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Effect of Brown Algae on Germination, Growth and Biochemical Composition of Pepper Leaves (*Capsicum annuum*)

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Abstract

In this study, we have evaluated the effects of three brown algae (*Cystoseira gibraltarica*, *Bifurcaria bifurcata,* and *Fucus spiralis*) as biostimulants on the germination and *in vitro* growth of pepper (*Capsicum annuum*) as well as in greenhouse. Two different treatments (Amendment/spray) have been tested accordingly, both with varied concentrations (0.5%, 1% and 2%) for extract and (C1=0.5 g/pot, C2=1 g/pot and C3= 2.5 g/pot) for amendment). Results showed that the seeds which were treated by *C. gibraltarica*, and *B. bifurcata* with a low concentration (0.5%) have slightly germinated with a high length of hypocotyls. Similar results have been obtained for the radicle length. On the other hand, *F. spiralis* extract improved the percentage of germination, the length of the radicle, hypocotyls, and seedling length and dry weights for the three tested concentrations. Also, all concentrations have a favorable effect on seedling biomass. In the greenhouse, the growth rate (roots' length, aerial part length, total length, and dry weights) was higher for the plants which received higher concentrations of aqueous extracts of the three species *B. bifurcata*, *F. spiralis*, and *C. gibraltarica*. Additionally, the maximum quantity of chlorophyll 'a' has been recorded in the plants treated with *B. bifurcata* and *C. gibraltarica* at 2% and *F. spiralis* extracts at 0.5%. In regard to the protein content, the maximum values were recorded at the level of the plants that were treated with *F. spiralis* extract at 0.5%. This study has provided important information about *B. bifurcata* and *F. spiralis* species could be considered as a good biostimulant to improve pepper's growth.

Keywords: Algae extracts, Amendment, Biostimulants, Germination, Growth.

Introduction

The fertilizers amelioration have become nowadays, one of the most serious issues for the whole countries of the world due to the fast growth of the population as well as the negative impact of using chemical fertilizers on the soil's fertility, and its risks on the environment, too. A significant portion of the algae which is produced annually is used as a nutrient supplement or plants growth regulator, fertilizer or pesticide (khan et al., 2009). Several studies have shown that the use of products based on seaweed improves significantly the productivity and quality of crops, even when they are used at low doses. According to (Marfaing and Lerat, 2007), algae is a very rich source of multiple nutrients such as (fiber, minerals and proteins), vitamins and fatty acids which are occupying a unique place in nutrition compared to other plants and more generally to other

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sources of nutrients. A chemical analysis of algae and their extracts revealed the presence of a wide variety of plant growth promoting substances such as auxins, cytokinins, and betaines (Khan et al., 2009). These substances can influence the development of the stem and the root's system of a plant (Stirk et al., 2004). In addition, macronutrients and micronutrients can help improve the growth of various vegetables, fruits and other crops, (Blunden, 1991; Crouch and Van Staden 1993; Möller and Smith, 1998). Similarly, various researches maintain that organic fertilization with seaweed has tremendous effects on the qualitative and quantitative productivity of various plant species (Augier and Santimone, 1978).

Indeed, market gardening is a staple food for the populations by contributing to the satisfaction of consumption needs. According to FAO (2017) statistics, world production of pepper in 2014 is estimated at about 32.3 million tonnes for an area of 1.9 million ha yielding an average yield of 17 t/ha. Accordingly, we have tested the effect of two treatments (algal extract and amendment) with brown algae on a socio-economically important plant which is pepper.

Materials and Methods

Three species of brown algae, *Cystoseira gibraltarica*, Bifurcaria bifurcata and *Fucus spiralis* had been collected during tide time at the coastal zone Cap Ghir, (30°38'37"N, 09°53'20"O), located about 43 Km West of Agadir, Morocco. In the laboratory, the algae species were being carefully cleaned of parasites they contained; they were also dried and then transformed into a fine powder.

Treatments Preparation

Aqueous Extracts

Five grams of each algal species were added to 100 ml of sterile distilled water. The obtained mixture was left for 24 hours to macerate and then centrifuged at 3000 tour/minute for 15 minutes. The recovered supernatant was filtered on Whatman nbr 1 paper. Also, the obtained extracts were stored at 4°C for different analysis and preparation of various watering concentrations.

Amendments

The obtained powder was used for the preparation of the three concentrations, C1 (0.5 g/pot), C2 (1g/pot) and C3 (2.5 g/pot) based on the concentrations used in organic farming 25kg/100 m2.

Germination Tests

The experiments were carried out using pepper seeds (Capsicum. annuum) of the Roldan variety. Ten treatments were tested with 100 seeds divided into four Petri dishes (25 seeds per a dish). Before treating the seeds with the aqueous extracts, they were treated with a solution of sodium hypochlorite 6% for 3 minutes. The tested seeds were placed on a paper filter in Petri dishes and then treated with 5 ml of distilled water (control) or different concentrations of the aqueous extracts (0.5%, 1% and 2%). The dishes are incubated at 24 ± 1°C. The germination is under a daily observation over a period of eight days.
The variables which were measured, were the germination percentage (GP), the germination index (GI), the mean germination time (MGT) and the seedling vigor index (SVI), as well as the length of the hypocotyl, the length of the radicle, the total height of the plant and dry weight of the seedlings. The effect of aqueous extracts on seed germination and plant growth was measured after eight days of germination.

Greenhouse Growth

The two hundred pepper plants that were cultivated in the greenhouse had been selected and planted in pots that contain a mixture of soil and peat (3:1). The plants were watered with aqueous algae extracts (50 ml per week), meanwhile, the fertilization with the amendment was done at the time of plant transplantation with the three different concentrations, determined previously. All pots were irrigated separately with water every two days. The potted plants were growing for seven weeks in the greenhouse, each treatment was represented by ten repetitions. The plants of each treatment were chosen randomly for diverse analyses. After 45 days of transplantation, the plants were removed and analyzed at the level of their growth parameters (length of root, length of the aerial part, total length, dry weight) and their biochemical parameters (pigments, proteins, total sugars).

Algae's Physicochemical Contents

The determination of the content of the three algae in mineral elements is carried out according to Page et al. (1982).

Dosage of Total Sugars

The dosage of total sugars was carried out according to Dubois et al. (1956). Briefly, twenty mg of dry plant material was homogenized with 2 ml of 70% ethanol (v/v), the mixture was centrifuged at 2000 rpm for 10 min. The supernatant was recovered and the pellet was rinsed twice with 70% ethanol (v/v). For the supernatants, they were combined; 16 ml of distilled water was added to them. 200 µl of a solution to be dosed and added to 200 µl of a 5% aqueous phenol solution, then 1 ml of concentrated sulfuric acid was rapidly introduced into the reaction medium. The homogenized vortex mixture was allowed to stand for 10 minutes and then placed in a water bath, for 10 to 20 minutes at a temperature of 30 ° C. The optical density is taken at 490 nm using the visible IC 6400 spectrophotometer.

Dosage of Chlorophylls and Carotenoids

A quantity of 0.5 g of fresh frozen leaves are crushed, then homogenized with 50 ml of acetone 90% (v/v), the extract is then centrifuged at 3000 rpm. The OD (optical density) was

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read at different wavelengths: 470 nm for carotenoids, 645 nm for chlorophyll "b" and 663 nm for chlorophyll "a".
The concentrations were calculated on the basis of the following formulas (Lichtenthaler, 1987):
Chlorophyll "a" (chl "a") (mg/g FM) =(11.75×DO663-2.35×DO645)×50/500
Chlorophll "b" (chl "b") (mg/g FM) = (18.61×DO645-3.96×DO663)×50/500
Carotenoids (mg/g FM) = ((1000×DO470)-(2.27×Chl "a")-((81.4×Chl "b"))/227)×50/500.

Dosage of Proteins

The dosage of protein content was carried out according to Lowry et al. (1951). Briefly, the Lowry method consists in forming a complex between the peptide bonds and the copper sulphate, in an alkaline medium. This complex after reduces the phosphomolybdic and phosphotungstic acids of the Folin-Ciocalteu reagent, to give a second blue complex, as measured by spectrophotometer (Frolund and Griebe, 1995).

The assay reagent (solution R) is prepared from three solutions in the order of addition of the reagents and stirring after each addition:
-Solution A: sodium carbonate (Na_2CO_3) at 20 g/ and sodium hydroxide (NaOH) 0.1 mol / l.
-Solution B: sodiumtartrate/potassium (Na/K) at 20 g/l.
-Solution C: copper sulphate at 10 g/l.
An amount of 0.1 g of leaf powder is milled in 1 ml of lysis buffer to extract the proteins. The extract is centrifuged at 13000 g for 10 min. The lysis buffer is prepared by mixing 8 ml of 1M Tris-HCl pH = 6.8, 2 ml of β-mercaptoethanol, 10 ml of SDS and 80 ml of water.
10 µl of the supernatant are added, 990 µl of water and 5 ml of solution R (3 ml of solution C, 3 ml of solution B and 300 ml of solution A). The tubes are incubated for 10 min in the dark, then 0.5ml of a solution of Folin-Ciocalteu 50% (v/v) reagent is added, the mixture is vortexed. Stabilization of the color takes a few minutes. The intensity of the coloration obtained is evaluated by measuring the absorbance at 750 nm using visible spectrophotometer IC 6400.

Statistical Analysis

The data has been processed by the STATISTICA software, version 6.0. The analysis of variance (ANOVA) was used to determine the degree of significance. The averages were compared using Duncan's tests at the probability level (P <5%).

Results

Physicochemical Analysis

The results obtained from the physicochemical analysis of the aqueous extracts of the three brown algae (*C. gibraltarica, B. bifurcata and F. spiralis*) showed the presence of various micro and macroelements (Table 1). The concentration of N and Ca is higher in *B. bifurcata* (1.75 and 3.24 g/100g DM, respectively), whereas P and Na are rather high in *F. spiralis* extract (0.2 and 1.85 g/100g DM respectively). The Potassium is well-represented in the extracts of the three algae with a higher content in *B. bifurcata* (5.02 g/100g DM) followed by F. spiralis (4.96 g/100g DM) and *C. gibraltarica* with a value of (4, 12 g/100g DM). In contrast to K, P is poorly concentrated in all three algae (Table 1). The concentration of Fe, Cu and organic matter is higher in both *F. spiralis* and *C. gibraltarica* algae (55.94, 25.76, 73.74 mg/kg DM respectively). While the concentration of Zn is higher in *B. bifurcata* extract (13.6 mg/kg DM). The Mn is higher in *C. gibraltarica* and *B. bifurcata* extract (18.44 mg/kg DM), the same result is revealed for Mg which represents a high concentration in *F. spiralis* (1.03 g/100g DM) (Table 1).

On the other hand, the pH values for the three aqueous algae extracts are greater than 6 except for the aqueous extracts of *B. bifurcata* at 1 and 2% (Table 2). The value of the electrical conductivity (EC) increases proportionally with concentration of the aqueous extracts of the three algae (Table 2). The same finding is recorded for salinity, which increases with increasing concentration of the extract and is also lower for *F. spiralis* extract (Table 2).

Effect of Aqueous Extracts of Algae on Seed Germination and Growth of Pepper Seedling

The pepper plants treated with the aqueous extracts of these three brown algae showed an improvement in the germination percentage (GP), germination index (GI), mean germination time (MGT) and seedling vigor index (SVI) (Table 3). Three brown seaweeds *B. bifurcata, C. gibraltarica, and F. spiralis* with the exception of the 2% concentration of *B. bifurcata* and *C. gibraltarica* showing a minimum value of the germination percentage and the vigor index of the seedlings respectively (Table 3). According to statistical calculations the aqueous extract of *B. bifurcata* at 0.5% obtained significantly high results in the other extracts. The maximum value of the germination percentage is presented by the concentrations 0.5 and 1% of *F. spiralis and B. bifurcata* (87.3), whereas the minimum value

Table 1. Minerals elements content of the three algae studied.

	g/100g							mg/kg			
species	**MO**	**N**	**Ca**	**K**	**Mg**	**P**	**Na**	**Fe**	**Mn**	**Cu**	**Zn**
B.B	69,68b	1,75 ±0,1a	3,24±0,3a	5,02±0,02a	0,66±0,06c	0,16±0,01b	1,65±0,4b	37,28±0,4b	18,44±0,1a	12,87±0 08b	13,6±0,1a
C.G	73,44a	1,38±0,1b	2,46±0,1b	4,12±0,03c	0,74±0,1b	0,12±0,01c	0,98±0,2c	55,92±0,5a	18,45±0,1a	25,76±0,09a	5,45±0,1b
F.S	73,74a	1,33±0,8c	2,34±0,2c	4,96±0,01b	1,03±0,08a	0,20±0,02a	1,85±0,3a	55,94±0,9a	9,22±0,1b	25,76±0,08a	5,46±0,2b

The values represent the mean ± SD (n = 3). *BB: B. bifurcata*, *CG*: *C. gibraltarica*, and *FS*: *F. spiralis.*

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Table 2. Physicochemical characterization of aqueous algae extracts from treatments of *B. bifurcata* (BB), *C. gibraltarica* (CG), and *F. spiralis* (FS).

Treatments (%)	pH	EC (dS/m)	Salinity (g/l)
Control	7,0±0,01 a	0 e	0,05±0,01 e
BB 0,5	6,02±0,01 f	2,16±0,02 g	1,12±0,01 g
BB 1	5,9±0,01 h	4,3±0,02 d	2,24±0,01 d
BB 2	5,82±0,02 g	7,98±0,01 a	4,32±0,02 a
CG 0,5	6,22±0,11 c	2,01±0,01 h	1,03±0,01 h
CG 1	6,13±0,01 d	3,7±0,1 e	2,01±0,01 e
CG 2	6,09±0,01 e	6,59±0,01 b	3,53±0,01 b
FS 0,5	6,29±0,01 b	1,46±0,02 k	0,76±0,01 k
FS 1	6,29±0,02 b	2,80±0,02 f	1,44±0,03 f
FS 2	6,29±0,03 b	4,6±0,04 c	2,44±0,01 c

The values represent the average ± SD (n = 3). EC: electrical conductivity.

is recorded by the aqueous extract of *B. bifurcata* at 2% (Table 3). Similarly, all the aqueous extracts of the algae show a significant difference to the control with the exception of the 2% aqueous extract of *F. spiralis* which has a low germination index (8.9), whereas the maximum value is shown by the aqueous extract of *B. bifurcata* at 0.5 % (16.1) (Table 3). While all concentrations of these three brown algae recorded a mean germination time, not significantly different from the control. The vigor index of the seedlings is significantly improved by all the aqueous extracts of the algae with the exception of the concentration 2% of *B. bifurcata* and *C. gibraltarica* (261 and 265 respectively), the maximum value of the seedling vigor index is recorded by the aqueous extract of *B. bifurcata* at 0.5% (490.1) (Table 3).

In addition, whatever the alga or its concentration, there is a positive effect on the parameters of the growth of pepper seedlings (Table 4). It should also be noted that low concentrations are more effective than high concentrations for all three algae. Thus, the highest mean radicle length is recorded by the aqueous extract of all *B. bifurcaria, C. gibraltarica* and *F. spiralis* algae at 0.5% (3.06 and 2.83 and 2.96 cm, respectively) (Table 4). In addition, these results indicate that the aqueous extracts of these three algae promote the growth of hypocotyl length with the exception of the aqueous extract of *B. bifurcata* and *C. gibraltarica* at 2% which show an inhibitory effect on the length of the hypocotyl (1.41 and 1.31 cm, respectively). The length of the highest mean hypocotyl is presented by the aqueous extracts of *C. gibraltarica* and *B. bifurcata* at 0.5% (2.59 and 2.57 cm, respectively) (Table 4). The maximum length of the seedlings is recorded by the aqueous extract of *B. bifurcata* at 0.5% (5.66 cm), while the minimum length is presented by the concentration 2% of *C. gibraltarica* and *B. bifurcata* (3.07 and 3.22 cm, respectively) (Table 4). Aqueous extracts of all algae showed a significant effect on the dry weight of pepper seedlings. The maximum dry weight is shown by the aqueous extract of *F. spiralis* at 0.5 (3.72mg) (Table 4).

Effect of Algae on Pepper Seedling Growth in Greenhouse

After germination the seedlings are transported under greenhouse and we noticed that all the aqueous extracts of the three algae showed an improvement in the length of the aerial part, the root and the total length of the plant (Table 5). Plants treated with aqueous extracts of *B. bifurcata* at 1% and *C. gibraltarica* at 2% showed an increase in the length of the aerial part (24.55 and 24.35 cm, respectively). Aqueous extract of *B. bifurcata* at 2% recorded high values of root length and total plant length (10.32 and 34.35 cm, respectively) (Table 5). Fertilization by amendment shows an improvement in the length of the aerial part with the exception of C3 amendment of *C. gibraltarica* and two concentrations C2 and C3 of *F. spiralis* which show low values (Table 5). The C1 Amendment of *B. bifurcata* has the maximum length of the aerial part (22.39 cm), while the minimum length of the aerial part is recorded by C3 amendment of *C. gibraltarica* (15.5 cm). All amendments show an improvement in root length with the exception of *C. gibraltarica* at C3 amendment and *F. spiralis* at C1 amendment which shows a minimum length (6.4 cm) (Table 5). No noticeable effect was detected by the amendments, except for the C1 and C2 concentrations of *B. bifurcaria* and *C. gibraltarica* which show significantly different values to the control (Table 5). The maximum total length is recorded by the C1 amendment of *B. bifurcaria* (32.69 cm) (tab 5). Regarding the dry weight, we noted that the aqueous extract of *C. gibraltarica* at 2% has a

Table 3. Effects of aqueous algae extracts (AAEs) treatments on the germination parameters of pepper seeds: germination percentage (GP), germination index (GI), mean germination time (MGT), and seedlings vigor index (SVI).

Aqueous Extract (%)	GP (%)	GI	MGT (Days)	SVI
Control	82,3±3,11 ab	9,2±1,00 a	8,5±0,30 a	281,4±28,6 ab
BB 0,5	86,3±1,11 a	16,1±2,97 c	8,6±0,65 a	490,1±9,9 c
BB 1	87,3±3,77 a	14,6±3,6 b	8,7±0,78 a	398,4±29,3 bc
BB 2	78,3±1,11 b	9,3±2,65 a	9,1±0,73 a	261,9±20,2 a
CG 0,5	86,3±3,77 ab	13,6±2,60 ab	8,7±0,65 a	430,1±10,0 c
CG 1	82,3±1,11 ab	14,4±1,27 ab	8,5±0,32 a	387,6±12,4 abc
CG 2	84,3±3,55 ab	13,6±1,69 ab	8,8±0,49 a	265,6±14,8 a
FS 0,5	87,3±1,11 a	12,5±1,46 ab	8,8±0,39 a	453,6±30,7 bc
FS 1	82,6±3,11 ab	10,7±3,14 a	8,7±0,10 a	370,2±26,4 abc
FS 2	82,3±1,11 ab	8,9±3,49 a	8,9±0,03 a	383,5±15,2 abc

The values represent the average ± SD (n = 300 seeds). The values indicated by a different letter are significantly different P≤0.05. *BB: B. bifurcaria, CG: C. gibraltarica, FS: F. spiralis.*

Table 4. Effects of aqueous algae extracts *C. gibraltarica* (CG), *B. bifurcata* (BB) and *F. spiralis* (FS) on the length of the radicle, length of the hypocotyls and dry weight of pepper seedlings at different concentrations (0.5%, 1% and 2%).

Aqueous Extracts (%)	Radicle Length (cm)	Hypocotyl Length (cm)	Seedling Length (cm)	Dry Weight (mg)
Control	1,78±0,07 a	1,69±0,02 ab	3,47±0,13 abc	2,61±0,03 a
BB 0,5	3,06±0,51 b	2,59±0,36 a	5,66±0,84 d	3,28±0,18 ab
BB 1	2,77±0,44 ab	2,37±0,51 ab	5,16±0,05 abd	3,19±0,32 ab
BB 2	1,73±0,31 a	1,48±0,22 ab	3,22±0,73 ac	3,67±0,54 b
CG 0,5	2,83±0,25 ab	2,57±0,54 a	5,49±0,07 bd	3,50±0,33 ab
CG 1	2,47±0,41 ab	2,18±0,69 ab	4,62±0,12 abcd	2,96±0,64 ab
CG 2	1,74±0,35 a	1,31±0,58 b	3,07±0,15 c	2,95±0,59 ab
FS 0,5	2,96±0,69 b	2,16±0,43 ab	5,14±0,04 abd	3,72±0,27 b
FS 1	2,7±0,47 ab	1,87±0,38 ab	4,62±0,10 abcd	3,15±0,14 ab
FS 2	2,68±0,73 ab	1,99±0,54 ab	4,68±0,12 abcd	3,33±0,42 ab

The values represent the average ± SD (n = 300 seeds). The values indicated by a different letter are significantly different P≤0.05.

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Table 5. Effect of aqueous algae extracts *Bifurcaria bifurcata* (BB), *Cystoseira gibraltarica* (CG), and *Fucus spiralis* (FS) treatments applied as soil spraying and soil amendment on the length of the aerial part, the length of the roots, total length and biomass of pepper plants at different concentrations.

Spraying	Plant Length (cm)			Dry Weight in (g)		
AE (%)	Aerial Part	Root	Total Length	Aerial Part	Root	Total Weight
Control	17,32±0,49 b	6,52±0,88 c	23,84±0,41 d	0,21±0,03 d	0,08±0,02 c	0,29±0,06 d
BB 0,5	23,35±1,27 a	7,30±0,38 b	30,65±0,31 bc	0,49±0,01 c	0,11±0,01 bc	0,60±0,01 c
BB 1	24,55±2,6 a	7,39±0,65 b	31,91±1,05 bc	0,64±0,03 ab	0,2±0,01 ab	0,85±0,1 ab
BB 2	24,03±2,47 a	10,32±,32 a	34,35±0,72 a	0,63±0,01 ab	0,23±0,02 a	0,86±0,05 a
CG 0,5	23,91±2,73 a	9,56±0,65 b	33,47±0,97 a	0,59±0,02 b	0,20±0,07 ab	0,8±0,02 ab
CG 1	23,93±1,91 a	8,79±0,70 b	32,72±2,12 bc	0,64±0,01 ab	0,21±0,05 a	0,86±0,01 a
CG 2	24,35±2 a	8,58±0,77 b	32,93±2,01 ab	0,65±0,02 a	0,24±0,01 a	0,90±0,05 a
FS 0,5	23,54±1,82 a	8,09±0,73 b	31,63±1,24 ab	0,6±0,04 ab	0,22±0,05 a	0,82±0,01ab
FS 1	22,95±1,27 a	7,79±0,84 bc	30,74±1,46 c	0,54±0,07 c	0,20±0,06 ab	0,74±0,16 b
FS 2	23,62±2,64 a	7,50±0,48 bc	31,12±0,83 c	0,59±0,07 b	0,23±0,02 a	0,83±0,01ab
Amendment						
Control	17,32±0,29 ab	6,52±0,36 bcd	23,84±0,77 de	0,21±0,01 cd	0,08±0,01 ed	0,29±0,02cd
BB C1	22,39±0,85 c	9,95±0,68 a	32,69±1,26 a	0,39±0,05 a	0,19±0,02 a	0,58±0,01 a
BB C2	20,03±0,91 bc	8,73±0,88 ab	28,50±0,78 b	0,31±0,03 b	0,12±0,01 bc	0,44±0,01 b
BB C3	17,78±0,89 ab	6,97±0,75 cd	24,62±0,78 ef	0,31±0,01 b	0,11±0,01 bcd	0,43±0,01 b
CG C1	18,76±0,75 b	8,35±0,22 bc	27,17±0,67 c	0,34±0,02 b	0,14±0,01 c	0,48±0,01 b
CG C2	18,73±0,50 b	7,71±0,35 d	26,14±0,32 e	0,33±0,01 b	0,14±0,01 c	0,47±0,02 b
CG C3	15,50±0,24 a	6,40±0,98 d	21,83±0,79 g	0,20±0,01 d	0,07±0,01 de	0,28±0,01 d
FS C1	18,09±0,50 ab	6,4±0,54 cd	24,02±0,32 f	0,26±0,01 c	0,09±0,01 de	0,35±0,01 c
FS C2	15,79±0,61 a	7,13±0,31 cd	22,13±0,64 g	0,19±0,01 d	0,06±0,01 e	0,24±0,01 d
FS C3	15,88±0,64 a	6,60±0,30 d	22,33±0,62 g	0,20±0,01 cd	0,09±0,02 bde	0,30±0,01cd

The values are the average ± SD (n = 10). The values indicated by a different letter are significantly different P≤0.05. C1 (0.5 g/pot), C2 (1g/pot) and C3 (2.5 g/pot).

Table 6. Effect of aqueous algae extracts *B. bifurcata* (BB), *C. gibraltarica* (CG), and *F. spiralis* (FS) treatments applied as soil spraying and soil amendment on protein content, the quantity of total sugars and chlorophyll pigments content of pepper leaves at different concentrations.

Spraying	Proteins	Total of Sugars	Pigments (mg/g FM)			
AE (%)	mg/g DM	mg/g DM	Chl a	Chl b	Carotenoids	Chl Total
Control	27,1±1,51 ef	18,47±1,16 c	0,50±0,13 cd	0,30±0,07 bc	0,10±0,02 e	0,81±0,20 bc
BB 0,5	24,65±1,04 f	22,33±1,05 b	0,45±0,02 d	0,26±0,01 c	0,16±0,02 cd	0,71±0,03 b
BB 1	35,84±0,92 c	30,08±0,55 a	0,50±0,04 cd	0,32±0,08 abc	0,15±0,02 cd	0,82±0,13 bc
BB 2	32,78±1,31 d	18,16±0,38 c	0,72±0,10 abcd	0,42±0,07 abc	0,22±0,03 ab	1,15±0,17 abc
CG 0,5	43,54±1,57 a	14±0,88 d	0,62±0,17 abcd	0,39±0,12 abc	0,19±0,05 abc	1,02±0,30 abc
CG 1	35,32±1,18 c	18±0,27 c	0,62±0,10 abcd	0,29±0,02 bc	0,16±0,02 bcd	0,92±0,11 abc
CG 2	27,54±1,31 e	9,36±0,7 e	0,80±0,18 abc	0,46±0,11 ab	0,20±0,20 abc	1,26±0,29 ac
FS 0,5	44,67±2,62 a	23,44±0,96 b	0,88±0,09 a	0,50±0,07 a	0,25±0,03 a	1,38±0,16 a
FS 1	27,45±0,92 e	23,36±0,46 b	0,82±0,21 ab	0,44±0,16 abc	0,20±0,04 abcd	1,26±0,37 ac
FS 2	40,39±1,04 b	22,75±0,27 b	0,55±0,19 bcd	0,29±0,14 bc	0,13±0,04 d	0,85±0,13 bc
Amendment						
Control	27,1±1,51 c	18,47±0,46 e	0,50±0,01 b	0,30±0,03 c	0,10±0,01 ab	0,81±0,02 ab
BB C1	31,03±1,06 b	21,13±0,46 d	0,63±0,09 a	0,35±0,03 abc	0,09±0,01 b	0,98±0,1 a
BB C2	31,73±1,31 b	35,19±0,42 b	0,67±0,09 a	0,38±0,07 a	0,13±0,02 a	1,05±0,16 a
BB C3	26,75±1,04 c	44,47±1,25 a	0,46±0,03 bc	0,27±0,04 bde	0,09±0,01 b	0,74±0,07 abcd
CG C1	22,55±1,04 d	35,5±0,05 b	0,65±0,15 a	0,27±0,09 bde	0,09±0,01 ab	0,93±0,1 abc
CG C2	23,08±2,62 d	13,88±0,31 f	0,40±0,02 c	0,24±0,01 e	0,07±0,01 b	0,64±0,04 d
CG C3	13,98±1,51 e	11,88±0,92 g	0,4±0,11 c	0,23±0,06 e	0,07±0,01 b	0,62±0,17 d
FS C1	28,15±1,32 c	21,94±0,7 d	0,41±0,08 c	0,25±0,05 de	0,09±0,01 b	0,66±0,14 d
FS C2	22,47±1,06 d	25,61±0,14 c	0,45±0,17 bc	0,25±0,09 de	0,07±0,01 b	0,7±0,27 bd
FS C3	38,47±1,09 a	26,72±0,18 c	0,43±0,03 bc	0,26±0,01 de	0,10±0,03 ab	0,69±0,04 d

Values average is ±SD (n = 3). The values indicated by a different letter are significantly different P≤0.05. Cl (0.5 g/pot), C2 (1g/pot) and C3 (2.5 g/pot).

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significant effect on the dry weight of the aerial part, root and on the total weight (0.65, 0.24 and 0.9 g, respectively) (Table 5). The fertilization by amendment showed a marked improvement of the aerial biomass, the root part and the total weight of the pepper plant with the exception of C2 amendment of *F. spiralis* which has low values of dry weight (Table 5). The C1 amendment of *B. bifurcaria* has a maximum dry weight of the aerial part, the root part and the total weight (0.39, 0.19 and 0.58 g, respectively) (Table 5).

Effect of Algae on Protein Content, Total Sugars, and Chlorophyll Pigments

All aqueous extracts of these three brown algae improved the protein content of pepper plants with the exception of *B. bifurcata* at 0.5%, which is the lowest level (24.65 mg/g DM). The plants watered with the aqueous extracts of the three brown algae had significantly higher levels than the control up to 44.67 mg/g DM (Table 6). *F. spiralis* with its three concentrations has the highest values, followed by the three concentrations of *C. gibraltarica*. The fertilization by amendment has affected the protein content less, indeed only the concentrations C1 and C2 of *B. bifurcata* and C3 of *F. spiralis* are significantly higher than the content of the control. The highest value is obtained by *F. spiralis* at C3 (38.47 mg/g DM), while the minimum content is presented by C3 amendment of *C. gibraltarica* (Table 6).

The aqueous extracts of the three brown algae improved the total sugar content of the pepper plants with the exception of *C. gibraltarica* at 0.5 and 2% (14 and 9.36 mg/g MS, respectively) which had the lowest levels, significantly different from the witness. The plants watered with the aqueous extracts of the three brown algae had significantly higher levels than the control up to 30.08 mg/g DM (Table 6). But unlike proteins, the aqueous extract of *C. gibraltarica* has no effect on the total sugar content. Fertilization by amendment has also improved the total sugar content. Indeed, all the treatments by amendment show significantly different values of the control except for once again C2 and C3 of *C. gibraltarica* which have the lowest levels (13.88 and 11.88 mg/g MS respectively). The highest values are obtained as for the algal extracts, by the three concentrations of *B. bifurcata* (Table 6). The maximum value of the total sugar content is recorded by C3 amendment of *B. bifurcata* (44.47 mg/g DM).

All aqueous extracts of these three brown algae showed an improvement in the amount of chlorophyll 'a', chlorophyll 'b', carotenoids and total chlorophyll (Table 6). Plants irrigated with 0.5% aqueous extract of *F. spiralis* had significantly higher levels of up to 0.88 mg/g MF for chlorophyll 'a' 0.50 mg/g MF, chlorophyll 'b' 0.25 mg/g MF carotenoids and 1.38 mg/g MF total chlorophyll (Table 6). The amendment treatments have no generally noticeable effect on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids content with the exception of *B. bifurcata* at C1 and C2 which have significantly different values from control. The maximum quantity of chlorophyll 'a', chlorophyll 'b', carotenoids and total chlorophyll are obtained by amendment C2 of *B. bifurcata* (0.67, 0.38, 0.13 and 1.05 mg/g MF respectively) (Table 6).

Discussion

The physicochemical analysis of Bifurcaria bifurcata, *Cystoseira gibraltarica*, and *Fucus spiralis* extracts revealed the richness of these brown algae in macroelements (Ca, K, P, Na, N), which was similar to the previous work of (Hong et al., 2007) (Kalaivanan and Venkatesalu, 2012), (Hernandez-Herrera et al., 2013). Likewise, these extracts have a pH which is slightly acidic and electrical conductivity and salinity which increased with the increase of the concentration of the algal extract. These last two parameters can influence tomato's germination and growth (Booth, 1969; Henry, 2005). Thus, the highest germination rate of pepper seeds was obtained with the low concentrations of the extracts of the three algae which have a conductivity that does not exceed 2.16 dS m/1. According to Reinhardt and Rost, (1995), most plants are sensitive to salinity during germination and seedling growth. However, a low concentration of salts in the medium enables the seeds to imbibe water and thus favor the rate of germination. Numerous studies have shown that the low concentrations of algal extracts have no harmful effect on plant germination and growth (Sridhar and Rengasamy, 2010; Kumari et al., 2011; Kalaivanan and Venkatesalu, 2012; Kumar et al., 2012; Ganapathy Selvam et al., 2013). However, aqueous extracts of *B. bifurcata* and *C. gibraltarica* algae at a higher concentration of 2% (7.98 and 6.59 dS/m, respectively) showed a negative effect on seeds germination, inhibiting them to absorb water. Similar results have been reported by (Hernandez-Herrera et al., 2013).

Whatever the type of treatment (Extract or amendment), the two algae of *B. bifurcata* and *C. gibraltarica* show maximum values of the length of the aerial part, the root, the total length and the biomass the pepper plant. According to Crouch et al., 1990, aqueous extracts of algae improved nutrient uptake in the roots. This stimulates root activity, increasing the absorption of water and nutrients, which improves plant growth and vigor.

Whatever the type of treatment (Extract or amendment) *B. bifurcata* had a significant effect on the length of pepper's root, this probably happens due to the richness of this alga in macroelements (N, Ca, K, P, Mg, Na). Algae contains macronutrients and microelements, amino acids, vitamins, cytokinins, auxins and abscisic acid that affect the cellular metabolism of treated plants, resulting in increased growth with crop yield (Crouch and Van Staden, 1993; Stirk et al., 2004). In addition, the presence of polysaccharides in aqueous extracts of algae as known sugars can enhance plant growth in a similar manner to hormones (Rolland et al., 2002). The aqueous extracts of brown algae contain various betaine type compounds (Blunden et al., 1986; Ghoul et al., 1995). This molecule acts as a compatible solute that alleviates salinity induced osmotic stress, and functions as a nitrogen source when provided in a low concentration and as an osmolyte at higher concentrations (Naidu et al., 1987).

Our study showed that treatment with aqueous extracts is more effective than amendment treatment for improving photosynthetic pigments in pepper leaves. Similar results have been shown by Whapham et al., 1993, where aqueous extracts of Ascophyllum Nodosum improved chlorophyll content in tomato leaves. Similarly, the aqueous extract of Sargassum was effective for the improvement of chlorophyll synthesis on two Zea mays and Phaseolus mungo plants (Lingakumar et al., 2004). A large content of chlorophyll contained in leaves treated with a low concentration of aqueous extracts of algae might be the consequence of the absorption of magnesium, which is a major constituent of chlorophyll (Whapham et al., 1993).

The protein content of the plants that were treated with the aqueous extracts is greater, compared to the treatment with an amendment. All aqueous extracts of these three brown algae improved the protein content of pepper plants. The maximum value of the protein content is obtained by *F. spiralis* at C3. Such an increase in protein content could be attributed to the increased availability and uptake of elements (N, K, Ca, Na, Mg, Cu, and Zn) which algal fertilizers contained. The same results were obtained in Zea mays and Phaseolus mungo which had high protein content when treated with concentrations 0.5% and 1% of the aqueous extract of Sargassum. sp (Lingakumar et al., 2004). Other results showed that the aqueous extracts of Sargassum johnstonii resulted in an increase in the protein content (Kumari et al., 2011), the aqueous extracts of Hydroclathrus clathratus at 1.5% also lead to a significant increase at the level of Sorghum vulgare proteins (Ashok and Douglas, 2004). The increase in protein content in low concentrations of aqueous extract may be due to the absorption of most necessary elements (N, K, Ca, Na, Mg, Cu, and Zn) for seedling growth (Anantharaj and Venkatesalu, 2001).

The plants watered with the aqueous extracts of the three brown algae showed significantly higher total sugar contents than the control. Fertilization by amendment has also improved the total sugar content. The highest values for total sugars are obtained by C3 amendment of *B. bifurcata*. Aqueous algae extracts stimulate various biological processes that increase carbohydrate levels in tomato plants (Kumari et al., 2011). Similar observations were recorded at Vigna Catajung treated with aqueous extracts of Caulerpa racemosa (Anantharaj and Venkatesalu, 2001). A significant amount of total sugars was also observed in the plants treated with the aqueous extract of Sargassum johnston (Kumari et al., 2011).

Conclusion

The study highlighted the positive effect of algae fertilizers on germination, growth parameters and biochemical composition of pepper. Plants which were treated with low concentrations (0.5%) of three brown algae *B. bifurcata, F. spiralis* and *C. gibraltarica* showed greater growth. Similarly, the chlorophyll content was higher when the plants were treated with these 0.5% algal extracts. Meanwhile, the quantity of total sugars reached the maximum when fertilized with amendment or extract of *B. bifurcata*. The maximum growth parameters (root length, length of the aerial part, total length, and dry weight) were recorded in the plants treated with the aqueous extracts of the three brown algae *B. bifurcata, F. spiralis* and *C. gibraltarica* at high concentrations. In addition, the plants treated with aqueous extract of *F. spiralis* showed maximum values in the content of proteins.

Based on these results, both *F. spiralis* and *B. bifurcata* algae can be used as fertilizer for pepper or other vegetables, but at low concentrations to increase agricultural production. These results also had shown that a small content of aqueous algae extract can be used or even mixed with available fertilizers exist in the marketplace to enhance plant growth. Moreover, we found that treatments with algal extract were more effective than the one conducted by amendment. In conclusion, *B. bifurcata* and *F. spiralis* had also shown outstanding results in relation to *C. gibraltarica*. Therefore, more studies are needed to determine the potential of *B. bifurcata* and *F. spiralis* as growth biostimulants that can be used as organic biofertilizers in Morocco.

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