The effect of *Enteromorpha intestinalis* and *Corallina elongata* on physiological parameters of *Zea mays* L.

Mohammed Bouhadi[1,2*], Ouafa Cherifi[3,4], Nadia Bahammou[3,4], Khalil Cherifi[5], Mohammed Talbi[2], M'hammed Elkouali[2], Hassan Fougrach[1]

[1] *Laboratory of Ecology and Environment, Faculty of Sciences Ben Msik, Hassan II University of Casablanca, BP 7955, Casablanca 20660, Morocco.*

[2] *Laboratory of analytical and molecular chemistry, Faculty of Sciences Ben Msik, Hassan II University of Casablanca, BP 7955, Casablanca 20660, Morocco.*

[3] *Laboratory of Water Biodiversity and Climat Change, Cadi Ayyad University, Marrakech, Morocco*

[4] *National Center for Studies and Research on Water and Energy, Cadi Ayyad University, Marrakech, Morocco*

[5] *Laboratory of Biotechnology and Valorization of Natural resources, Biology Department, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco*

*E-mail: mohammed.bouhadi-etu@etu.univh2c.ma

Received: 17/12/2021
Revised: 05/06/2022
Accepted: 22/06/2022

Abstract

Purpose: Algae are living organisms capable of photosynthesis and live mainly in an aquatic environment (marine or freshwater), and considered as plants like those growing on the soil. It can therefore be used to enrich the soil in organic matter, and mineral elements, as for composting green waste, for example. The objective of this study was to investigate the effect of marine macroalgae *Enteromorpha intestinalis* (Ulvophycae) and *Corallina elongata* (Corallinacae) as biofertilizers on the growth of *Zea mays* L.

Method: After rinsing, the algae were ground into a fine powder. The powder obtained from each seaweed was divided into two batches. For the first batch, the seaweed powder (AP), was used directly in the pots one week before planting. Two concentrations, 5% and 10%, were studied. The second batch was used to prepare liquid algal fertilizer (ALF). To determine the mineral composition of each seaweed, X-ray fluorescence analysis was performed. the biofertilizing potential of *Enteromorpha intestinalis* and *Corallina elongata* was studied on corn plants, evaluating the effect of these two algae at different growth stages (15, 25, 45 and 60 days) and the chlorophyll a and b content.

Results: The elemental analysis of the two algae by X-ray Fluorescence shows that the main elements are water, chlorine, potassium, calcium, silicon, natrium, magnesium, iron and sulfur. The results show that *E.intestinalis* is richer in minerals compared to *C.elongata*, except for Strontium and calcium, where *C.elongata* is more rich.

Conclusion: The results of this study showed that the macroalgae *Enteromorpha intestinalis* and *Corallina elongata* from the Atlantic coast of Morocco are valuable biofertilizers. On the other hand, the application of seaweed extract is more effective than the application of seaweed powder, and *Enteromorpha intestinalis* can affect the growth parameters at a low concentration (5%), unlike *Corallina elongata*. The different positive roles of algae in agriculture should be clarified, as our results showed that they can be used as an excellent fertilizer.

Keywords: Macroalgae, *Enteromorpha Intestinalis*, *Corallina Elongata*, Biofertilizers, Maize.

Introduction

During their growing cycle, crops need inputs in mineral elements, to ensure their physiological functions. Fertilizers are chemical substances, most often mixtures of mineral elements, intended to provide plants with nutrient supplements, to improve their growth, and to increase the yield and quality of crops. These last decades with the intensive farming, however, many studies show that the uses of chemical fertilizers unfortunately have more disadvantages than benefits inducing health risks and tremendous environmental costs. Also, in developing countries, there is a lack of fertilizers and inadequate agricultural practices leading to bad quality crops. To overcome these problems and to increase crop yields in the absence of resources for obtaining costly fertilizers, efficient and sustainable practices are needed to allow cost-efficient production of adequate nutrition for the growing populations, especially indeveloping countries. Thus, many researchers encourage the use of biofertilizers. Among them the macroalgae. This type of fertilizer eliminates the use of harmful chemicals. Morocco, with more than 3400km of coastal areas, has a large richness of macroalgae. Indeed, 438 species have been inventoried but only few red species are used in agro-industrial field (Riadi, 1998). Marine macroalgaeare one of the important living resources of the world. Indeed, many compounds, isolated from these seaweeds, haveseveral applications in pharmacy, cosmetic, nutrition, and agriculture and many other biotechnology related industries. They have already proved their efficiency as biofertilizer by many authors around the world (Pouset, 2002; Hibar et al., 2006; Divya et al., 2015) and many companies improving algal biofertizer products have emerged.

The aim of this study is to improve the use of two dominant macroalgae *Enteromorpha intestinalis* and *Corallina elongata* from the Atlantic coast of Morocco in agricultural field as biofertilizer.

Materials and Methods

Sampling stations

The harvest is done during spring and autumn 2017 at low tide in two Moroccan coastal areas, ElOualidia (32°45’N, 9°30’W) and Eljadida (33°13′52″N, 8°32′51″W) where *Corallina elongata* and *Enteromorpha intestinalis* were dominant, respectively. The samples were washed in seawater at the sampling station, placed in plastic bags and transported to the laboratory in an icebox.

Experimental procedures

At the laboratory, we trilled and rinsed the sample thoroughly and several times with seawater to eliminate impurities (sand and debris of algae). The drying was done in shadow open air. The seaweeds were reduced to fine powder in a mechanic grinder. The powder obtained from each seaweed was divided into two lots subjected to specific conditions to study the fertilizing activity of the algae. For the first lot, the algal powder (AP), was used as a treatment, directly in the pots a week before planting. Two concentrations, 5% and 10%, were studied. The second one was used to prepare the Algal liquid fertilizer (ALF).

Seaweed liquid fertilizer preparation

The seaweed powder was added with distilled water in a ratio 1:20(w/v) and autoclaved. After that, the extract was filtrate and stored at 4°C. The extract was used to prepare two concentrations of ALF: 5% and 10% by adding distilled water.

Plant material

Maize seeds were sterilized with ethanol 70% and washed by sterile distilled water, then dried in shadow open air. For the AP treatment, the seeds were planted in 30 cm diameter earthen pots containing 1 Kg of an autoclaved clay loam soil (Table 1) with each algal powder/ extract algal. They were daily watered with tap water. For the ALF, the sowing seeds were soaked, in each concentration of SLF and in distilled water for the control, for 24hours. Then, the seeds were planted and irrigated as AP experiment. In addition, they were treated once a week with a volume of 10 ml of the ALF. The experiments were carried out under natural conditions of temperature (23-28 °C).

Table 1. Physicochemical properties of soil used in this study

Texture	Clay loam
Clay (%)	65
Sand (%)	20
Silt (%)	15
EC (dS/cm)	1.3
pH	6.85
Organic matter (%)	1.1
HCO_3 (mmol/l)	2.89
Available P (mg/kg)	5.5
K^+(mmol/l)	0.14
Na^{2+} (mmol/l)	2.6
Cl^- (mmol/l)	1.59

Chlorophyll contents

The chlorophyll a and b contents were analyzed in the fresh leaves of maize (*Zea mays*) by following the method by (Metzner et al., 1965).The extract was taken in acetone (80%) solution. After that absorbance was noted at optical densities (OD) of 662 and 645 nm on spectrophotometer. Final calculations were made using the following relations. The chlorophyll a and b was calculated according to the coefficients and equations given by (Lichtenthaler, 1987)

$$\text{Chlorophyll a (mg/g)} = 11{,}24 \times DO662 - 2{,}04 \times DO645$$

$$\text{Chlorophyll b (mg/g)} = 20{,}13 \times DO645 - 4{,}19 \times DO662$$

$$\text{Total chlorophyll} = \text{chl a} + \text{chl b}$$

Analysis of growth parameters

Shoot and root lengths were recorded at different growth stages; 15, 25, 45 and 60 days. At the end of the experiments, plant dry weight was recorded, and chlorophyll content was analyzed.

The plants were oven dried at 80°C for 24h to determine the dry matter. The content of chlorophyll a was estimated using the method of (Metzner et al., 1965). Pigment concentrations are expressed as µg/g FW (Fresh Weight).

Fluorescence X Analysis

The work presented here was conducted in 2017 using a portable X-ray fluorescence spectrometer (XRF) (Fondis, Niton XLt).

Statistical analysis

All the measurements were triplicates. The analysis of variance (ANOVA) was carried out using a version 24 of SPSS and by Tukey's post-hoc test between treatment means (Khadraji et al., 2020).

Results

Growth parameters

Growth parameters of Maize, such as shoot length, leave length, and dry weight, were significantly influenced by the addition of algal powder/extract to soil. A gradual increase in shoot length, leave length and dry weight were observed. Under algal powder application, the maximum of shoot length (Figure 1a), Leave length (Figure 2a) and dry weight (Figure 3), was at10% of Corallina elongate and 10% of *Enteromorpha intestinalis*. Unlike to algal powder application, the application of algal extract, increased significantly the shoot length (Figure 1b), Leave length (Figure 2b) and Dry weight (Figure 3) and we obtained the maximum at 5%, 10% of *E.intestinalis* and 10% of *C.elongata*.

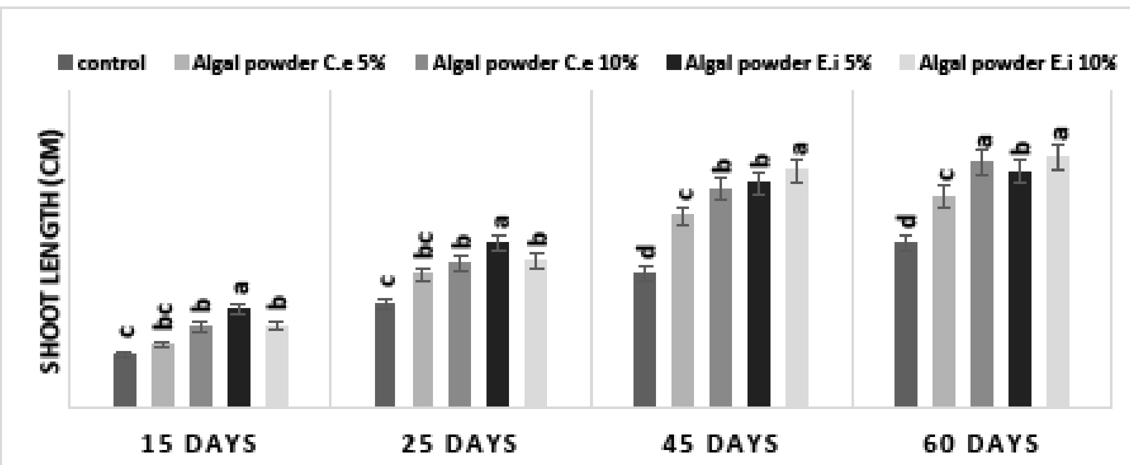

Figure 1a. Shoot length of Maize plant treated by Algal powder of C.e.; *Corallina elongata*, Ei: *Enteromorpha intestinalis*

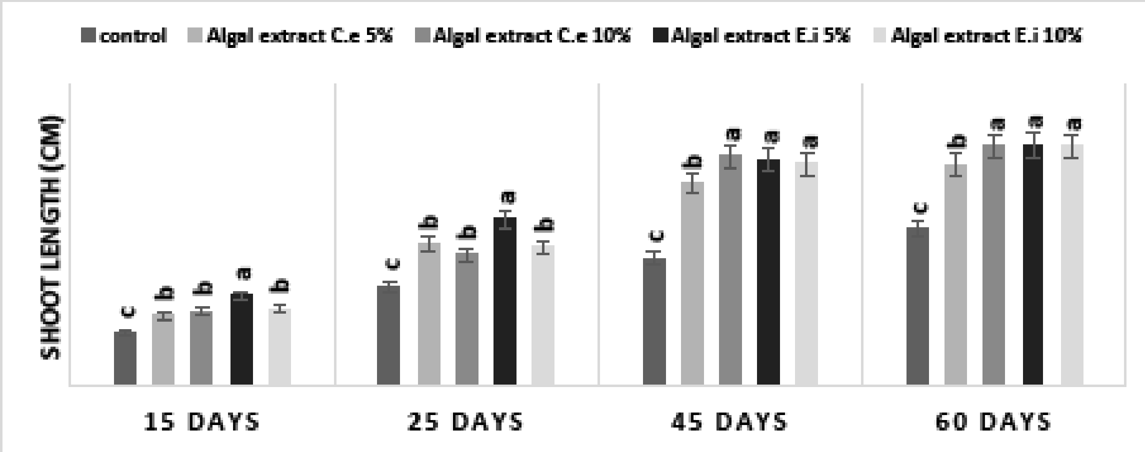

Figure 1b. Shoot length of Maize plant treated by algal extract of C.e.; *Corallina elongata*, Ei: *Enteromorpha intestinalis*

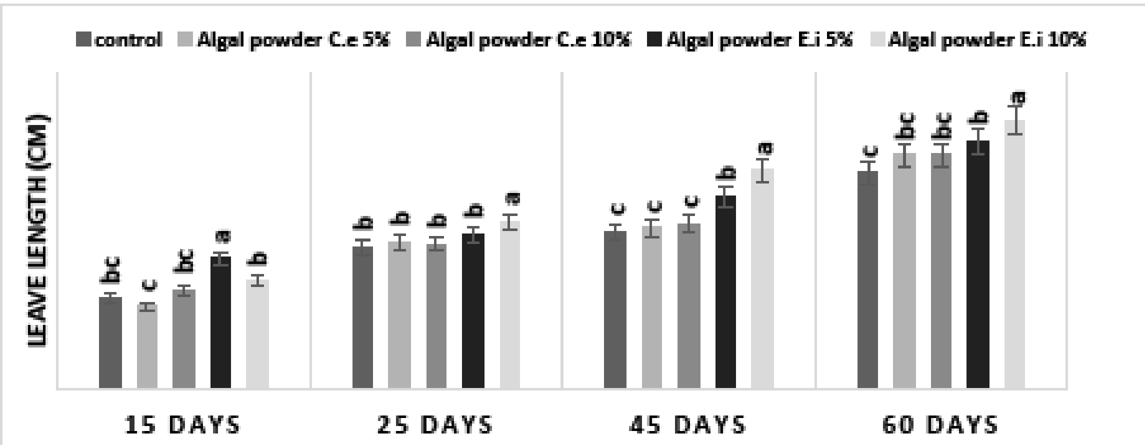

Figure 2a. Leave length (cm) of Maize plant treated by Algal powder

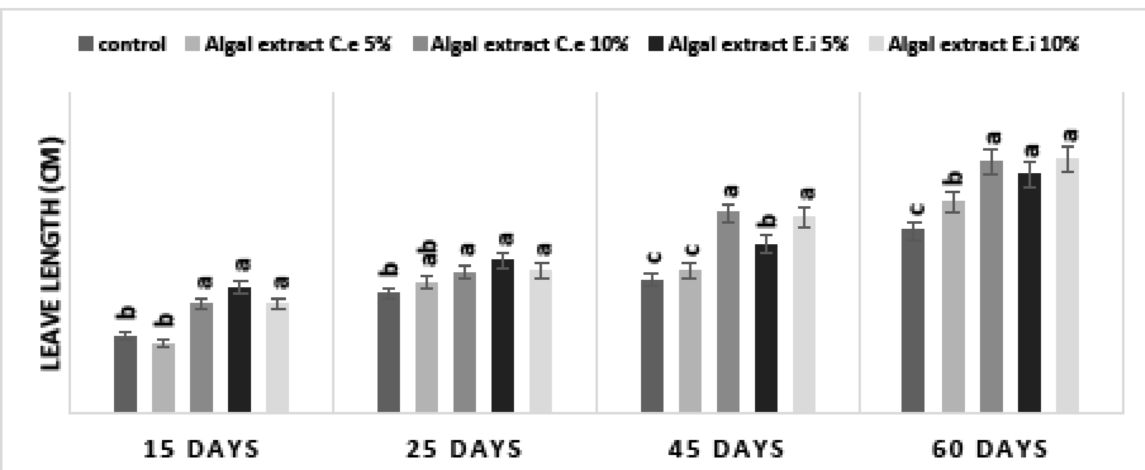

Figure 2b. Leave length (cm) of Maize plant treated by Algal extract

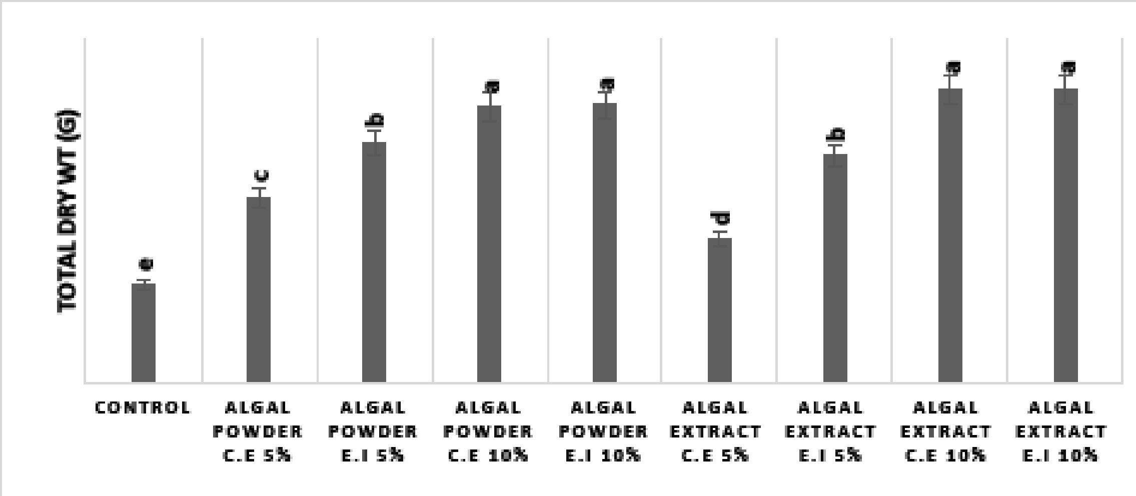

Figure 3. Total dry Weight of Maize plant

Chlorophyll content

After 60 days algal treatment, the results were shown in (Figure 4).The chlorophyll contents have showed a highly significant increase with the increase of algal treatment concentration. For algal powder treatment, the maximum chlorophyll content was registered at *C.elongata*10% following by *E.intestinalis*10% as compared to control plant. For algal extract, the results showed that the maximum chlorophyll content was registered at *C.elongata*10% and *E.intestinalis* 10% following by *E.intestinalis* 5%.

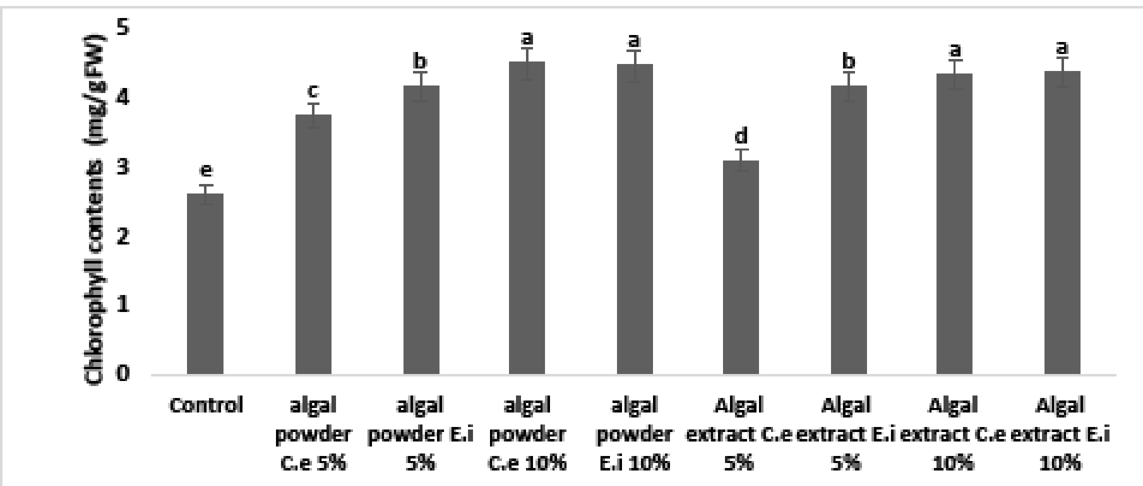

Figure 4. The chlorophyll contents of Maize

Fluorescence X Analysis

The results of the elemental analyses of the two algae by Fluorescence X are presented in (Table 2). The main elements are water, chlorine, potassium, calcium, silicon, natrium, magnesium, iron and sulphur. The results show that *E.intestinalis* is richer in minerals compared to *C.elongata*, with the exception of Strontium and calcium, the analysis showed that *C.elongata* is more richer.

Table 2. Elemental composition by Fluorescence X of the two algae

Element (ppm)	*C.elongata*	*E.intestinalis*
Cu	29	15
Zn	45	26
Sr	263	1416
Zr	11	8
Rb	39	17
Y	18	12
LE	89.06%	83.61%
Cl	5.56%	2.96%
Al	1956	1498
Ca	3.32%	12.18%
Si	5058	4648
K	7590	2760
P	1403	1259
Fe	3257	261
S	1215	544

Discussion

In the present study, shoot length, leave length, dry weight and Chlorophyll contents have significantly increased with application of algal powder/extract (Figure 1, 2, 3 and 4). (Bouhadi at al., 2019) reported that Corallina officinalis powder improve the growth parameters and the chlorophyll content in the leaves of Vicia faba plant. Seaweeds induced increase in plant growth has been reported by many researchers in many plant species including Maize (Agnieszka et Katarzyna 2018; Safinaz and Ragaa 2013). (Ouhssine et al., 2007) showed a positive correlation between the percentage of the algal powder in the soil and the development of the different parts of the Maize plant (the length, the diameter of the stems, and number it sheets., This biomass production increase due to application of seaweeds powder has also been observed in many other plants. Indeed, (Filipkowska et al., 2008) observed that the growth rates of tomato plants growing in the soil supplemented by macroalgae (Sargassum spp., Turbinaria spp., Caulerpa chemnitzia, *Enteromorpha intestinalis*) were conspicuously faster than in the control. Also, they reported that these seaweed extracts contain trace elements, vitamins, amino acids, cytokinines and growth-promoting hormones. Moreover, (Sivasankari et al. 2006; Hong et al., 2007) found that their concentration in seaweeds are superior to other fertilizer sources. (Gupta and Lata 1964) observed that cyanobacteria increase seed germination and sponsored seedling growth. Also, they have supplementary positive roles, such as liberating of bioactive substances like biological control, stimulators and hormones (Lugtenberg et al., 1991). However, (Filipkowska et al.,2008) noted that the decomposition of macroalgae is very rapid when mixed with soil. Significant increase in soil polysaccharides, phosphatase activities, urease, and dehydrogenase was documented. Studies of Burns and Davies (1986) suggested soil polysaccharides as major component responsible for soil stabilization. (Safinaz andRagaa 2013) reported that the application of Laurencia obtusa + Jania rubens caused 48.21% increase in plant length, 61.84% increase in potassium content and increase the leaves number, as compared to control plant. Additionnally, the application of Laurencia obtusa + *Corallina elongata* increased by 90.86% the plant fresh weight. (Sosnowski et al., 2019) observed that Ecklonia maxima extract had multi-directional effect on photosynthetic activity of the plants and in relation to the control plant there was a significant increase in chlorophyll content in Alfalfa leaves (12%) as a response to E. maxima extract. (Mzibra et al., 2018) showed that six seaweed species (*Ulva rigida, Codium decorticatum, Fucus spiralis, Chondracanthus acicularis, Bifurcaria bifurcata and Gigartina sp.*) were a promising source of polysaccharide-enriched extracts (PEEs) with beneficial effect on germination, plant biomass, and chlorophyll content. However, it is obvious that some of their advantageous properties and beneficial effects influence plant/soil-systems. Two important potential uses of soil microalgae in crop production are as biofertilizers or soil conditioners.

Conclusion

In this study, the results show that the two macroalgae, *Enteromorpha intestinalis* and *Corallina elongata* macroalgae from the Atlantic coast of Morocco,and their products are valuable biofertilizers. In the other hand, the algal extract application is more efficient than the algal powder application, and *Enteromorpha intestinalis* can influenced the growth parameters at a small concentration (5%), unlike to *Corallina elongata*. An effort was supported to throw some light on the different positive roles of algae in agriculture, with respect to the association between algae and plants. Our results showed that can be utilized as excellent fertilizers.

References

Agnieszka, D., Katarzyna, C., (2018) Algae as Fertilizers, Biostimulants, and Regulators of Plant Growth. Developments in Applied Phycology, pp.115-122 DOI: https://doi.org/10.1007/978-3-319-74703-3_10

Bouhadi, M., Ainane, A., M’hammed, E.L., Talbi, M., Cherifi, O., El Yaacoubi, A., & Ainane, T. (2019) Role of the macroalgae Corallina officinalis in alleviating the toxicity of hexavalent chromium on Vicia faba L. Journal of Analytical Sciences and Applied Biotechnology, 1(2), 60-64. DOI: https://doi.org/10.48402/IMIST.PRSM/jasab-v1i2.18976

Burns, R.G., Davics, J.A., (1986) The microbiology of soil structure, Biol. Agric. Hortic. 3. 95-113. DOI: https://doi.org/10.1080/01448765.1986.9754465

Filipkowska, A., Lubecki, L., Szymczak-Żyła, M., Kowalewska, G., Żbikowski, R., Szefer. P., (2008) Utilisation of macroalgae from the Sopot beach (Baltic Sea). OCEANOLOGIA, pp. 255–273.

Gupta AB, Lata K (1964) Effect of algal growth hormones on the germination of paddy seeds. Hidrobiologia, 24(1-3): 430-434. DOI: https://doi.org/10.1007/BF00170414

Hong D.D., Hien H.M., Son P.N., (2007) Seaweeds from Vietnam used for functional food, medicine and biofertilizer, J. Appl. Phycol., 19 (6), 817–826. DOI: https://doi.org/10.1007/s10811-007-9228-x

Khadraji, A., Bouhadi, M. and Ghoulam, C. (2020) Effect of Soil Available Phosphorus

Levels on Chickpea (Cicer arietinum L.) - Rhizobia Symbiotic Association. Legume Research. 43(6): 878-883. DOI: http://dx.doi.org/10.18805/LR-524

Lichtenthaler, H.K., (1987) Chlorophylls and carotenoids pigments of photosynthetic biomembranes. In: In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods. Enzymol. 148. pp. 350–382. DOI: https://doi.org/10.1016/0076-6879(87)48036-1

Lugtenberg, BJJ., Weger, LA., de Bennett, JW., Deweger, LA., (1991). Microbial stimulation of plant growth and protection from disease. Curr. Opin. Biotechnol., 2(3): 457-464. DOI: https://doi.org/10.1016/S0958-1669(05)80156-9

Metzner, H., Rau, H., Senger, H., (1965) Untersuchungen zur Synchronisierbakeit einzelner Pigmentmangel-Mutation von Chlorella. Planta 65, 186–194. DOI: https://doi.org/10.1007/BF00384998

Mzibra, A., Aasfar, A., El Arroussi, H., Khouloud, M., Dhiba, D., Kadmiri, I. M., & Bamouh, A. (2018) Polysaccharides extracted from Moroccan seaweed: a promising source of tomato plant growth promoters. Journal of Applied Phycology, Vol 30, Issue 5, pp 2953–2962

Ouhssine, K., Ouhssine, M., Elyachioui, M., (2007) L'application des déchets traités de l'algue Gelidium sesquipédale dans la culture du Maïs. Afrique SCIENCE 03(2) (2007) 259 – 270.

Sivasankari S., Venkatesalu V., Anantharaj M., ChandrasekaranM., (2006) Effect of seaweed extracts on the growth and biochemical constituents of Vignasinensis, Bioresource Technol., 97 (14), 1745–1751. DOI: https://doi.org/10.1016/j.biortech.2005.06.016

Safinaz, A F, Ragaa, A H. (2013) Effect of some red marine algae as biofertilizers on growth of maize (Zea mayz L.) plants. International Food Research Journal Vol. 20 N° 4 : 1629-1632.

Sosnowski, J., Jankowski, K., Truba, M., & Malinowska, E. (2019) Effect of Ecklonia maxima extract on photosynthesis activity and chlorophyll content of Medicago × varia Martyn leaves. Chilean Journal of Agricultural Research, 79(2), 257–265. DOI: http://dx.doi.org/10.4067/S0718-58392019000200257

تأثير *Enteromorpha intestinalis* و *Corallina elongata* على البارامترات الفسيولوجية لـ *Zea mays* L

محمد بوهادي[1، 2*]، وفاء شريفي[3، 4]، نادية باحمو[3، 4]، خليل شريفي[5]
محمد طالبي[2]، محمد القوالي [2]، حسن فوكراش[1]

[1] مختبر الإيكولوجيا والبيئة، كلية العلوم بن مسيك، جامعة الحسن الثاني بالدار البيضاء،
ص. ب 7955، الدار البيضاء 20660، المغرب.

[2] مختبر الكيمياء التحليلية والجزيئية، كلية العلوم بن مسيك، جامعة الحسن الثاني بالدار البيضاء،
ص. ب 7955، الدار البيضاء 20660، المغرب.

[3] مختبر الماء، التنوع البيولوجي والتغير المناخي، جامعة القاضي عياض، مراكش، المغرب.

[4] المركز الوطني للدراسات والأبحاث حول الماء والطاقة، جامعة القاضي عياض، مراكش، المغرب.

[5] مختبر التكنولوجيا الحيوية وتثمين الموارد الطبيعية، قسم الأحياء، كلية العلوم، جامعة ابن زهر، أكادير، المغرب.

* البريد الإلكتروني: mohammed.bouhadi-etu@etu.univh2c.ma

تاريخ استلام البحث: 2021/12/17
تاريخ تعديل البحث: 2022/06/05
تاريخ قبول البحث: 2022/06/22

المُستَخلَص

الهدف: الطحالب كائنات حية قادرة على التمثيل الضوئي وتعيش بشكل أساسي في بيئة مائية (بحرية أو مياه عذبة)، وبالتالي فهي نباتات بنفس الطريقة التي تنمو على الأرض. لذلك يمكن استخدامها لإثراء التربة بالمواد العضوية، والعناصر المعدنية، كما هو الحال مع سماد النفايات الخضراء، على سبيل المثال.

الطريقة: بعد شطف الطحالب، تم طحنها إلى مسحوق ناعم. تم تقسيم المسحوق الذي تم الحصول عليه من كل عشب بحري إلى مجموعتين. بالنسبة للدفعة الأولى، تم استخدام مسحوق الأعشاب البحرية (AP) مباشرة في الوعاء الزراعي قبل أسبوع من الزراعة. تمت دراسة تركيزين 5٪ و10٪. تم استخدام الدفعة الثانية لتحضير سماد الطحالب السائلة (ALF). لتحديد التركيب المعدني لكل عشب بحري، تم إجراء تحليل مضان بالأشعة السينية. تمت دراسة إمكانية التسميد الحيوي لكل من *Enteromorpha intestinalis* و*Corallina elongata* على نباتات الذرة، وتقييم تأثير هذين الطحلبين في مراحل نمو مختلفة (15، 25، 45 و60 يومًا) ومحتوى الكلوروفيل أ وب.

النتائج: يظهر التحليل الأولي للطحالبين بواسطة الأشعة السينية أن العناصر الرئيسية هي الماء والكلور والبوتاسيوم والكالسيوم والسيليكون والناتريوم والمغنيسيوم والحديد والكبريت. أظهرت النتائج أن *E.intestinalis* أكثر ثراءً بالمعادن مقارنةً بـ *C.elongata*، باستثناء السترونتيوم والكالسيوم، حيث *C.elongata* أكثر ثراءً. وقد تأثرت معاملات النمو، مثل الطول والوزن الجاف للبراعم والأوراق، معنوياً بإضافة مسحوق/مستخلص الطحالب إلى التربة. تم تحقيق الحد الأقصى لجزء الجذع وطول الأوراق والوزن الجاف باستخدام 10 بالمائة من *Corallina elongata* و10 بالمائة من *Enteromorpha intestinalis*. كما أظهر محتوى الكلوروفيل زيادة معنوية مع زيادة تركيز معاملة الطحالب في التربة.

الاستنتاج: أظهرت النتائج في هذه الدراسة أن الطحالب الكبيرة *Enteromorpha intestinalis* و *Corallina elongata* من ساحل المحيط الأطلسي في المغرب من الأسمدة الحيوية القيمة. من ناحية أخرى، فإن تطبيق مستخلص الطحالب أكثر كفاءة من تطبيق مسحوق الطحالب، ويمكن أن يؤثر *Enteromorpha intestinalis* على معاملات النمو بتركيز صغير (5٪)، على عكس *Corallina elongata*. يجب إلقاء بعض الضوء على الأدوار الإيجابية المختلفة للطحالب في الزراعة، حيث أظهرت نتائجنا أنه يمكن استخدامها كسماد ممتاز.

الكلمات المفتاحية: الطحالب الكبيرة، *Corallina elongata*، *Enteromorpha intestinalis*، الأسمدة الحيوية، الذرة.