Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 1863 – 1878 (2024)
www.ejabf.journals.ekb.eg

Exploring New Ways for Cereal Planting: Implications of Different Algal Extracts and Hydrogel on Germination and Biochemical Content of Wheat Plants

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ARTICLE INFO

Article History:
Received: Aug. 20, 2024
Accepted: Oct. 13, 2024
Online: Oct. 19, 2024

Keywords:
Algae,
Biostimulants,
Agricultural management,
Hydrogel,
Wheat plants

ABSTRACT

Prompt seed germination and emergence, along with excellent seedling vigor, are highly desired traits for ensuring optimal crop establishment and subsequent vegetative growth. This study aimed to evaluate the effects of different algal biostimulants derived from the freshwater macroalgae *Compsopogon caeruleus* (Rhodophyta) and *Pithophora roettleri* (Chlorophyta) on the germination and seedling vigor of wheat plants (cultivar Mawaddah). Both algal species were identified using a polyphasic approach that combined morphotaxonomy and the nuclear-encoded SSU rRNA gene. The effects of each algal extract, as well as the combined impact of both extracts, were tested at three different concentrations (0.5, 1, and 2%) in conjunction with 2 and 4% hydrogel. Major nutrients, total carbohydrates, and total proteins were estimated. The experiment was conducted under controlled environmental conditions. Our findings revealed that the 2% aqueous extract of *C. caeruleus* generally improved nutrient levels and biochemical content in wheat plants compared to other treatments. Additionally, applying 2% hydrogel (2g/ kg of soil) showed the most significant positive effects on germination traits and soil properties. The bioactive constituents in both algal biostimulants were characterized using gas chromatography-mass spectrometry (GC-MS), revealing 20 different bioactive compounds in *C. caeruleus* compared to 13 compounds in *P. roettleri*. We believe that algal biostimulants, particularly the 2% aqueous extract of *C. caeruleus*, enhance germination and biochemical content in wheat plants due to their richness in bioactive compounds, which make nutrients more available to the seeds. Furthermore, the 2% hydrogel retained soil moisture and nutrient availability for a longer period, subsequently reducing water drainage—a critical factor under conditions of water scarcity. In conclusion, we recommend the application of algal biostimulants in conjunction with hydrogel as an eco-friendly technique for sustainable agricultural management.

INTRODUCTION

Algae are a sustainable global resource, with annual production exceeding 35.1 million tons **(FAO, 2022)**. It could be used as human food and animal feed since they are rich in dietary fibers, omega-3 fatty acids, essential nutrients, vitamins, essential amino acids, and polysaccharides **(Rashad & El-Chaghaby, 2020)**. Algae are a rich source of different bioactive compounds, including plant hormones, antioxidants, antimicrobials, osmoprotectants, essential minerals, and many other organic matters, which make them a good candidate in biofertilizers' industry **(Nabti *et al.*, 2016; Galal *et al.*, 2024)**. Previous studies assessed the feasibility of algae-based extracts as biofertilizers and biostimulants. The application of algal extracts offers several significant benefits for enhancing agriculture since they can improve plant growth and productivity through various mechanisms, including 1) algae containing several bioactive constituents that protect crops from fungal and microbial diseases **(Elastal *et al.*, 2005; El-Sheekh *et al.*, 2021)**; 2) providing essential macro- and micro-elements for growth and physiological functions **(Sirbu *et al.*, 2020; Yusuf *et al.*, 2021)**; 3) enhancing soil structure and retaining water capacity when added to the soil **(Errati *et al.*, 2022)**. This property improves crops' ability to withstand drought and salinity **(Hashem *et al.*, 2019; Hamed *et al.*, 2024)**. In this context, mechanisms through which algae enhance plant growth include 4) the presence of various phytohormones, viz. auxins, gibberellins, and cytokinins **(El Shoubaky & Salem, 2016; Al-Katib *et al.*, 2017)**.

Among the protocols used in algal-based biofertilization techniques, seeds can be soaked in the algal extracts to fasten the germination rate, or soaking the bases of cuttings before planting in nurseries to improve the rooting rate, and then added to the soils **(Almaleky *et al.*, 2013)**. It has been emphasized that the use of algal biostimulants in general promotes the plant growth, chlorophylls and carbohydrate contents, leaf area, and absorption of major and minor nutritional ions **(Mahmoud *et al.*, 2019)**. Algae can be used as natural biostimulants, instead of traditional synthetic fertilizers, in the form of liquid extracts. A wide range of substances that promote plant growth, such as auxins and cytokines, are found in these algal biostimulants. The algal biostimulants have been established to improve the seed germination **(Layth *et al.*, 2015)**.

Iraq is now considered a declining country for growing the first wheat crop in the world in terms of the vitality of the cultivated area, production as well as consumption. Therefore, it has become one of the most important and a widespread crop, as it is grown and harvested everywhere on the surface of the Earth **(Awwad, 2009)**. Wheat is considered one of the most important cereal crops in the world because it contains a high percentage of proteins (~20%) and calories at (21%). It is considered a food source for the majority of human population in many parts of the world **(Brennan & Acosta-Martinez, 2017)**. Hydrogel is a group of thick, water-loving colloidal substances produced by all plants and microorganisms. Aloe vera, succulents, and flax seeds are considered rich sources of hydrogel **(Villellas *et al.*, 2013)**. Hydrogel has been used in several medical and health applications due to its unique characterization and ability to retain water **(Rani *et al.*, 2021)**. Recently, few studies investigated the potential of hydrogel on agricultural management **(Al-Abbar *et al.*, 2021)**.

This study aimed to ascertain the impact of applying liquid extracts derived from the freshwater macroalgae *Compsopogon caeruleus* (Rhodophyta) and *Pithophora roettleri* (Chlorophyta), as natural biofertilizers, on germination and seedling vigor of wheat plants (cultivar Mawaddah) at various concentrations (0.5, 1, and 2%) and in the presence of 2 and 4% hydrogel.

MATERIALS AND METHODS

Algal materials

The specimens of *Compsopogon caeruleus* and *Pithophora roettleri* investigated in the present study were collected on September the 15th 2023 from the Tigris River in the Qayyarah area, Mosul District, Nineveh Governorate. The specimens were collected manually and with forceps in 100ml sterile, clean polyethylene terephthalate (PET) bottles and were transported chilled in an ice-box to the laboratory for further studies. In the field, the specimens were rinsed with the river water to remove any contaminants and epiphytes. In the lab, the specimens were washed again with distilled water to be completely free of epiphytes and debris. This step was verified by microscopic examination. The specimens were shade-air dried at room temperature. Using an electric blender, the dried algal specimens were homogenized into a fine powder and were then stored in sterile clean plastic bags.

Morphological characterization

The specimens were morphologically identified based on the taxonomic classification systems adopted by **Sheath and Sherwood (2011)** for *Compsopogon caeruleus* and by **Škaloud *et al.* (2018)** for *Pithophora roettleri*.

DNA sequencing and phylogenetic analysis

Specimens for DNA analysis were cleaned of visible epiphytes and were then preserved in silica desiccant. Specimens were extracted for DNA by grinding a mortar and pestle with liquid nitrogen and using the NucleoSpin® extraction kit (Clontech, Mountainview, CA, USA) according to the manufacturer's protocols. SSU rDNA was amplified using primers and cycles previously described **(Milstein & Oliveira, 2005; Conklin *et al.*, 2009)**. The resulting PCR products were purified using the UltraClean™ PCR Clean-up DNA purification kit (MoBio, Carlsbad, CA, USA), Wizard SV Clean-up System (Promega, Madison, USA), or GFX® PCR (GE HealthCare Life Sciences, Buckinghamshire, UK), all according to manufacturer's protocols. Phylogenetic analyses of the 18S rRNA gene data were conducted using Geneious **(Drummond *et al.*, 2012)**. The best-fit model of sequence evolution was determined using the Akaike Information Criterion (AIC) implemented in ModelTest 3.7 **(Posada & Crandall, 1998)**, which is part of the PAUP*4.0 plugin for Geneious. Maximum likelihood (ML) topologie from 1000 replicates were inferred using PhyML **(Guindon & Gascuel, 2003)**. The new 18S rDNA sequences of *Compsopogon caeruleus* and *Pithophora roettleri* were submitted to the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers PP955312 and PP955313, respectively.

Preparation of the algal extracts

The dried algal samples were mixed at 3000-5000rpm for 15min with sterilized distilled water (1:10 v/v) for one hour until a smooth mixture was obtained to prepare the aqueous extracts. The mixture was then squeezed and filtered through cheesecloth to further filter the supernatant, removing any remaining solid matters. The collected extracts were stored as stock solutions and used to make further concentrations (0.5, 1, and 2%) by mixing them with sterilized distilled water. Hydrogel was purchased from local markets, and the different concentrations (2 and 4%) were prepared by dilution.

Origin of the wheat plants grains and hydrogel

Grains of the wheat plants (cultivar Mawaddah) were obtained from the Seed Certification Center in Nineveh (Iraq), and tested for their viability at a temperature of 20 ± 2°C.

Experimental design

Aqueous extracts the of two macroalgae *Compsopogon caeruleus* and *Pithophora roettleri* were prepared and applied at concentrations of 0.5, 1 and 2%, as described in the study of **Mal Allah and Al-Katib (2024)**. The soil was obtained from the village of Al-Kasr, southeast of Mosul, Al-Hamdaniya district, and was sieved to get rid of the large stones hindering the growth of seeds.

Estimation of plant elements

Dried plant samples of 90-day-old growing wheat plants were taken and crushed using an electric grinder. 0.5gm of each plant was weighed and digested using the wet digestion method. 10ml of conc. sulfuric acid was added for 24h, after which 3ml of conc. perchloric acid was added and filtered after digestion, and then the macro- (N, P and K) and micronutrients (Mg) were spectrophotometrically measured **(Abbas *et al.*, 2022)**.

Estimation of total proteins and total carbohydrates

The total protein content was quantified using the Folin-Ciocalteu reagent, following the protocol proposed by **Daughaday *et al.* (1952)**. The absorbance was measured at 700nm with Unico S-1201 spectrophotometer (TEquipment Comp., NJ, USA). Total carbohydrates were estimated according to the method described by **Blakeney and Mutton (1980)**.

Identification of the bioactive compounds in the algal extracts

The bioactive constituents in the *C. caeruleus* and *P. roettleri* extracts were analyzed using gas chromatography–mass spectrometry (GC–MS) using a Perkin Elmer Elite-5MS capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness) in a 7890B gas chromatograph system (Agilent Technologies Co., Santa Clara, CA, USA), coupled with a 5977A mass selective detector. Helium was the carrier gas, with a 1.8mL min^{-1} flow rate. The algal extract injected was 1.0µl. The fractioned bioactive constituents were unraveled by comparing their mass spectra with those in the standard databases.

RESULTS AND DISCUSSION

Morphological characterization

To aid morphology-based identification process (Fig. 1), the 18S rRNA phylogenetic assessment placed our *Compsopogon caeruleus* strain in a good supported subclade with phylogenetic proximity of other available *C. caeruleus* sequences (Fig. 2). Our *Pithophora roettleri* isolate also formed a strongly supported lineage with all sequences of *P. roettleri* (Fig. 3).

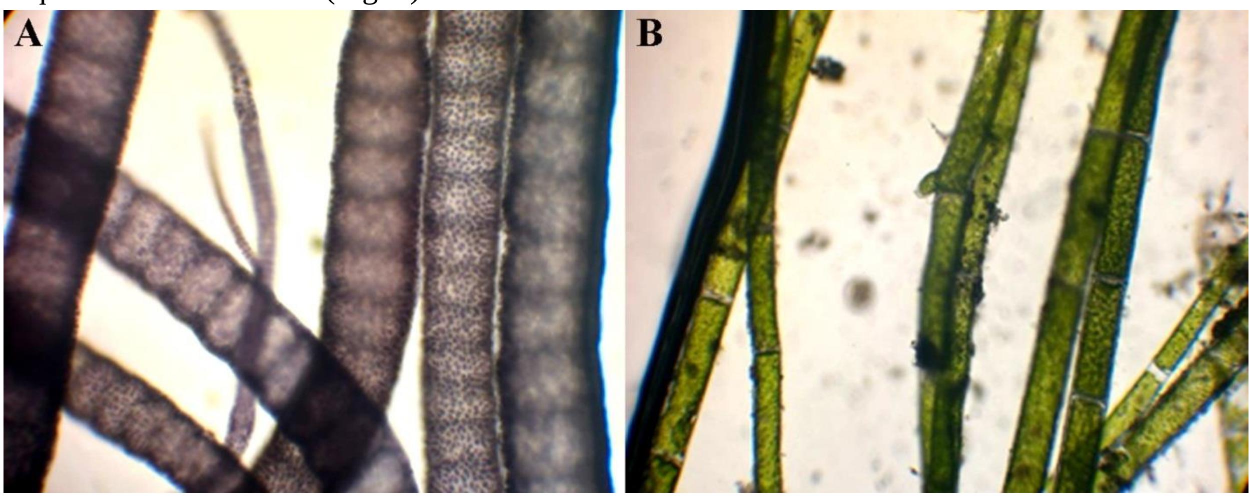

Fig. 1. Macroscopic images of *Compsopogon caeruleus* and *Pithophora roettleri*

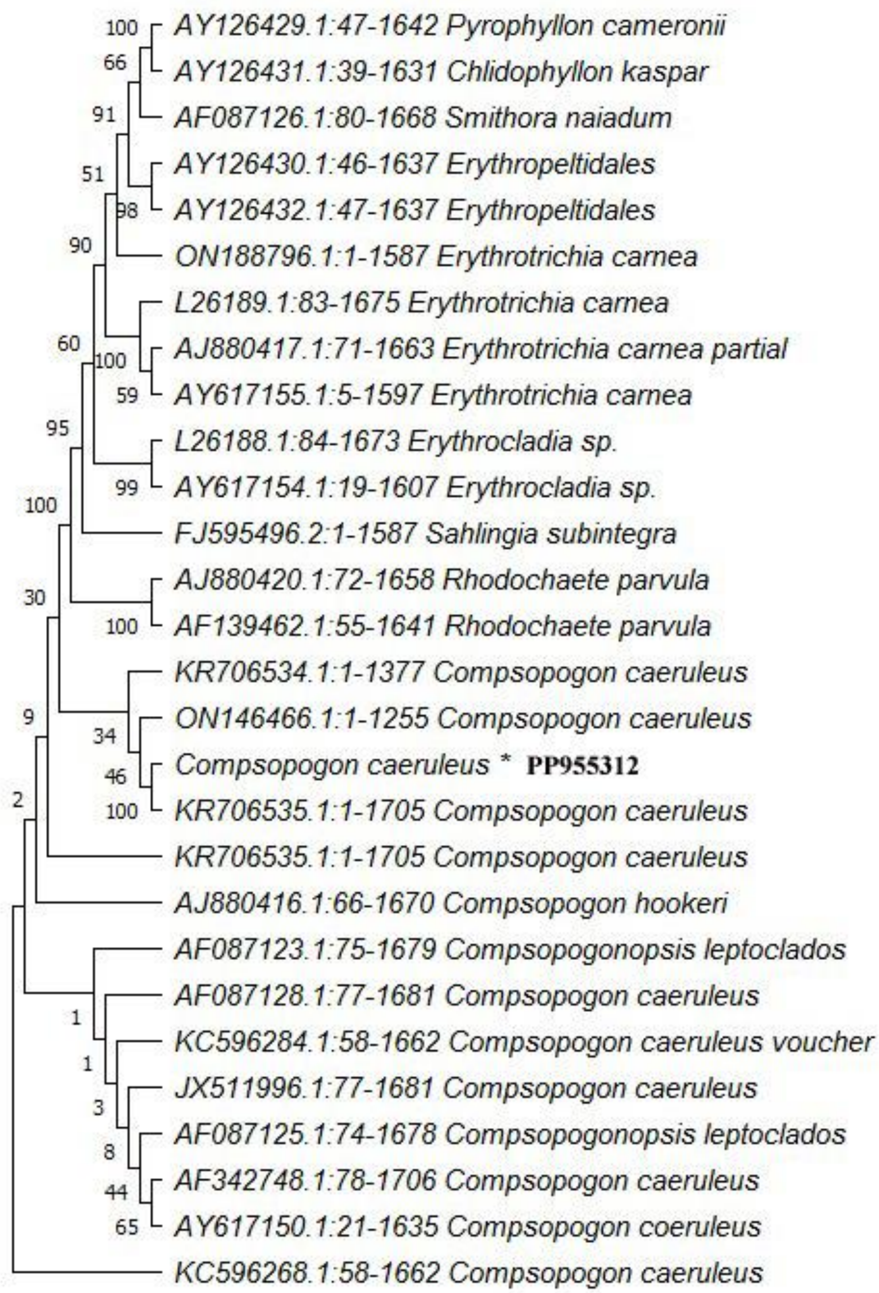

Fig. 2. ML phylogenetic tree inferred from 18S rRNA gene for *Compsopogon* taxa showing the phylogenetic position of our *C. caeruleus* isolate. ML bootstrap values (1000 replicates) are indicated above the branches

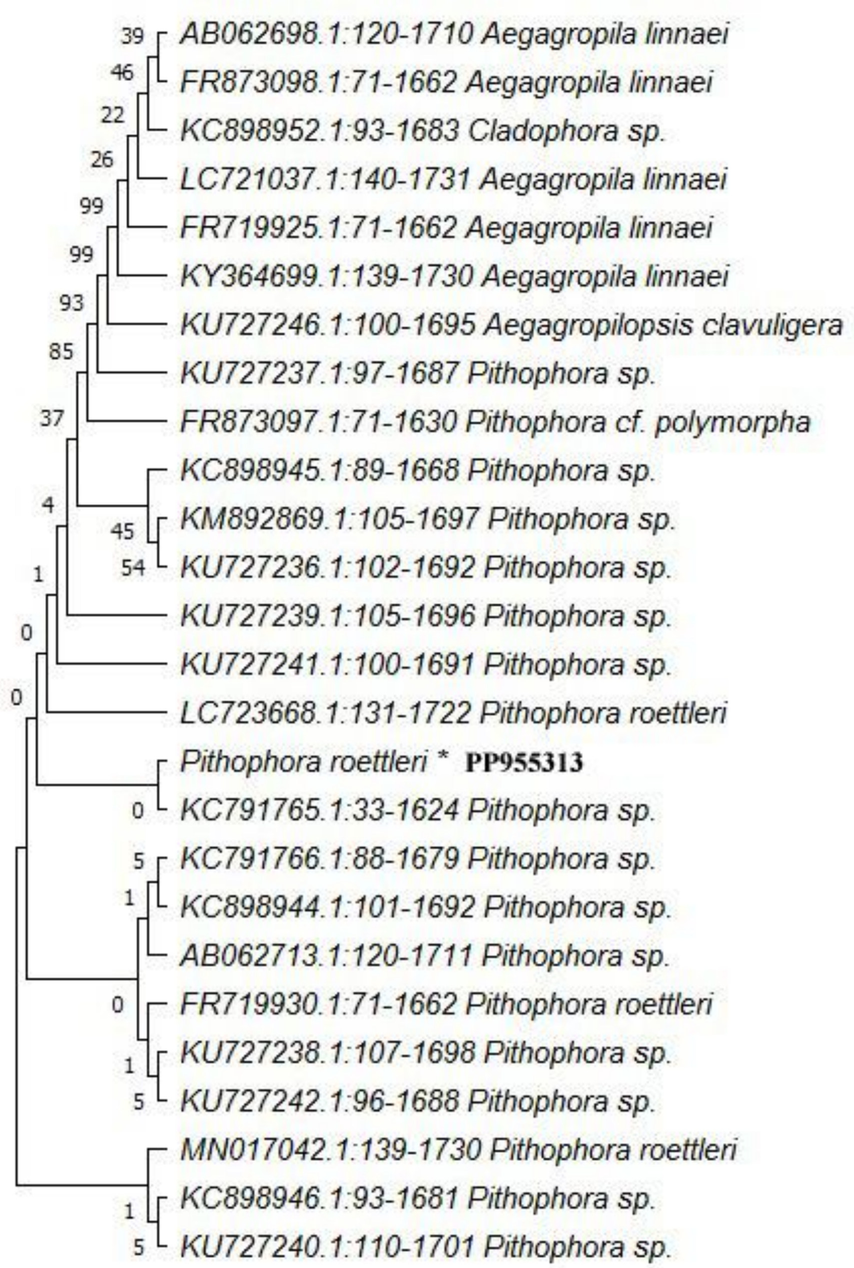

Fig. 3. ML phylogenetic tree inferred from 18S rRNA gene for *Pithophora* taxa showing the phylogenetic position of our *P. roettleri* isolate. ML bootstrap values (1000 replicates) are indicated above the branches

Characterization of the bioactive compounds using GC-MS

The GC-MS analysis revealed the presence of 20 different bioactive compounds in the *C. caeruleus* extract, varying in structure and molecular weight. The major compounds identified were: 1,4-benzenedicarboxylic acid bis(2-ethylhexyl) ester, eucalyptol, n-hexadecanoic acid, phytol, cis-vaccenic acid, 9-octadecenamide (Z), hexadecanoic ethyl ester, and α-pinene. Among these, 1,4-benzenedicarboxylic acid bis(2-ethylhexyl) ester had the largest surface area (49.31%), followed by eucalyptol (15.26%). Other components included n-hexadecanoic acid (9.47%), cis-vaccenic acid (5.31%), and phytol (5.33%) (Table 1 & Fig. 4).

In the *P. roettleri* extract, 13 different bioactive compounds were identified (Table 2 & Fig. 5). Among these, seven constituents showed clear surface areas, particularly eucalyptol (28.22%) and desmosterol (31.13%), followed by oleic acid (14.82%) and n-hexadecanoic acid (4.26%).

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Table 1. The bioactive compounds identified in the *C. caeruleus* extract

Peak#	Retention time	Area	Area (%)	Compounds
1	6.401	1336938	2.10	alpha-Pinene
2	7.708	9727222	15.26	Eucalyptol
3	14.964	471040	0.74	Decane. 1.1-diethoxy
4	17.968	510067	0.80	Heptadecane
5	19.129	312947	0.49	Hexadecanal
6	19.583	516681	0.81	Neophytadiene
7	19.892	464661	0.73	Cycloteradecane
8	20.788	6036625	9.47	n-hexadecanoic acid
9	20.883	703443	1.10	Ethyl 9-hexadeeenoate
10	21.141	1766683	2.77	Hexadecanoic acid.ethyl ester
11	22.407	3397781	5.33	Phytol
12	22.506	763394	1.20	9-12-Octadecadienoic acid(Z,Z)-
13	22.586	3388164	5.31	Cis-Vaccenic acid
14	22.808	1080548	1.69	1,5-Cyclododecadiene,(Z,Z)
15	22.896	461351	0.72	E-11- Hexadecenoic acid,ethyl ester
16	22.959	660253	1.04	E-11-Hexadecenoic acid .ethyl ester
17	24.556	1453760	2.28	9-Octadecenamide.(Z) -
18	24.696	396311	0.62	Cis-7-cis-11-Hexadecadien-1-yl acetate
19	25.002	784278	1.23	Hexanedioic acid,bic(2-ethylhexyl)ester
20	27.962	29527776	46.31	1,4-Benzendicarboxylic acid.bis(2-ethylhexyl) ester
		63759923	100.00	

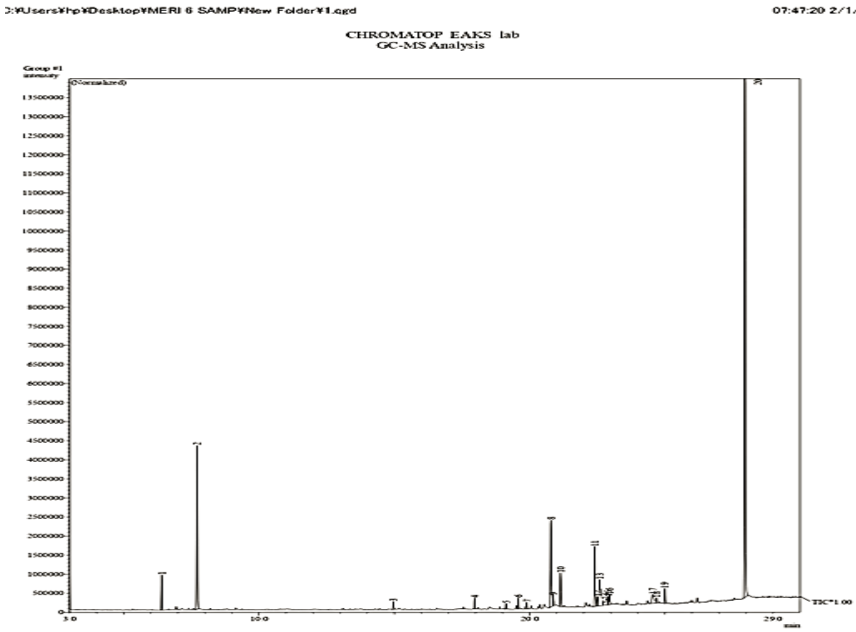

Fig.4. GC–MS chromatograph of the bioactive constituents of the *C. caeruleus* extract

Table 2. The bioactive compounds identified in the *P. roettleri* extract

Peak#	Retention time	Area	Area (%)	Compounds
1	7.701	5700870	28.22	Eucalyptol
2	10.909	201221	1.00	4-(2-Methoxypropan -2-yl)1-methylcyclobox 1-ene
3	15.658	70227	0.35	Arachidonoyl amide N,N-bis(tert-butyldimethysiyl)
4	20.396	152293	0.75	1-Hexadecanaminium N,N,N trimethyl-octadecanoat
5	20.772	1870257	9.26	n-Hexadecanoic acid
6	22.511	319112	1.58	Methyl 10.11-octadecadienoate
7	22.585	2993946	14.82	Oleic Acid
8	22.859	727413	3.60	Octadecanoic acid
9	22.967	47439	0.23	3-Buten-1-ol,3-methylene
10	24.658	6325831	31.13	Desmosterol
11	25.793	878913	4.35	Cholestane-3,5-diol,5-acetate,(3beta.,5.alpha).
12	25.917	155401	0.77	1,1,1,5,7,7,7.Heptamethyl-3,3bis(trimethylsiloxy)tetrasiloxane
13	26.310	758679	3.76	Erucic acid
		20201602	100.00	

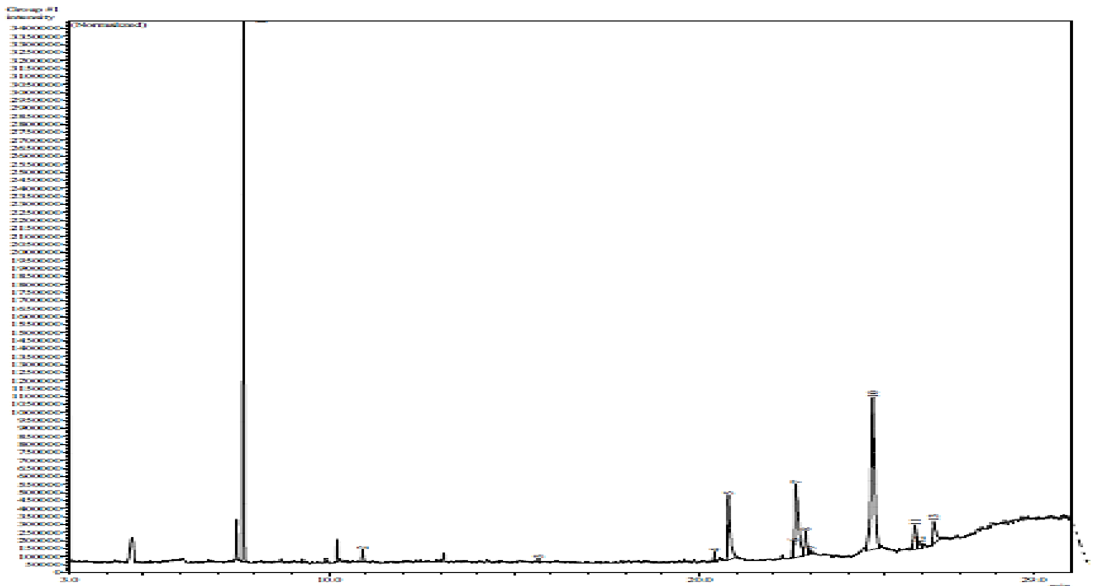

Fig. 5. GC–MS chromatograph of the bioactive constituents in the *P. roettleri* extract

Estimation of chemical elements in shoots of wheat plants

Nitrogen concentration (%)

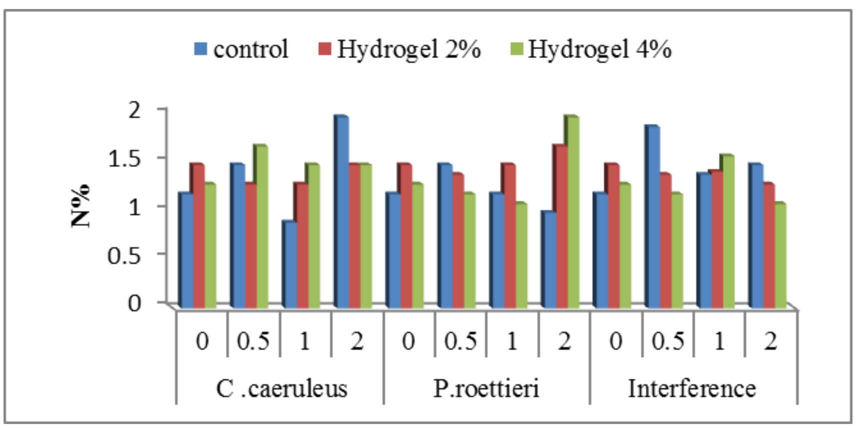

Fig. 6. Effect of the algal treatments with hydrogel on nitrogen concentrations in Mawaddah wheat variety

Phosphorus concentration (%)

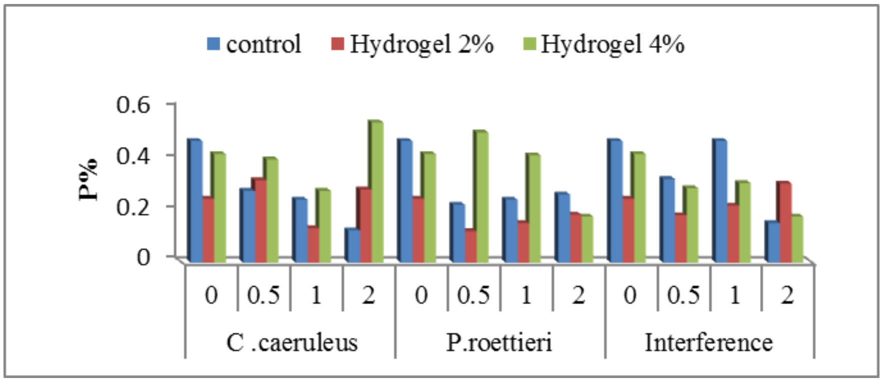

Fig. 7. Effect of the algal treatments with hydrogel on P concentrations in Mawaddah wheat variety

Potassium concentration (%)

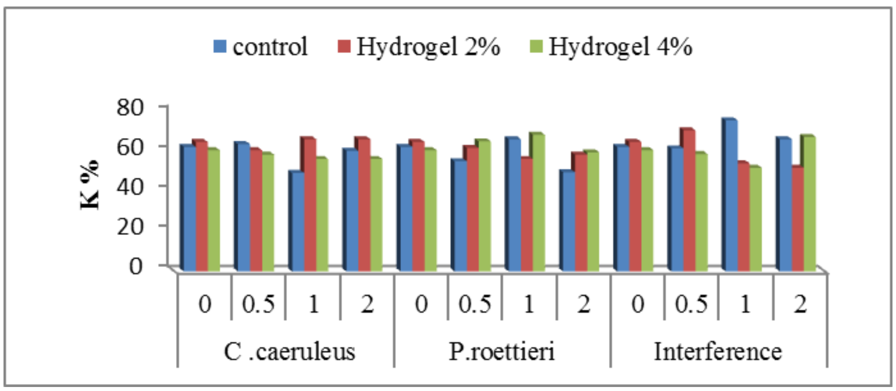

Fig. 8. Effect of the algal treatments with hydrogel on potassium concentrations in Mawaddah wheat variety

Magnesium concentration (%)

As demonstrated in Fig. (9), the algal treatments with hydrogel showed a significant effect on the magnesium level in the wheat plants. The highest Mg concentration (1.11%) was recorded with 1% combined algal treatment and in the presence of 2% hydrogel. Algae extracts contain growth hormones that stimulated the roots to absorb nutrients in the soil and increased their efficiency and concentration including magnesium **(Al-Taee *et al.*, 2017)**.

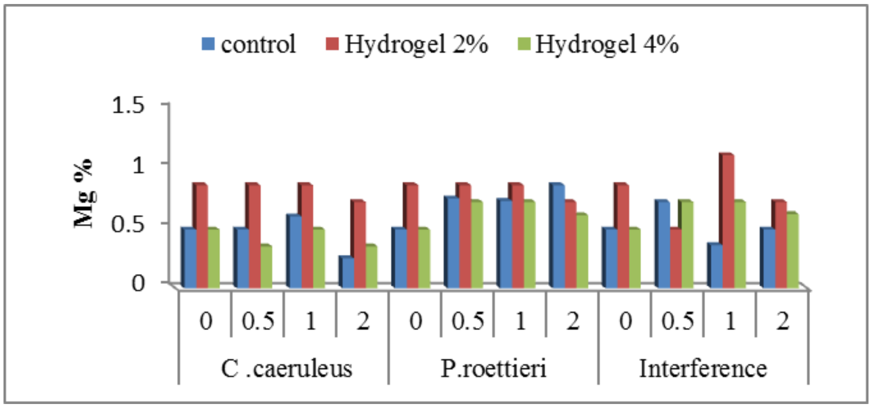

Fig. 9. Effect of the algal treatments with hydrogel on magnesium concentrations in Mawaddah wheat variety

Total proteins

Significant differences appeared in the protein concentrations between the treatments (Fig. 10). The highest protein amount (10.19mg/ g) was obtained by applying 1% *C. caeruleus* and 2% hydrogel (2gm/ kg of soil). This might be attributed to the efficacy of gel in storing nutrients and increasing their levels in the plant, and consequently increasing the protein concentration **(Al-Abbar *et al.*, 2021)**.

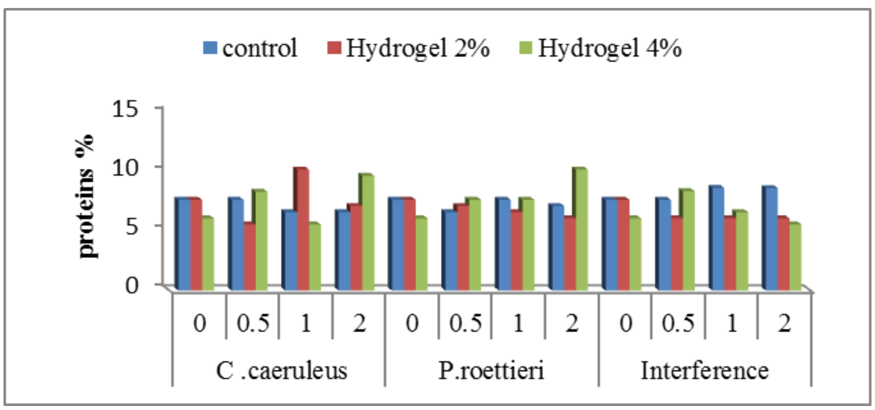

Fig. 10. Effect of the algal treatments with hydrogel on the protein concentration in Mawaddah wheat variety

Total carbohydrates

The highest total carbohydrates concentration (29.31mg/ g) was obtained by applying 2% algal extracts and 2% hydrogel (Fig. 11). The algal extracts contain both micro- and macronutrients, and subsequently increased the concentration of chlorophyll (*a* & *b*) in the leaf tissues, resulting in an increase in the concentration of total carbohydrates **(Anatharaj *et al.*, 2001)**. The reason for the increase in carbohydrates in the presence of hydrogel at a concentration of 2g/ kg of soil is its roles in providing nutrients to the plant and carrying out the process of photosynthesis, leading to a remarkable increasing in carbohydrates in the wheat plant **(Abbas *et al.*, 2022)**.

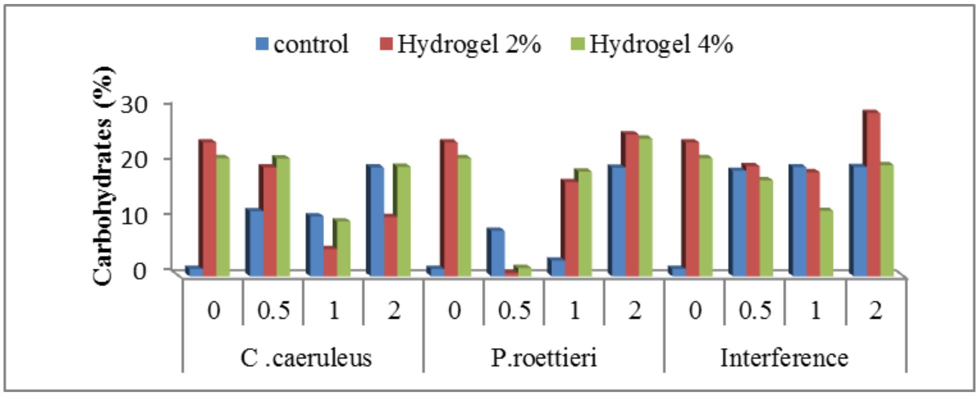

Fig. 11. Effect of the algal treatments with hydrogel on total carbohydrates in Mawaddah wheat variety

CONCLUSION

Biostimulants derived from the macroalgae *Compsopogon caeruleus* and *Pithophora roettieri*, particularly a 2% aqueous extract of *C. caeruleus*, significantly improved the germination and biochemical content of wheat plants (var. Mawaddah) due to their richness in bioactive compounds, which enhance nutrient availability to the seeds. Additionally, a 2% hydrogel (2g/ kg of soil) retained soil moisture and nutrient availability for an extended period, thereby reducing water scarcity. In conclusion, we recommend applying algal biostimulants in conjunction with hydrogel as an eco-friendly technique for sustainable agricultural management.

ACKNOWLEDGEMENTS

We are grateful to the President of the University of Mosul and the Dean of the College of Education for Pure Sciences for the arrangement and management of the chemicals, and allowing us to carry out the analysis in the university laboratory. We also thank the Ministry of Higher Education and Scientific Research, Mosul, Iraq. We also are deeply thankful to Abdullah A. Saber, Assoc. Professor of Phycology at the Botany Department, Faculty of Science, Ain Shams University, Egypt for his kind supervision on the experimental work and for editing the manuscript.

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