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Macroalgae as Soil Conditioners or Growth Promoters of *Pisum sativum* (L)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IJD and SHAH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ALI and ARC managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Mexico has long littorals that receive great amounts of marine algae that are wasted despite being potentially useful as growth promoters without the inconveniences of agrochemicals. Some macroalgae have been used with excellent results as soil conditioners and fertilisers in agriculture. In this study nine macroalgae from the coasts of Veracruz (Gulf of Mexico) and one from Guerrero (Mexican Pacific), Mexico, were tested. They were added to the soil in the form of fragments and of silage in liquid around *Pisum sativum* plants. A two-way random design with four replicas was followed. Two controls, hormones and water, were also used. Growth was recorded every third day. Results showed that four algae promoted growth ($p < 0.05$) *Ulva fasciata* (ensiling treatment) and *Ulva lactuca* (ensiling treatment) and *Gracilaria caudata* (fragment treatment) and *Palisada perforata* (fragment treatment), compared with the hormones and water controls. The activity of *Ulva fasciata*

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and *Palisada perforata* is recorded for the first time. Since the control plants treated with hormones grew much less than those treated with the four algae, we conclude that the release of all algae compounds was responsible for the growth, and not the hormone mimetics.

Keywords: Macroalgae; Mexico; *Pisum sativum*; fertilizer; soil conditioner.

1. INTRODUCTION

Today, marine algae and the products obtained from them constitute an object of interest for their use in sustainable agriculture [1]. Marine algae are the most important group of organisms that have been widely used as biostimulants of plant growth and as fertilisers of soil for crops [2].

Algae have been used over millennia as fertilisers and soil conditioning agents, to improve soil fertility and crop productivity [3,1,4,5]. The number of species involved is small, but the volume of algal biomass may be considerable [4].

Marine algae components, such as macro and microelements, amino acids, vitamins, cytokinins, gibberellins, auxins and abscisic acid (ABA), stimulate growth, as they affect the cellular metabolism of plants improving growth speed and crop yield [6,7,1].

Algae extracts act as biostimulants, due mainly to the presence of plant hormones [8]. The main phytohormones identified in algae extracts are: auxins (key regulators in most aspects of plant growth and development; [9,10]), cytokinins (essential in cellular division, they control the root-shoot relationship, they interrupt bud latency, they affect the movement of nutrients, apical dominance, floral development and seed germination; [7,11,10]), gibberellins (they have an effect on diverse physiological processes like seed germination, stem elongation, leaf expansion and seed and flower development; [12]), abscisic acid and ethylene [8,2]. The effects on crops include an improved germination and establishment of seeds, an increase in growth and flower and fruit production, a resistance to biotic and abiotic stress, and a better post-harvest shelf life [3,13,1,4,14,5]. These effects have often been attributed to the presence of plant growth hormones and compounds like trace elements, macro and microelements, amino acids and vitamins present in the extracts [15,16].

Plants grown on soil treated with extracts, or that have their leaves treated, present a wide range

of positive responses that have been well documented [17,18,19,20,21,22,23,24,3,25,26,27,15,28].

Marine algae have been widely used in agriculture as a result of their biostimulant activity. Among these are, of the Division Rhodophyta: *Coralina mediterranea*, *Jania rubens* and *Pterocladia pinnata*, of the Chlorophyta: *Cladophora dalmatica*, *Ulva intestinalis* (= *Entromorpha intestinalis*) and *Ulva lactuca*, and of the Phaeophyta: *Ascophyllum nodosum*, *Ecklonia maxima* and *Saragassum* spp. [29]. [2] obtained excellent biostimulant results with *Ascophyllum nodosum*, *Laminaria hyperborea*, *Laminaria digitata*, *Fucus vesiculosus*, *Durvillea potatorum* and *Ecklonia maxima*. Recent studies have recorded an improved growth and yield in agricultural and horticultural crops like wheat [30] and tomato [31], among others. The growth of roots and shoots in the model plant *Arabidopsis thaliana* also improved with an algae extract treatment [13].

Several authors have stated that the presence of various trace metals (Cu, Co, Zn, Mn, Fe, Ni, Mo and B) in soluble form in algae extracts is responsible for the growth of treated plants [32,33]. [3] concluded that the growth in length of the plant, the shoot and the root of *Vigna sinensis* followed the presence of macroelements (K, Na, Mg and Ca), microelements (I, Fe, Mn, Co, Zn, Cu, Mo, Bo, Pb, Cd, Cr and Sr) and vitamins (vitamin A and vitamin C) in the algae extracts under study. On the same hypothesis, [34] coincided in holding macro and micronutrients accountable, and the possible presence of growth hormones such as auxin, gibberellin and cytokinins, as generating the growth stimulus. A second characteristic feature of algae is the presence of several types of mucopolysaccharides, carbohydrates and organic matter that improve soil fertility and its capacity to retain humidity.

The test organism selected for this study was the common pea *Pisum sativum* (Linnaeus), a legume amply adaptable to soils of various types, from sand to clay. The pea has a relatively

shallow root system that is highly efficient in the use of water. It is thus used to obtain a good crop rotation in regions where water conservation is a problem [35,36,37]

2. MATERIALS AND METHODS

2.1 Collection of Algal Material

Macroalgae were collected from different localities along the coasts of Mexico (Table 1): along the Gulf of Mexico, in the state of Veracruz, Playa Muñecos (19°44'45"N - 96°24'24"W), Costa de Oro (19°8'40"N - 96°6'27"W) and La Mancha (19°35'42"N - 96°23'10"W), and along the Pacific, in the state of Guerrero, Playa Papanoa (17°19'28"N - 101°02'28"W). The phycological material was collected manually, stored in hermetic plastic bags, frozen and transported to the laboratory in solid CO₂ to prevent decomposition and enzymatic metabolism. Once in the laboratory, it was washed under running water. Epiphytes, organic matter and sand were removed under an optic microscope. The clean samples were kept in a freezer at -20°C until used.

2.2 Preparation of the Algae

Two treatments were carried out in order to liberate algal components: one to obtain a silage (Ensiling Treatment) and another, to contrast this treatment, in which the algal biomass was fragmented (Fragment Treatment).

2.3 Ensiling Treatment

The algae were unfrozen at room temperature, then 150 g of each species were crushed and the

algal material of each species was placed in a plastic container. To this crushed material were added 500 ml of water. The container was covered with a gauze mesh and stored in the shade for three months. The silages were stirred every third day. The final concentrate was diluted at a 1:10 ratio and stored in the shade at room temperature until used.

2.4 Fragment Treatment

One hundred grams of each algal species were cut with scissors to obtain fragments of approximately 4 mm long. These processed algae were stored at -20°C until used in the tests.

2.5 Germination of *Pisum sativum*

Approximately 150 *Pisum sativum* seeds were placed in an outdoor germinator and watered every third day, until 3.35 ± 0.52 cm long seedlings, measured from the base of the stem to the apical meristem, were obtained. Eighty plants of the same size were selected to start the treatments.

2.6 Experiments

2.6.1 Ensiling

For the preparation of the *Pisum sativum* seedlings, 150 g of earth were mixed with 100 g of sand and placed in a pot, in order to ensure good drainage and the conservation of humidity, and to avoid waterlogging and the resulting fungal infestation in the root. The pots were kept outdoors throughout the experiment. Then, 6 ml of silage mixed with 10ml of running water, for a total of 16 ml, were given every third day to each

Table 1. Average growth (± SD) of *Pisum sativum* obtained with the Ensiling and Fragment treatments, and the hormones and water controls

Species	Average ± SD (Ensiling)	Average ± SD (Fragment)
² <i>Gracilaria caudata</i> J. Agardh	9.8 ± 5.03	10.4 ± 4.4
² <i>Gracilaria cervicornis</i> (Turner) J. Agardh	9.1 ± 3.9	9.7 ± 4.4
² <i>Palisada perforata</i> (C. Agardh) D.J. Garbary & J.T. Harper	9.9 ± 4.8	10.6 ± 5.2
³ <i>Ulva lactuca</i> Linnaeus	10.4 ± 5.1	9.2 ± 4.9
¹ <i>Ulva fasciata</i> Delile	10.8 ± 4.5	10.2 ± 4.9
¹ <i>Codium taylorii</i> P.C. Silva	9.1 ± 4.9	10.008 ± 4.6
¹ <i>Padina gymnospora</i> (Kützting) Sonder	9.9 ± 3.9	9.8 ± 4.7
⁴ <i>Padina durvillaei</i> Bory Saint-Vincent	9.6 ± 4.7	9.2 ± 4.3
¹ <i>Sargassum polyceratium</i> Montagne	8.2 ± 4.3	10.3 ± 5.2
Hormones control		8.5 ± 4.4
Water control		8.1 ± 3.3

Collection localities: 1. La Mancha, 2. Costa de Oro, 3. Playa Muñecos, 4.Papanoa

plant in the treatment. Plant length was recorded every third day as has been indicated.

2.6.2 Fragments

For this treatment, 100 g of earth, 100 g of sand and 50 g of algal fragments were mixed. Running water (16 ml) was provided every third day. Plant length was recorded as is indicated above.

2.6.3 Controls

Two controls were used. In the first, the seedlings were watered only with running water, pH 7.0, 16 ml every third day, and in the hormones control they were treated with a mixture of Agromil V® commercial hormones with the following composition: cytokinins 81.90, gibberellins 31.0, auxins 30.5, folic acid 0.92, pantothenic acid 12.53, riboflavin 0.86, nicotinamide 0.16, choline 748.81, niacin 84.56 and thiamin 100.11, all p.p.m. The product was applied to the leaves following the manufacturer's recommendations. The experiment lasted 21 days.

2.7 Experimental Design

Three algae species of each of the three Divisions were used in each of the two treatments, with a replica factor of four plants per treatment per algae species. The design used randomised blocks. The water and hormones controls were carried out in quadruplicate.

2.8 Statistical Analyses

The justification of the assumptions underlying the ANOVA model was reviewed with the Omnibus and Levene tests. The experimental design was analysed with a two-way ANOVA and four replicas, using the NCSS statistical software. LSD multiple comparisons tests were carried out.

2.8.1 Results

The average length values of the *Pisum sativum* plants during the 21 days of the experiment with the ensiling, fragment and the control treatments are presented in Table 1. The greatest average value with the silage was recorded for *Ulva fasciata* (10.8, s = 4.5 cm) and the minimum value was obtained for *Sargassum polyceratum* (8.2, s = 4.3 cm). In the case of the fragments, the greatest average value was recorded for *Palisada perforata* (10.6, s = 5.2 cm) and the minimum value was obtained for *Padina durvillaei* (9.2, s = 4.3 cm). The maximum

average length in the hormones control was 8.5, s = 4.4 cm and that in the water control was 8.1, s = 3.3 cm.

The ANOVA indicated significant differences for the factor growth ($p < 0.05$) in both treatments (Ensiling and Fragments). The variance analysis of all treatments also indicated significant differences ($p < 0.05$).

The LSD a posteriori tests ($p < 0.05$) indicated that the silages of *Ulva lactuca* and *U. fasciata* formed a group with the greatest growth. The growth averages of the other species were not different among species. The least growth was obtained for *Sargassum polyceratum*, *Gracilaria cervicornis* and *Codium taylori*. Similarly, the controls presented the least seedling growth, as was recorded for these last three algae species.

The differences in average growth recorded for the ensiling and fragment treatments are presented in Figs. 1 and 2 respectively.

3. DISCUSSION

The results obtained in this study indicated that the growth of the *P. sativum* plants treated with macroalgae extracts is due to the algal compounds and not only to phytohormones. This conclusion was arrived at since, when comparing the growth of the plants treated with silage and fragments, with that of the plants treated with phytohormones (Agromil V®), the first plants grew best. These results coincide with the proposals of [1,4,38,39,13,40].

The ensiling and fragment treatments promoted a greater growth in the *P. sativum* plants, since macronutrients, micronutrients and other macroalgal substances were available for the roots to absorb. This coincides with the findings of [1,32,5,4] who, using commercial extracts prepared with a combination of several algae without hormones, also increased the growth of plants treated with extracts and ascribed this effect to the presence of macro and micro-element nutrients, such as amino acids and vitamins. In addition to promoting plant growth, macroalgae affect the physicochemical and biological condition of the earth, enhancing its health by promoting a greater capacity to retain liquids and increasing the growth of beneficial microorganisms [1,32].

It was observed that the plants treated with hormones presented the least stimulus for growth, as occurred with the water control. These

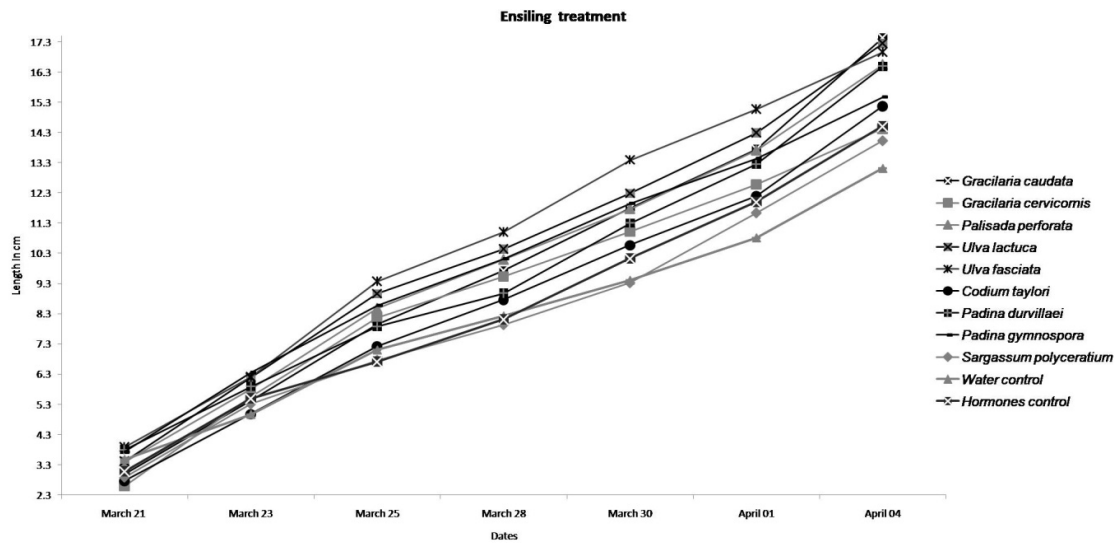


Fig. 1. *Pisum sativum* growth with the ensiling treatment

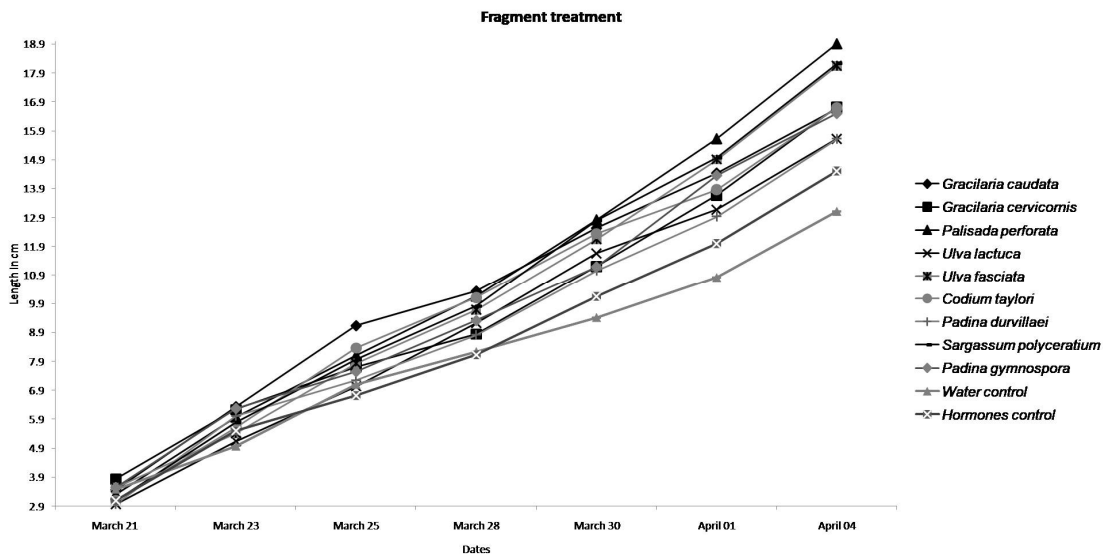


Fig. 2. *Pisum sativum* growth with the fragment treatment

results contrast with those of [32,1,41,42] who considered that, apart from nutrients, substances similar to growth hormones, such as cytokinins, auxins or gibberellins, could be present.

The growth promoting activity of the species *Padina durvillaei* and *Sargassum polyceratum* of the Division Phaeophyta has not yet been reported in the literature. Of note is that *S. polyceratum* stimulated growth only with the fragment treatment, and not with the ensiling of the alga compared with hormone control. The

doubt remains whether there are substances similar to hormones in this alga, since the presence of these substances has been previously reported for some species of the Division Phaeophyta, particularly *Sargassum* spp. and *Sargassum muticum* [43,44].

In the case of the species of the Division Rhodophyta that were analysed, *Gracilaria caudata*, *G. cervicornis* and *Palisada perforata* had a positive effect on growth when fragments were provided, with *G. caudata* and *P. perforata*

producing the greatest growth. Different authors have worked with various species of Rhodophyta. Of these, [41] reported that after treating wheat with macroalgae extracts, the yield and nutritional quality (carbohydrates, proteins and minerals) of the grains improved when the plants were treated with pulverized extracts, at ratios of 0.25%, 0.50% and 1.0% of *Kappaphycus alvarezii*. These same authors, sometime later, concluded that not all commercial extracts come from brown marine algae, but they also come from red algae such as *K. alvarezii* which, given to tomato plants, result in a 60-89% improved growth and yield of the fruit in comparison with control plants sprayed with only water [45]. In our case, the red alga that produced the greatest growth of *P. sativum* was *Palisada perforata*, Order Ceramiales, with the fragment treatment. Of all marine algae, *Ascophyllum nodosum* (Phaeophyta) is the species that has been most studied and used in agriculture, since it has been proven that its extracts are the most biologically active of all the commercial products, and it is also the most used in Europe and North America [20]. Commercial extracts available today are prepared mainly from Phaeophyta species that include: *A. nodosum*, *Laminaria* spp., *Ecklonia maxima*, *Sargassum* spp. and *Durvillaea* spp.. However, other species like *Fucus serratus*, *Enteromorpha intestinalis*, *Ulva lactuca* and *Kappaphycus alvarezii* have been used with the same results [46,13,47,48]. We can highlight that the species we report for the first time as having a growth promoting activity are: *Ulva fasciata* (Chlorophyta), *Gracilaria caudata* and *Palisada perforata* (Rhodophyta).

The use of algae as growth promoters or even as agricultural soil conditioners has a great potential in Mexico. Littoral currents deposit great amounts of algae on the shores, however they are wasted, particularly in the Mexican shores of the Gulf and Caribbean where they are treated as rubbish and are buried or burnt without giving them any use [49].

4. CONCLUSIONS

We can conclude that some macroalgae promoted significantly the growth of *Pisum sativum* L., particularly two algae from fragment treatment (*Palisada perforata* and *Gracilaria caudata*) and two from ensiling treatment (*Ulva lactuca* and *Ulva fasciata*). The best growth was obtained with ensiling treatment reached with *Ulva fasciata* as the better growth promoter. This

is the first report on growth promoter activity of *Palisada perforata*, *Gracilaria caudata* and *Ulva fasciata*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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