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Production of bio-fertilizer from *Ascophyllum nodosum* and *Sargassum muticum* (Phaeophyceae)

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Abstract Oceans and seas form a large body of water that contains a natural biodiversity. For humans, represents a resource, which makes this a point of interest, from researches to improve the economy. Seaweeds produce many compounds and secondary metabolites that can be used in different fields of industry such as food, agricultural, pharmaceutical and health. Even though seaweeds are ancestral resources, recently it was notorious a global interest in knowing more about its potentials, where biotechnology plays an important role in research. Studies showed that seaweed has many bioactive compounds beneficial to plant development, giving them a great potential as an agricultural fertilizer. Adding seaweeds to the soil provides organic matter, minerals, trace elements, growth plant regulator, metabolites, vitamins, and amino acids and it can work as a soil conditioner. In Portugal, the use of seaweeds for agriculture is important since long time ago. In the past, populations that lived near coastal zone depended on the seaweeds as a family subsistence but, throughout the years, synthetic fertilizers replaced seaweeds. Our work aimed to assess the potential of the extracts obtained from *Ascophyllum nodosum* and from *Sargassum muticum* as an agricultural fertilizer. This evaluation was carried out with rice plants (*Oryza sativa*) and lettuce (*Lactuca sativa*), in germination bioassays, the culture of rice and lettuce plants in pots, and culture of lettuce plants in hydroponics. For that, seaweed liquid extracts were used in different concentrations in different bioassays. Results show that extracts obtained from two seaweeds, *A. nodosum* and *S. muticum*, can be promissory plant biofertilizer at a concentration of 25% and had a positive effect on seed germination, plant development, and production.

Keyword: fertilizer; bioactive compounds; *Ascophyllum nodosum*; *Sargassum muticum*; *Oryza sativa*; *Lactuca sativa*

1 INTRODUCTION

The seaweeds are benthic organisms, usually attached to solid substrates, ranging from shallow coastal waters to around 90 m deep (Akila and Jeyadoss, 2010; Pereira and Correia, 2015). Despite being devoid of leaves, stems, roots or vascular system, seaweeds are photosynthetic organisms, primary producers and one of the main sources of organic matter for aquatic ecosystems, playing a crucial role in the carbon cycle as they fix it through photosynthesis. This permits to make an analogy between the values of seaweeds in marine ecosystems as good as higher plants in terrestrial ecosystems (Cunha-Santino et al., 2008; Silva, 2009; Akila and Jeyadoss, 2010).

Belonging to Eukarya Domain and Kingdoms Plantae (green and red algae) and Chromista (brown algae), seaweeds exhibit a wide variety of shapes, sizes, and colours, not found combined in a homogeneous group. Based on their colour and chemical characteristics, they are classified into three phyla: Chlorophyta (green algae), Rhodophyta (red algae) and Heterokontophyta or Ochrophyta (class Phaeophyceae) (brown algae) (Silva, 2009; Pereira and Correia, 2015).

As benthic organisms, seaweeds are exposed to

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different variations of environmental factors, such as the concentration of dissolved gases, light intensity, salinity and nutrient concentration, temperature, UV rays, various pollutants, pathogens (fungi, viruses, and bacteria) and predators. Thus, it is therefore essential for seaweeds to produce a wide variety of secondary metabolites in order to ensure their adaptation and survival (Silva, 2009; Gressler, 2010).

In general, seaweeds are rich in polyunsaturated fatty acids, steroids, terpenes, carotenoids, sulphated polysaccharides, sesquiterpene hydroquinins, mycosporins, acetogenins, phenols, amino acid derivatives, various vitamins (A, B_1, B_{12}, C, D), as main part of other biochemicals (Gressler, 2010; Safinaz and Ragaa, 2013). Many of these molecules show different biological activities such as antiviral, antibacterial, antifungal, antitumor, anticoagulant, hypotensive and antioxidant, revealing the great potential seaweeds could have in the different industries (Gressler, 2010; Limberger and Gheller, 2012).

Seaweeds provide a wide range of products for different industries, such as the food industry, pharmaceutical, and cosmetics industry, agriculture (fertilizers and bio-stimulants), water and effluents treatment (Pereira, 2010; Pereira and Correia, 2015). The beneficial effect of seaweed extracts was tested in many crops: black gram and green gram (Venkataraman et al., 1993), sesame (Gandhiappan and Perumal, 2001), and capsicum (Veeragurunathan et al., 2011). Being a cheap and abundant source of nutrients, minerals, and natural antioxidants, seaweeds can be beneficial in improving seedling emergence and vigor.

The fertilizer potential of seaweeds, mainly from the Phaeophyceae class, has been explored for many centuries (McHugh, 2003; Thirumaran et al., 2009). Recently there has been an increased interest regarding the applications of seaweeds as an agricultural fertilizer and how they can be applied, as whole seaweed, in small pieces or as a liquid extract (Thirumaran et al., 2009).

The addition of seaweeds matter to the soils improves their physical, chemical and biological properties (Bruno et al., 2007), providing organic matter, improves soil structure and promotes the aeration, increasing the moisture-holding capacity, adjusting the pH and acting as a source of trace elements (McHugh, 2003; Thirumaran et al., 2009).

The seaweed extract contains regulators, plant growth hormones, carbohydrates, auxins, gibberellins and vitamins and helps to maintain soil fertility. It is cost effective and eco-friendly for sustainable agriculture (Erulan et al., 2009).

The extraction methods are purpose dependent. There is not much information regarding the processes of seaweed extraction technologies for agricultural purposes, mostly because the manufacture methods are rarely published and held as proprietary information (Craigie, 2011). However, in the work published by Briceño-Domínguez et al. (2014), we can find the complete extraction methodology from the brown alga *Macrocystis pyrifera*.

Several extraction procedures are adopted for agricultural biostimulant production from seaweeds. In most cases, extracts are produced by processes using water, alkalis or acids, or physically by disrupting the seaweed by low-temperature milling to give a micronized suspension of fine particles (Shekhar Sharma et al., 2014; Kawakita et al., 2015). As seaweed extracts can be used as fertilizer and biostimulant, water extraction seems to be the most cost-effective and practical tool for better release of micro- and macroelements from the biomass (Michalak and Chojnacka, 2015). The fertilizer obtained from seaweed extract is biodegradable, non-polluting, non-toxic and non-hazardous to humans and other animals, including birds (Vijayanand et al., 2014).

In reality, it is not yet clear how seaweeds and their compounds act on the physiological processes of plants, it is only known that they potentiate their function in different levels (McHugh, 2003). In particular, seaweeds and their compounds, influence early seed germination, root and plant growth, confer resistance to biotic stresses, tolerance to freezing and increase the plant nutrient absorption capacity (Akila and Jeyadoss, 2010; Safinaz and Ragaa, 2013).

In Portugal, since the fourteenth century, seaweeds are used as a fertilizer and its use was first regulated by the King D. Dinis (Pereira, 2010; Pereira and Correia, 2015). According to these authors, until the mid-twentieth century, the use of seaweeds as biofertilizer was a very important socio-economic activity, but recently practically abandoned and restricted to the north of Portugal (Póvoa de Varzim and Viana do Castelo).

Ascophyllum nodosum, commonly known as "Norwegian Kelp", is a perennial brown seaweed (Phaeophyceae class) with slow growth that can live

up to fifteen years in locations protected from the waves. It grows in the intertidal zone along the Atlantic coast and can be found on the north coast of Portugal (Fernandes and de Oliveira Silva, 2011; Pereira, 2018a). It contains high amounts of carbohydrates and other substances, such as amino acids, natural hormones, alginates, minerals, trace elements, and has biostimulant properties, acting positively on metabolic processes in plants (Fernandes and de Oliveira Silva, 2011; Plantytec, 2014). Different studies show that *Ascophyllum nodosum* as a biofertilizer, like other brown algae, has important functions in terms of physiological and biochemical processes, such as stimulation of cell division, control, and promotion of apical growth, improving cellular elasticity and high resistance to various abiotic factors (Fernandes and de Oliveira Silva, 2011). The use of slow-release fertilizers based on the extract can enhance efficiency and minimize environmental pollution (Wang et al., 2017).

Sargassum muticum, commonly known as "Japanese Weed" is a pseudo-perennial brown seaweed (Phaeophyceae) with fast-growing and highly regenerative capacity, living up to four years and forming dense floating masses that occupy large portions of water surface (Pereira, 2018b). Its invasive and colonizing potential is related to the great reproductive capacity and long fertile period, combined with the ability to multiply in substrates polluted by other species of seaweeds (Sánchez and Fernández, 2005; Monteiro et al., 2009), modifying the structure and functioning of these ecosystems. Economically, species of the genus *Sargassum*, including the species *Sargassum muticum*, are used as a source of alginate and bioactive compounds that can be applied in the textile, food and pharmaceutical industry. McHugh (2003) and Coimbra (2006) reported that, in certain parts of the world, this species could be used as agricultural fertilizer.

This work aimed to evaluate extracts obtained from two seaweeds, *Ascophyllum nodosum*, and *Sargassum muticum* as a biofertilizer for plants.

2 MATERIAL AND METHOD

2.1 Plant material

Plants used in this work were two lettuce commercial varieties (*Lactuca sativa*), "Maravilhas de Inverno" (green lettuce) and "Maravilhas das Quatro Estações" (purple lettuce).

2.2 Seaweed extracts

Two seaweeds, *Ascophyllum nodosum*, and *Sargassum muticum* were used as a source for biofertilizer. The algal biomass used in this experiment was collected in the Praia Norte (Viana do Castelo) and in the Buarcos Bay (Figueira da Foz), respectively.

Two types of seaweed extract were used, from each seaweed: 1) Aqueous crude extract (ACE), obtained from fresh seaweeds triturated with distilled water and filtered, to remove the larger particles (Mayara et al., 2015); 2) Aqueous extract processed (AEP), obtained through chemical hydrolysis with ethanol and distilled water (Kawakita et al., 2015).

2.2.1 Obtaining the ACE

The ACE is obtained hiyanby reducing the algal material to small particles suspended in distilled water, where all the constituents of the algae are present. Initially, the algae were cut using food scissors, in order to reduce its size. The algae were placed in a vessel and distilled water was added in the proportion of 1 600 mL of distilled water per kilogram of seaweed. The algae together with the distilled water were then placed in a Bimby® until a "juice" was obtained, repeating the process until all algae were liquefied.

The obtained juice was crushed again using a 750-W power wand and later filtered by a filter set consisting of a metal mesh strainer and two cloth strainers coupled to remove the particles larger dimensions. The obtained filtrate solution is then the ACE of the algae (pH 6, 3); this was stored at a temperature between 2 and 4°C in properly labeled plastic bottles and sealed with parafilm.

2.2.3 Obtaining the AEP

The initial protocol consisted of a sequential extraction, where the same sample is used from the beginning to the end of the process and first passes through a phase where it is extracted with hexane, another where it is extracted with methanol and finally an extraction with distilled water at 100°C.

As this protocol entails high costs due to the required volumes of hexane and methanol, it has been determined that these solvents would be replaced with ethanol since, theoretically, the residue obtained in this solvent extraction has a similar content and, using a rotary evaporator with vacuum pump, it is possible to reuse the ethanol. At each extraction, a volume of 2 500 mL of aqueous extract was obtained,

this process being repeated whenever more quantity was needed.

Twenty-five grams of dry seaweed were added to 500 mL of ethanol (1:20 ratio) in a goblet with a magnet, stirring for 20 min at room temperature. After this time, the sample is allowed to stand for 1 min and filtration is carried out. The filtration is carried out in a vacuum, with the aid of funnel silica filters with G_3 porosity coupled to a Kitasato. Thus, under vacuum, the contents of the goblet are poured into the filter, where the solvent is recovered and stored into a hermetically sealed bottle, and the initial sample is retained in the silica filter. The recovered solvent is transferred into the Kitasato and into a properly labeled vial and closed with filter paper in order to avoid solvent losses. The dried sample retained in the silica filter is again placed in the goblet with the magnet is subjected to further extraction with ethanol, and this process is repeated until the ethanol solution is translucent. It should be noted that initially, the sample has a rather intense greenish coloration but as the process is repeated it becomes translucent because the solvent removes a large part of the polar and nonpolar compounds in the sample, to which the chlorophylls are associated and therefore the greenish color will "disappear".

After extraction with ethanol, the aqueous extraction step was followed, in which distilled water was added to the sample in a ratio of 1:100. For this purpose, a graduated glass beaker is used to measure 2 500 mL of distilled water that is poured into an Erlenmeyer flask. The Erlenmeyer is placed on a heating plate until the distilled water reaches 100°C.

At 100°C, the sample was added to the water and left for 2 h, with gentle stirring, for extraction to take place. After that time, the solution is allowed to cool and vacuum filtration using a silica filter with G_2 porosity coupled to a Kitasato (in the same manner as for ethanol extraction). The part of the sample that was trapped in the silica filter was discarded and the solution obtained (pH 6, 5) is recovered and stored at 4°C into a hermetically sealed bottle.

2.3 Germination bioassays

Seeds from two lettuce varieties were used. Two extracts, ACE and AEP, from each seaweed, were used, in three concentrations (100%, 75%, and 25%) and distilled water as a control. Germination was evaluated in Petri dishes (Φ12 cm) where 25 seeds were spread on a filter paper adding 50 mL of the extracts used as a germination media, with 4 replicates. Petri dishes were incubated in dark at 25°C for 7 days. The evaluated parameters are:

a) Percentage of seed germination (PG) (the difference between germinated and not germinated seeds),

b) Seed germination index (SGI) regarding (Maguire, 1962):

$$SGI=(G_1/N_1)+(G_2/N_2)+\cdots+(G_n/N_n),$$

where G_1=number of germinated seeds at the first observation account; N_1=number of days till the first observation; n=number of observation;

c) Percentage (%) of dry matter (PDM)

% dry matter=dry biomass (g)×100/fresh biomass (g).

The following nomenclature was assigned for each solution: T0: distilled water (0% algal extract); T1: ACE *Ascophyllum nodosum*; T2: ACE *Sargassum muticum*; T3: AEP *Ascophyllum nodosum*, and T4: AEP *Sargassum muticum*.

2.4 Bioassays with lettuce plants in pots

Green and purple lettuce plants were used and seaweed extracts (25% concentration), from both seaweeds, were applied. Distilled water was used as a control. Lettuce plants were planted in plastic pots (Φ20 cm) with sterilized soil (3:1, soil: sand). One plant per pot in four replications (5 plants/variety) was used. Two types (ACE and AEP) of seaweed extracts were applied, as fertilizer, on Day 10 and Day 24 of the culture. Plant head diameter, length of the main root, and total fresh and dry matter (leaves and roots) were evaluated.

To facilitate the identification of the solutions used, the following nomenclature was assigned for each solution: T0: distilled water (0% algal extract); T1: ACE *A. nodosum*; T2: ACE *S. muticum*; T3: AEP *A. nodosum*; T4: AEP *S. muticum*.

2.5 Bioassays with lettuce plants in hydroponics (NFT)

Plants from two lettuce varieties were grown using an aqueous extract crude from *S. muticum* (25% concentration). The electrical conductivity (EC) was adjusted using distilled water. A fertigation solution (data not available) from the enterprise "Nutrimondego" was used as a control. For each extract and control, fifteen green and purple plants were grown using the NFT-hydroponic system. Parameters evaluated during the experiment were: a plant head diameter, length of the main root, total fresh and dry matter of leaves and roots, and

Table 1 Analysis of the percentage of germination (PG); seed germination index (SGI) and percentage of dry matter (PDM) for green and purple lettuce with treatments (% extract)

Treatment	Extract concentration	PG		SGI		PDM	
		Green	Purple	Green	Purple	Green	Purple
T0	0%	94	93	43.76	42.95	2.4	2.5
T1	100%	72	87	31.62	39.43	5.4	6.2
T1	75%	81	93	36.90	40.90	5.0	4.8
T1	25%	95	94	43.90	42.95	4.5	3.5
T2	100%	86	88	38.28	40.9	11.7	5.7
T2	75%	94	89	42.76	41.05	2.1	4.2
T2	25%	99	95	46.81	44.57	2.2	3.3
T3	100%	74	79	38.57	37.62	8.3	4.0
T3	75%	90	79	42.52	36.95	3.0	2.7
T3	25%	93	92	43.62	43.48	3.1	2.9
T4	100%	90	89	42.19	41.71	3.8	3.7
T4	75%	92	93	42.48	42.95	2.4	2.2
T4	25%	95	93	45.24	43.95	2.9	2.0

T0: distilled water (0% algal extract); T1: ACE *A. nodosum*; T2: ACE *S. muticum*; T3: AEP *A. nodosum* and T4: AEP *S. muticum*.

percentage of leaves dry matter.

The following nomenclature was used for the applied treatments: T4: ACE *S. muticum* 25%; T4 CEC: ACE *S. muticum* 25%, with correction of the electrical conductivity by addition of distilled water; T6: fertigation solution, the formulation used in the grower company "Nutrimondego".

The percentage of dry matter corresponds to the percentage of dry matter accumulated in the plants during their growth, allowing determining the amount of water that they had accumulated.

2.6 Soil and extract analysis after lettuce cultivation

The soil samples and the seaweed extracts (Zero: without any treatment, T0: distilled water only; T1: ACE 25% *A. nodosum*; T2: ACE 25% *S. muticum*; T3: AEP 25% *A. nodosum*, and T4: AEP 25% *S. muticum*) were analysed in the soil laboratory of ESAC, where were determined: pH values, EC, % of organic matter, N, P, K, Ca and Mg contents.

2.7 Statistical analysis

For the statistical treatment of the data, a variance analysis (ANOVA) was performed and the means and standard deviations were compared using the Tukey test for a significance level of $P<0.05$. All results expressed as percentages were first converted to arcsine values and then treated statistically.

3 RESULT

3.1 Germination bioassays

In this assay were used ACE and AEP from each seaweed, in three concentrations (100%, 75%, 25%), in order to evaluate their influence on different parameters as percentage of germination (PG), Seed Germination Index (SGI) and percentage of dry matter (PDM).

Seeds from two lettuce varieties (green and purple) germinated in different concentrations had the maximum values of PG in the extracts with 25% concentration of the treatments (Table 1) and the lowest in the media with 100% concentration. Comparing these values, at 25% concentration, with the values obtained with 0% concentration (control), they were very similar.

SGI varied in the same way as the PG values, the highest values were observed in treatments with 25% concentration and the lowest values in treatments with 100% concentration (Table 1).

PDM allows determining the amount of water accumulated in the seedlings. Analyzing the PDM of both lettuce varieties, the highest value appeared with 100% concentration of the different treatments and the lowest in treatments with 25% concentration (Table 1). Comparing the values obtained with the treatments with control seeds in two lettuce varieties, all PDM increased with all treatments.

3.2 Bioassays with lettuce plants in pots

Regarding the plant diameter, T1 and T2 treatments influenced, in the same way, the green lettuce plants, differing to T0 treatment (Table 2). The same was not observed with the purple lettuce plants. When root length was analyzed, there were slight differences: the purple plants had shorter roots.

In green lettuce plants grown without seaweed extracts (T0), fresh leaves presented the highest value, 78.5 g, and with T4 the lowest value (Table 3). Plants treated with T4 presented the heaviest roots, differing significantly from the other treatments. In the case of purple lettuce, the highest values were found in plants treated with T4, for fresh leaves and roots.

Analyzing the leaf and root dry matter of a green lettuce was possible to verify that the plants treated with T4 produced the highest values, 12.07 g, and

Table 2 Influence of four treatments on the development of green and purple lettuce plants (plant diameter, root length, a fresh and dry matter of leaves and roots)

Treatment	Lettuce	Plant diameter	Root length (cm)	Fresh matter		Dry matter	
				Leaf (g)	Root (g)	Leaf (g)	Root (g)
T0	Green	26.30±5.25^{a}	35.30±6.40^{a}	78.15±14.38^{a}	83.03±14.19^{b}	9.79±1.55^{a}	19.03±0.11^{a}
	Purple	21.80±1.32^{a}	36.30±4.95^{a,b}	50.80±8.17^{b}	43.54±5.43^{b,c}	5.47±1.02^{b}	8.22±1.78^{c}
T1 (25%)	Green	19.60±3.03^{b}	36.70±5.52^{a}	47.21±3.62^{b}c	56.36±15.73^{b}	7.47±2.19^{a}	11.67±4.70^{b}
	Purple	21.10±2.85^{a}	35.80±2.82^{a,b}	55.64±13.52^{a}	47.02±8.22^{b}	7.51±1.53^{a}	9.64±3.06^{b,c}
T2 (25%)	Green	21.40±2.91^{b}	40.15±4.47^{a}	53.69±15.77^{a}	49.24±14.36^{b}	7.64±1.95^{a}	10.94±3.80^{b}
	Purple	22.00±2.31^{b}	40.00±3.27^{a}	54.21±9.37^{a}	38.03±6.62^{b,c}	5.39±0.95^{b}	7.50±1.76^{c}
T3 (25%)	Green	22.70±3.56^{a,b}	36.90±4.25^{a}	52.22±13.58^{a}	52.70±16.60^{b}	8.05±2.03^{a}	11.93±5.46^{b}
	Purple	19.30±2.71^{a}	37.00±4.97^{a,b}	49.33±6.65^{b}	34.68±7.40^{c}	4.94±1.01^{b}	5.35±1.31^{a}
T4 (25%)	Green	25.70±2.26^{a}	36.80±4.42^{a}	36.80±4.42^{c}	105.99±25.9^{a}	12.07±2.05^{a}	24.79±10.38^{a}
	Purple	22.50±3.60^{a,b}	34.20±3.22^{b}	62.94±9.66^{a}	63.61±14.68^{b}	6.40 ±1.26^{b}	12.29±5.59^{b}

T0: distilled water; T1: ACE *A. nodosum*; T2: ACE *S. muticum*; T3: AEP *A. nodosum*; T4: AEP *S. muticum.* Values of the measurements are the mean±standard error (n=20). In the same column, values with the same letter are not significantly different (Tukey test, P<0.05).

Table 3 Parameters that were analyzed in the soil samples, before starting the experiment (Zero) and at the end of cultivation, lettuce plants in pots (T0, T1, T2, T3, T4)

Parameter	Sample					
	Zero	T0	T1	T2	T3	T4
Organic matter (%)	26.42	26.51	24.40	34.78	31.53	26.06
pH (H_2O)	6.6 N	6.6 N	7.1 N	7.2 N	7.1 N	7.1 N
EC (mS/cm)	0.21 N	0.20 N	0.11 N	0.12 N	0.10 N	0.12 N
P_2O_5 (mg/L)	138.6 M	78.6 L	78.8 L	80.1 L	64.0 L	60.4 L
K_2O (mg/L)	621MH	312 H	342 H	312 H	192 M	183 M
CaO (mg/L)	87.5 L	76.9 L	70.0 L	65.8 L	61.6 L	44.1 ML
MgO (mg/L)	14.94 ML	14.94 ML	11.62 ML	12.45 ML	13.28 ML	8.30 L

T0: distilled water (0% algal extract); T1: ACE 25% *A. nodosum*; T2: ACE 25% *S. muticum*; T3: AEP 25% *A. nodosum*, and T4: AEP 25% *S. muticum*; N: neutral; H: high; L: low; M: medium; ML: medium low; MH: medium high.

24.79 g, respectively. In purple lettuce plants, the respective weights were lower with almost all treatments (dry leaves and roots, respectively).

3.3 Soil and extract analysis after lettuce cultivation

Results of the substrate analysis of the samples treated with different extracts allowed to verify that the treatments slightly increased the pH values and decreased the EC values (Table 3), whereas in the control sample (T0) there were no changes in the values of these parameters, in comparison with the initial substrate sample (Zero).

In terms of percentage of organic matter present in the samples, T0, T1, and T4 presented similar values as the Zero sample, while samples T2 and T3 presented higher values (Table 3).

In relation to the mineral content, all treatments (T1, T2, T3, and T4) presented the lower amount of minerals than the Zero sample. Comparing the number of minerals (P, K, Ca) present in the soil samples, treated with different extracts, it was verified that T1 and T2 showed similar values but higher than those presented by T3 and T4 (Table 3). From all treatments, only T3 increased (13.28 mg/L) and T4 contributed for the lowest Mg values in the soil (8.3 mg/L).

A nutrient difference between Zero and the four treatments (Table 3), shows that plants grown with different seaweed extracts absorbed a high amount of nutrients (almost half of P, K, Ca, Mg) compared with the nutrients from the Zero substrate.

Table 4 Influence of three treatments on the development of green and purple lettuce plants grown in NFT (plant diameter, root length, a fresh and dry matter of leaves and roots)

Treatment	Lettuce	Plant diameter	Root length (cm)	Fresh matter		Dry matter	
				Leaf (g)	Root (g)	Leaf (g)	Root (g)
T4	Green	13.61±1.39^{b}	6.05±1.21^{c}	13.18±3.81^{b}	8.79±3.74^{c}	1.59±0.44^{b}	1.19±0.55^{c}
	Purple	11.29±1.39^{b}	5.26±0.89^{c}	13.60±2.91^{b}	10.65±2.03^{c}	1.53±0.25^{b}	1.50±0.28^{c}
T4 CEC	Green	14.15±1.99^{b}	13.08±2.62^{b}	16.07±3.70^{b}	19.37±4.54^{b}	1.26±0.28^{b}	1.84±0.45^{c}
	Purple	15.58±2.79^{b}	12.73±12.73^{b}	17.72±6.49^{b}	12.01±4.31^{c}	1.11±0.40^{b}	1.20±0.48^{c}
T6	Green	29.54±2.02^{a}	23.21±4.26^{a}	106.24± 14.48^{a}	36.53±7.24^{a}	6.53±1.10^{a}	3.43±0.47^{b}
	Purple	26.71±1.93^{a}	24.43±24.43^{c}	72.83±13.87^{a}	35.13±6.13^{a}	4.96±1.09^{a}	3.87±0.43^{a}

T4: ACE *S. muticum* 25%; T4 CEC: ACE *S. muticum* 25%, with correction of the electrical conductivity by addition of distilled water; T6: fertigation solution, the formulation used in the grower company Nutrimondego. The values of the measurements are the mean±standard error (n=20). In the same column, values with the same letter are not significantly different (Tukey test, P<0.05).

Table 5 Percentage of dry matter in green and purple lettuce plants with three treatments, grown in NFT (plant diameter, root length, a fresh and dry matter of leaves and roots)

Treatment	Green lettuce (%)	Purple lettuce (%)
T4	12.43	11.39
T4 CEC	7.86	6.30
T6	0.54	6.30

T4: ACE *S. muticum* 25%; T4 CEC: ACE *S. muticum* 25%, with correction of the electrical conductivity by addition of distilled water; T6: fertigation solution, the formulation used in the grower company Nutrimondego.

3.4 Bioassays with lettuce plants in hydroponics (Nutrition Film Technique (NFT))

In this essay, T4 was used as it showed the best results in the previous analysis, with lettuce plants.

After the development of lettuce plants in hydroponics, T6 treatment showed the highest results and the treatment T4 is the one that presented the lowest values (Table 4). Treatment T4 CEC presented intermediate values, between the other two treatments.

Green and purple lettuce plants showed similar behavior with the three treatments. Plants from both varieties, treated with T4, had a higher percentage of dry matter showing that these plants accumulated less water (Table 5).

4 DISCUSSION

4.1 Bioassays of germination

The germination bioassays, carried out under controlled conditions, had as objective to evaluate a seed germination potential in substrates composed of ACE and AEP of the seaweeds *A. nodosum* and *S. muticum* in different concentrations.

High concentration of the extracts had an inhibitory influence on the seed germination (Eyras et al., 2008). This inhibition can be related with the high concentration of the growth regulatory substances with phytoactive properties, such as auxins, cytokinins, ethylene, gibberellins, abscisic acid (Arioli et al., 2015) and combined effects induced by the presence of soluble sugars, amino acids and various mineral elements (Hernández-Herrera et al., 2014) that make part of the seaweed extracts (Anisimov and Chaikina, 2014).

Germination is a process that occurs in three phases (Erreira, 2004): a) phase I, when water enters in the seed by difference of water potential; b) phase II, continuity of water absorption and activation of the metabolic processes responsible for the embryo development; c) phase III, when the elongation of the primary root occurs. During this process, the osmotic potential of the seed is determinant for the ability of the embryo to absorb water. If a seed is placed on an osmotic substrate it is possible to block the germination process in phase II. The pH of the solutions, as well as the presence of substances such as amino acids, sugars, and organic acids, osmotically active compounds, usual constituents of crude extracts, may influence germination and physiological processes (Erreira, 2004). Considering this, as the substrate concentration increased the percentage of germination and the SGI decreased, it is apparent that these two parameters probably can be conditioned by the presence of osmotically active compounds in the extracts. Thus, the higher the substrate concentration the lower the water content in the plants. This should be due to the difficulty that seedlings had in absorbing water probably due to the presence of osmoprotectant agents in algal extracts (Briceño-Domínguez et al., 2014).

4.2 Bioassay of lettuce plants in pots

Comparing the values of the different parameters with the treatments applied in the two lettuce varieties, including the control, seaweed extracts used in this bioassay stimulated the plant development. Lower amounts of seaweed extract concentration affected the plant cell metabolism, positively influencing the development of the plant structures (Moreira et al., 2006; Craigie, 2011). These results are in accordance with Dantas et al. (1998) who applied *Sargassum vulgare* extracts in lettuce and coriander and found that plants treated with 25% extract exhibited significant growth compared to the control plants.

4.3 Analysis of the effect of extracts on soil after lettuce cultivation

Until now, no severe general limitations of the application of seaweeds as biofertilizer are known. However, due to the high salt content of seaweeds (Na, Cl, K, and Ca), the long-term or excessive application of seaweeds may contribute to the development of saline soils and may increase the salt content in plant tissues (Nabti et al., 2017). Analysis of the different substrate samples showed that the addition of the seaweed extracts slightly decreased the EC values, were not harmful to the plants (Nabti et al., 2017). In our experiment, compared to control, pH increased; so, according to Eyras et al. (2008) increased exchangeable Ca and decreased an exchangeable Al. Regarding the percentage of organic matter present in the samples, it is clear that seaweeds can increase the organic matter in the soils and thus the plant yield.

Regarding the mineral content level, the soil samples, at the end of the cultivation, showed less mineral content than the initial sample. Comparing the number of minerals present in the samples, it was verified that the treatments with ACE presented values similar to each other but mainly higher than other extracts. Thus, these extracts must have bioactive compounds capable of promoting the absorption of minerals by the plants and therefore the mineral content in the soil was lower.

The growth rate of the plant is closely related to N supply (Zheng et al., 2016). Hernández-Herrera et al. (2014) found that phosphorous in seaweed extracts facilitates root proliferation increasing the root/shoot ratio and that the K in seaweed extracts has a positive effect on enhancing photosynthesis and meristematic growth and regulating water status in treated plants. The Ca present in seaweed extracts facilitates cell elongation improving, cell stability and enzyme activation in plants (Zheng et al., 2016). Besides, it has been confirmed that foliar application of seaweed extracts enhances root development in varieties of crops (Calvo et al., 2014). Our results show that the nutrient content was modified in the soil samples treated with seaweed extracts.

4.4 Bioassays of lettuce plants in hydroponics

The greenhouse bioassays with green and purple lettuce plants in NFT showed that, although the values differ between the two varieties, the differences observed between the treatments (AEP *S. muticum*) were close. The fertigation solution influenced the production, as expected.

In the treatment where the EC was corrected, the values of plant parts development were intermediate between the other two treatments. This showed that the EC should be carefully controlled and monitored. This is in accordance with Eyras et al. (2008) who noted that plants increase their resistance to osmotic stress when cultivated in pure seaweed extract. The existence of osmoprotectants in seaweeds can increase survival of plants treated with its extracts in media with high EC that can introduce stress in plants. As seaweeds have been proven as a source of antioxidants, plant growth hormones, osmoprotectants, mineral nutrients and many other organic compounds including novel bioactive molecules (Nabti et al., 2017) they introduce tolerance to plants to stress provoked by high EC.

Analyzing the values of the percentage of dry matter in green and purple lettuce, it was verified that they were similar to ones from the germination bioassays, the more concentrated the treatment, and the greater the percentage of dry matter. This is in accordance with Briceño-Domínguez et al. (2014), who stated that the algal extracts affected water absorption in the plants as a result of the presence of osmoprotectant agents in their extracts. There are no works related with the application of seaweed extracts as a nutrient medium in hydroponic crops, mainly NFT, so similar works can contribute, not only as information but also as a source for more research.

5 CONCLUSION

Improving organic farming and producing organic foods had increased the demands of looking for another alternative source that can be used to feed the

plants and thus to protect the human health and environment. Here seaweeds can take part as an organic, healthy and nutrient source.

This work was developed in the field and applied in the real production unit. This study shows that the use of seaweed extracts in low concentration (25%) can influence crop production.

Thus, seaweed cultivation and its utilization as a biofertilizer is an economically promissory approach in agricultural production.

Seaweeds harvested at different seasons could be an interesting challenge for the future studies of seaweed extract application as biofertilizer.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the repository "Estudo Geral" from the University of Coimbra, with the identifier http://hdl.handle.net/10316/32863 (in Portuguese).

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