



Evaluation of the newly recorded alga *Phyllymenia gibbesii* compared with six Egyptian native species as biofertilizers to enhance growth and yield of potato cultivars

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Abstract

Seaweeds are acknowledged for their potential as biofertilizers and biostimulants, contributing to enhanced growth and yield of various crops globally due to their appropriate amount of macro- and microelements, plant growth regulators, and their richness in bioactive compounds. In the current study, the seaweeds *Ulva lactuca*, *Ulva intestinalis*, *Corallina officinalis*, *Ellisolandia elongata*, *Jania rubens*, *Colpomenia sinuosa*, and the newly recorded red alga *Phyllymenia gibbesii* were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) from April 2015 until June 2016 and were evaluated as biofertilizers to enhance the growth and yield of potato cultivars (cv. Hermes and cv. Bellini). The chemical contents of macroelements (Ca, Na, N, P, and K) and the microelements (Zn, Cu, Ni, Cd, Co, Pb, and Fe) were determined in the dry seaweed powder and its water extracts. For short-term use of SWEs, the results showed that all the measured microelements and Na in seaweed extracts were below the permissible levels according to FAO/WHO. For long-term use of SWEs, the Co concentration exceeded the permissible level in all SWEs according to FAO/WHO. The same pattern was observed for Cd, except for *C. officinalis* and *P. gibbesii*. The concentration of Cu and Ni exceeded the permissible levels in *U. intestinalis* and *C. officinalis* (0.28 and 0.36 mg L⁻¹), respectively, for Cu and (0.41 and 0.31 mg L⁻¹), respectively, for Ni. For the analyses of carbohydrates and protein in seaweeds, *P. gibbesii* had the highest concentration of carbohydrates ($56.2 \pm 3.8\%$), followed by *U. lactuca* ($53.6 \pm 5.1\%$), while the protein content showed the inverse pattern. The growth-regulating hormones (IAA, Kinetin, GA₃, and ABA) were quantified in the seaweeds. *Ulva lactuca* had the highest concentrations of (IAA, GA₃, and ABA) (1 µg g⁻¹ FW, 0.12 µg g⁻¹ FW, and 0.006 µg g⁻¹ FW), respectively. The highest concentration of kinetin occurred in *C. sinuosa* (0.168 µg g⁻¹ FW). In the greenhouse experiment, the seaweed extracts were applied (autoclaved and non-autoclaved) to evaluate their effects on the potato cultivars. The algal extracts, particularly *C. sinuosa*, *U. lactuca*, and *P. gibbesii*, were promising for potato growth and yield parameters for both cultivars. Due to the high concentrations of some microelements in seaweed extracts, which exceeded the permissible levels for long-term use according to FAO/WHO, they are recommended to be used only for short-term irrigation.

Keywords Seaweeds · Biochemical contents · Growth-regulating hormones · Short-term · Long-term use · Microelements

Introduction

The potato plant (*Solanum tuberosum* L.) is considered one of the most important vegetable crops in the world because of its high yield potential and great nutritional content (Koch et al. 2020). They are an excellent source of calories, minerals, proteins, lipids, and vitamins (King and Slavin 2013). Potatoes serve as more than just a staple food (Jagatee et al. 2015). They have a wide range of applications, which sets them apart from most other crops and increases the tendency to produce them (Koch et al. 2020). Potato production ranks fourth in the world, after rice, wheat, and maize (FAOSTAT

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2013). Every year, 378 million tonnes of potatoes are produced worldwide (FAOSTAT 2017). Egypt is one of the top 20 potato growers in the world and a major exporter of potatoes in Africa, producing 5.2 million tonnes of potatoes annually (Rabia et al. 2021). To be highly competitive in the global marketplace, one of the goals of the Egyptian Ministry of Agriculture is to improve the efficiency of potato production and to attain better quality and higher yields (Rabia et al. 2021).

The potential yield is never achieved in natural production systems due to the negative interference of biotic and abiotic factors (Nowicki et al. 2012; Koch et al. 2020), which can be reduced by using balanced agronomic management strategies. Adequate nutrient management is one of the crucial agronomic strategies for potato production, in addition to cultivar selection, plant protection, and a steady water supply (Koch et al. 2020). The sustainability of its production has been seriously threatened by its dependence on chemical fertilizers needed to fulfill its nutrient requirements (Prajapati et al. 2016).

As an inexpensive natural source for promoting plant growth and the most effective, safe, and eco-friendly biofertilizers, seaweeds have been applied as an alternative or complement to chemical fertilizers and/or biostimulants in the agriculture domain (Shukla et al. 2021; Ammar et al. 2022; Vaghela et al. 2022, 2023). They have been used for several decades as they contain essential and trace elements, amino acids, fatty acids, and growth biostimulant agents (Van Oosten et al. 2017; Li et al. 2022; Vaghela et al. 2022, 2023; Meena et al. 2025), such as gibberellins, auxins, cytokinins, and abscisic acid (Mondal et al. 2015; Wani et al. 2016; Vaghela et al. 2022, 2023). These plant growth stimulant compounds enhance plant growth and improve plant resistance to pathogens (Van Oosten et al. 2017; Gupta et al. 2021; Ribeiro et al. 2024). Seaweeds contain bioactive compounds such as sulfated polysaccharides (eg., alginates, ulvan, agar, and carrageenan), polyphenols, and terpenoids (Shukla et al. 2021). These sustainable sources of stimulants activate flowering and fruit setting by stimulating the synthesis of polyamines (viz., thiazolidine, putrescine, tyramine, and tryptamine) (Vaghela et al. 2023), which play a role in plant growth, development, defense mechanisms, and enhancement of plant stress tolerance (Cansev et al. 2017; Leonard et al. 2022). The application of the brown alga *Ascophyllum nodosum* as a foliar spray significantly improved the yields of three varieties of potatoes (Kuisma 1989). Bio-Algen S-90, produced from *A. nodosum* and *Laminaria digitata*, enhanced the starch content in potato tubers (Prisa 2020). The usage of the red algae *Kappaphycus alvarezii* or *Gracilaria edulis* extracts (10%) supplemented with the recommended fertilizer dose significantly improved the growth attributes of the potato crop (Prajapati et al.

2016; Garai et al. 2021). Combined with the nano-fertilizer, seaweed extracts improved potato yield and growth parameters, rather than using nano-fertilizer or the growth regulator (hypertonic) alone (Al-Juthery et al. 2018).

In Egypt there have been some trials based on commercial seaweeds for enhancing potato production. Two extracts were used for this purpose, Alga 600 (a mixture from *A. nodosum*, *Laminaria* spp., and *Sargassum* sp., N $\geq$ 1%, K₂O $\geq$ 18.5%, alginic acid $\geq$ 10%, Mg $\geq$ 0.42% S $\geq$ 2.2%, natural PGRs, vitamins, amino acids, betaine, and mannitol) and Sea Force 2 (alginate 20–25%, laminarin + pheophytin 10–20%, mannitol 8–10%, vitamin and amino acid 2–3%, fatty acid 2–4%, and large-particle micronutrients 15–18%) (Sarhan 2011). An algal extract of 'Oligo-x' (a mixture from *Ascophyllum*, *Laminaria*, *Sargassum*, *Fucus* spp., oligosaccharides 3%, alginic acid 5%, phytin 0.003%, menthol 0.001%, cytokinin 0.001%, indole acetic acid 0.0002%) was successfully applied (Abido et al. 2020). Likewise, TAM® extract (a mixture from *Ulva lactuca*, *Jania rubens*, and *Pterocladia capillacea*, K 12%, P 2%, N 0.14%, Cu 0.39 ppm, Fe 16.18 ppm, Mg 19.72 ppm, Zn 1.19 ppm, Mn 3.72 ppm) was used to evaluate these algae products as biostimulants (Ammar et al. 2022).

However, introduced algal species may have the potential to displace native species, affect ecosystem processes, change community structure, impact human health, decrease native biodiversity, and become invasive species when established, causing substantial economic losses (Simberloff 2005). The red alga *Grateloupia doryphora* was recorded for the first time in the Eastern Harbor, Alexandria, Egypt (Mediterranean Sea) (Shafik and Taha 2008). The taxonomic position of the species was revised by Rodríguez-Prieto et al. (2021) and re-identified as *Grateloupia gibbesii* Harvey. The species is currently regarded as a synonym of *Phyllophora gibbesii* (Harvey) Showe M. Lin, Rodríguez-Prieto, De Clerck & Guiry (Rodríguez-Prieto et al. 2022). It can be considered as an introduced species rather than an invasive one, causing no problems in the introduced area since it contributed at a negligible rate to the main algal bulk (Shams El-Din et al. 2025).

Thus, the objectives of the current study were to analyze the chemical composition of the newly introduced alga *P. gibbesii* compared with six common native algal species and to assess their application (autoclaved and non-autoclaved extracts) as alternative, safe, and eco-friendly biofertilizers on the growth and yield of two common potato cultivars (Hermes and Bellini) in Egypt. This study is also an effort to recycle *P. gibbesii* by using it as a biofertilizer, which is one suitable strategy for its introduction on our coasts to avoid the changes probably occurring in the future due to its presence.

Materials and methods

Collection of seaweeds

Macroalgal samples were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (31°16'14"-31°12'20" N and 30°3'41"-29°53'1"E) from April (2015) until June (2016). The samples were washed with seawater at the sampling sites to remove the adhered sediments and impurities and then placed in polyethylene bags in an ice box for transport to the laboratory, where they were quickly rinsed with tap water on the same day to remove the remaining impurities and epiphytes. Macroalgal sub-samples were preserved in 4% formalin-seawater and/or pressed for herbarium specimen sheets (Dhargalkar and Kavlekar 2004).

The six algal species, *Ulva intestinalis* Linnaeus, *Ulva lactuca* Linnaeus, *Corallina officinalis* Linnaeus, *Ellisolandia elongata* (J. Ellis & Solander) K.R. Hind & G.W. Saunders, *Jania rubens* (Linnaeus) J.V. Lamouroux, and *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier were identified based on morphology using guides and keys to identification, according to Aleem (1993) and Cabioch et al. (2014). The six macroalgal species were updated according to the taxonomic database site algaebase.com (Guiry and Guiry 2024).

Preparation of seaweed extracts (SWEs)

The algal samples were shade-dried at ambient temperature (25–30 °C) on blotting paper for about 2–6 days. After completely drying, the samples were ground with a mortar and pestle, then packed and labeled in self-sealing bags for further use.

Non-autoclaved aqueous extract

The seven seaweed species were separately soaked in distilled water (20 g DW) in 100 ml for five days and filtered through muslin cloth to obtain 20% aqueous algal extract. The extract was stored at 4 °C until use (Pise and Sabale 2010).

Autoclaved aqueous extract

As the 20% autoclaved extract turned into a very thick paste that was very hard to filter, a stock with a lower appropriate concentration was prepared (10%). Thus, the seven seaweed species were separately soaked in distilled water (10 g DW in 100 mL) for 30 min before autoclaving at 121 °C, 15 psi for 30 min. The hot extract was filtered through a double

layer of muslin cloth and allowed to cool to room temperature, and then the extracts were stored at 4 °C, with their initial concentrations being 10% (Rama Rao 1990).

Both the autoclaved and the non-autoclaved algal extracts were applied with the same concentration (0.5%), where the autoclaved stock extracts (10%) were diluted 20 times and the non-autoclaved stock extracts (20%) were diluted 40 times to reach a concentration of 0.5%.

Analysis of macroelements in seaweeds

Nitrogen

The nitrogen (N) content was determined according to macro-Kjeldahl by the colorimetric method of Hevia and Cioccia (1988) and Khouli et al. (2002). The algal samples (0.5 g DW) were digested by 25 mL of concentrated sulfuric acid and 1 mL hydrogen peroxide (30%) at 270 °C. A volume of 0.1 mL was blended with 7.5 mL nitroprusside reagent and 7.5 mL buffer solution. After 1 h the sample absorbance was measured at 662 nm against the reagent blank. Three replicates were prepared for nitrogen measurement. The results were calculated from the N standard curve, using ammonium sulfate as the standard solution, and were expressed as a percentage of dry weight.

Phosphorus

The algal samples (0.5 g DW) were digested by the modified HNO₃/HClO₄ method (Pequerul et al. 1993). The phosphorus content (P) was estimated by the ascorbic colorimetric method (APHA 1992), in which an acidified molybdate mixed reagent was added to a 2 mL aliquot of digested sample (between 10–30 min) for complete color development. The absorbance of the sample was measured against the reagent blank at a wavelength of 880 nm. Three replicates were prepared for phosphorus measurement. The results were compared to a standard curve with a range of 0.5 to 5 ppm phosphorus (P) in solution and were expressed as a percentage of dry weight.

Determination of (Na, K, Ca) in dry seaweed powder

The macroelements potassium, calcium, and sodium were estimated according to Miller (1998), where each dry powder algal sample (0.5 g) was digested with 6 mL nitric acid and 2 mL perchloric acid at room temperature (25 °C). The digested material was filtered through Whatman No. 40 filter paper and the filtrate was made up to a suitable volume with deionized water and then injected into an Atomic Absorption Spectrophotometer (Perkin Elmer 2380). Three replicates for each element were taken, and

the concentration was expressed as a percentage of dry weight.

Analysis of microelements in dry seaweed powder

A weight of homogeneous dry powder algal samples (0.5 g of each species) was digested with 5 mL concentrated HNO_3 at room temperature (25°C) overnight and then the mixture was gently boiled to 70 °C with the addition of 1 mL perchloric acid until reaching a clear solution. After cooling, solutions were then made up to 25 mL with distilled water (Schuhmacher and Domingo 1996; Hseu 2004). The analysis of zinc, copper, cobalt, cadmium, nickel, lead, and iron was performed with a flame atomic absorption spectrophotometer (Model Perkin Elmer 2380) and the concentration was expressed as a percentage of dry weight.

Analysis of microelements in seaweed extracts

The microelements (Zn, Cu, Co, Cd, Ni, Pb, and Fe) were assessed in liquid aqueous extract (0.5% of each species), following the same procedure as used in the analysis of microelements in dried powder seaweeds. The concentration of one replica for each element was expressed as mg L^{-1} .

Estimation of carbohydrate content in seaweeds

The total carbohydrate content was estimated by the phenol/sulfuric acid method (Dubois et al. 1956) using glucose as a standard. The absorbance of blank, standards, and algal samples was measured at 490 nm. Three replicates were taken for each algal sample, and the results were calculated from the glucose standard curve as mg mL^{-1} . The percentage of total carbohydrates present in algal samples was determined according to Sadasivam and Manickam (2005).

Estimation of protein content in seaweeds

The total protein content was determined according to the method described by Naguib (1964), cited in Mohy El-Din (2015). An aliquot of water extract (3 mL) was digested with 3 mL 50% nitrogen-free H_2SO_4 . Thereafter, 1 mL H_2O_2 (30%) was added and heating was continued until the fumes disappeared and the mixture turned pale green. After cooling, the solution was made into a volume. An aliquot was neutralized and its ammonia content was estimated according to Fawcett and Scott (1960). Three replicates were taken for each algal sample and the results were calculated according to Lourenço et al. (2002), where total protein = $\text{N} \times \text{N-protein conversion factor}$. The N-protein factor for green algae was 5.13, for red algae the N-protein factor was 4.59 and 5.38 for brown algae.

Quantification of plant growth regulators (PGRs) content in seaweeds

Based on the effect of seaweeds on potato plant growth parameters, the most effective three seaweeds (*U. lactuca*, *P. gibbesii*, and *C. sinuosa*) were chosen to analyze their content of plant growth regulators. The PGRs analysis; indole acetic acid (IAA), kinetin (Kin), gibberillic acid (GA_3), and abscisic acid (ABA) was performed according to Shindy and Smith (1975) using high-performance liquid chromatography (HPLC) (Agilent Technologies 1200). The mobile phase of HPLC was methanol: water in the ratio of 50:50 v/v, with a flow rate of $200 \mu\text{L min}^{-1}$ and a temperature of 50 °C. Each algal sample (5 g FW) was ground and soaked in 80% aqueous methanol (v/v) and allowed to extract for 72 h. The mixture was filtered through filter paper and the residue was re-extracted three times with aqueous methanol. The extracts were evaporated into the aqueous phase, which was partitioned into two equal volumes (about 15 mL each). The first part was adjusted to pH 8.6 with 1% NaOH and extracted three times with ethyl acetate, then evaporated to dryness, and the residue was dissolved in 1 mL methanol to be used for the quantification of kinetin at 269 nm. The second part of the aqueous phase was adjusted to pH 2.6 with 1% HCl, extracted similarly to the first part, and used for the quantification of indole acetic acids (IAA), gibberellic acid (GA_3), and abscisic acid (ABA) at 254 nm. The methanolic extracts were filtered through a membrane filter (0.45 μm) before injection of 10 μL into the HPLC system, taking into consideration that all the extractions were done at a temperature below 4 °C in dark vials. The PGRs analysis was based on one replica for each algal sample.

Greenhouse studies on potato plants treated with seaweed extracts (SWEs)

Growth parameters

To evaluate seaweed extracts (SWEs) as bio-fertilizers, nine treatments (7 different algal extracts, NPK, and the control) were used in the experiment for each potato cultivar. A greenhouse trial was constructed using a randomized complete block design (RCBD) in 20 cm pots where a sterilized mixture of sand and peat moss (1:1) was added, where sand facilitates excess water drainage while peat moss helps keep an even moisture level (Karlatti et al. 1997; Mohamed 2015). Two potato seed cultivars, Hermes and Bellini, were sown separately. The pots were irrigated with 300 mL seaweed extracts at a concentration of 0.5% every 3 days (during approximately 90 days). For the control, the pots were irrigated with tap water instead of algal extract. To compare the seaweed extracts to the commercial chemical

fertilizer Zain™ (NPK; nitrogen, phosphate, and potassium in a ratio of 20:20:20), it was applied similarly to the seaweed extracts at a concentration of 0.5% (0.5 g NPK dissolved in 100 mL of water). A duration of 110 days from sowing was needed to reach the mature phase of potato plants to determine the impact of the seaweed extracts on (the number of tubers/plants, tuber size, and tuber weight). After this period, three replicates for each growth parameter were taken: shoot and root length, shoot and root fresh weight, shoot and root dry weight, leaf surface area, number of tubers, tuber weight average, chlorophyll in leaves, and carbohydrates %.

Chlorophyll and carbohydrate analyses in the leaves of potatoes

The total chlorophyll content was estimated in the 4th leaf from the top of the potato plants by the method of Arnon (1949) at 662 nm. The results of three replicates were expressed as average mg g⁻¹ fresh weight for each treatment. The carbohydrate content in the leaves was estimated colorimetrically by anthrone reagent at 630 nm (Hedge and Hofreiter 1962). The results of three replicates were expressed as average percentages.

Statistical analysis

The t-test was applied for each growth parameter to compare the responses of the two potato cultivars (Hermes & Bellini) to the algal treatments (n = 28) and to compare the effects of the two methods (autoclaved and non-autoclaved) on the two cultivars (n = 28). Significantly, different means were separated at $p \leq 0.05$ levels by the t-test. Analysis of variance (one-way ANOVA) and Tukey test were performed for each growth parameter to compare the seven seaweeds and their influence on the two cultivars and also to compare the

seaweeds and the control and NPK (n = 36). Significantly different means were separated at $p \leq 0.05$ levels by the Tukey test. All the statistical analysis was performed using the Minitab 14 program (Blair and Taylor 2007).

Results

Algal contents of some macroelements

Estimation of macroelements in seaweeds revealed that the highest nitrogen content was found in the green alga *U. lactuca*, followed by the red alga *P. gibbesii*, while the phosphorus content was the highest in the brown alga *C. sinuosa*, followed by *U. lactuca*. The highest potassium and sodium content was found in *U. intestinalis* and the lowest content in *P. gibbesii*. Finally, the highest calcium content was reported in the two coralline algae *E. elongata* and *J. rubens*, while the lowest content was recorded in *P. gibbesii* (Table 1).

Microelements content

Microelements content in dry seaweed powder

The analysis of microelements in dry seaweed powder revealed that the two green algae recorded the highest quantities of almost all of them; viz. Zn, Co, Pb in *U. intestinalis*, and Cd in *U. lactuca*. The red alga *C. officinalis* had the highest Cu content, while the brown alga *C. sinuosa* had the highest Ni concentration and Fe (Table 2).

Microelements content in seaweed extracts used for irrigation

The concentration of microelements (Zn, Cu, Co, Cd, Ni, Pb and Fe) in the seaweeds extracts (SWEs) used in the

Table 1 Concentration of some macroelements (nitrogen, phosphorus, potassium, calcium and sodium) in dry seaweed powder

Seaweed sp.	N	P	K	Ca	Na
Green algae					
<i>Ulva intestinalis</i>	2.02 ± 0.10	0.56 ± 0.03	0.51 ± 0.11	0.07 ± 0.06	0.53 ± 0.26
<i>Ulva lactuca</i>	3.51 ± 0.15	0.69 ± 0.11	0.50 ± 0.09	0.07 ± 0.09	0.48 ± 0.10
Red algae					
<i>Corallina officinalis</i>	2.47 ± 0.20	0.33 ± 0.09	0.07 ± 0.07	0.27 ± 0.12	0.26 ± 0.15
<i>Ellisolandia elongata</i>	2.30 ± 0.02	0.31 ± 0.06	0.12 ± 0.07	0.32 ± 0.17	0.30 ± 0.21
<i>Jania rubens</i>	3.32 ± 0.18	0.51 ± 0.14	0.09 ± 0.03	0.31 ± 0.20	0.29 ± 0.12
<i>Phyllymenia gibbesii</i>	3.49 ± 0.15	0.35 ± 0.07	0.08 ± 0.10	0.03 ± 0.07	0.02 ± 0.009
Brown algae					
<i>Colpomenia sinuosa</i>	2.97 ± 0.17	0.72 ± 0.12	0.43 ± 0.12	0.24 ± 0.21	0.27 ± 0.11

Data are expressed as % of dry weight (mean ± SD). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015–June 2016)

*Data are means of three replicates ± SD

Table 2 Trace elements (zinc, copper, cobalt, cadmium, nickel, lead and iron) concentrations in dry seaweed powder

Seaweed sp.	Zn	Cu	Co	Cd	Ni	Pb	Fe
Green algae							
<i>Ulva intestinalis</i>	31.5	5.6	408.9	4.5	13.8	30.8	226.5
<i>Ulva latuca</i>	11.8	3.2	354.8	4.8	13.7	21.9	233.6
Red algae							
<i>Corallina officinalis</i>	26.9	17.2	264	1.6	15.3	13.9	354.9
<i>Ellisolandia elongata</i>	11.7	1.3	400.4	3.8	10.5	23.1	335.1
<i>Jania rubens</i>	10.4	3.7	402	3.2	5.2	22.5	466.9
<i>Phyllymenia gibbesii</i>	9.7	3.1	277	2.1	11.4	15.8	242.7
Brown algae							
<i>Colpomenia sinuosa</i>	17.9	3.4	354.1	3.3	16.9	21.4	652.8

Data are expressed as $\mu\text{g g}^{-1}$ dry weight. Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea)(April 2015—June 2016)

*Data are for one replicate

irrigation of the two potatoes cultivars were compared with the permissible limits of trace metals in irrigation water set by Rowe and Abdel-Magid (1995) and FAO/WHO (2003). The results showed that all the seaweeds extracts fell within the permissible dose for short-term use of SWEs as biofertilizers (Table 3).

For long-term use of SWEs as biofertilizers, the Co concentration exceeded the permissible levels in all SWEs according to FAO/WHO. The same pattern was observed for Cd, except for *C. officinalis* and *P. gibbesii*. The concentrations of Cu and Ni exceeded the permissible level in *U. intestinalis* and *C. officinalis* adopted by Rowe and Abdel-Magid (1995) and FAO/WHO (2003) (Table 3).

Carbohydrate and protein content in dry seaweed powder

Carbohydrate content was the highest in the red alga *P. gibbesii*, followed by *U. lactuca*, while protein content showed the inverse pattern. The brown alga *C. sinuosa* was in the third rank for the two biochemical contents (Fig. 1).

Plant growth regulators content in dry seaweed powder

The green alga *U. lactuca* had the highest concentration of IAA, GA₃, and ABA acid, while *C. sinuosa* showed the highest concentration of kinetin (Fig. 2).

Table 3 Trace elements concentration (zinc, copper, cobalt, cadmium, nickel, lead and iron) in seaweed extracts (SWE) used in irrigation, data are expressed as mg L^{-1}

Seaweed sp.	Zn	Cu	Co	Cd	Ni	Pb	Fe
Green algae							
<i>Ulva intestinalis</i>	1.58	0.28	1.03	0.03	0.41	1.54	6.35
<i>Ulva lactuca</i>	0.94	0.16	0.43	0.02	0.18	1.15	6.91
Red algae							
<i>Corallina officinalis</i>	1.45	0.36	0.72	0.01	0.31	1.45	7.36
<i>Ellisolandia elongata</i>	0.68	0.19	1.54	0.04	0.25	1.10	7.42
<i>Jania rubens</i>	0.86	0.19	0.91	0.05	0.13	1.12	11.17
<i>Phyllymenia gibbesii</i>	0.49	0.16	0.43	0.01	0.22	0.79	6.13
Brown algae							
<i>Colpomenia sinuosa</i>	1.32	0.24	0.34	0.02	0.26	1.15	12.21
* short-term use limits	10	5	5	0.05	2	10	20**
* long-term use limits	2	0.2	0.05	0.01	0.2	5	5**

Data are compared with short and long-term use limits. Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea)(April 2015—June 2016)

* Recommended limits for heavy metals in irrigation water. (Adapted from FAO/WHO (2003) and Rowe and Abdel-Magid (1995)

** Not toxic to plants in aerated soils but can contribute to soil acidification and loss of essential phosphorus and molybdenum

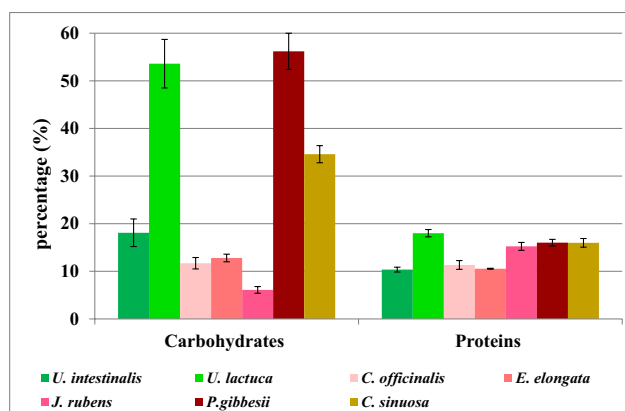


Fig. 1 Carbohydrates and protein percentage in dry seaweed powder (mean $\pm$ SD% dry wt.). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)

Evaluation of seaweed extracts as bio-fertilizers

The results of the greenhouse experiment for growth parameters are shown in (Table 4A, B, C), (S1–S3), and (Fig. 3, 4, 5, 6).

One-way ANOVA followed by Tukey test revealed that SWE extracts in both forms (autoclaved and non-autoclaved) significantly improved the growth parameters of the potato cultivars (Hermes and Bellini) ($p \leq 0.05$), except shoot length, root length, and root fresh weight (Table 4C). Based on Tukey test at $P \leq 0.05$ level, the enhancement of the growth parameters was mostly achieved by using *C. sinuosa*, *U. lactuca*, and *P. gibbesii* along with the chemical fertilizer NPK over the control treatment ($p \leq 0.05$) (Table 4C). On the other hand, the green alga *U. intestinalis* was less significant than NPK and more significant than control (Table 4C), while the

Fig. 2 Total content of plant growth regulators: indole acetic acid (IAA), kinetin (KIN), gibberellic acid (GA3) and abscisic acid (ABA) ($\mu\text{g g}^{-1}\text{dwt}$) of the most effective seaweeds on potato plant growth parameters (*U. lactuca*, *P. gibbesii* and *C. sinuosa*), collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)

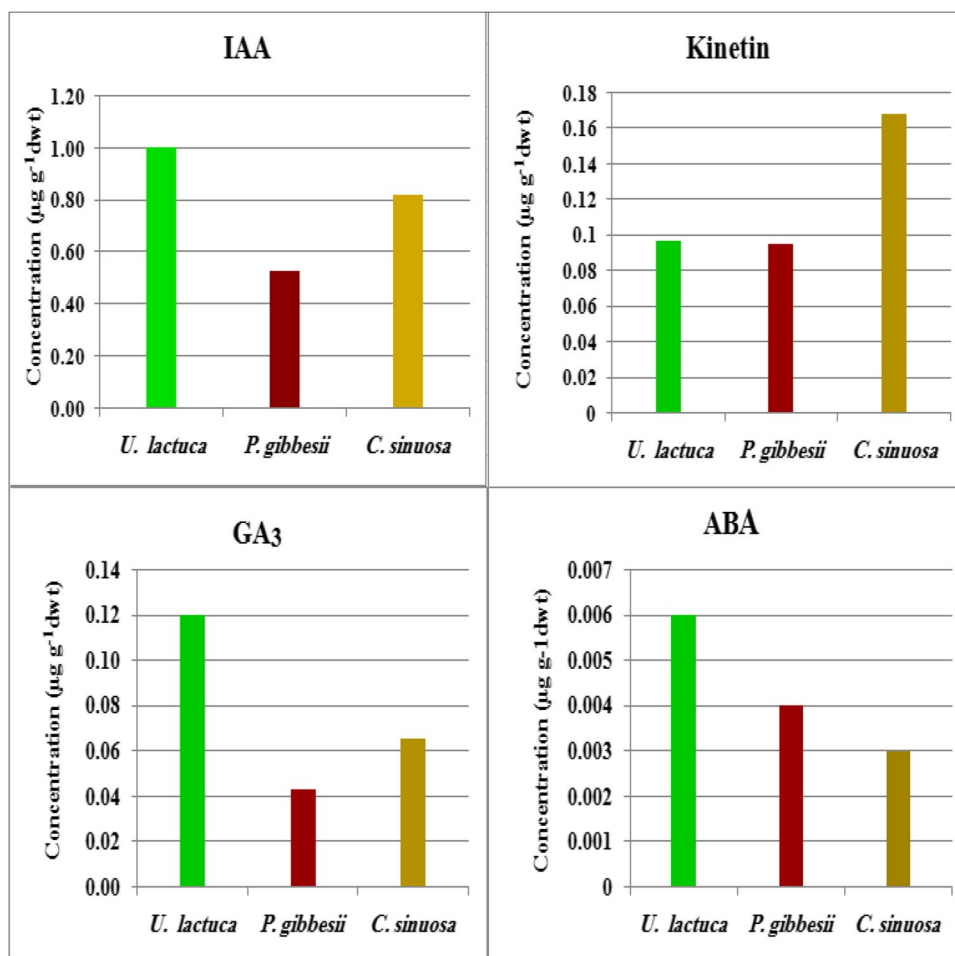
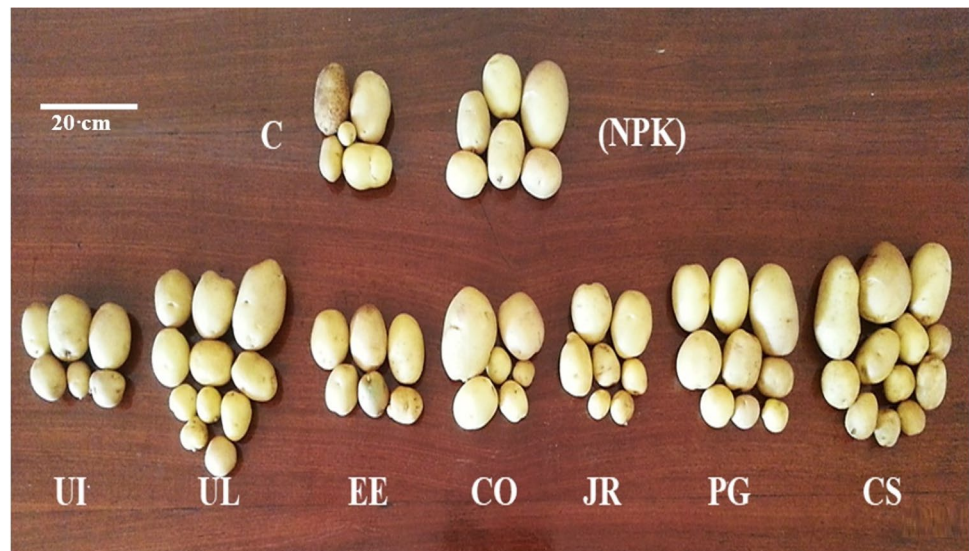


Fig. 3 Effect of seaweed auto-claved extracts as biofertilizers on potato yield cv. Hermes. C: control, UI: *Ulva intestinalis*, UL: *Ulva lactuca*, EE: *Ellisolandia elongata*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, CS: *Colpomenia sinuosa*, and NPK: commercial fertilizer (nitrogen, phosphorus and potassium). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)



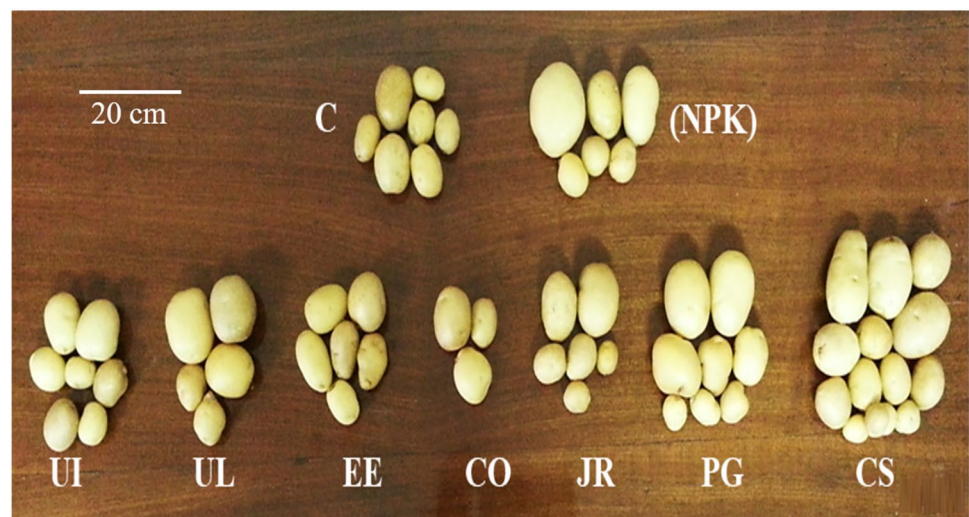
three coralline algae *J. rubens*, *C. officinalis*, and *E. elongata* were less significant than NPK and showed different degrees of significance compared to the control ($p \leq 0.05$) (Table 4C).

Comparing the two potato cultivars, the t-test showed significantly better performance of Hermes cultivar over Bellini, for all growth parameters ($P < 0.05$), except for leaf area, shoot dry weight, tuber No, tuber weight, and carbohydrates% (Table 4A). The application of autoclaved extracts was more effective than non-autoclaved in influencing only the tubers No. and carbohydrates%, while for the other parameters there were no significant differences between the two methods ($p \leq 0.05$) (Table 4B).

Discussion

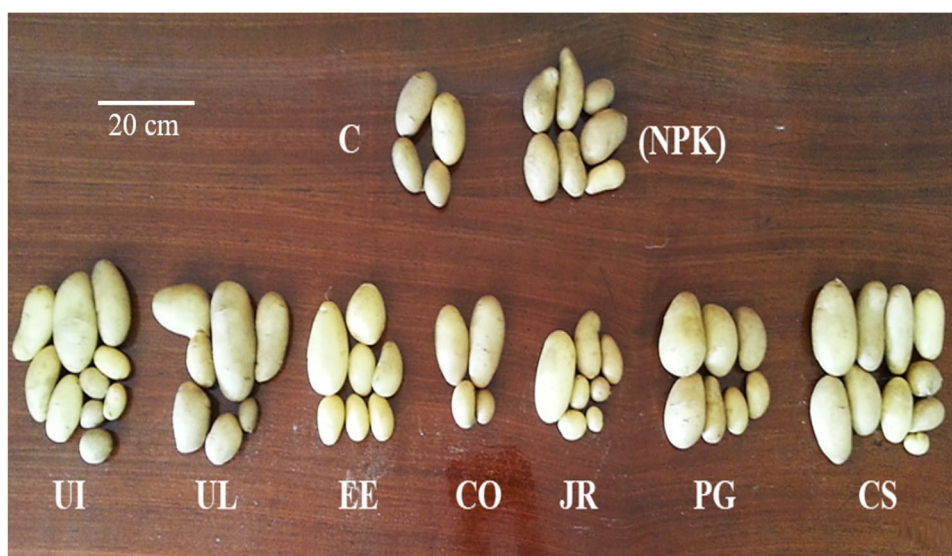
The use of seaweed extracts for potato plants aims to evaluate them as biofertilizers, in comparison with the commercial chemical fertilizer (Zain™ NPK). As shown in this study, the *C. sinuosa*, *U. lactuca*, and *P. gibbesii* extracts, along with NPK (at a concentration of 0.5%) were the most effective on the growth and yield of the two potato cultivars, coming in the first rank, exceeding the values of the control as confirmed by the one-way ANOVA and Tukey test at $p \leq 0.05$. Thus, the three algal extracts can be alternatives to NPK. The effects of the other four algal extracts—*J. rubens*, *E. elongata*, *C. officinalis*, and *U. intestinalis*—were

Fig. 4 Effect of seaweed non-autoclaved extracts as biofertilizers on potato yield cv. Hermes. C: control, UI: *Ulva intestinalis*, UL: *Ulva lactuca*, EE: *Ellisolandia elongata*, CO: *C. officinalis*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, CS: *Colpomenia sinuosa*, and NPK: commercial fertilizer (nitrogen, phosphorus and potassium). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)



less pronounced than those of NPK ($p \leq 0.05$). As a result,

Fig. 5 Effect of seaweed autoclaved extracts as biofertilizers on potato yield cv. Bellini. C: control, UI: *Ulva intestinalis*, UL: *Ulva lactuca*, EE: *Ellisolandia elongata*, CO: *C. officinalis*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, CS: *Colpomenia sinuosa*, and NPK: commercial fertilizer (nitrogen, phosphorus and potassium). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)



these four algal extracts can be supplemented with NPK to improve plant performance as previously recommended by Yusuf et al. (2021) and Ammar et al. (2022). The varied effect of the seaweed extracts may be attributed to the type of algae involved, their different bioactive compounds (viz. biochemicals, polysaccharides), plant growth regulators, mineral content (Vaghela et al. 2023), the various sites where the seaweeds were harvested, the sampling season, and the environmental factors (Hernández-Herrera et al. 2016; Sambhwani et al. 2020).

Seaweed proteins fulfill the requirements of plants for amino acids for growth and development (Ghaderiardakani et al. 2019; Vaghela et al. 2023). The high carbohydrate content of seaweeds has the benefit of increasing their scavenging capacity (Yang et al. 2011), which fortifies their antioxidant and anti-inflammatory properties and promotes plant growth (Garcia-Vaquero et al. 2017). In the present study, the carbohydrates and protein contents were high, particularly in *P. gibbesii* (56.2% & 16.02%), *U. lactuca* (53.6% & 18.01%), and *C. sinuosa* (34.6% & 15.98%), respectively.

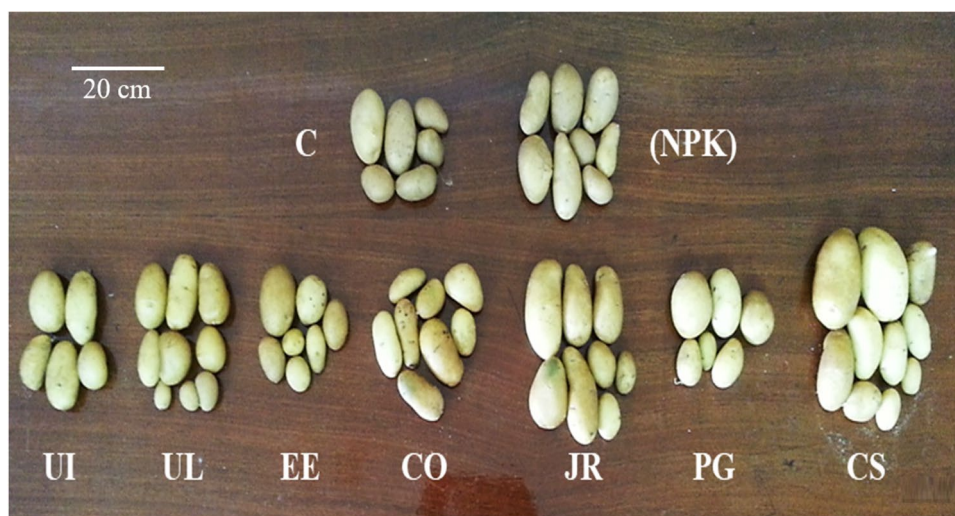


Fig. 6 Effect of seaweed non-autoclaved extracts as biofertilizers on potato yield, Bellini. C: control, UI: *Ulva intestinalis*, UL: *Ulva lactuca*, EE: *Ellisolandia elongata*, CO: *C. officinalis*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, CS: *Colpomenia sinuosa*, and NPK: commercial fertilizer (nitrogen, phosphorus and potassium). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)

Effect of seaweed non-autoclaved extracts as biofertilizers on potato yield, Bellini. C: control, UI: *Ulva intestinalis*, UL: *Ulva lactuca*, EE: *Ellisolandia elongata*, CO: *C. officinalis*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, CS: *Colpomenia sinuosa*, and NPK: commercial fertilizer (nitrogen, phosphorus and potassium). Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015– June 2016)

Table 4 (A, B & C): Statistical analysis for each growth parameter to compare between the two potato cultivars (Hermes & Bellini), the two methods (autoclaved and nonautoclaved) using the t-test ($p \leq$ 0.05), and between the algal species and NPK by using one-way ANOVA, followed by the Tuckey test ($p \leq 0.05$)

Type of statistical test	4A Hermes & Bellini T test	4B Autoclaved & nonautoclaved T test	4C Algae One way ANOVA—Tukey test
1- Shoot Length(cm)	t = 3.89 p = 0.001*	t = 1.48 p = 0.15	F = 1.95 p = 0.09
2- Root Length(cm)	t = 5.53 p = 0.00*	t = 1.30 p = 0.21	F = 0.72 p = 0.67
3- Leaf area (cm ²)	t = -0.87 p = 0.39	t = -0.70 p = 0.49	F = 12.9 p < 0.001* UL ^a > CS ^b > UI ^c > PG ^d > NPK ^e > JR ^{fg} > Con ^g . > EE ^h > CO ⁱ
4-Shoot Fresh weight (g)	t = 4.19 p = 0.00*	t = 0.02 p = 0.99	F = 3.18 p = 0.01* CS ^a > UL ^b > PG ^c > NPK ^d > UI ^e = JR ^e > Con ^f . > CO ^g > EE ^h
5-Root Fresh weight (g)	t = 12.92 p = 0.00*	t = -0.46 p = 0.65	F = 0.27 p = .97
6-Shoot Dry weight (g)	t = -0.14 p = 0.89	t = 0.67 p = 0.51	F = 5.47 p < 0.001* UL ^a > CS ^b > UI ^c > PG ^d > NPK ^e > EE ^f > JR ^g = Con ^g . > CO ^h
7-Root Dry weight (g)	t = 3.19 p = 0.004*	t = 0.06 p = 0.95	F = 2.66 p = 0.027* CS ^a > PG ^b > NPK ^c > JR ^d > UL ^e > CO ^f > UI ^g > EE ^h = Con ^h
8-Tubers No	t = 1.70 p = 0.11	t = 2.14 p = 0.04*	F = 2.29 p = 0.05* CS ^a > JR ^b > UL ^c > NPK ^d > PG ^e > U ^f = CO ^f > EE ^g > Con ^h
9-Tubers weight/plant	t = 0.18 p = 0.86	t = -1.11 p = 0.28	F = 11.92 p < 0.001* CS ^a > NPK ^b > UL ^c > PG ^d > UI ^e > JR ^f > Con ^g . > CO ^h > EE ⁱ
10-Chlorophyll mg g ⁻¹	t = 2.38 p = 0.025*	t = 1.27 p = 0.21	F = 6.27 p < 0.001* NPK ^a > CS ^b > UL ^c > JR ^d > UI ^e > PG ^f > CO ^g > Con ^h . > EE ⁱ
11-Carbohydrates (%)	t = 1.85 p = 0.08	t = 2.33 p = 0.03*	F = 2.56 p = 0.03* NPK ^a > UL ^{ab} > CS ^c > PG ^d > Con ^e . > UI ^f > CO ^g = EE ^g > JR ^h

UI: *Ulva intestinalis*, UL: *Ulva lactuca*, CO: *Corallina officinalis*, EE: *Ellisolandia elongata*, JR: *Jania rubens*, PG: *Phyllymenia gibbesii*, and CS: *Colpomenia sinuosa*. Seaweeds were collected from the eastern coast of Alexandria, Egypt (Mediterranean Sea) (April 2015–June 2016). The asterisk means that there is a significant difference. Small letters show statistical differences between algal species for each growth parameter, while the same letter (s) are statistically equal according to different means separated at $p \leq 0.05$ levels by the Tukey test

Using seaweeds as biostimulants, lower carbohydrates and protein contents were reported in *Kappaphycus alvarezii* and *Sargassum wightii* than those of the present study (Elangoan and Anantharaman 2019; Vaghela et al. 2022, 2023).

Amino acids play a key role in regulating cell differentiation and development, as well as growth (Basu et al. 1989). They are used in the synthesis of a wide range of chemicals, such as proteins, amines, and plant hormones that control many plant functions (Ibrahim et al. 2010). The amino acids profile was investigated in *P. gibbesii* samples that were taken simultaneously with the current study (spring, 2015, and summer, 2016) (Shams El-Din et al. 2022). The results revealed that arginine, lysine, leucine, valine, threonine, and phenylalanine were the dominant essential amino acids (EAA), while alanine, glycine, aspartic acid, and glutamate were the dominant non-essential amino acids (NAA), with seasonal variations (spring 2015 > summer 2016). A previous study of the amino acid composition in *U. lactuca* (collected in 2017) (Egyptian Mediterranean Sea) revealed similar dominant constituents of EAA and NAA (Elmosallamy et al. 2021). Similar findings demonstrated the significance of these EAA and NAA detected in both *K. alvarezii* and *S. wightii* for increasing plant immunity, strengthening

pathogen defense mechanisms, and improving plant growth and yield (Vaghela et al. 2023).

One important class of bioactive substances is polysaccharides, among which are ulvan, carrageenan, and alginates (Mettwally et al. 2022; Moawad et al. 2022). A parallel study characterized the sulfated polysaccharide in *P. gibbesii* (spring, 2016). It was composed of galactose (predominant sugar) and traces of xylose and glucuronic acid (Mettwally et al. 2022). The characterization of ulvan isolated from *U. fasciata* (currently known as *U. lactuca*) (collected in 2018) (Egyptian Mediterranean Sea) revealed the presence of monosaccharides: galactose, rhamnose, and uronic acids (Moawad et al. 2022). These monomeric or oligomeric forms can protect against stress by serving as carbon skeleton donors, osmoprotectants, or signaling molecules for several defensive mechanisms (Lukaszuk et al. 2017). The interaction of these sugars with microbiomes may produce secondary advantageous compounds for both the plants and the rhizosphere's microorganisms (Mishra et al. 2009). There was neither available literature on the amino acids profile nor polysaccharides characterization of *C. sinuosa* (Egyptian Mediterranean Sea).

Seaweed extracts have high levels of natural plant growth regulators, especially auxins, cytokinins, and gibberellins (Ali et al. 2019). Auxins are best known for their positive effect on rooting. Cytokinins stimulate rapid cell division and the production of new cell walls. Gibberellins have a positive influence on stem elongation and flower development and increase chlorophyll content and leaf numbers (Ali et al. 2019). In the present study the PGRs (IAA, GA₃, abscisic acid, and kinetin) were assessed in the three most effective seaweeds, and the results revealed that the green alga *U. lactuca* had the highest content of the first three PGRs (1 µg IAA g⁻¹ FW, 0.12 µg GA₃ g⁻¹ FW, and 0.006 µg ABA g⁻¹ FW), respectively, while *C. sinuosa* had the highest concentration of the last (0.168 µg kinetin g⁻¹ FW). Application of GA₃-free *Kappaphycus* at two concentrations (5% and 10%) resulted in lower potato growth and yield attributes than *Kappaphycus* at the same concentrations (Garai et al. 2021), confirming the important role of GA₃ in the photosynthesis process, biomass accumulation, and other physiological activities (Miceli et al. 2019). The current study did not, however, examine the hormone content in potato plants using seaweed extracts.

In general, the Hermes cultivar showed significantly better performance over Bellini for all the growth parameters ($p \leq 0.05$), except for leaf area, shoot dry weight, tuber yield, and carbohydrates%. This may be attributed to the differences in genetic characteristics of the two types, giving more affectedness of the Hermes cultivar for seaweed fertilizers. The type of cultivar is a significant factor in potato production (Koch et al. 2020).

Autoclaving is effective in removing the undesired microbes that may interfere negatively with plant growth (Koch et al. 2020). In the present study, the effect of both forms, the autoclaved and the non-autoclaved extracts, on the growth parameters was not significantly different regardless of the type of potato cultivar. These results were compatible with those of Mondal et al. (2015) who applied treatments of *K. alvarezii* extract for enhancement of *Zea mays* crop yield. The results revealed similar effect of both autoclaved and non-autoclaved treatment on growth attributes and grain yield and quality, assuming that the active compounds present in the autoclaved extract must be thermally stable, such as quaternary ammonium, glycine betaine, and choline.

Recently, rules limiting the quantities of trace elements have been set in seaweed-based biostimulants (Vaghela et al. 2023). In the present study, the contents of Zn (9.7–31.5 ppm), Cu (1.3–17.2 ppm), Cd (1.6–4.8 ppm), and Pb (13.9–30.8 ppm) in the dry seaweeds powder were below the permissible limits set by Fertilizer Control Order (FCO 2021) for biostimulants (1000, 300, 5, and 100 ppm, respectively). The cobalt content (264–408.9 ppm) was higher than the permissible limit (50 ppm) set by FAO/WHO (2001). Trace elements in dry seaweed powder are at their maximum

concentrations, which, when they are applied to plants in liquid diluted forms, their concentrations would be three orders of lower magnitude, such as in the present study, and hence negligible (Vaghela et al. 2023).

The observed benefits of added liquefied seaweed extracts to the growth, health, and yields of plant crops may be attributed to the supply of essential nutrients such as K, P, Ca, and Mg (Basmal 2009). These nutrients, particularly potassium, were highlighted as being crucial for boosting photosynthesis and improving growth and yield parameters (Sahai 2008; Layek et al. 2015).

In the current study, applying seaweed extracts increased the yield of potato tuber roots, had a positive impact on nutrient availability, enhanced the efficiency of macroelements, and fulfilled potato crop microelements (Sarhan 2011; Haider et al. 2012; Garai et al. 2021). Using the commercial seaweed extract (SPRINT) for enhancing potato crops gave good results in terms of growth attributes such as leaf number, leaf area, shoot and root dry weight and fresh weight, and plant yield (Issa et al. 2019), which were compatible with those of the current study. In another study, combining an algae-based commercial biofertilizer TAM® with the traditional NPK fertilization practices in different concentrations significantly improved potato yield in field conditions and strengthened the composition of its essential constituents (Ammar et al. 2022), which were similar to the findings of the current study. The effects of different algal treatments, namely foliar application of (*Spirulina platensis*, *Acanthophora spicifera* extracts), dried biomasses of *Turbinaria ornata*, and a mixture of *Caulerpa racemosa* and *Cystoseira myrica* (1:1), were evaluated on potato yield (Hamed et al. 2023). The algal extracts significantly increased potato yield at all locations and phosphorus uptake (Hamed et al. 2023). Liquid fertilizers derived from seaweeds are superior to chemical fertilizers because of their high levels of organic matter, micro- and macroelements, vitamins, fatty acids, and growth regulators (Sathya et al. 2010).

In terms of heavy metal accumulation, adding seaweeds to soils is safer than sewage sludge application to agricultural soils (Eid et al. 2018, 2019). For the present study, the seven tested seaweeds had a suitable concentration of macroelements: nitrogen, phosphorus, potassium, and calcium, in addition to the microelements. Furthermore, the presence of a high content of carbohydrates in the tested seaweeds, especially *P. gibbesii*, followed by *U. lactuca* and *C. sinuosa*, may be useful for increasing the water-holding capacity and improving the soil texture, especially in light sandy soils (Ghazal et al. 2013).

In the present study, the concentrations of microelements in the seaweed extracts (SWEs) were compared to the permissible limits of microelements in irrigation water set by Rowe and Abdel-Magid (1995) and FAO/WHO (2003). According to the short-term use of SWEs as biofertilizers, all SWEs had

permissible concentrations of microelements. For long-term use of SWEs, Co concentration exceeded the permissible levels (0.05 mg L^{-1}) in all SWEs, as well as Cu and Ni exceeded the permissible levels in *U. intestinalis* and *C. officinalis* (0.28 and 0.36 mg L^{-1}) for Cu, respectively, and (0.41 and 0.31 mg L^{-1}) for Ni, respectively. However, Fe exceeded the permissible limits for the long-term use of all SWEs, but it did not contribute to any toxicity effects on plants. For that, it is recommended to utilize the SWEs only for the short term. Further studies should be carried out on the uptake of these trace elements by the potato plant and assess their concentrations in the soil to determine their safety for long-term usage.

Conclusion

Seaweed aqueous extracts are promising for using them as biofertilizers to enhance the growth and yield of the two potato cultivars (Hermes and Bellini cv.). The two native species, *U. lactuca* and *C. sinuosa*, and the newly recorded alga *P. gibbesii* were the most effective seaweeds for the two potato cultivars, as their extracts contain considerable concentrations of growth regulators: IAA (0.53 – $1.00 \mu\text{g g}^{-1}$ FW), kinetin (0.095 – $0.168 \mu\text{g g}^{-1}$ FW), gibberellic acid (0.043 – $0.12 \mu\text{g g}^{-1}$ FW), and Absciscic acid (0.003 – $0.006 \mu\text{g g}^{-1}$ FW), minerals, as well as their effects exceeded the control and were at the first rank along with the chemical fertilizer (NPK) according to ANOVA-one way, followed by the Tukey test ($p \leq 0.05$). Due to the high concentrations of some microelements in SWEs, which exceeded the permissible levels for Ni (0.2 mg L^{-1}), Cu (0.2 mg L^{-1}), Co (0.05 mg L^{-1}), and Cd (0.01 mg L^{-1}) for long-term use according to FAO/WHO, SWEs are recommended to be used only for short-term irrigation. It is of vital importance to apply new technologies for seaweed cultivation in Egypt to guarantee their safe usage as biofertilizers, which are more useful than synthetic agricultural fertilizers. This alternative would be essential to maintain the seaweed resources and prevent overexploitation, and extinction in their natural ecosystems.

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Data Availability Data are available from the corresponding author upon reasonable request.

Declarations

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Competing interests The authors declare no competing interests.

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