

Potential assay of two green algae *Ulva lactuca* and *Ulva intestinalis* as biofertilizers.

Ensayo potencial de dos algas verdes *Ulva lactuca* y *Ulva intestinalis* como biofertilizantes.

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ABSTRACT

In recent years, development has been witnessed in the market expansion of agricultural inputs and products including substances and materials that improve the functioning of the soil, plants, and the interactions between soil and plant. This is a very broad category of products and substances that often bring innovative solutions in the field of fertilization and crop protection. The objective of this study is to improve the production of the market garden where tomato plants in nurseries from powders of two green algae *Ulva lactuca* and *Ulva intestinalis*, harvested as biofertilizers in the west coast of Mostaganem province at North-Western Algeria. They have minimal effects on the environment, including soil, subterranean water, river, and estuary ecosystems such as the Cheliff river, biodiversity, and global warming. The experimental part was carried out following the method of extraction of algae and used as a biofertilizer on the tomato to its simplicity and accuracy, which is the subject of the study of the germination, growth, and the development of young plants. The results show that the powders obtained from the *Ulva lactuca* algae and used as biofertilizers are the most suitable to increase the growth of plants in the case of tomato in our study, which means a remarkable increase in stem length by compared to the other treatments with averages of 17.75 cm, 16 cm length of stem/plant, while the other treatments (*Enteromorpha intestinalis*, mixture, and reference) gave 14.75; 13.87; 13.65 cm length of stem/plant.

Keywords: seaweed, Ulvaceae, biofertilizers, sustainable agriculture, North-Western Algeria.

RESUMEN

En los últimos años, se ha observado un desarrollo en la expansión del mercado de insumos y productos agrícolas, incluidas sustancias y materiales que mejoran el funcionamiento del suelo, las plantas y las interacciones entre el suelo y las plantas.

Se trata de una categoría muy amplia de productos y sustancias que a menudo aportan soluciones innovadoras en el campo de la fertilización y la protección de cultivos. El objetivo de este estudio es mejorar la producción de la huerta donde se encuentran las plantas de tomate en viveros a partir de polvos de dos algas verdes *Ulva lactuca* y *Ulva intestinalis*, recolectadas como biofertilizantes en la costa oeste de la provincia de Mostaganem en el noroeste de Argelia. Tienen efectos mínimos sobre el medio ambiente, incluidos los ecosistemas de suelos, aguas subterráneas, ríos y estuarios como el río Cheliff, la biodiversidad y el calentamiento global. La parte experimental se llevó a cabo siguiendo el método de extracción de algas y se utilizó como biofertilizante en el tomate en su sencillez y precisión, que es objeto de estudio de la germinación, crecimiento y desarrollo de plantas jóvenes. Los resultados muestran que los polvos obtenidos a partir del alga *Ulva lactuca* y utilizados como biofertilizantes son los más adecuados para incrementar el crecimiento de las plantas el caso del tomate de nuestro estudio, lo que supone un notable incremento en la longitud del tallo respecto a los otros tratamientos con medias de 17,75 cm, 16 cm de longitud de tallo / planta, mientras que los demás tratamientos (*Enteromorpha intestinalis*, mezcla y referencia) dieron 14,75; 13,87; 13,65 cm de longitud de tallo / planta.

Palabras clave: algas, Ulvaceae, biofertilizantes, agricultura sostenible, noroeste de Argelia.

INTRODUCTION

Algae (González et al 2001; Wariaghli et al 2005; Sirbu et al., 2006; Chiheb et al., 2009; El-Gamal, 2010; Zubia et al., 2014) have long been used as organic fertilizers. It has been reported that algae contain high levels of minerals, vitamins, essential amino acids, non-digestible carbohydrates, and dietary fiber (Jimenez-Escriq & Goli Isabel, 1999; Brault et al 2005, 2006). Therefore, we were interested in studying the effects of biofertilizers based on the species of green algae *Ulva lactuca* and *Ulva intestinalis* on plant production cases of tomato. In this experimental protocol, the techniques used for the preparation of the powders of two green algae *Ulva lactuca* and *Ulva intestinalis* harvested on the west coast of the Mostaganem wilaya are described, and the use of these powders As a "biofertilizer" for the tomato plant production.

MATERIAL AND METHODS

Sampling site: Stidia is located 15 km west of the city of Mostaganem, in the center of the Gulf of Arzew, bounded by Cape Carbon to the west and cape of Salamandre to the east (Figure 1), and extends along the linear coast of 14.5 km.



Figure 1. (A) in 2004 and (B) in 2015 are Geographical location of the study area (Stidia) «Google Earth Pro». (C) Stidia site in 2016.

The east coast, which is flooded by Atlantic water, supports the dispersal of possible sources of pollution and allows for a relatively large development of the entire food chain (Safer, 2013; Taleb et al., 2015; ASAL, 2015; Azza, 2015). Algae harvesting took place in the Mediterranean Sea Mostaganem coast: Stidia during April 2016. For this study, two species of algae belonging to the class of macroalgae Chlorophyceae.

Steps for sampling and grinding green algae: The green algae represented by the two species *Ulva lactuca* and *Enteromorpha intestinalis* were rinsed with tap water and then with distilled water to remove the various parasites (Small animals, shell debris) and pollutants.

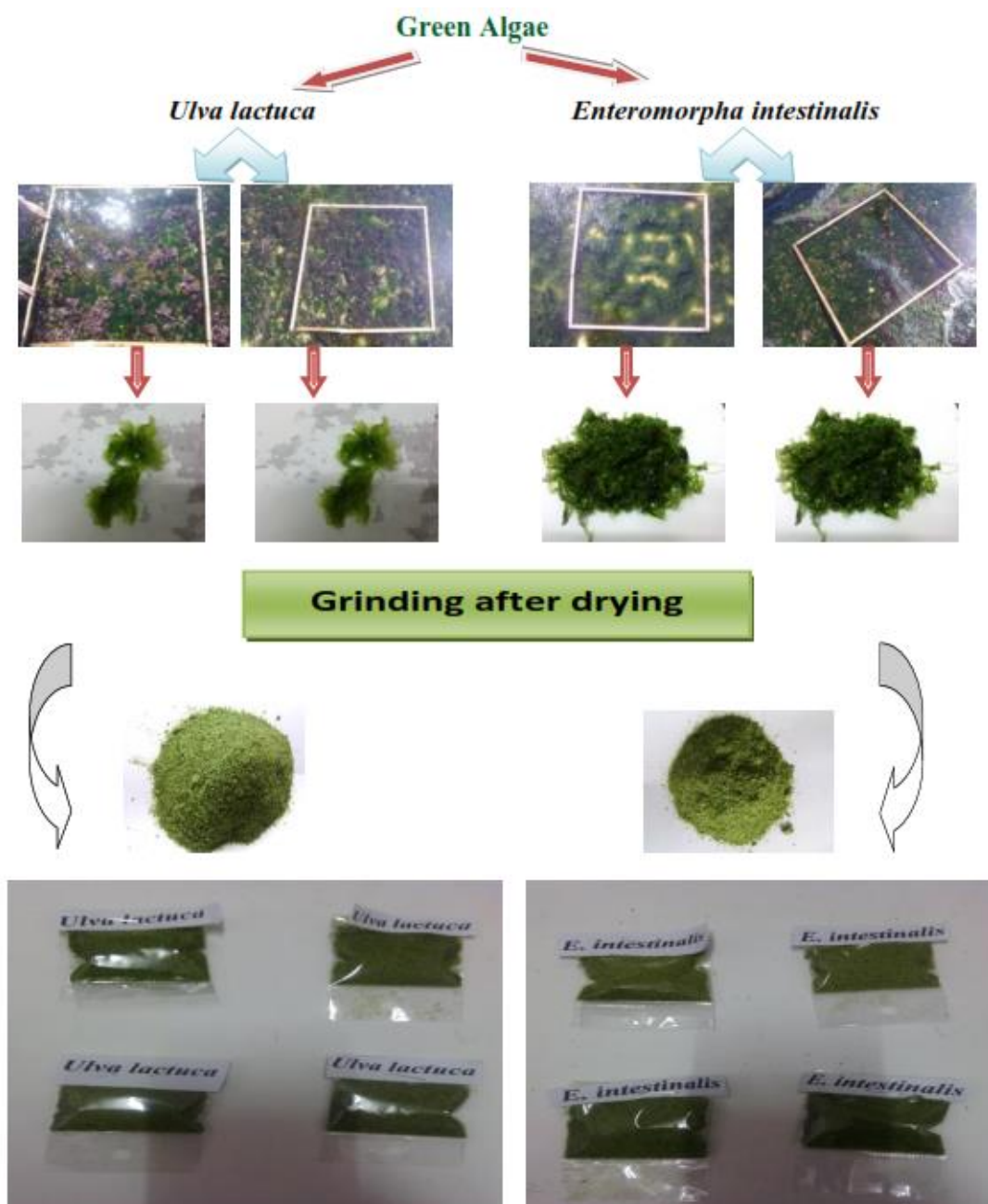


Figure 2. Stages of sampling and grinding of green algae.

Thereafter, they were dried at room temperature in the dark for a few days. Finally, they are ground to prepare the powders which are stored in the dark and in a dry place (Farid et al., 2009). These powders will be used as much as biofertilizers in tomato growing which is a new experiment made in the field of eco-agriculture in Algeria (Figure 2).

Potential assay of algal biofertilizers in tomato culture. Objectives

This trial is to study the effectiveness of green algae (*Ulva lactuca* and *Enteromorpha intestinalis*), which improves soil quality and fertilization of plant production, and finally, improve production at the level of the tomato plant as well as the protection.

Principle: The experimental device takes a square form and is subdivided into 4 blocks, each comprising four treatments, and the number of repetitions for each treatment in the different blocks (Figure 3)

Treatments (1) = *Ulva lactuca*, number of repeats = 4 seedlings (1, 2, 3, 4).

Treatments (2) = *E. intestinalis*, number of repeats = 4 seedlings (5, 6, 7, 8).

Treatments (3) = Mixture of species (70% green algae and 30% red algae), number of repetitions = 4 seedlings (9, 10, 11, 12).

Treatments (4) = Reference, number of references = 4 seedlings (13, 14, 15, 16).

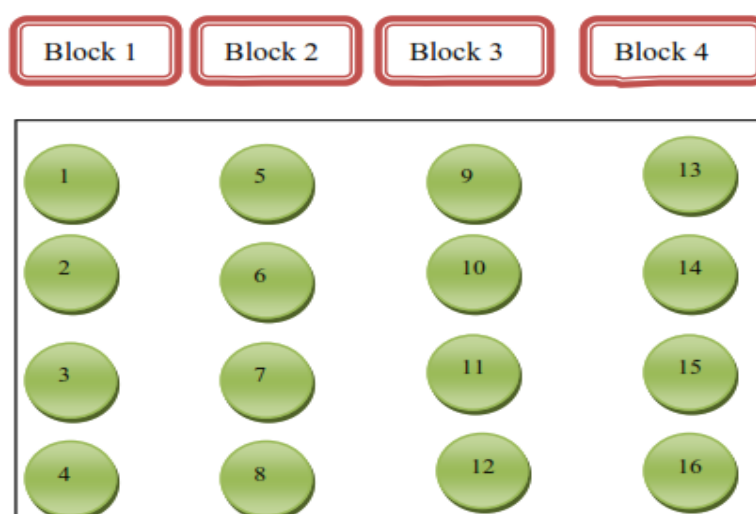


Figure 3. Experimental device (blocks).

Materials used: The tomato seeds: We worked on the seeds of the tomato (Figure 4).



Figure 4. Tomato seeds.

The types of containers: We used the alveoli and potting soil (Figure 5).



Figure 5. Soil and alveoli.

Transplantation and Treatment Condition: After sowing, watering was carried out, depending on the moisture state of the substrate, generally (3) times per week. The doses of watering were the same for all substrates. Transplanting of the seedlings to the pots took place 3 to 6 weeks after seeding (Figure 6). One week before transplanting (mean stem length is 10 cm), seedlings should be weaned by reducing watering, but 12 to 14 hours before removal from the seedbed, they should be watered extensively to avoid excessive damage to roots when they are unwrapped. This work should be done only during the afternoon or on a cloudy day to reduce transplant shock, and it will be necessary to water immediately. The treatments of 07/05/2016 at 9 am with powders: *Ulva lactuca* and *E. Intestinalis*, and the mixture, on 16 seedlings (mix the powder 4 g with the soil). The temperature was of the order of 27 ° C.



Figure 6. The transplanting of the seedlings

Watering: The tomato is not resistant to aridity. The yield decreases considerably after short periods of water deficiency. It is important to water the plants regularly, especially during periods of flowering and fruit formation. The amount of water required depends on the type of soil and weather conditions (precipitation, humidity, and temperature). On sandy soils, it is particularly important to water regularly (e.g. 3 times a week). Under good conditions, weekly watering should suffice. It takes about 20 mm of water per week when the weather is cool, but about 70 mm during arid periods. Water intake plays a major role in achieving uniform maturity and avoiding apical rot, a physiological disease associated with an irregular water supply, and calcium deficiency in the resulting fruit.

Fertilizing the tomato: The tomato is a "voracious" plant. It requires good fertilization to yield acceptable yields. In this study, algae powders of green species (*Ulva lactuca* and *Enteromorpha intestinalis*) are used as a biofertilizer to improve the production of tomato plants. To study the effects of these powders on the tomato plants, 16 pots were planted in total. In the seedlings were added 4 g of algal powder of each species (Figure 7). In parallel, a

mixture of 70%, corresponding to 2.8 g of green algae (*Ulva lactuca* 1.4 g, *Enteromorpha intestinalis* 1.4 g), and 30% red algae were prepared for 4 pots. Corresponds to 1.2 g (*Janira rubens* 0.6 g, *Laurancia obtusa* 0.6 g), with a reference test (without any addition) in the remaining (4) pots. The last 8 pots planted in tomatoes were prepared as much as references to be able to later compare them with the previous 8 pots.

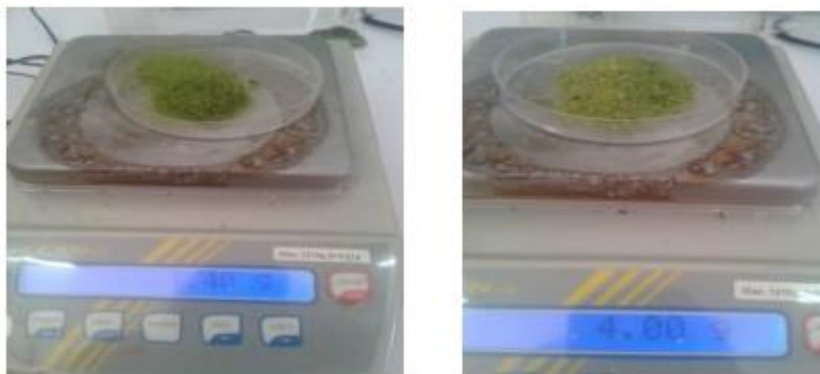


Figure 7. Measurement of algae powders in the laboratory.

RESULTS AND DISCUSSIONS

The number of plant and length of stems: Following the sowing of the tomato seeds (Figure 8), the length of the seedlings was measured at the end of each ten days for one month, to confirm whether these seedlings are growing or not growth. During this period estimated at one month, nothing is added to the seedlings except for the watering that takes place every two days.

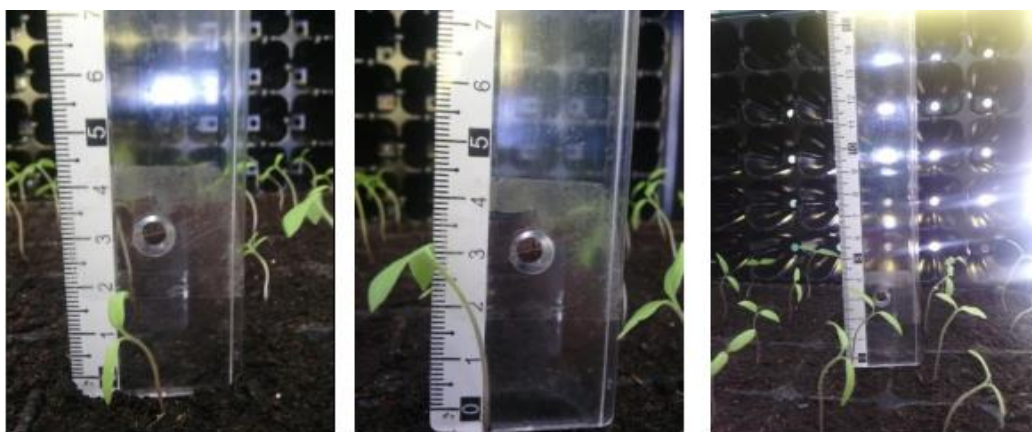


Figure 8. Measurement of stems.

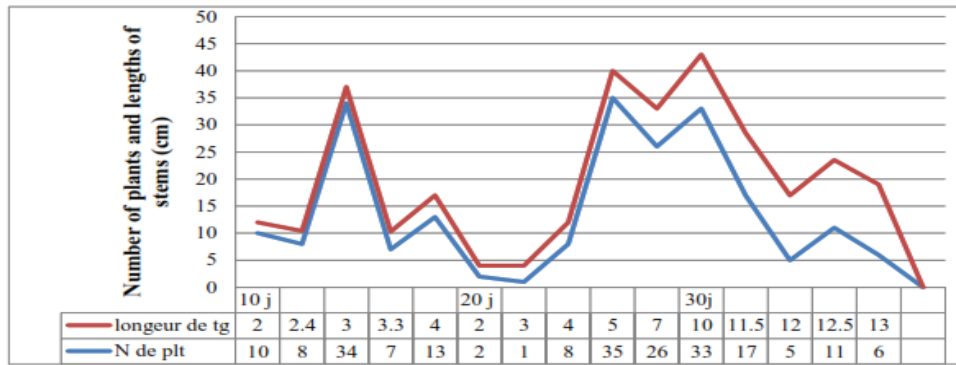


Figure 9. Seedling number curve and stem length.

After the sowing of the tomato seeds, the stem lengths were measured instead of the test (Figure 9), for one month from 7 April to 6 May of 2016, to be able subsequently to select the seedlings which have the same length and this for the preparation in the following step which is the transplanting. In our experiment, 72 seedlings were obtained; of which 33 seedlings were the same size (10 cm). Thus, for our study, 16 seedlings were selected for treatment and subsequent follow-up. According to Figure 9, there are three different decades. In the first decade, we obtained seedlings of an average size equivalent to 3.00 cm (with a minimum of 2 cm and a maximum of 4 cm). In the second decade, the length of the stems reached an average of 4.50 cm (with a minimum of 02 cm and a maximum of 07 cm). Finally, a third decade was propitious for a selection of seedlings intended for the next stage which is the transplanting; the latter was of an average length of 11.00 cm (with a minimum of 10 cm and a maximum of 13 cm).

Measurement of stem length after transplanting and use of biofertilizers: The next step is the transplanting (Figure 10) of the 24 seedlings of the same stems (ten centimeters 10 cm), adding to each pot bio-powder fertilizers of the green algae *Ulva lactuca*, *Enteromorpha intestinalis*, a mixture of the 2 algal species and the references, to know the effects of the powders of each alga used as biofertilizers on the growth of tomato, while comparing it with the pots or the mixture of the algae powder and the reference to which no chemical or organic substance has been added. The measurements of stems are made every ten days.



Figure 10. Measurement of stems every 10 days.

Statistical treatments of stem length per plant

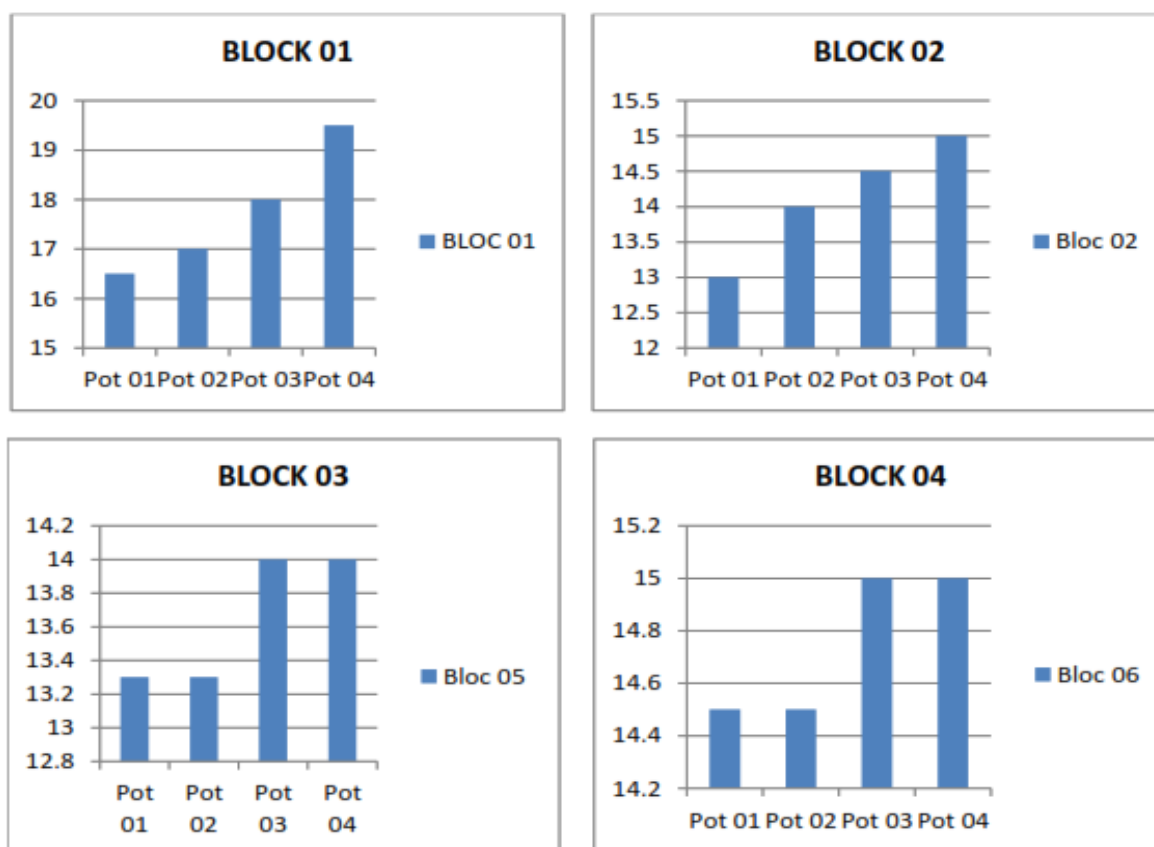


Figure 11. Histogram of the length of the stems in (cm) according to the 4 treatments.

The histogram (Figure 11) indicates the length of the stems as a function of four treatments (Figure 12), in which treatment in block one of the green alga species *Ulva lactuca*, or a stem length which varied from 16.50 cm to 19.50 cm. Thus, the treatments cited above are beneficial (remarkable) results compared to the other blocks (other treatments by the two remaining algal species, the mixture, and the reference).

For the green species *Enteromorpha intestinalis*, a stem length from 13.00 cm to 15.00 cm was observed.

For the mixture, a stem length from 13.30 cm to 14.00 cm was noted, while for the reference it was from 14.50 cm to 15.00 cm.



Figure 12. Measurement of stem lengths.

Comparison between reference and *Ulva lactuca*

Table 1. Comparison of the statistical parameters of the reference with biofertilizers of *Ulva lactuca*.

	<i>Number</i>	<i>Average</i>	<i>Variance</i>	<i>Standard deviation</i>
<i>Reference</i>	6	14,75	0,08	0,28
<i>Ulva lactuca</i>	6	17,75	1,75	1,32

We conclude from Table 1 that the average length of the stems of the plants treated with *Ulva lactuca* is higher. It is estimated to be 17.75 cm relative to the average length of the reference plant stems which is 14.75 cm (Figure 13). Noting a minimum of a stem languor of 16.50 cm and a maximum of 19.50 cm for this treatment (with the powder of *Ulva lactuca*). The standard of deviations value mentioned that there is a very large variability between the sets of treatment data by *Ulva lactuca* compared to the reference which is evaluated at 1.75 compared to the reference which is 0.08.



Figure 13. Results obtained from treatments (A) Reference (B) *Ulva lactuca*

Comparison between Reference and *Enteromorpha intestinalis*

Table 2. Comparison between Reference and *Enteromorpha intestinalis*

	<i>Number</i>	<i>Average</i>	<i>Variance</i>	<i>Standard deviation</i>
<i>Reference</i>	6	14,75	0,08	0,28
<i>E. intestinalis</i>	6	13,87	0,72	0,85

It can be said from Table 2 that the mean length of the stems of plants treated with *Enteromorpha intestinalis* (Figure 14) is small and is evaluated at 13.87 cm from the average

length of the reference plant stems which is 14.75 cm, noting a minimum of 13.00 cm long stalks and a maximum of 15.00 cm for this treatment (with *Enteromorpha intestinalis* powder). The variance analysis shows that there is considerable variability between the sets of treatment data by *Enteromorpha intestinalis* which is evaluated at 0.72 compared to the reference which is 0.08.



Figure 14. Results obtained from treatments (A) Reference (B) *Enteromorpha intestinalis*.

Comparison between reference and mixture

Table 3. Comparison between the results obtained from treatments reference and mixture

	<i>Number</i>	<i>Average</i>	<i>Variance</i>	<i>Standard deviation</i>
<i>Reference</i>	6	14,75	0,08	0,28
<i>Mixture</i>	6	13,65	0,16	0,40

From Table 3, it can be seen that the average stem length of the plants treated by the powder mixture of the different algal species is small and is estimated to be 13.65 cm from the average length of reference plant stems is 14.75 cm (Figure 15). It is noted a minimum length of 13.30 cm stems and a maximum of 14.00 cm for this treatment (the mixture of powder of different algal species). By analyzing the growing average, a slight variability is observed between the sets of the mixing treatment data which is evaluated to 13.65 cm compared to the reference which is 14.75 cm.



Figure 15. Results obtained from treatment reference and mixture of the powders of the 2 algae.

Comparison and confirmation of the effects of different biofertilizers: Considering the results obtained for the various treatments extracted from green algae. We can assert that among the four treatments T1, T2, T3, and T4 respectively, *Ulva lactuca*, *Enteromorpha intestinalis*, algae mixture, and the reference, that the treatment of *Ulva lactuca* T1 is the best "biofertilizer".

The results show that the powders obtained from the algae and used as biofertilizers are the most suitable to increase the growth of plants the case of tomato in our study, which means a remarkable increase in stem length by compared to the other treatments with averages of 17.75 cm, 16 cm length of stem/plant, while the other treatments (*Enteromorpha intestinalis*, mixture, and reference) gave 14.75; 13.87; 13.65 cm length of stem/plant.

Our results are confirmed by recent studies, where it has been shown that green algae extracts have beneficial effects on germination, root, and stem elongation in the plants (Ei-Seekh & Ei-Saied, 2000; Hashem et al., 2019). Our results remain inferior to those obtained by EI-Sheekh & EI-Saied, 2000, which are explained by adverse climatic and physiological conditions such as (Climatic changes at the time of germination, the phenomenon of alternation).

In conclusion, the use of algae as an amendment has given way to the use of flours and liquid extracts based on algae. While solid fertilizers improve the physical, biological, and chemical properties of soil, liquid extracts occur at different stages of plant production by influencing seed germination, plant growth and resistance, crop yields, quality, and the conservation of crops. For this reason, we are interested to study the effect of *biofertilizers* based on some Mediterranean algae in the coastal of Mostaganem "Stidia" on the production of seedlings or floral plants.

This study aimed to develop biofertilizers to produce tomato plants from bio-based *algae fertilizers*. In our case, we have taken two green algal species which are *Ulva lactuca* and *Ulva intestinalis*. The results of this study showed that tomato plants sown under *Ulva lactuca* treatment gave a higher stem length compared to other treatments, and for *Ulva intestinalis* treatments, the witness. The experimental results obtained by our study; show that organic fertilizers based on the species of *Ulva lactuca* are solid fertilizers that improve and promote the germination and growth of plants.

Finally, this type of fertilizer should be encouraged and applied to have good plant production that is desirable for human consumption and which does not hurt people's health (Hashem et al., 2019). The use of this biofertilizers does not contribute to the pollution of waters near agricultural land such as rivers, underground waters, and no longer to ecosystem disturbance of the ocean coast "*eutrophication phenomenon*" from agricultural activities (Kies & Kerfouf, 2014a,b; Kies, 2015; Kies et al., 2020). The macroalgae (*green algae*) used; *Ulva lactuca* and *Ulva intestinalis* collected from the Mediterranean Sea in these biofertilizers have remarkable anti-bacterial effects, which is confirmed by the *second part* of this experiment.

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REFERENCES

- Agence Spatiale Algerienne (ASAL)., 2015. Rapport sur matérialisation par GPS du Méridien de Greenwich traversant la ville de Stidia (Wilaya de Mostaganem).
- Azza, Z., 2015. Utilisation de l'oursin *Arbacia lixula* comme bioindicateur macrobenthique pour évaluer la qualité microbiologique au niveau de la plage de Stidia. Diplôme de master, université de Mostaganem, Algérie.
- Brault, D., Duche, D., Deniaud, E., Dion, P., Le Marrec, M-C., 2005. Algues et alimentation animale. *Rapport CEVA Algorythme n°72, 4eme trimestre.*
- Brault, D., Duche, D., Robic, A., Menuy, A., Le Marrec, M-C., 2006. Les algues Source de médicaments. *Rapport CEVA, Algorythme n° 76, 4eme trimestre.*
- Chiheb, I., Riadi, H., Martinez-Lopez, J., Dominguez Seglar, J-F., Gomez., Vidal, J-A., Bouziane, H., Kadiri, M., 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco, *African Journal of Biotechnology Vol. 8 (7), pp. 1258-1262, ISSN 1684-5315.*
- Ei-Sheekh & Ei-Saied., 2000. Les extraits d'algues en agriculture vers une meilleure compréhension de leur mécanisme d'action, 1p.
- El-Gamal, A., 2010. Biological importance of marine algae, *Saudi Pharmaceutical Journal, Vol 18.*
- Farid, Y., Etahiri, S., Assobhei, O., 2009. Activité antimicrobienne des algues marines de la lagune d'Oualidia (Maroc) : Criblage et optimisation de la période de la récolte, *Journal of Applied Biosciences 24 : 1543 – 1552, ISSN 1997-5902.*
- Gonzalez Del Val, A., Gerardin, P., Farny, K., Simac, C., Laurenta, A-F., Grandbastiend, B., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo, E., Jimenez Del Rio, M., Reina, G-G., Pelaez, F., 2001. Screening of antimicrobial activities in red, green, and brown macro algae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.* 4.
- Hashem, H. A., Mansour, H. A., El-Khawas S. A., and Hassanein, R. A. 2019. The Potentiality of Marine Macro-Algae as Bio-Fertilizers to Improve the Productivity and Salt Stress Tolerance of Canola (*Brassica Napus L.*) Plants. *Agronomy* 9 (3):1-18. <https://doi.org/10.3390/agronomy9030146>
- Jimenez-Escrig, A., Goni Isabel, C. A., 1999. Évaluation A nutritionnel y efectos fisiológicos de macroalgas marinas comestibles. *Archivas Latino americanos de Nutrición*, 49 :114-120p.

- Kies, F., & Kerfouf, A., 2014a. State of the algal biodiversity of the Mostaganem coast (West of Algeria), Conference paper: Monitoring of Mediterranean Coastal Areas: Problems and Measurement Techniques, volume: a fifth international symposium. Livorno (Italy) 17-18.
- Kies, F., & Kerfouf, A., 2014b. Impact of the climate change on the west coast of Algeria: Gulf of Oran, Arzew, and Mostaganem. *Journal of Sustainability, Agri, Food and Environmental Research*, 2(3): 1-15. KIES, F., KERFOUF, A. 2014b. Physico-chemical characterization of surface waters of the west coast of Algeria: Bay of Mostaganem and Cheliff estuary. *Journal of Sustainability, Agri, Food and Environmental Research*, 2(4): 1-10.
- Kies, F., 2015. Assessment of surface water chemistry and algal biodiversity in the Bay of Mostaganem and the Cheliff estuary: North-western Algeria. *Journal of Sustainability, Agri, Food and Environmental Research*, 3(1): 1-16.
- Kies, F., Monge-Ganuzas, M., De Los Rios, P., Elegbede., I & Corselli, C., 2020. Integrated Coastal Zone Management (ICZM) Framework and Ecosystem Approach: eutrophication phenomenon at the Mediterranean Sea. *Bulletin de la Société Royale des Sciences de Liège* [En ligne], Volume 89: 55-73, <https://popups.uliege.be:443/0037-9565/index.php?id=9493>
- Safer, I., 2013. Etude des analyses physico-chimiques et microbiologiques de la lagune de Stidia. Diplôme d'ingénieur d'état, Université de Mostaganem.
- Sirbu, R., Sava, C., Ghergic, D-L., Nadine, A-F, Mouima, P., 2006. Caractérisation de certains principes actifs de *Ulva lactuca* et *Ulva rigida* algues vertes du littoral roumain de la mer noire. Université « Ovidius » Constanta, scientific study & research vol. vii (1), Issn 1582-540x.
- Taleb, M-K., Tahraoui, F., Kerfouf, A., 2015. Impact of Anthropic Activity on a Coastal Environment of Ecological Interest: Stidia (Mostaganem - Algerian West). Article in the international journal of sciences: basic and applied search (IJSBAR).
- Wariaghli, F., El Ghzaoui, G., Al Amouri, M., 2005. Les algues et leur intérêt en écotoxicologue. Thèse de doctorat, Université Mohamed V Faculté des sciences Rabat-Agdal.
- Zitouni, H., 2015. Valorisation nutritionnelle d'algues marines du littoral Algérien chez le ruminant via des méthodes chimiques, biologiques et moléculaires. Thèse de doctorat, Université des Frères Mentouri Constantine.
- Zubia, M., Freile-Pelegrin, Y., Robledo, Y., 2014. Photosynthesis, pigment composition, and antioxidant defenses in the red alga *Gracilariopsis stenuifrons* (Gracilariales, Rhodophyta) under environmental stress, IV Latin American Congress of Algae Biotechnology (CLABA) and IV Redealgas Workshop.

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