via the elevated activity of the enzyme PAL (Cappellari et al. 2020). Thus, SA regulates the expression of the genes corresponding to the enzymes associated with secondary metabolite biosynthesis, including the key enzymes; PAL and isochorismate synthase (Ali 2021). *Cicer arietinum* plants responded very quickly to SA at 1.5 mM and showed higher induction of peroxidase and polyphenol oxidase activities, besides the higher accumulation of phenols, H₂O₂ and proteins (War et al. 2011). Accordingly, foliar spray of SA significantly increased vegetative growth because of its positive influences on cell membrane functions by raising nutrient uptake, ion absorption, and nucleic acid (Es-sbihi et al. 2020). CO₂ fixation was augmented via enhancing ribulose diphosphate carboxylase activity as a result of affording SA (Tan et al. 2020), hence photosynthesis, respiration, vegetative growth, thermogenesis, flower formation, seed production, and senescence were regulated (An and Mou 2011). Additionally, SA enhanced flowering and flower longevity, while suppressed ethylene biosynthesis and reversed abscisic acid action (Rocher et al. 2009). SA treatment resulted in significant increases of intracellular protein and the amounts of DNA and RNA while decrease of proteolytic activity (Kirillova et al. 2011). Thus, using SA gave the better growth and flower production of marigold plant (Choudhary et al. 2016; Basit et al. 2018), while increased biomass production (Pacheco et al. 2013).

As for essential oil content, Mirzajani et al. (2015) recorded an increase in total essential oil production in the leaves and the stems of sweet basil (*Ocimum basilicum*), in response to 1 mM dose of SA. SA application not only

affects essential oil content but also oil composition. Herein, foliar application of SA significantly enhanced the content and yield of essential oil in *Thymus daenensis* plants while carvacrol, α -thujene, α -pinene and p-cymene increased and thymol and, β -caryophyllene declined (Pirbalouti et al. 2014). Limonene, linalool and linalyl acetate of essential oil of *Citrus aurantium* leaves were elevated due to SA treatment (Sarrou et al. 2015). SA at 1.00 mM increased the yield of artemisinin up to 50.0% in *Artemisia annua* plants (Aftab et al. 2010). Monoterpene oxygenated and sesquiterpenes in lemon balm (*Melissa officinalis*) plants were considerably enhanced by SA spray, while essential oil yield was not affected (Pirbalouti et al. 2019).

Regarding the use of natural materials in crop growing, it is worthily to notice that seaweed extract (SW) had several significant compounds involving nutrients and growth regulators (Table 2) that are useful for plant growth. Seaweed extract contains amino acids, laminaran, fucoidan, alginate, and betaine, which stimulate metabolic activity and thus, increase plant growth and yield (Khan et al. 2009). It has been reported that alginic acid significantly enhanced radish growth with adjusting the osmotic potential (Wang et al. 2020). Alginate oligosaccharide improved photosynthetic performance and accumulation of sugars in citrus (Li et al. 2023). Thus, the exogenous supply of seaweed was expected to promote French marigold plants. However, the appropriate concentration was not well known. This research elucidated that SW at the concentration of 6 ml L⁻¹ was the promising treatment for enhancing physiological status, hence growth and yield. In this regard, spraying the vegetative part of *Amaranthus tricolor* plant with two concentrations of seaweed caused significant increases in plant height and number of branches in addition to the fresh and dry weights of shoot and root (Abdel-Aziz et al. 2011). Similar results were obtained in *Tagetes erecta* plants treated with seaweed extract at 2.0, 4.0, 6.0 and 8.0 ml L⁻¹ after 30 days of germination (Sridhar and Rengasamy 2010). Similarly, significant increases in chlorophyll content, stomatal conductance, photosynthetic rate, and transpiration rate were recorded in asparagus plants treated with seaweed extract (Al-Ghamdi and Elansary 2018). Treatment of willow (*Salix* sp.) plants with an extract of *Ecklonia maxima* enhanced the electron transfer rates of both photosystems (Digruher et al. 2018). Applications of seaweed extract on cottonwood (Fei et al. 2017) and mustard (Stasio et al. 2017) significantly increased leaves potassium uptake. The physiological status was adjusted in favor of plant development by *Ecklonia maxima* seaweed extract via enhancing stomata opening and conductance, chlorophyll content assimilation of CO₂, while raising transpiration and nutrient uptake (Rouphael et al. 2017; Lefi et al. 2023). Therefore, application of seaweed extracts in maize showed that leaves

were able to significantly absorb more Zn, Fe, B, Cu, Mo, S, Mg, Ca, and Mn than the control treatment (Ertani et al. 2018). Accordingly, seaweed extract is represented a promising natural source in nourishing medicinal crops such as marigold plants.

5 Conclusions

In potted-cultivated French marigold plants, foliar salicylic acid or seaweed extract applications exhibited promising findings on vegetative and flowering growth, physiological response, nutrients and essential oil. Leaves biomass, which is the most significant product for obtaining oil of marigold plants, remarkably increased with increasing salicylic acid and seaweed extract concentrations. Salicylic acid or seaweed extract concentrations not only affected yield biomass and essential oil content but also, oil profile. Accordingly, using salicylic acid (200 mg L⁻¹) and seaweed extract (6.0 ml L⁻¹) is advisable to gain better profits from marigold plants cultivation. Since salicylic acid and seaweed extract achieved individually distinctive improvements in French marigold physiology and end marketable product, further studies should be implemented to assess their combined application effect.

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Author Contributions This study was designed and implemented by its authors. All authors contributed to writing the manuscript, interpreting the information presented, and have read and agreed to the final version of the manuscript.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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