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PRODUCTION AND EVALUATION OF SEAWEED-CONTAINING PLANT GROWTH ADJUVANT FORMULATION

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ABSTRACT

The beneficial effects of seaweed on soil and crops have been widely demonstrated. Few studies, however, have examined the effects of seaweed as a component of plant growth adjuvant formulation (PGAF). This work aimed to review the performance of brown seaweed (*Cystoseira baccata*) or green seaweed (*Ulva* sps.) for application as PGAF . Analyses of its efficacy showed both types of seaweed to be suitable for use as plant growth formulation although their fertilizing effect was moderate. None of the substrates were phytotoxic but those containing the highest proportion of *C. baccata* led to high plant mortality at an early stage of seedling growth. Substrates containing *C. baccata* improved growth in cucumber culture; whereas *Ulva*-based substrates exhibited no substantial differences in performance from that of the controls. Seaweed can be a useful component of PGAF, but should not be added an excessive amount in order to avoid toxic effects of high concentration of biostimulants.

Key words: soil-less cultivation, macro-seaweed, *Cystoseira baccata*, *Ulva*, biostimulant.

Abbreviations

AC	aeration capacity
AGR	germination rate
ARLP	average root length per plant
AWP	average weight per plant
C. baccata	*Cystoseira baccata*
EAW	easily available water
EC	electrical conductivity
GeI	germination inhibition
GrI	growth inhibition
MLV	Munoo–Liisa vitality
PGAF	Plant Growth Adjuvant Formulation
S	shrinkage
sps.	species
UW	unavailable water
v:v	volume:volume = volumetric fraction
WBC	water buffer capacity

CONTENTS

1. INTRODUCTION

Seaweed has long been known to be an effective fertilizer improving soil quality and also a biostimulant of plant growth. Seaweed contains all the macro and micronutrients needed for plant growth (Verkleij, 1992). Also, it has nitrogen (N) content similar to that of most animal manures, high content in potassium (K)(especially brown seaweed) and a modest amount of phosphorus (P) (Stephenson, 1974; Senn and Kingman, 1978). Seaweed improves soil quality, mainly as a result of its high content in phycocolloids resulting in increased structure, water retention and exchange capacity (Metting, 1990; Lynn, 1972; Khan, 2009). By virtue of its high Calcium (Ca) content and the presence of alginates capable of binding aluminium, seaweeds additionally have liming effects (Crouch et al., 1990; López-Mosquera and Pazos, 1997; Eyras et al., 1998). Also, it facilitates the growth of beneficial microbiota, thereby increasing biological activity in soil (Kuwada et al., 2000, 2006; Chen et al., 2003; Khan, et al., 2009).

In addition to the previously mentioned benefits, seaweeds have bio-stimulating effects owing to their high contents of vitamins (Bourgougnon et al., 2011), plant hormones, quaternary ammonium compounds (e.g., proline, betaines), trace elements, and lipid-base molecules, having a favourable impact on seed germination (Hernández-Herrera et al., 2019), aerial growth (Basak, 2008), root development (Finnie and Van Staden, 1985; Crouch and van Staden, 1991), nutrient uptake (Castaings et al., 2011; Di Stasio, 2017), flowering (Basak, 2008), and tolerance of abiotic stress such as that caused by salinity (Yildiztekin et al., 2018) or drought (Xu et al., 2015). These effects influence the improvement of plant yield and quality of crops (Chouliaras et al., 2009; Rouphael et al., 2018), the extension of their lifespan and the increase of their nutrient contents

(Kamel, 2014), especially in crops growing in below optimal conditions (Crouch and Van Staden, 1994; Craigie,2011).

In agriculture, seaweed has traditionally been used as an amendment or fertilizer. At present, a number of commercial fertilizers include seaweed flour, compost or extract as a biostimulant in their formulations. Although soil-less cropping has grown steadily in recent decades, few studies have addressed the impact of the presence of biostimulants in growing media (Rady, 2016), even though they improve crop growth while minimizing the needed for fertilizers and phytosanitary products. Seaweed can in fact be an interesting component of substrates for soil-less cultivation since it is a natural, biodegradable, nutrient- and biostimulant-rich, and pathogen- and weed-free material that is toxic to neither humans nor animals, so it can facilitate soil-less cropping while helping fight pests and prevent plant diseases. Also, the rheological properties of seaweed could increase the water retention capacity of substrates. Finally, seaweed occasionally accumulates in unwanted zones as waste that could be valorized as a growth media.

However, using seaweed as a substrate component can pose problems arising from its high content in salts and, in polluted areas, also in heavy metals (García and Martel, 2000; Sudharsan et al., 2012). Growth hormones, they possess, can also produce phytotoxic effects if they are present in high amounts. Besides, untreated seaweed is highly biologically unstable and can raise problems such as poor nitrogen fixation, anoxia and the release of phytotoxic substances such as organic acids (Zucconi et al., 1981a, 1981b) or hydrogen sulphide (Craigie, 2011).

The aim of this work was to produce and evaluate the agricultural performance of seaweed-based growing substrates. The substrates were made with fresh brown seaweed of *Cystoseira baccata*, a species widely distributed in the NW Atlantic region (García-Fernández and Bárbara, 2016), and beach wrack collected from shellfish cultivation areas on the coast of Galicia (NW Spain) consisting mainly of two *Ulva* sps.

2. DETAILS OF EXPERIMENTATION

2.1. Preparation of substrates

The raw material used to prepare the substrates was coir consisting of coconut peat and fibre (PeleMix ¾"), which was inert enough to clearly expose the chemical and biostimulating properties of seaweed. The material was supplied with two types of seaweed, namely:

– *Cystoseira baccata* (S.G. Gmelin) P.C. Silva, a brown seaweed species-rich in growth hormones (especially cytokinins) that was obtained in fresh form from a coastal area in A Coruña, NW Spain (43° 21′ 47.5″ N, 8° 20′ 46.5″ W).
– *Ulva* sps. obtained from shellfish cultivation areas in the Ría of Pontevedra, NW Spain (42° 25′ 32″ N, 8° 41′ 07″ W), and consisting largely of *Ulva rigida* C. and *Ulva intestinalis* L. This beach wrack was partly stable as it had been air-dried and stored for about 6 months after have been removed from the beach and prior to be used.

The high salinity of *C. baccata* required washing the seaweed before use. Both materials were finely chopped and mixed with PeleMix ¾", which was also used, unmixed, as a control substrate. The seven treatments used were as follows:

– CC. 100% coconut coir (PeleMix ¾").
– 10C. 10% *C. baccata* / 90% coir (v:v).
– 25C. 25% *C. baccata* / 75% coir (v:v)
– 50C. 50% *C. baccata* / 50% coir (v:v)
– 10U. 10% *Ulva* / 90% coir (v:v)
– 25U. 25% *Ulva* / 75% coir (v:v)
– 50U. 50% *Ulva* / 50% coir (v:v)

After mixing, the substrates were supplied with water to the container's capacity and allowed to stand for 1 month, moisture levels being maintained by adding water as required, the mass being turned over at 2-day intervals to facilitate aeration and the temperature being measured periodically. Then, the substrates were assessed for stability by using the self-heating test of Brinton et al. (1995).

2.2. Chemical characterization of substrates

Once the substrates were checked to be stable, they were characterized in chemical terms for pH, electrical conductivity (EC), and contents in organic matter (OM) and elements soluble in water and $CaCl_2$ + DTPA ($N\text{-}NH_4^+$, K^+, Ca^{2+}, Mg^{2+}, Na^+, Cl^- and PO_4^{3-}) according to European standards (viz. EN 13037, 2011; EN 13038, 2011; EN 13039, 2011; EN 13652, 2001). Total C and N were determined with a Lecco 2000 autoanalyzer following attack with H_2SO_4 (Thomas et al., 1967); total Ca, Mg, Na, and K by ICP-OES and P colorimetrically (Chapman and Pratt, 1997); and heavy metals (Cd, Cu, Cr, Hg, Mn, Ni, and Zn) determined by ICP-MS after attack with HNO_3.

Dry bulk density, particle density, total porosity, water retention properties, and shrinkage were determined in accordance with standard EN 13041. Water retention measurements were made at a suction pressure of 1, 3, 5, 7.5, and 10 kPa, and moisture contents were expressed a volumetric fractions (v:v). Aeration capacity (AC) was calculated as the difference between total porosity and volumetric moisture content (v:v) at 1 kPa; and easily available water (EAW), water buffer capacity (WBC), and unavailable water (UW) were taken to be the volumetric fractions of moisture retained by each substrate at 1–5 kPa, 5–10 kPa and > 10 kPa, respectively (Felipó et al., 1979).

2.3. Agronomic evaluation of substrates

The agronomic potential of the substrates was assessed, and their interactions with plants were examined, with the tests described below.

2.3.1. Germination

Phytotoxicity test

It was carried out following the methodology described in EN 16086-2 (2011) using cress (*Lepidium sativum*) as an indicator species. Screened, wetted samples of the different substrates were placed together with 10 cress seeds in Petri dishes in triplicate, using peat as control treatment. The dishes were tilted vertically by 70–80° and incubated at 25 ± 5 °C for 72 h. Then, they were used to determine the average germination rate (AGR), average root length per plant (ARLP), and Munoo–Liisa vitality (MLV) index, the last being calculated as follows:

$$MLV\ (\%) = \frac{(GR_{s1} \times RL_{s1}) + (GR_{s2} \times RL_{s2}) + (GR_{s3} \times RL_{s3})}{3 \times (GR_c \times RL_c)} \times 100$$

where GR_{si} is the germination rate of replicate *i* in each treatment, GR_c that of the control treatment (peat), RL_{si} the average root length in replicate *i* in each treatment and RL_c that of the control treatment.

Growth test

It was performed on Chinese cabbage (*Brassica rapa* spp. pekinensis) following the methodology "pot experiment with direct use of the prepared sample" of the EN 16086-1 (2011) Thus, a total of 20 Chinese cabbage seeds were sown in triplicate in the different substrates and grown under controlled conditions. After 5 and 12 days, each pot was

assessed for average germination rate (AGR) or the average number of germinated seeds in the 3 replicates for each treatment. The results were used to calculate germination inhibition as follows:

$$GeI\ (\%) = \frac{\left(AGR_{control} - AGR_{sample}\right)}{AGR_{control}} \times 100$$

2.3.2. Growth

Plant growth tests were performed on two different crops, namely: Chinese cabbage and cucumber.

Chinese cabbage (*Brassica rapa spp. pekinensis*)

One month after cabbage seeds were sown in the previous test, the influence of the different substrates on plant growth was assessed in terms of plant survival, number of leaves per plant, aerial and root length, and fresh and dry weight of the aerial and root portions. The average weight per plant (AWP) for the substrate and control treatments were used to calculate growth inhibition as follows:

$$GrI\ (\%) = \frac{APW_{control} - APW_{sample}}{APW_{control}} \times 100$$

Cucumber (*Cucumis sativus*)

Cucumber plants were used to compare growth in the different substrates with or without added fertilizer in order to isolate the potential fertilizing effect of the seaweed-enriched substrates. The experiment was conducted in two seed trays of 14 × 9 cells each. One tray was filled with seven substrates unfertilized (18 cells per substrate) and the other with the substrates that were supplied with 1.5 g L^{-1} substrate of NPK liquid fertilizer 15-10-20. After 45 days, each treatment was assessed for number of leaves per plant, length of the aerial and root portions, and fertilizer efficiency, the last being calculated from the following equation:

$$Efficiency = \frac{average\ length\ of\ fertilized\ plants - average\ length\ of\ unfertilized\ plants}{Average\ length\ of\ unfertilized\ plants}$$

2.4. Statistical analysis

Raw data were processed with the software SPSS Statistics v. 23.0 from IBM. Means for different treatments were compared via analysis of variance (ANOVA) and significant

differences ($p < 0.05$) identified by using Duncan's test after checking for normality with the Kolmogorov–Smirnov test and variance homoscedasticity with Levene's test. Data resulting in non-homoscedastic variances were subjected to the Games–Howell test.

3 EXPERIMENTAL RESULTS

3.1. Raw materials

Both types of seaweed contained greater amounts of nutrients than coconut coir. Thus, *C. baccata* was richer in K and P, and so was *Ulva* in Ca and Magnesium (Mg). *Cystoseira baccata* additionally had a high content in Na —much higher than that of *Ulva*, which was even poorer in this element than was coir. Both materials had low contents in heavy metals —by exception *C. baccata* had an increased content in Cd (2.55 mg kg^{-1}). In any case, washing reduced the electrical conductivity (EC) of the two materials below the maximum thresholds for use as growing substrates (Table 23.1).

Table 24.1. Chemical characterization of the raw materials used to prepare the mixed substrates (seaweed washed and chopped).

	Cystoseira baccata	*Ulva* sps.	Coconut coir
CBD (g L^{-1})[1]	423.05	538.33	488.94
EC (dS m^{-1})[2]	0.47	0.25	0.39
C (%)	40.31	37.63	51.09
N (%)	2.12	2.32	0.73
C/N	19.00	16.20	69.99
P (%)	0.14	0.01	0.06
K (%)	1.15	0.41	0.93
Ca (%)	1.64	2.11	0.42
Mg (%)	0.76	0.90	0.16
Na (%)	0.73	0.19	0.28
Cd (mg kg^{-1})	2.55	0.02	0.39
Cu (mg kg^{-1})	0.77	1.30	7.57
Ni (mg kg^{-1})	3.44	2.27	4.01
Pb (mg kg^{-1})	3.07	0.00	10.28
Zn (mg kg^{-1})	11.07	8.67	44.82
Hg (mg kg^{-1})	0.02	0.01	0.06
Cr (mg kg^{-1})	4.37	10.15	20.05

All values except those for CBD and EC are expressed on a dry weight basis

[1] Laboratory compacted bulk density according to UNE 13040

[2] Electrical conductivity

3.2. Characterization of mixed substrates

As can be seen from Table 23.2, adding either type of seaweed increased the pH of the substrates. This was especially so with *C. baccata*, a 50% proportion of which raised the pH to 7.28. This proportion of seaweed additionally increased electrical conductivity (EC) to 1.16 dS m^{-1}. The amount of organic matter —and hence carbon— in the substrates decreased with increasing proportion of seaweed but remained above the threshold recommended by Raviv et al. (1986), 80%, in all cases. Both types of seaweed facilitated N release to a similar extent, so they decreased the C/N ratio in similar proportions.

Table 24.2. Chemical characterization of the substrates.

	CC	10C	25C	50C	10U	25U	50U
Moisture (%)	86.91	86.48	85.84	84.76	86.47	85.80	84.68
pH	6.03	6.24	6.84	7.28	6.31	6.69	6.99
EC (dS m^{-1})	0.39	0.37	0.64	1.16	0.30	0.33	0.39
C (% dm)	51.09	51.05	47.92	48.20	51.42	50.02	49.21
OM (% dm)	87.87	87.80	82.42	82.91	88.44	86.03	84.64
N (% dm)	0.73	0.87	1.08	1.43	0.89	1.13	1.53
C/N	69.99	58.68	44.37	33.71	57.77	44.26	32.16
P (% dm)	0.06	0.07	0.08	0.10	0.06	0.05	0.04
K (% dm)	0.93	0.96	0.99	1.04	0.88	0.80	0.67
Ca (% dm)	0.42	0.54	0.73	1.03	0.59	0.84	1.26
Mg (% dm)	0.16	0.22	0.31	0.46	0.23	0.34	0.53
Na (% dm)	0.28	0.32	0.39	0.51	0.27	0.26	0.23
Soluble elements extracted by $CaCl_2$+DTPA (mg L^{-1} substrate)							
NH_4^+	7.75	3.95	4.30	8.65	10.25	12.10	11.70
NO_3^-	10.55	13.70	7.30	2.20	13.85	4.95	1.05
Mg^{2+}	63.72	125.83	194.62	352.39	114.33	353.30	501.72
K^+	1945.8	2742.8	2267.8	2868.4	3061.8	1527.90	1264.9
Na^+	203.77	396.54	467.23	685.77	357.34	151.31	181.78
PO_4^{3-}	7.58	4.93	2.45	4.75	13.35	33.33	17.98
Heavy metals (mg kg^{-1} dm)							
Cd* (mg kg^{-1})		0.61	0.93	1.47			

* Calculated from its proportion in the substrate

As regards nutrients, *C. baccata* increased the contents in P and K, and both seaweed species increased those in Ca, Mg and Na —the last was especially markedly raised by *C. baccata*. Adding either seaweed to coir increased the content in nitrate and decreased that in ammonium ion among species soluble in $CaCl_2$–DTPA. Also, both seaweed species increased the content in soluble K with increasing proportion in the mixed substrates. *Cystoseira baccata* additionally raised the content in Na —and hence EC. As regards heavy metals, *C. baccata* substantially increased Cd levels, which must be borne in mind to avoid its potential hazards, if any.

Table 24.3. Physical characterization of the substrates.

	CC	10C	25C	50C	10U	25U	50U
BD (g L^{-1})	86.65	81.95	73.93	76.23	78.45	72.68	74.07
PD (g L^{-1})	1944.9	1945.3	1977.5	1974.6	1941.5	1964.1	1955.8
Porosity (% v/v)	95.54	95.79	96.26	96.14	95.96	96.3	96.21
CA (% v/v)	39.6	37.71	38.83	42.46	39.99	43.19	48.17
EAW (% v/v)	17.88	18.34	18.5	16.6	19.05	17.46	15.11
WBC (% v/v)	1.65	2.96	4.09	3.67	1.99	2.01	1.81
UW (% v/v)	3642	36.77	34.85	33.41	34.93	33.64	31.13
R (kPa)	2.14	2.44	2.31	1.93	2.05	1.73	1.05
S (% v/v)	11.77	15.32	18.36	21.11	16.61	18.43	19.59

BD bulk density, PD particle density, CA aeration capacity, EAW easily available water, WBC water buffer capacity, UW unavailable water, *R* suction that equalizes the water and air contents, S shrinkage.

As can be seen from Table 23.3, the physical properties of the substrates were not appreciably altered by the addition of either type of seaweed. Porosity was high (96% on average) in all mixed substrates. *Ulva* decreased the total amount of water retained and, especially, that of unavailable water (UW), which was 5.2% smaller in the substrate containing 50% seaweed than in coir. The presence of either seaweed increased the aeration capacity of the substrates, which was 8.6% higher with a proportion of 50% *Ulva*. *R*, which is a measure of water availability at low pressures, ranged from 1 to 3 kPa in the mixed substrates. *R* values above 3 kPa are suggestive of root anoxia by the effect of excessive moisture, and values below 1 of moisture scarcity (Ansorena, 1994). As can be seen in Table 23.3, *R* decreased and aeration increased as a result of increasing proportion of green seaweed. The presence of either seaweed, but particularly *C. baccata*, increased shrinkage (*S*) with increasing proportion in the mixed substrates; in any case, *S* was invariably lower than 30 %, the maximum recommended value (Abad et al., 1992).

3.3. Agronomic evaluation

3.3.1. Germination

No signs of phytotoxicity were detected with any of the substrates in either experiment. Also, the germination results exhibited no significant differences. Thus, although germination was always greater with the mixed substrates, the differences were not significant enough for a biostimulating effect to be inferred (Tables 23.4 and 23.5).

As can be seen from Table 23.4, the presence of seaweed in the substrates increased root growth and the Munoo–Liisa vitality index in cress relative to both coconut coir and the control treatment (peat).

Table 24.4. Phytotoxicity test on cress. Means ± standard deviation for 3 replicates.

Substrate	AGR (%)	ARLP (%)	MLV (%)
Peat	97.7 ± 4.2 a	46.0 ± 9.8 b	1.01 ± 13.6 b
CC	93.3 ± 4.4 a	29.9 ± 10.0 a	0.63 ± 7.4 a
10C	100.0 ± 0.0 a	50.9 ± 6.3 bc	1.15 ± 6.3 bc
25C	100.0 ± 0.0 a	54.5 ± 11.5 bc	1.23 ± 11.5 bc
50C	97.7 ± 4.2 a	45.6 ± 3.6 b	0.99 ± 5.4 b
10U	100.0 ± 0.0 a	58.7 ± 10.3 c	1.32 ± 10.3 c
25U	97.7 ± 4.2 a	54.7 ± 8.6 bc	1.20 ± 12.2 bc
50U	100.0 ± 0.0 a	56.5 ± 9.6 bc	1.27 ± 9.6 bc

AGR average germination rate, ARLP average root length per plant, MLV Munoo–Liisa vitality index. Different letters in each column denote significant differences between treatments (rows) as per Duncan's test at $p < 0.05$.

With Chinese cabbage (Table 23.5), the highest germination rate after 5 days was obtained in comparison with the control (peat); however, differences from the other treatments were not significant. On the other hand, inhibition of germination was very negligible in most cases. This trend ceased after 10 days, where an increased number of seeds had germinated —several close to the value for the control treatment or even greater.

Table 24.5. Germination test on Chinese cabbage. Means ± standard deviation for 3 replicates.

	GR (%)		GeI (%)	
Substrate	5 days	10 days	5 days	10 days
Peat	85.0 ± 8.7 a	95.0 ± 0.0 a	0.0 ± 10.2 a	0.0 ± 0.0 abc
CC	81.7 ± 5.8 a	90.0 ± 0.0 a	3.9 ± 6.8 a	5.3 ± 0.0 bc
10C	78.3 ± 2.9 a	91.7 ± 2.9 a	7.8 ± 3.4 a	3.5 ± 3.0 bc
25C	83.3 ± 5.8 a	95.0 ± 0.0 a	2.0 ± 6.8 a	0.0 ± 0.0 abc
50C	81.7 ± 2.9 a	98.3 ± 2.9 a	3.9 ± 3.4 a	–3.5 ± 3.0 ab
10U	70.0 ± 13.2 a	98.3 ± 2.9 a	17.6 ± 15.6 a	–3.5 ± 3.0 a
25U	78.3 ± 5.8 a	93.3 ± 7.6 a	7.8 ± 6.8 a	1.8 ± 8.0 a
50U	70.0 ± 13.2 a	93.3 ± 2.9 a	17.6 ± 15.6 a	1.8 ± 3.0 c

GR germination rate, GeI, germination inhibition. Different letters in each column denote significant differences between treatments (rows) as per Duncan's test at $p < 0.05$.

3.3.2. Plant growth

Chinese cabbage

As can be seen in Fig.23.1, there were visible differences in growth between the Chinese cabbage plants sown in the substrate containing 10% *C. baccata* and those sown in substrates containing high proportions of this seaweed, as well as with plants grown in the *Ulva*-containing substrates. These last, where salinity level was no limiting unlike mixtures with more than 10% of *C. baccata,* grew to a similar extent as the plants sown in peat or coir; their aerial portion exhibited an increased fresh and dry weight, but the differences were not significant in any case (Table 23.6).

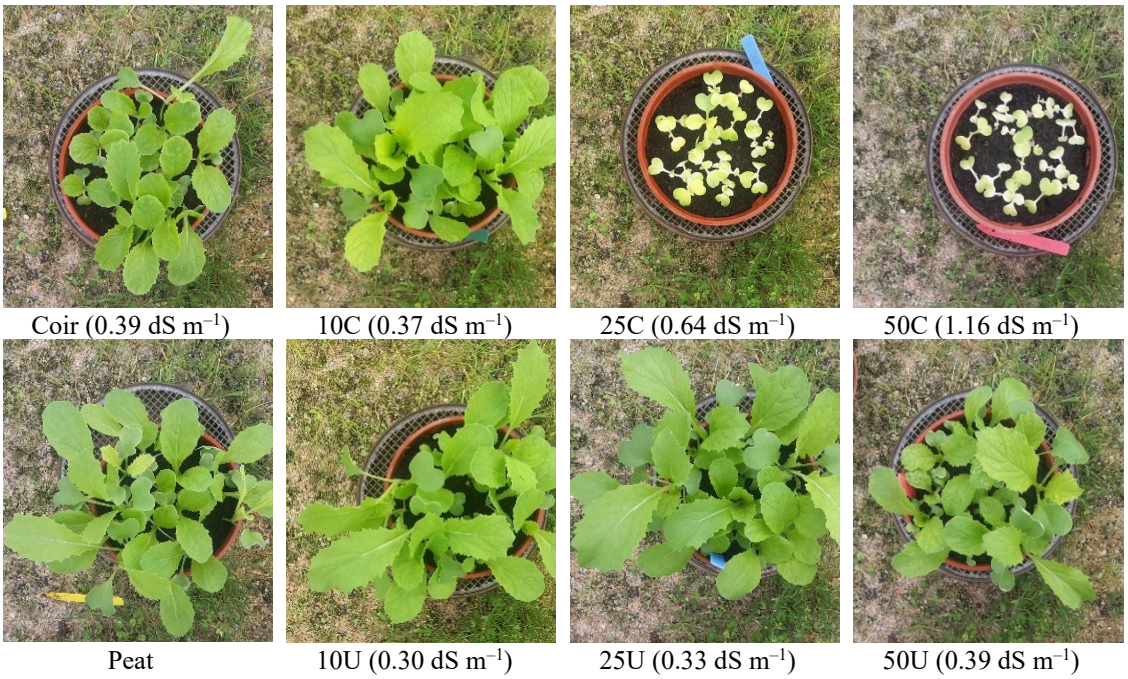

Fig.24.1. Chinese cabbage growth 20 days after sowing in various substrates. The electrical conductivity of the substrates is shown in brackets.

Table 24.6. Growth test on Chinese cabbage. Mean ± standard deviation.

	Plants per pot	Total length (cm)	Root length (cm)	Root dry weight (g)	Number of leaves	Aerial length (cm)	Aerial dry weight (g)
Peat	19.00 ± 0.00b	23.02 ± 5.71a	8.19 ± 2.54bc	0.16 ± 0.23a	5.35 ± 0.97a	14.82 ± 3.73a	0.28 ± 0.21a
CC	16.00 ± 1.00b	22.48 ± 6.91a	9.41 ± 3.90c	0.03 ± 0.01a	5.10 ± 1.08a	13.07 ± 3.83a	0.36 ± 0.07a
10C	18.33 ± 0.58b	22.22 ± 6.54a	8.60 ± 2.95bc	0.03 ± 0.01a	5.24 ± 1.04a	13.62 ± 4.12a	0.44 ± 0.02a
25C	2.50 ± 0.71a	22.00 ± 6.11a	7.80 ± 2.80abc	0.03 ± 0.02a	6.20 ± 1.10a	14.20 ± 3.55a	0.36 ± 0.30a
50C	–	–	–	–	–	–	–
10U	19.67 ± 0.58b	21.81 ± 7.96a	8.34 ± 3.26bc	0.04 ± 0.00a	5.00 ± 1.29a	13.47 ± 5.13a	0.43 ± 0.03a
25U	18.67 ± 1.53b	21.22 ± 7.12a	6.56 ± 2.31a	0.04 ± 0.00a	5.43 ± 1.25a	14.66 ± 5.19a	0.51 ± 0.05a
50U	17.00 ± 2.65b	20.90 ± 8.52a	6.96 ± 2.89ab	0.04 ± 0.01a	5.47 ± 1.33a	13.94 ± 5.82a	0.52 ± 0.12a

Different letters in each column denote significant differences between treatments (rows) as per Duncan's test at $p < 0.05$.

Cucumber

As can be seen from Table 23.7, all fertilizer treatments substantially enhanced the overall growth in the cucumber plants, which suggests that the fertilizing effect of the seaweed was visible, though limited. The greatest effect was that on the aerial part, where the lowest proportions of the two types of seaweed increased growth by 105 and 104%, respectively (Table 23.8). The increase was much greater than that observed with coconut coir despite the very small amounts of nutrients supplied by the seaweed in such low proportions (Table 23.2). The presence of *C. baccata* in the substrates additionally increased root growth, the substrates containing it exhibiting a fertilizer efficiency of up to 67%.

Table 24.7. Growth-related parameters for cucumber. Mean ± standard deviation.

Treatment	Total length (cm)	Leaves per plant	Root length (cm)	Aerial length (cm)
CC	21.39 ± 3.40 bc	2.39 ± 0.50 ef	9.58 ± 1.64 abc	11.81 ± 2.84 b
10C	16.08 ± 3.45 a	1.56 ± 0.51 a	7.89 ± 2.34 a	8.19 ± 1.86 a
25C	24.29 ± 2.38 cde	2.29 ± 0.47 def	10.53 ± 1.80 bcd	13.76 ± 2.06 cd
50C	26.81 ± 3.39 ef	2.56 ± 0.51 f	10.36 ± 2.40 bcd	16.44 ± 1.76 ef
10U	18.36 ± 4.71 ab	1.78 ± 0.43 ab	10.53 ± 3.85 bcd	7.83 ± 1.42 a
25U	17.89 ± 2.56 a	1.89 ± 0.47 bc	8.83 ± 1.62 ab	9.06 ± 1.50 a
50U	18.47 ± 3.50 ab	2.00 ± 0.00 bcd	9.00 ± 2.72 ab	9.47 ± 2.31 a
F-CC	29.03 ± 3.60 fg	2.39 ± 0.50 ef	12.08 ± 1.76 def	16.94 ± 2.81 f
F-10C	29.94 ± 5.12 g	2.33 ± 0.49 def	13.17 ± 2.95 def	16.78 ± 3.12 f
F-25C	33.08 ± 6.84 h	2.61 ± 0.50 f	13.42 ± 2.88 f	19.67 ± 4.27 g
F-50C	31.72 ± 7.69 gh	2.61 ± 0.50 f	11.50 ± 3.65 cde	20.22 ± 4.71 g
F-10U	26.58 ± 4.43 ef	2.32 ± 0.48 def	10.58 ± 2.90 bcd	16.00 ± 2.82 ef
F-25U	23.11 ± 4.75 cd	2.17 ± 0.38 cde	11.03 ± 2.68 cd	12.08 ± 2.88 bc
F-50U	25.08 ± 3.69 de	2.33 ± 0.49 def	10.33 ± 2.01 bcd	14.75 ± 2.42 de

F- fertilized substrate. Different letters in each column denote significant differences between treatments (rows) as per Duncan's test at $p < 0.05$.

Table 24.8. Fertilizer efficiency as relative growth increase by effect of fertilization. Mean ± standard deviation.

Treatment	Total length	Root length	Aerial length
F-CC	0.36±0.17 ab	0.26±0.18 b	0.44±0.24 ab
F-10C	0.86±0.32 c	0.67±0.37 c	1.05±0.38 c
F-25C	0.36±0.28 ab	0.27±0.27 b	0.43±0.31 ab
F-50C	0.18±0.29 a	0.11±0.35 ab	0.23±0.29 a
F-10V	0.45±0.24 b	0.00±0.28 a	1.04±0.36 c
F-25V	0.29±0.27 ab	0.25±0.30 b	0.33±0.32 ab
F-50V	0.36±0.20 ab	0.15±0.22 ab	0.56±0.26 b

F- fertilized substrate. Different letters in each column denote significant differences between treatments (rows) as per Duncan's test at $p < 0.05$.

Comparing treatments with or without added fertilizer separately, plants grown on substrates containing *C. baccata* invariably grew more than those sown on coir; however, the mixed substrates unfertilized never reached the effects of the coir + fertilizer combination —not even in treatment 50C, which was the one that grew the most. Seaweed appreciably increased root growth relative to the control treatment. The increase occurred mainly in the aerial portion, in which the substrates containing a 25 or 50% proportion of *C. baccata* compared to the control and control + fertilizer treatments, respectively.

4. IMPLICATIONS OF OUR STUDIES

4.1. Characterization of seaweeds and mixed substrates

No appreciable change in temperature —and hence no potentially adverse effect on the integrity of bio stimulating substances— was observed during the stabilization period in the mixed substrates. In fact, at the end all mixtures were stable according to the self-heating test. This result excluded the presence of strong biological changes but not necessarily that of a slow degradation process.

Mixed substrates should be able to efficiently deliver plant nutrients. The initial characterization analyses showed that both types of seaweed were suitable for use in plant growing substrates; in fact, both contained substantial amounts of nutrients such as N, K, Ca and Mg. *Cystoseira baccata* additionally contained some P, which is interesting given the increasing scarcity of sustainable phosphorus sources some authors (Cordell et al., 2009) have proposed using seaweed as a potential source of P in the future. One potential chemical constraint on the selection of new materials to be used as growth media component is their content in heavy metals (Chong, 2005); in fact, cultivation in a substrate avoids the dilution effects of soil and raises toxic risks for plants and humans. Because seaweed is a bioaccumulator, it can increase such risks to an extent dependent on the degree of pollution at the collection site. The ability of seaweed to accumulate toxic substances depends on the composition of its cell walls and increases with the presence of alginates, which are abundant in brown seaweed such as *C. baccata* (Davies et al., 2003). European legislation in force (Regulation (EU) 2019/1009) has set maximum tolerated levels for heavy metals and As in substrates to be CE marked. Spanish law has additionally set domestic limits for heavy metals in growing media and discriminated between those which can be used with edible horticultural crops and those which cannot. Here, only the *C. baccata*-based substrates contained substantial amounts of Cd that increased with increasing proportion in the substrate —so much so that 50C contained 1.5 mg kg^{-1}, which is near the limit for CE marking. Also, only substrate 10C had a Cd content below the Spanish legal threshold for use with edible crops (Class A, Cd < 0.7 mg kg^{-1}); by contrast, 25C and 50C should only be used for other purposes (Class B, Cd < 2 mg kg^{-1}).

The physical properties of a growing substrate are especially important because, unlike its chemical properties, they are very difficult to alter. Some deficiencies or excesses can be offset by combining two or more materials to improve the structure and ensure an appropriate air-water balance during irrigation for optimal plant growth in the absence of anoxia or drought stress. Phycocolloids, which are matrix polysaccharides exclusively present in seaweed, can form grids capable of retaining large amounts of water (Verkleij, 1992; García and Martel, 2000). The main phycocolloids in green and brown seaweed are ulvans and alginates, respectively (Lahaye et al., 2007; Kumar et al., 2008). The latter can alter the distribution of soil moisture (Nabti et al., 2017). However, brown seaweed has very little effect on the physical properties of coconut coir despite its high aeration capacity and low water retention capacity (Abad et al., 2005). As expected,

C. baccata increased the total water retention capacity (EAW + WBC + UW) of the substrates, mainly by increasing WBC (by 5–10 kPa); on the other hand, *Ulva* decreased the water retention capacity —by up to 8% in the mixture containing 50% of this seaweed— to the benefit of aeration.

4.2. Substrate performance

The Chinese cabbage experiment revealed inhibited germination relative to peat 5 days after sowing. The effect disappeared at the second sampling (10 days), which suggests that some unknown factor may have not completely suppressed germination but rather delayed it. One such factor might be salinity, which is especially important in seedlings (Sharma et al., 2004). However, there was no correlation between substrate EC and germination. The addition of *C. baccata*, which is highly saline, improved germination and root growth. Some authors have found seaweed to induce resistance against abiotic stresses such as salinity by virtue of its containing biostimulating and osmoprotective substances (Neily et al., 2008; Nabti et al., 2017) that additionally improve root development (Finnie and Van Staden, 1985; Crouch and van Staden, 1991). In the longer term, the high enzyme contents of seaweed can facilitate enzymatic hydrolysis of insoluble substances, and hence demineralization and desalination of the medium (Canales-López, 1999).

Despite the good results obtained with both cress and Chinese cabbage, the latter developed increasing problems in substrates 25C and 50C and eventually died in most cases. Also, plant development and EC were correlated, the plants grown in the most saline substrates being those surviving the least. However, the facts that the previous treatment had no apparent adverse effect on germination, that the initial salinity was not too high and that daily irrigation facilitated leaching —and hence rapid removal of excess salinity— suggest that the adverse effect observed was not due to salinity. One plausible reason for the low survival rate may have been the presence in the seaweed of excessive amounts of biostimulants, which promote growth at low concentrations but inhibit it at high concentrations (Battacharyya, 2015). Also, the organic matter may have been unstable and decomposed during cropping —one of the greatest shortcomings of using fresh seaweed in growing substrates. In fact, the stability of a substrate is governed by the resistance of its organic matter to microbial degradation (Komilis, 2015). The decomposition of fresh organic matter produces secondary metabolites such as low-molecular weight organic acids (e.g., phenolic acids), ammonium ion and some salts high

concentrations of which can hinder plant growth (Zucconi et al., 1981a; Luo et al., 2018). Also, an immature growing medium can lead to poor N fixation and an oxygen-deficient rhizosphere (Raviv et al., 1986), thereby also adversely affecting crop growth.

The results of the cucumber experiment, which was conducted on robust plants after germination, were rather different. Thus, plant growth was optimal with *C. baccata*, particularly at the highest proportions in the mixed substrates, and worse with *Ulva*, both with and without added fertilizer. As regards fertilization efficiency, the substrate containing 10% of *C. baccata* clearly excelled all others. Several studies have shown substrates containing seaweed extracts to improve nutrient uptake by plants (Battacharyya et al., 2015).

5. CONCLUSIONS AND FUTURE PROSPECTS

Our results confirm the starting hypothesis that seaweed can be a useful component of plant growing substrates. Seaweed is a natural, biodegradable, pathogen- and weed-free, non-toxic material capable of enriching common substrates such as coconut coir with nutrients. Also, the biostimulating properties of seaweed boost crop growth and nutrient uptake. As with extracts, however, it is very important not to add excessive amounts of seaweed to the substrates to avoid toxic effects. Additional studies are needed to determine both the most advantageous types of seaweed and the most appropriate proportions to obtain the best effects on the crop, depending on the intended use of the substrates

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SUBJECT INDEX