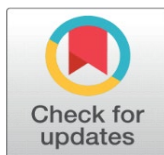


# EFFECTS OF THE SEAWEED EXTRACT OF GRACILARIA EDULIS ON THE GROWTH OF ZEA MAYS L. IN A GREENHOUSE

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## ABSTRACT

The application of marine macroalgae-based biostimulants has gained increased momentum in terms of plant growth and crop production. Seaweed has biostimulatory activities that result in improved plant growth and crop growth in various important commercial crops. They contribute to resistance to various abiotic and biotic stresses. In the present study, *Gracilaria edulis* was collected from the west coast of India. The compositions of fibre, protein, carbohydrate, moisture, ash, vitamins and other minerals were determined. The biostimulant properties of the seaweed extract were tested on *Zea mays* L. in a greenhouse. *Z. mays* L. seedlings were treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract improved the elemental composition and chlorophyll content of the leaves. These findings revealed that seaweed extracts are suitable for environmentally friendly crop management.

**Keywords:** Seaweeds, Water Extract, Biostimulant, Plant Growth

## 1. INTRODUCTION

The unrestricted use of chemical fertilizers causes environmental pollution and affects the ecosystem. In recent years, greenhouse gas emissions have increased, causing global warming and water pollution, which are associated with climatic changes and affect agricultural production (Sharma et al., 2014). Seeking eco-friendly, safe, and more sustainable is a top-most priority for modern crop production. Alternative approaches, including the application of plant bioeffectors, also referred to as plant biostimulants, have become highly adopted and have become commonly used in several agricultural practices, showing a number of benefits in stimulating plant growth and mitigating biotic and abiotic stresses. Seaweed extracts are naturally available and common biostimulants utilized for sustainable agricultural practices (Kergosien et al., 2023). The use of seaweed extracts as natural biostimulants in agriculture is very old. On the basis of their beneficial roles, seaweeds represent a treasury of proteins, polyunsaturated fatty acids, minerals, polysaccharides and antioxidants, as well as several types of biomolecules. In addition, seaweed has several important ecological functions and is a significant component of the marine environment (Melo et al., 2020). In addition, seaweed extracts could be applied in several biological processes in the pharmaceutical industry. However, the application of marine macroalgae and their extracts as aquaculture feeds improves immunity and aquatic growth. Seeds are used for the preparation of biofertilizers, not only because they have an ecological effect but also because of their compatibility, as they generally share several common secondary metabolites with terrestrial plants. This is the major advantage of

seaweed and facilitates sustainable and organic agriculture. Seaweed extracts are useful for the preparation of plant biostimulants and have been reported previously. Seaweed extracts include *Ascophyllum nodosum*, *Pterocladia capillacea*, *Sargassum* spp., *Ecklonia maxima*, *Laminaria* spp., *U. lactuca*, *Sargassum liebmannii*, *Padina gymnospora*, *Caulerpa sertularioides*, *P. gymnospora*, *C. sertularioides*, *S. johnstonii*, *Laminaria* spp., *Durvillaea potatumum*, and *S. liebmannii* (Sangha et al., 2014). In addition, the application of macroalgae improves the production of phytohormones in plants. Generally, the foliar spray method is useful for the administration of seaweed extracts to improve the growth and yield of commercial crops. The application of seaweed extract improved the total yield, chlorophyll content, and root system. Seaweed extracts are eco-friendly, harmless and biodegradable (Michalak et al., 2016; Carillo et al., 2020). With promising approaches, marine macroalgae extracts are highly suited for application in improving organic agriculture (Lakshmi and Meenakshi, 2022; Kholssi et al., 2022; Chanthini et al., 2022; Arias et al., 2024). The main objective of this study was to analyse the growth performance of a cucumber plant, *Cucumis sativus*, in a greenhouse via the application of a foliar spray of seaweed extract.

## 2. MATERIALS AND METHODS

### 2.1. SEAWEED EXTRACT PREPARATION AND APPLICATION

*Gracilaria edulis* was collected from the west coast of India. The collected seaweeds were washed with tap water to remove debris. It was dried under sunlight and ground by a mixer grinder with steel blades at room temperature. Approximately 100 g of seaweed powder was used for the preparation of liquid fertilizer. The mixture was transferred to a 2 L Erlenmeyer flask, and 1 L of demineralized water was added and heated at 60 °C for 1 h via a magnetic stirrer. The final solution was stored in a plastic container until otherwise stated. It is considered a seaweed fertilizer or biostimulant. The seaweed extract was applied in the early morning. Foliar application was performed in the morning. The biostimulants were not added to the control plants. For the experimental groups, foliar spray was performed at various concentrations.

### 2.2. DETERMINATION OF LIPID AND PROTEIN CONTENTS

The lipid content of the seaweed was evaluated as described previously by Mehlenbacher (1960). The total protein content of the algae was determined via the micro-Kjeldahl method (Sarker et al., 2022).

### 2.3. ANALYSIS OF FIBRES

The crude fibre content of the alga was evaluated as described previously via the AOAC method (AOAC, 2000). To a 10 g dried sample, 100 mL of 0.255 N sulphuric acid was added. The mixture was boiled for 20 min, 100 mL of 0.313 N sodium hydroxide was added, and the mixture was further boiled for 25 min. The filtrate was collected and weighed after drying. Then, the mixture was kept in a muffle furnace at 500 °C for 4 h. The sample was cooled and weighed, and the weight difference was observed.

### 2.4. MOISTURE

The moisture content of each sample was tested via a moisture meter. Briefly, 2 g of seaweed powder was placed into the tray of a moisture meter for 3 min.

### 2.5. ASH

The ash content of each sample was determined via the AOAC method. Briefly, 10 g of dried alga was weighed into a porcelain crucible and further incinerated at 600 °C for 4 h. The obtained ash content was cooled in desiccators and weighed, and the percentage ash content was calculated.

### 2.6. CARBOHYDRATE DETERMINATION

The amount of carbohydrate in the alga was calculated via the following formula (Sarkiyayi and Agar, 2000):

$$\text{Total carbohydrates (\%)} = 100 - (\text{moisture (\%)} - \text{crude fibre (\%)} + \text{crude protein (\%)} + \text{crude lipid (\%)} + \text{ash (\%)})$$

## 2.7. MINERALS AND TRACE ELEMENTS

The amounts of Mg, Ca, Cu, Fe and P and heavy metals such as lead (Pb) were determined via an atomic absorption spectrophotometer.

## 2.8. GREENHOUSE EXPERIMENT

The present study was performed in a greenhouse from 14 January to 29 May 2020. The relative humidity and temperature were registered during the experimental conditions. The relative humidity ranged from 30% to 76%. The temperature ranged from 24.5 to 32 °C.

## 2.9. GROWTH CONDITIONS OF ZEA MAYS L.

A total of 60 seeds were counted and soaked in double distilled water for 18 h. The surface was sterilized in (v/v) 5% sodium hypochlorite solution for 10 min. The seeds were allowed to germinate in the dark for three days at 25 °C. It was wrapped in moist filter paper. The germinated seeds were subsequently transplanted into 5 L pots containing nutrient medium. A total of 10 plants were seeded in a single pot, and the nutrient medium was composed of (μM) Ca(NO<sub>3</sub>)<sub>2</sub>-200, KH<sub>2</sub>PO<sub>4</sub>-40, MgSO<sub>4</sub>-200, KNO<sub>3</sub>-200, FeNaEDTA-10, CuCl<sub>2</sub>-0.036, H<sub>3</sub>BO<sub>3</sub>-4.6, MnCl<sub>2</sub>-0.9, NaMoO<sub>4</sub>-0.01 and ZnCl<sub>2</sub>-0.09. The plants were cultivated for three weeks in a 12 h dark and 12 h light cycle. The approximate temperature was 28 °C, and the average humidity was 80%. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%), 1.5%, or 2.0%). The seaweed extract was not added to the control. The plants were harvested after five weeks and were also dried and powdered. The powdered sample was used for the determination of sugars and element analysis.

## 2.10. STATISTICAL ANALYSIS

One-way analysis of variance was performed to determine the significance level. A p value<0.05 was considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1. PROXIMATE ANALYSIS OF SEAWEED

The compositions of fibre, protein, carbohydrate, moisture, ash, vitamins and other minerals were determined (Table 1). Seaweed extracts are well known to contain several minerals because marine macroalgae bioaccumulate minerals from seawater. Seaweeds accumulate minerals such as K, N, and Mg. In addition, Zn and Fe have also been reported. Marine macroalgae, including Ulva species as liquid extracts or dried powders, are utilized as biofertilizers or biostimulants for various agronomically important crops because they are rich in inorganic matter (Ahmed et al., 2021; Sekhoua et al., 2021).

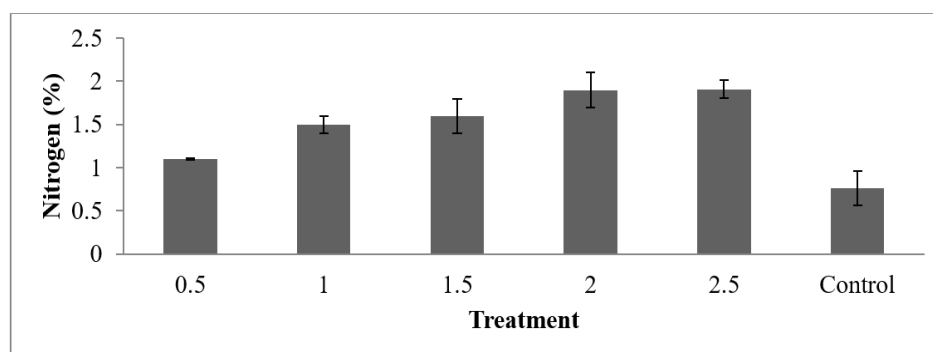
**Table 1.** Biochemical composition of seaweed

Components	Results
Crude fibre (%)	4.3 ±0.1
Crude protein (%)	25±2.3
Carbohydrate (%)	32±1.1
Moisture (%)	5±0.2
Ash (%)	9±0.3
Calcium (mg/100 g)	400±11.4
Phosphorus (mg/100 g)	150±2.0
Magnesium (mg/100 g)	4.2±0.3

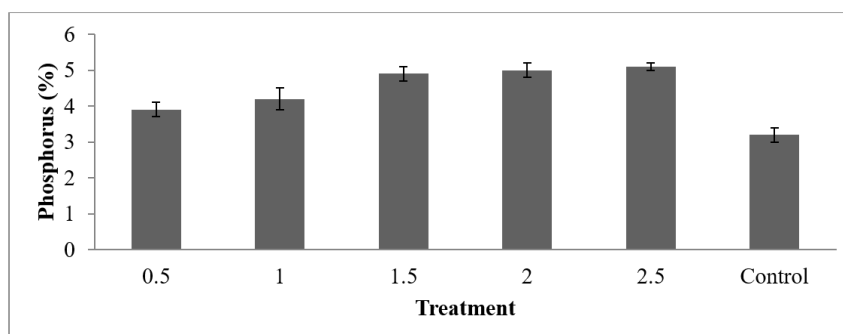
Iron (mg/100 g)	105±2.4
Lead (mg/kg)	0.021±0.001
Copper (mg/kg)	2.2±0.2
Vitamin C (mg/kg)	3.4±0.2
Total energy (kcal/100 g)	270±1.7

### 3.2. EFFECTS OF PLANT EXTRACTS ON THE NUTRIENT PROFILE OF PLANTS

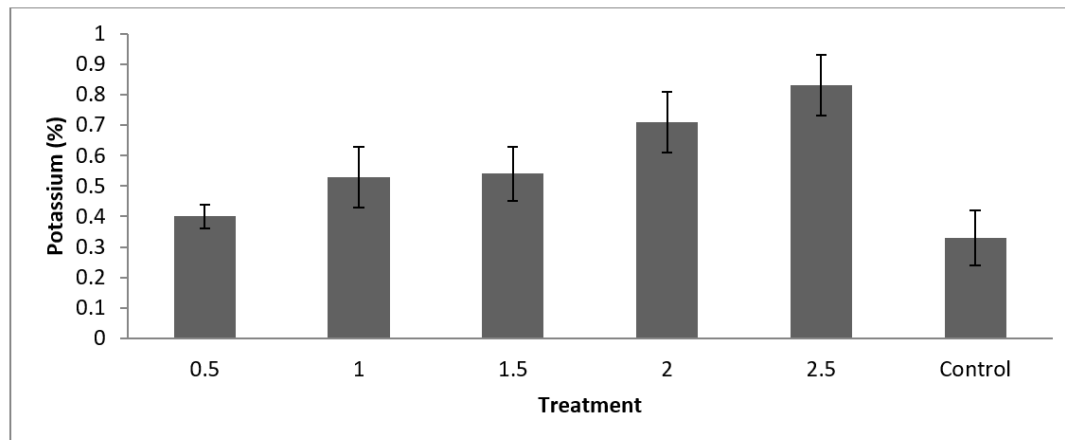
In this study, the plant extracts were treated with various concentrations of seaweed extract (0.5%, 1%, 1.5%, 2.0%, and 2.5%). The applied seaweed extract improved the macro- and micronutrient contents of the plants in the greenhouse (Figs. 1--6). Seaweed extract improves the growth of plants and increases their chlorophyll content, leaves, and photosynthetic capacity (Soppelsa et al. 2018). Seaweed extracts promoted the seed germination rate and improved seedling vigour by increasing both the root density and the root size. Seaweed extracts also protect seedlings from transplantation shock in cabbage, marigold, and tomato (Crouch et al., 1993; Aldworth et al., 1987). The available phytohormones, e.g., auxins in the algal extracts, improved rooting architecture as well as stimulatory processes in plants. An improvement in the root system of plants treated with several seaweed extracts was observed. For example, cutting from floricultural plants such as marigolds treated with macroalgal extracts from *E. maxima* increased the root density (Crouch and van Staden, 1993). *K. alvarezii* and *A. nodosum* extracts improved nutrient and water uptake, which improved the overall vigour and growth of the plants (Crouch et al., 1990). Compared with the control, the application of *Laminaria* spp. and *A. nodosum* extracts to maize significantly improved the contents of Fe, Zn, Cu, B, S, Mg, Mn, and Ca (Ertani et al., 2018). The application of *A. nodosum* improved potassium uptake in *A. nodosum* (Fei et al., 2017). In addition, the potassium level increased in the leaves of mustard plants treated with *E. maxima* (Di Stasio et al., 2017). Seaweed extracts contain several cellular materials, including polysaccharides, vitamins, minerals, fats, oils, antioxidants, acids, hormones and pigments (Craigie, 2011). Hence, understanding their molecular mechanism of action is highly complex and requires a multidisciplinary approach because several interactions occur within the same extract. Seaweed extract can be used on plants as a foliar spray. When seaweeds are subjected to positively regulated remediation, soil retention, and soil microflora, they improve nutrients, and they have hormonal effects (Craigie, 2011).



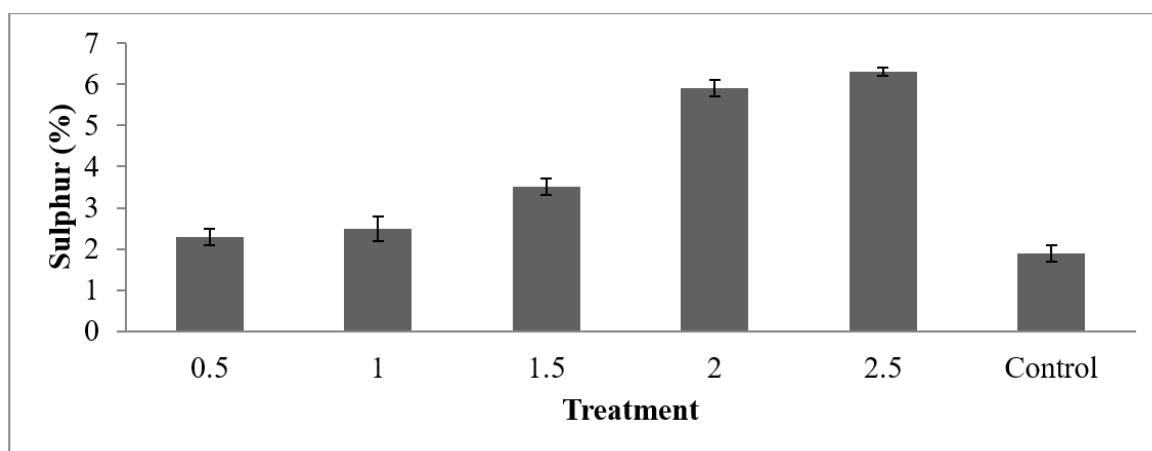
**Fig. 1.** Nitrogen content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract was not added to the control.



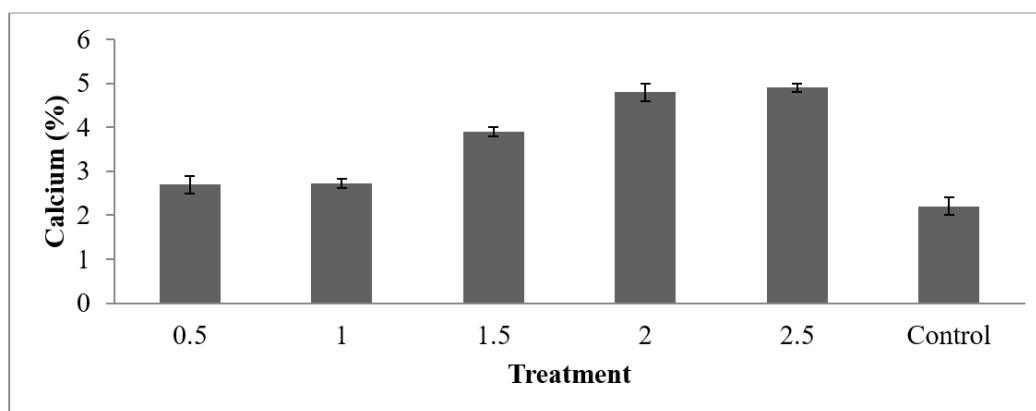
**Fig. 2.** Phosphorus content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract was not added to the control.



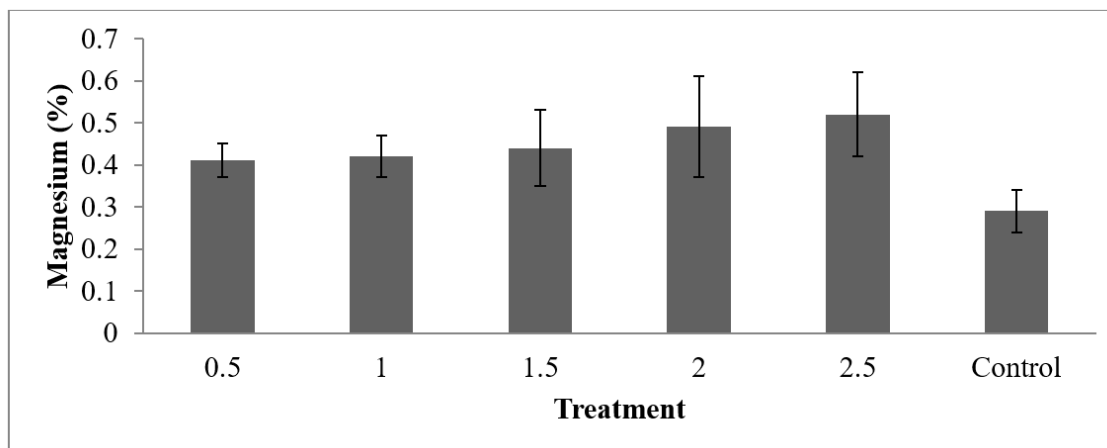
**Fig. 3.** Potassium content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract was not added to the control.



**Fig. 4.** Sulphur content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract was not added to the control.



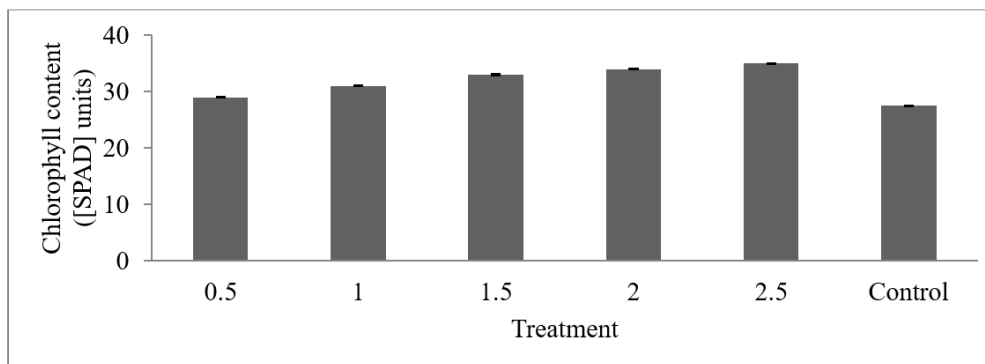
**Fig. 5.** Calcium content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%), 1.5%, or 2.0%). The seaweed extract was not added to the control.



**Fig. 6.** Magnesium content in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%), 1.5%, or 2.0%). The seaweed extract was not added to the control.

### 3.3. EFFECTS OF SEAWEED EXTRACTS ON ZEA MAYS L. CHLOROPHYLL

In the present study, the amounts of nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium improved in the plants treated with the algal extract compared with those in the control plants after foliar spraying. The nutrient parameters of the plants improved marginally after the addition of 0.5% macroalgal extract. However, the biostimulant properties of macroalgal extract-treated plants were greater than those of the control plants ( $p < 0.05$ ). The mixture of algae-based biostimulants is highly complex and may promote plant growth. In our study, alga extract increased the chlorophyll content in plants, and the growth-promoting effect of algae extract was reported previously (Ali et al., 2021). In this study, the seaweed extracts improved the chlorophyll content in *Zea mays* L. leaves (Fig. 7). These findings are consistent with those of Spinelli et al. (2009), who reported steep increases in the chlorophyll level and photosynthetic ability after the application of seaweed extract to trees. Seaweed extracts increase the chlorophyll content of grape leaves (Sabir et al., 2014). Over the past few decades, seaweed extracts have been frequently explored for potential applications in crop production to improve yield and crop quality. These algal extracts positively regulate seed germination and plant growth and are useful for postharvest management (Ali et al., 2020). In addition, the algal extract induced the expression of cytokinin genes, revealing growth promoters. The algae extract-treated plants presented improved chlorophyll concentrations, revealing that the algae extract significantly influenced leaf growth. In a previous study, Rathore et al. (2009) reported increases in P, N, K and S  $> 20\%$  in soybean seeds sprayed with *K. alvarezii*. Commercial algal extracts improve the P content in leaves (Nelson and Staden, 1984). Turan and Köse (2004) reported increases in P, K, N, Mg, Ca, Zn, Fe, Cu and Mn in grapes when algae extracted from *A. nodosum* were used.





**Fig. 7.** Chlorophyll content ([SPAD] units) in the leaves of 35-day-old *Zea mays* L. treated with seaweed extract. The values represent the means  $\pm$  standard deviations. After three weeks, the plants were divided into four different groups and treated with seaweed extracts (0.5%, 1%, 1.5%, or 2.0%). The seaweed extract was not added to the control.

#### 4. CONCLUSION

Seaweed extracts are useful for the preparation of eco-friendly biofertilizers that are used to mitigate the environmental impacts caused by chemical fertilizers. The present study revealed the positive impact of seaweed extracts on plants. The plant growth-promoting activity of various seaweed extract concentrations in this study allowed us to identify suitable seaweed extracts with improved biostimulant properties. The aqueous extract of the seaweed improved the macro- and micronutrient contents and chlorophyll content in *Zea mays* L.

#### CONFLICT OF INTERESTS

None.

#### ACKNOWLEDGMENTS

None.

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