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Effect of algal fertilization on the biochemical and phytochemical composition and antioxidant activity of tomato and pepper plants

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Abstract. *Baroud S, Tahrouch S, Hatimi A. 2024. Effect of algal fertilization on the biochemical and phytochemical composition and antioxidant activity of tomato and pepper plants. Cell Biol Dev 8: 36-44.* The aim of our study was to evaluate the effect of three brown algae, *Bifurcaria bifurcata, Cystoseira gibraltarica* and *Fucus spiralis*, on the biochemical and phytochemical composition of tomato and pepper plants. The algae were applied in two forms and at different concentrations: aqueous extract (0.5%, 1% and 2%) and amendment (C1, C2 and C3). The aqueous extract of *B. bifurcata* with its three concentrations showed the highest protein content in tomato leaves (217, 200 and 196.9 mg/g DM) and all aqueous extracts of *F. spiralis* showed high levels of total sugars (83.13, 83.08 and 75.38 mg/g DM). For pepper, the highest protein content was recorded for the 1% *C. gibraltarica* aqueous extract (196.57 mg/g DM). High levels of total sugars in pepper leaves were induced by the 2% *C. gibraltarica* aqueous extract (52.22 mg/g DM). Furthermore, the photosynthetic pigment content of the leaves of both vegetable crops (tomato and pepper) was generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae. In addition, tomato and pepper plants treated with aqueous extracts (spraying) or by amendment, showed a significant improvement in all phytochemical parameters and antioxidant activity. These three algae proved to be good candidates for the effective development of biostimulants to improve biochemical composition and phytochemical parameters. This study could provide important information on the identification and use of Moroccan algal resources in agriculture.

Keywords: Amendment, aqueous extracts, biochemical, biofertilization, phytochemical

INTRODUCTION

The main aim of an organic farming system is to optimize the health and productivity of soil, plants, animals and people, and to create an ecological balance and better functioning of the agro-ecological system (Dumont et al. 2013). This type of agriculture is based on the use of biostimulators as organic fertilizers made from dead leaves, grass clippings, vegetable garden waste, ashes, plant extracts (Cisse 2014). Seaweed-based fertilization is one of the fertilizers used by farmers to improve germination and growth of vegetable crops.

In Morocco, sea currents and hydroclimatic conditions rapidly favor for the development and expansion of marine algae such as brown seaweed and many species of algae grow rapidly and efficiently, (green algae) especially compared with land plants (Kindleysides et al. 2012). Brown algae are very abundant along the Atlantic coast, especially in the Cap Ghir Region. This biomass, while promoting research aimed at exploiting these brown algae, notably *Cystoseira gibraltarica*, *Bifurcaria bifurcata* and *Fucus spiralis*, in a number of fields, particularly agriculture.

The greenhouse is a structure designed to house vegetable crops in more favorable or safer conditions than in the open air (Osentowski 2015). This structure protects plants by controlling the climate to obtain optimal growth conditions or minimize health risks. Greenhouse cultivation plays an important economic role in the marketing of off-season products. This technique makes it possible to grow plants in better conditions than those found in the natural environment, and therefore to obtain better-quality products.

Pot cultivation is the practice of growing plants, including vegetable plants, exclusively in pots instead of planting them in the ground (Mills 2012). Pot cultivation is a method used by farmers in areas where the soil or climate is unsuitable for the crop in question. As such, this method is useful for scientific trials before moving on to full-ground cultivation. In addition to the optimal conditions offered by the greenhouse, seaweed fertilization could significantly improve the growth and yield of greenhouse crops.

Some studies indicate that algal extracts can partially substitute fertilizers (Hong et al. 2007; Zodape et al. 2010) because they contain both minor and major mineral elements. Saccharides from algal extracts can act as elicitors of plant defensive mechanisms (Khan et al. 2009). Algae-based fertilizers contain 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 aerial and root parts of plants (Durand et al. 2003). In addition, macronutrients (N, P, K, Ca and Na) and micronutrients (Fe, Zn, Mn and Cu) can promote fruit growth and yield (Möller and Smith 1998). These algal extracts can also increase phytochemical

parameters (Lola-Luz et al. 2014). Positive responses include improved plant growth and fruit quality, as well as overall plant vigour and pathogen resistance (Khan et al. 2009). For example, Ali et al. (2016) showed that the application of aqueous extracts of *Ascophyllum nodosum* algae increased the chlorophyll 'a' and 'b' content of tomato plants. In this context, our objective is to carry out a greenhouse experiment to test the effects of three brown algae: *B. bifurcata, C. gibraltarica* and *F. spiralis* on the biochemical and phytochemical parameters of pepper and tomato leaves and fruit.

MATERIALS AND METHODS

Plant material

Three brown seaweeds, *C. gibraltarica, B. bifurcata* and *F. spiralis* were collected at low tide, in the coastal area of Cap Ghir, (30°38'37 "N, 09°53'20 "W), located about 43 kilometers northwest of Agadir Morocco. All algal species identified by algal specialist Prof Chfiri INRH Agadir. The algae species harvested are carefully washed and dried, before being ground to a fine powder.

The experiments were carried out using certified tomato seeds (*Solanum lycopersicum*) of the Campbell variety marketed by Technisem, and pepper seeds (*Capsicum annuum*) of the Roldan variety.

Treatment preparation

Two types of treatment are used: amendment and spraying.

Amendment: Seaweed powder is applied to the crops in specific concentrations: C1 concentration (2.5 grams of powder per pot), C2 concentration (5 grams of powder per pot) and C3 concentration (10 grams of powder per pot).

Algal extract: Tomato and pepper plants are regularly sprayed with an aqueous algal extract in three different concentrations (0.5, 1 and 2%).

Preparation of soil improvers

Three amendments are prepared based on the concentrations used in organic farming (25 kg/100 m^2). Each pot contains 5 kg of substrate made up of a mixture of 75% soil and 25% peat. Three amendments are determined: C1 (2.5 grams of powder per pot), C2 (5 grams of powder per pot) and C3 (10 grams of powder per pot).

Preparation of algal extracts

Five grams of powder of each algal species are added to 100 mL of distilled water under magnetic stirring for 24 hrs. The recovered supernatant is filtered, and the aqueous extracts obtained are then stored in a cool place. These extracts are designated as stock solutions and coded according to genus and species: *C. gibraltarica* (C g), *B. bifurcata* (B b) and *F. spiralis* (F s). The stock solution of each alga was diluted with water to three concentrations (0.5, 1 and 2%).

Setting up greenhouse cultivation

The seeds of the two vegetable plants (tomato and pepper) were germinated in honeycomb plates containing peat. After 25 days of germination, 400 plants were selected at the four-leaf stage and transplanted into five-liter pots containing 5 kg of a mixture of 75% soil and 25% peat. Ten pots were used for each algal treatment, with one plant per pot. Each pot receives 50 mL/week of algal extract in three concentrations (0.5, 1 and 2%). For the amendment, the treatment is also represented by three increasing concentrations C1 (2.5 g/pot), C2 (5g/pot) and C3 (10 g/pot). At the same time, we used a water-only control and a witness chemical fertilizer (Maxi Greene: N:20, P:20, K:20).

Spraying with aqueous extracts was applied two weeks after sowing at a rate of 50 mL/week for three months. Fertilization was carried out when the plants were transplanted, using the three different concentrations determined above. All pots were irrigated with 50 mL of water every other day during the growing period. After 90 days of cultivation (Figure 1), the tomato and pepper fruits were harvested and the plants carefully removed and washed. We then measured leaf biochemical parameters (proteins, total sugars and chlorophyll pigments) and phytochemical parameters (flavonoids, total phenols and antioxidant activity).

Figure 1. Tomato and pepper plants after 90 days of cultivation

Determination of biochemical parameters

Determination of total sugars (Dubois et al. 1956)

20 mg of algal powder homogenize with 2 mL of ethanol 70% (v/v), the mixture is centrifuged at 2000 g. After recovery of the supernatant, the pellet is rinsed twice with ethanol 70% (v/v). To the supernatants thus combined, 16 mL of distilled water are added. 200 µL of the solution to be determined is added to 200 µL of a 5% aqueous phenol solution, then 1 mL of concentrated sulfuric acid is quickly introduced into the reaction medium. The vortexed mixture is allowed to stand for 10 min and then placed in a water bath for 10 to 20 min at a temperature of 30°C. The optical density is read at 490 nm using the visible IC 6400 spectrophotometer. The blank is the reaction mixture without sample. The values obtained are converted into sugar content in mg/g of Dry Matter (DM).

Protein assay

The method of Lowry (Lowry et al. 1951) consists in forming a complex between the peptide bonds and copper sulfate in alkaline medium. This complex then reduces the phosphomolybdic and phosphotungstic acids of the Folin-Ciocalteu reagent to give a second complex of blue color, measured by spectrophotometer (Frolund et al. 1995).

The assay reagent (solution R) is prepared extemporaneously from three solutions, respecting the order of addition of the solutions and stirring after each addition:

- Solution C: copper sulfate at 10 g/L,
- Solution B: sodium/potassium tartrate (Na/K) at 20 g/L,
- Solution A: Sodium carbonate (Na_2CO_3) at 20 g/L and soda (NaOH) 0.1 mol/L.

Protein extraction *(Lowry et al. 1951)*

0.1 g of algal powder is ground in 1 mL of lysis buffer to extract the proteins. The extract is centrifuged at 13000 g for 10 min.

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

Assay method *(Lowry et al. 1951)*

To 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 0.5 mL of a 50% (v/v) Folin-Ciocalteu reagent solution is added and the mixture is vortexed. The stabilization of the color takes a few minutes. The intensity of the color obtained is evaluated by measuring the absorbance at 750 nm using the visible IC 6400 spectrophotometer. At the same time, a calibration line of Bovine Serum Albumin (BSA) (2mg/mL) is performed. Protein concentrations are expressed in milligram per gram of dry matter (mg/g DM) of sample.

Determination of chlorophylls and carotenoids

0.5 g fresh frozen leaves are ground and homogenized with 50 mL acetone (90:30; v/v), the extract is then centrifuged at 3500 g. The recovered supernatant is run through a visible IC 6400 spectrophotometer, either directly or after dilution. Optical Density (OD) is read at different wavelengths: 470 nm for carotenoids, 645 nm for chlorophyll "b" and 663 nm for chlorophyll "a" (Lichtenthaler 1987):

Concentrations are calculated from the following formulas (Lichtenthaler 1987):

$$Chlorophyll\ "a"\ (chl"a")(mg/gFM) = (11.75 \times DO663 - 2.35 \times DO645) \times \frac{50}{500}$$

$$Chlorophll\ "b"\ (chl"b")\ (mg/gFM) = (18.61 \times DO645 - 3.96 \times DO663) \times \frac{50}{500}$$

$$Carotenoids\ (mg/gFM) = \left((1000 \times DO470) - (2.27 \times Chl"a") - \frac{(81.4 \times Chl"b")}{227}\right) \times \frac{50}{500}$$

Determination of phytochemical parameters

Extraction

50 mg of algae powder, put in an Eppendorf tube, are homogenized in 1 mL of Methanol-water (8:2, v/v). The mixture is sonicated for 20 min and then centrifuged for 15 min at 10000 g. The extract obtained is used for the quantification of phenolic compounds (total phenols and total flavonoids) and for the determination of the antioxidant activity of the different algae.

Determination of total phenols

25 µL of algal extract, previously prepared, 110 µL of Folin-Ciocalteu reagent is added, shaking for 3 minutes and then 200 µL of sodium carbonate is added to the mixture. Then 1.9 µL of distilled water is added and vortexed. After a 30-minute incubation in the dark, the Optical Density (OD) of each sample is measured by spectrophotometer at 750 nm (Makkar 2003). The calibration range is performed by gallic acid. The OD values obtained are then transformed into the unit microgram of gallic acid equivalents per milligram of dry matter (µg GAE/mg DM).

Determination of total flavonoids

The dosage of flavonoids is carried out using two different methods:

Method of Andary (Andary 1990):

2 mL of algal extract are added with 100 µL of the reagent (2 amino-ethyl diphenyl borate) (Neu 1956). The OD reading is done at a wavelength of 404 nm. The flavonoid content is calculated according to the following formula:

$$T\ flavonoïdes = Aext \times 0.05 \times 100/Aq \times Cext$$

Where:

Aext: Absorption of the extract

Aq: Absorption of quercetin (0.05 mg/mL)

Cext: Concentration of the extract in mg/mL

The results are given in micrograms of quercetin equivalents per mg of dry matter (µg quercetin/mg DM).

Jay's method

The determination of flavonoids is performed according to the method of Jay (1975) as described by Harnafi et al, (2007) with a difference in the extraction solvent. To 1 mL of algal extract are added 0.5 mL of aluminum chloride ($AlCl_3$), left to stand for 30 minutes at room temperature, then the OD is measured at 430 nm by a visible IC 6400 spectrophotometer. The calibration range is performed by

quercetin. The OD values obtained are then transformed into the unit µg quercetin equivalents/mg Dry Matter (DM).

Determination of antioxidant activity

950 µL of a methanolic solution of DPPH (0.1 mM) are added to 50 µL of methanolic extract of the sample to be analyzed. After 30 min, the absorbance of the mixture is measured at 517 nm. The ability to trap the DPPH radical is calculated according to the following formula (Loo et al. 2008).

$P = (A1-A2)/A1X100$

Where:

P : Percentage of radical trapping

A1 : Absorbance of the control (DPPH solution without extract)

A2 : Absorbance in presence of extract

The DPPH- test is not quantitative, it allows to compare different extracts according to their capacity to trap DPPH- and thus, to appreciate the qualitative variations of phenolic compounds. The evaluation of the anti-free radical activity must be interpreted with precaution because the absorbance of DPPH- at 515-520 nm decreases under the action of light, oxygen, according to the pH and the type of solvent added to the antioxidant.

Statistical analysis

For each analysis three repetitions are carried out. The data are processed by the STATISTICA software, version 6.0. The Analysis of Variance (ANOVA) is used to determine the degree of significance. Means are compared using Duncan's tests at the probability threshold ($P<0.05$).

RESULTS AND DISCUSSION

Effect of algal fertilization on protein and total sugar content of tomato and pepper leaves

The protein contents of the two vegetable crops (tomato and pepper) were generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae (Table 1). However, the extracts had no effect on the pepper. In fact, we note that in tomatoes, the addition of extracts or amendments significantly increased protein content compared with the control. Tomato plants sprayed with aqueous extracts of the three brown algae had significantly higher protein contents than the control, up to 217 mg/g DM. The aqueous extract of *B. bifurcata* with its three concentrations showed the highest protein values in tomato leaves (217, 200 and 196.9 mg/g DM), followed by the three concentrations of *C. gibraltarica* (201, 197.65 and 192.07 mg/g, DM).

Fertilization with algal amendment also increased protein content in tomato plants. In fact, the results obtained show values significantly different from the untreated control. The highest values for protein content in tomato leaves were obtained with *F. spiralis* C1 amendment (208.91, 198.32 and 196.9 mg/g DM), followed by *B. bifurcata* (199.41, 198.66 and 191.9 mg/g DM).

In the case of peppers, treatment with aqueous extracts showed no significant effect on protein content, with the exception of the two concentrations of 1 and 2% *C. gibraltarica*. The highest protein content was recorded for the 1% aqueous extract of *C. gibraltarica* (196.57 mg/g DM). On the other hand, fertilization with algal amendments increased protein content (Table 1). In fact, the results obtained show values significantly different from the untreated control. The highest values for protein content in pepper leaves were obtained with *C. gibraltarica* C1 amendment (204.4 mg/g DM).

The three aqueous extracts of *F. spiralis* showed high levels of total sugars (83.13, 83.08 and 75.38 mg/g DM), followed by *C. gibraltarica* with its three concentrations (83.16, 82.75 and 43.77 mg/g DM). Fertilization by amendment also increased total sugar content, except for C3 amendment of *B. bifurcata* and *F. spiralis*. The highest statistical values for total sugar content in tomatoes were obtained with *F. spiralis* amendment C1 (83.08 mg/g DM).

Pepper plants sprayed with aqueous extracts of the three brown algae showed significantly lower levels than the control. The high levels of total sugars in pepper leaves were induced by the 2% aqueous extract of *C. gibraltarica* (52.22 mg/g DM). Fertilization by amendment also improved total sugar content. In fact, all *F. spiralis* amendments showed significantly lower values than the control. The highest values for total sugar content in pepper leaves were obtained with *F. spiralis* C3 (69.44 mg/g DM) (Table 1).

Effect of algae on the photosynthetic pigment content of tomato and pepper leaves

The photosynthetic pigment content of the leaves of the two vegetable crops (tomato and pepper) was generally significantly affected by the presence of aqueous extracts and amendments of the three brown algae (Table 2). The use of algal fertilizer in the form of an amendment produced significantly better results than the use of aqueous extracts for both tomato and pepper crops. The aqueous extracts of the three brown algae showed a clear improvement in the quantity of chlorophyll 'a', chlorophyll 'b' and carotenoids in pepper plants compared with tomato plants.

Algal extracts of *B. bifurcata* at 1% and 2% and *C. gibraltarica* at 1% significantly improve leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control in tomato crops. Indeed, the 2% *B. bifurcata* extract is significantly effective (1.34 mg/g FM for chlorophyll 'a' and 0.8 mg/g FM for chlorophyll 'b'), followed by the *C. gibraltarica* extract at 1% (0.92 mg/g FM for chlorophyll 'a' and 0.48 mg/g FM for chlorophyll 'b') and *B. bifurcata* extract at 1% (0.9 mg/g FM for chlorophyll 'a' and 0.49 mg/g FM for chlorophyll 'b'). In pepper, algal extracts of *C. gibraltarica* at 1% and *F. spiralis* at 1% and *B. bifurcata* at 0.5% and 2% significantly improved leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer. *C. gibraltarica* at 1% gives highly significant chlorophyll 'a' and 'b' contents (1.47 mg/g FM, for chlorophyll 'a' and 0.65 mg/g FM, for chlorophyll 'b'), followed by *F. spiralis* at 1% (1.13 mg/g

FM, for chlorophyll 'a' and 0.45 mg/g FM, for chlorophyll 'b') and finally *B. bifurcata* (1.07 mg/g FM, for chlorophyll 'a' and 0.44 mg/g FM, for chlorophyll 'b' at 2% 1.06 mg/g FM, for chlorophyll 'a' and 0.37 0.44 mg/g FM, for chlorophyll 'b' at 0.5%) (Table 2).

Table 1. Effect of the algae *C. gibraltarica* (Cg), *B. bifurcata* (Bb) and *F. spiralis* (Fs) in the two treatments (spraying and amendment) at different concentrations on the protein and total sugar content of tomato and pepper leaves

Spraying	Tomato		Pepper	
	Proteins	Total sugars	Proteins	Total sugars
	mg/g DM	mg/g DM	mg/g DM	mg/g DM
Control	191.15±0.75 g	56±0.16 e	186.06±0.62 d	23.8±0.12 f
Witness	191.4±0.25 g	31.6±0.12 h	198.66±0.5 a	10.44±0.25 k
BB 0,5 %	196.9±0.25 e	77.83±0.16 c	181.89±0.25 f	28.47±0.12 e
BB 1 %	217±0.25 a	46±0.2 f	183.81±0.38 e	40.58±0.08 c
BB 2 %	200±0.25 b	56.38±0.26 e	185.73±0.62 d	19.52±0.31 g
CG 0,5 %	201±0.38 b	83.16±0.08 a	171.88±0.25 l	50.77±0.12 b
CG 1 %	197.65±0.25 d	43.77±0.26 g	196.57±0.14 b	19.88±0.2 g
CG 2 %	192.07±0.38 f	82.75±0.25 b	191.48±0.76 c	52.22±0.41 a
FS 0,5 %	191.9±0.5 g	75.38±0.25 d	173.8±0.38 k	38±0.16 d
FS 1 %	193.32±0.38 f	83.13±0.12 a	180.81±0.62 g	17.16±0.16 h
FS 2 %	199.24±0.38 c	83.08±0.08 a	177.64±0.25 h	10.25±0.22 k
Amendment				
Control	191.15±0.75 g	56±0.16 f	186.06±0.62 f	23.8±0.12 e
Witness	191.4±0.25 g	31.36±0.12 k	198.66±0.5 b	10.44±0.25 l
BB C1	199.41±0.25 b	55.58±0.08 f	171.38±0.25 l	18.66±0.16 g
BB C2	198.66±0.5 c	80.36±0.17 b	189.23±0.14 e	31.97±0.2 b
BB C3	191.9±0.25 g	51.83±0.16 g	196.9±0.5 c	17.05±0.12 h
CG C1	194.49±0.38 e	62.75±0.08 e	204.4±0.25 a	10.36±0.12 l
CG C2	196.24±0.52 d	70.27±0.09 d	176.22±0.38 k	21.94±0.25 f
CG C3	193.4±0.5 f	62.66±0.16 e	177.14±0.25 h	11.83±0.16 k
FS C1	208.91±0.25 a	83.08±0.08 a	184.31±0.38 g	31.33±0.16 c
FS C2	198.32±0.38 c	77±0.16 c	192.82±0.38 d	25.69±0.25 d
FS C3	196.9±0.25 d	43.8±0.2 h	188.81±0.52 e	69.44±0.2 a

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different P≤0.05

Table 2. Effect of the algae *C. gibraltarica* (Cg), *B. bifurcata* (Bb) and *F. spiralis* (Fs) by the two treatments (soil watering and soil amendment) at different concentrations on the photosynthetic pigment content of tomato and pepper leaves

Spraying	Tomato			Pepper		
	Pigments (mg/g FM)			Pigments (mg/g FM)		
	Chl a	Chl b	Carotenoids	Chl a	Chl b	Carotenoids
Control	0.84±0.02d	0.27±0.04f	0.14±0.02 c	0.66±0.01g	0.25±0.01e	0.22±0.01 h
Witness	1.12±0.02b	0.89±0.02a	0.21±0.02 b	0.85±0.01d	0.47±0.01b	0.12±0.00 k
BB 0.5 %	0.8±0.02 d	0.41±0.01d	0.16±0.01 c	1.06±0.02c	0.37±0.13c	0.37±0.05 d
BB 1 %	0.9±0.03 c	0.49±0.01c	0.16±0.01 c	0.75±0.00e	0.28±0.01e	0.30±0.01 e
BB 2 %	1.34±0.07a	0.8±0.06 b	0.22±0.04 a	1.07±0.01c	0.44±0.01b	0.43±0.01 c
CG 0.5 %	0.79±0.02d	0.39±0.04d	0.17±0.02 c	0.6±0.01d	0.33±0.01d	0.54±0.01 a
CG 1 %	0.92±0.02c	0.48±0.01c	0.14±0.01 c	1.47±0.00a	0.65±0.01a	0.47±0.01 b
CG 2 %	0.71±0.07e	0.39±0.07d	0.13±0.03 c	0.73±0.00f	0.26±0.01e	0.27±0.01 f
FS 0.5 %	0.56±0.01f	0.28±0.01f	0.08±0.01 d	0.76±0.00e	0.30±0.01e	0.28±0.01 f
FS 1 %	0.6±0.01 f	0.33±0.01e	0.09±0.01 d	1.13±0.01b	0.45±0.01b	0.37±0.01 d
FS 2 %	0.71±0.04e	0.38±0.04d	0.10±0.01 d	0.71±0.01g	0.29±0.01e	0.24±0.01 g
Amendment						
Control	0.84±0.02h	0.27±0.04g	0.14±0.02 f	0.66±0.01k	0.25±0.01d	0.22±0.01 d
Witness	1.12±0.02f	0.89±0.02 b	0.21±0.02 c	0.85±0.01h	0.47±0.01c	0.12±0.00 e
BB C1	1.03±0.05g	0.59±0.01 e	0.16±0.01 e	0.73±0.03k	0.3±0.01 d	0.23±0.00 c
BB C2	1.5±0.15 d	0.8±0.03 b	0.22±0.05 c	1.21±0.01d	0.72±0.01a	0.37±0.01 b
BB C3	0.97±0.01g	0.55±0.01 f	0.11±0.02 g	0.93±0.01g	0.45±0.01c	0.26±0.02 c
CG C1	1.37±0.01e	0.72±0.02 c	0.16±0.01 e	1.51±0.02b	0.74±0.1 a	0.34±0.04 b
CG C2	1.85±0.07b	1.13±0.07 a	0.26±0.03 c	1.41±0.02c	0.75±0.11a	0.35±0.04 b
CG C3	1.96±0.08a	1.12±0.09 a	0.35±0.04 a	0.98±0.06f	0.59±0.07b	0.15±0.03 e
FS C1	1.7±0.01 c	0.55±0.08 f	0.18±0.01 d	1.72±0.01a	0.75±0.06a	0.41±0.03 a
FS C2	2.06±0.07a	1.3±0.07 a	0.29±0.02 b	1.15±0.01e	0.39±0.01c	0.36±0.01 b
FS C3	2.12±0.02a	1.22±0.02b	0.39±0.04 a	0.8±0.01 h	0.47±0.02c	0.24±0.01 c

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different P≤0.05

Table 3. Effect of *B. bifurcata* (BB), *C. gibraltarica* (CG) and *F. spiralis* (FS) algae by both treatments (spraying and amendment) at different concentrations on antioxidant activity (%), flavonoid content (µg/100 mg EqQ) and total phenols (µg/mg EAG) of tomato fruit and pepper

Spraying	**Tomato**				**Pepper**			
	Antioxidant activity	**Flavonoids by $AlCl_3$**	**Flavonoids by NEU**	**Total phenols**	**antioxidant activity**	**Flavonoids by $AlCl_3$**	**Flavonoids par NEU**	**Total phenols**
Control	8.67 k	13.23±0.43	4.61±0.29 e	1.76±0.08 f	47.76 g	40.95±0.59 e	5.72±0.03 h	1.76±0.08 ef
Witness	10.71 h	32.38±1.64 a	3.36±0.04 f	8.6±0.12 a	86.73 a	67.04±0.43 a	8.06±0.04 c	1.68±0.08 f
BB 0.5 %	20 g	15.42±0.28 e	4.56±0.03 e	3.06±0.04 c	73.77 c	56.28±0.28 c	7.05±0.04 d	1.76±0.16 f
BB 1 %	24.19 c	13.23±0.16 f	5.39±0.02 d	2.72±0.08 d	38.84 k	35.14±0.28 g	4.37±0.04 l	1.2±0.08 g
BB 2 %	38.87 a	19.23±0.16 d	10.45±0.02 a	3.46±0.04 b	54.2 f	40.85±0.28 e	15.39±0.08 a	1.68±0.08 f
CG 0.5 %	23.88 d	23.42±0.28 c	5.58±0.03 d	3.54±1.08 b	75.59 b	56.57±0.28 c	6.19±0.07 f	2.02±0.04 d
CG 1 %	21.36 f	23.9±0.16 c	7.61±0.06 b	2±0.08 e	75.06 b	43.42±0.57 d	6.81±0.05 e	1.84±0.08 d
CG 2 %	24.34 c	23.33±0.43 c	6.17±0.04 c	2.4±0.08 de	48.59 g	38.47±0.32 f	7.04±0.08 d	2.64±0.08 c
FS 0.5 %	37.85 b	32.38±1.64 a	7.73±0.08 b	2.4±0.08 de	70.08 d	40.76±0.71 e	10.4±0.05 b	8.38±0.12 a
FS 1 %	21.45 f	24.57±0.28 c	6.10±0.05 c	3.49±0.12 b	54.1 f	43.52±0.32 d	5.93±0.06 g	8.26±1.22 a
FS 2 %	22.71 e	30.47±0.32 b	5.97±0.08 c	1.94±0.12 e	68.6 e	64±0.28 b	4.59±0.08 k	7.12±0.08 b
Amendment								
Control	8.67 f	13.23±0.43 f	4.61±0.29 c	1.76±0.08 k	47.76 m	40.95±0.59 l	5.72±0.03 k	1.76±0.08 g
witness	10.71 d	32.38±1.64 a	3.36±0.04 f	8.6±0.12 d	86.73 d	67.04±0.43 e	8.06±0.04 e	1.68±0.08 g
BB C1	27.85 a	32.19±0.16 a	4.99±0.08 b	2.69±0.04 h	65.22 g	75.8±0.43 b	7.69±0.05 g	2.66±0.46 e
BB C2	27.51 a	27.61±0.43 b	3.83±0.08 e	8.98±0.12 c	71.56 e	73.61±0.16 c	7.89±0.09 f	3.73±1.22 b
BB C3	8.37 f	17.42±0.28 d	9.17±0.05 a	11.70±0.04 b	90.3 c	58.85±0.28 g	9.37±0.06 c	2.88±0.08 e
CG C1	17.6 b	25.71±0.28 c	4.22±0.02 d	13.44±0.08 a	60.66 h	59.33±0.43 g	9.21±0.08 d	2.85±0.04 e
CG C2	9.84 e	12.85±0.28 f	3.4±0.03 f	8.72±0.08 d	68.97 f	55.71±0.28 h	10.7±0.08 a	4.8±0.08 a
CG C3	9.51 e	15.71±0.28 e	3.88±0.04 e	8.66±0.04 d	57.8 k	49.14±0.28 k	7.92±0.08 f	1.01±0.12 h
FS C1	9.87 e	16±0.28 e	2.53±0.04 h	8.4±0.08 e	56.69 l	70.19±0.43 d	8.15±0.05 e	3.32±0.08 d
FS C2	11.01 d	17.33±0.43 d	2.9±0.05 g	7.84±0.08 f	91.99 b	85.23±0.16 a	6.43±0.04 h	3.52±0.08 c
FS C3	12.68 c	29.33±0.16 a	2.64±0.04 h	7.28±0.08 e	92.52 a	64.57±0.28 f	9.79±0.04 b	2.33±0.30 f

Note: Values show mean ± standard deviation (n=10). Values indicated by a different letter are significantly different $P \leq 0.05$. EqQ: Quercetin equivalent; EAG: Gallic Acid Equivalent

The *C. gibraltarica* and *F. spiralis* were also statistically effective in improving leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer in tomato cultivation. Indeed, *F. spiralis* at C3 and C2 and *C. gibraltarica* at C3 gave statistically the best results (2.12, 2.06 and 1.96 mg/g FM, respectively) for chlorophyll 'a'. For chlorophyll 'b' *F. spiralis* at C2 and *C. gibraltarica* at C2 and C3 (1.3, 1.13 and 1.12, mg/g FM, respectively) gave statistically the best results.

For the pepper crop, amendment with *F. spiralis* at C1 was statistically effective in improving leaf chlorophyll 'a' and chlorophyll 'b' content compared with the control and even with chemical fertilizer (1.72 and 0.75 mg/g FM, respectively). Followed by *C. gibraltarica* at C1 (1.51 mg/g FM, for chlorophyll 'a' and 0.74 mg/g FM, for chlorophyll 'b'), then the same alga at C2 (1.41 mg/g FM, for chlorophyll 'a' and 0.75 mg/g FM for chlorophyll 'b') and finally the amendment by *B. bifurcata* at C2 (1.21 mg/g MF, for chlorophyll 'a' and 0.72 mg/g MF, for chlorophyll 'b') (Table 2).

As far as carotenoids are concerned, the algal extracts of the three algae and the amendments significantly improved leaf content compared with the control and even with the chemical fertilizer for the pepper crop (Table 2). *C. gibraltarica* extract at 0.5% showed statistically maximum content (0.54 mg/g FM), followed by extract of the same alga at 1% (0.47 mg/g FM) and finally *B. bifurcata* extract at 2% (0.43 mg/g FM). As with chlorophyll 'a' and 'b', the 2% *B. bifurcata* extract was significantly effective on tomatoes (0.22 mg/g FM). *C. gibraltarica* and *F. spiralis* were statistically effective in improving leaf carotenoid content compared with the control and even with chemical fertilizers for tomato crops. Indeed, *F. spiralis* and *C. gibraltarica* at C3 gave statistically the best results (0.39 and 0.35 mg/g FM, respectively), followed by *F. spiralis* at C2 (0.29 mg/g FM). In peppers, *F. spiralis* at C1 was significantly effective (0.41 mg/g FM), followed by *B. bifurcata* at C2, *F. spiralis* at C2 and *C. gibraltarica* at C2 and C1 (0.37, 0.36, 0.35 and 0.34 mg/g FM, respectively).

Effect of algal fertilization on phytochemical parameters of tomato and pepper fruits

Both tomato and pepper plants grown in greenhouse pots, treated with aqueous extracts (spraying) or by amendment, showed a significant improvement in all phytochemical parameters (Table 3). Nevertheless, we note that the application of algal fertilizer in the form of aqueous extracts gives significantly better results than the amendment for tomato cultivation. On the other hand, the application of algal fertilizer in the form of an amendment gave significantly better results than the aqueous extract for peppers.

For tomato fruits, the aqueous extract of *B. bifurcata* at 2% showed a maximum value in antioxidant activity (38.87%), a maximum value in total flavonoids (10.45 µg EqQ/100mg DM, by NEU reagent) and a maximum content in total phenols (3.46 µg EAG/mg DM). On the other hand, the same alga (*B. bifurcata*) added as an amendment gave highly significant values for antioxidant activity (27.45%), total flavonoids (32.19 µg EqQ/100mg DM by $AlCl_3$; 9.17 µg EqQ/100mg MS by NEU reagent) and total phenols (11.7 µg EAG/mg DM). In addition, the 0.5% *F. spiralis* extract significantly improved antioxidant activity (37.85%) and total flavonoid content (32.38 µg EqQ/100mg DM by $AlCl_3$ and 7.73 µg EqQ/100mg DM by NEU reagent). Amendment with *F. spiralis* C3 also improved total flavonoid content (29.33 µg EqQ/100mg DM).

For pepper fruits, antioxidant activity was significantly enhanced by the 0.5% and 1% aqueous extracts of *C. gibraltarica* (75.59% and 75.06%, respectively) and by the amendment with *F. spiralis* at C2 and C3, which recorded maximum values (92.52% for C3 and 91.99% for C2). For total flavonoids, the aqueous extract of F. spiralis at 2% recorded a significantly high value for $AlCl_3$ (64 µg EqQ/100mg DM), while it was *B. bifurcata* at 2% that gave a maximum value with the NEU reagent (15.39 µg EqQ/100mg DM). On the other hand, amendment fertilization showed a significant improvement in total flavonoid content with the $AlCl_3$ reagent compared with the control, with a maximum value obtained by the *F. spiralis* C2 amendment (85.23 µg EqQ/100mg DM), followed by the *B. bifurcata* C1 amendment (75.8 µg EqQ/100mg DM).

Amendment with *C. gibraltarica* to C2 showed a very highly significant value for total flavonoid content with NEU reagent (10.7 µg EqQ/100mg DM), followed by C3 from *F. spiralis* (9.79 µg EqQ/100mg DM). Total phenol content was significantly improved with the 0.5% and 1% aqueous extracts of *F. spiralis* (8.38 and 8.26 µgEAG/mg DM, respectively), followed by the 2% extract of the same alga (7.12 µg µg EqQ/100mg DM/mg MS) (Table 3). On the other hand, fertilization by amendment also showed a significant improvement over the untreated control, with a maximum value obtained by the C2 amendment of *C. gibraltarica* (4.8 µg/mg DM), followed by *B. bifurcata* at C2 (3.73 µg/mg DM) and finally *F. spiralis* at C2 (3.52 µg/mg DM).

Discussion

In order to assess the effect of algal fertilization on the biochemical and phytochemical composition of plants, we tested increasing concentrations of extracts and amendments of three brown algae (*B. bifurcata, C. gibraltarica* and *F. spiralis*) on two vegetable crops, tomato and pepper. In general, the results obtained after three months of cultivation in pots (greenhouse) are very satisfactory. The results show a significant improvement in all biochemical parameters. The quantity of photosynthetic pigments (Chlorophyll 'a'; 'b' and carotenoids) in both tomato and pepper crops was significantly improved by the addition of aqueous extracts and amendments of the three brown algae. Pigments play a vital role in plant photosynthesis. It is thanks to this phenomenon that plants absorb CO_2, which could be responsible for increasing the sugar, protein and organic matter content of both crops. Several studies have shown that algal fertilization improves the chlorophyll and protein content of *Zea mays* and *Phaseolus mungo* leaves (Lingakumar et al. 2004).

According to Whapham et al. (1993), the increase in the quantity of these pigments is a consequence of the uptake of magnesium, a major constituent of chlorophyll. Furthermore, aqueous extracts of the three brown algae showed a notable effect on the protein content of tomato and pepper leaves. Such an increase in protein content may be contributed to the increased availability and uptake of mineral elements (N, K, Ca, Na, Mg, Cu and Zn) present in algal fertilizers. Our results concur with those of Ashok et al. (2004) who show that the protein content of *Sorghum vulgare* increases when this plant is treated with the aqueous extract of *Hydroclathrus clathratus*. Our study shows that the total sugar content of tomato and pepper leaves was enhanced by aqueous extracts of the three brown algae at low concentrations. This could be explained by the fact that algal extracts stimulate various biological processes that increase carbohydrate levels in plants (Kumari et al. 2011). Similar observations were recorded in *Vigna catajung* treated with aqueous extracts of *Caulerpa racemosa* (Anantharaj and Venkatesalu 2001).

Algae contain macronutrients and microelements, amino acids, vitamins, cytokinin, auxins and abscisic acid that affect the cellular metabolism of treated plants, resulting in enhanced crop growth (Crouch and Van Staden 1993; Stirk et al. 2004). In addition, the presence of polysaccharides in algal extracts can enhance plant growth in a similar way to hormones (Rolland et al. 2002). Brown seaweed extracts also contain various betaine-type compounds (Ghoul et al. 1995). This molecule acts as a compatible solute that mitigates salinity-induced osmotic stress, and functions as a nitrogen source when provided in low concentration and as an osmolyte at higher concentrations (Naidu et al. 1987). This could often improve the biomass and fruit quality of vegetable crops, notably tomatoes and peppers.

Our results also showed that the application of aqueous extracts or amendment increased the phenolic compound content and antioxidant activity of tomato and pepper fruits. In general, the aqueous extracts were more effective than the amendment treatment in providing maximum levels of total phenols and flavonoids, as well as antioxidant activity, particularly for the two algae *B. bifurcata* and *C. gibraltarica*. Similar results have shown that aqueous extracts of *A. nodosum* algae increase the total phenol and flavonoid content of fruit (Fan et al. 2011; Lola-Luz 2014). The same *A. nodosum* algae can act as a stressor due to its bioactive components. This stress enhances the defense system, leading to an increase in phenolic compound content, which explains the increase in phenolic compounds after addition of algal extracts (Alghamdi 2017). As a result, increased production of various phenolic compounds improves plant resistance to pathogen infection (Levine et al. 1994). We have also noted that algal extracts can be a promising source of new biologically active substances and compounds essential for human nutrition (Jimenez-Escrig et al. 2012).

Phenolic compounds undergo a redox reaction with the complex of phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent. This reaction varies according to the number of hydroxyl groups (OH) of the phenolic compounds (Singleton et al. 1999). However, this method is non-specific because the reagent can react with some amino acids (tyrosine and tryptophan), reducing sugars and sulfur compounds (Boizot and Charpentier 2006). Bruneton (1999) reported that phenolic compounds are generally soluble in polar organic solvents and aqueous solutions and are poorly soluble in apolar organic solvents, hence the choice to extract optimally with methanol-water (80-20; v/v). Similar results have been reported by several studies using the same extraction system and conditions from different plant parts (Ahmed et al. 2016).

In conclusion, this work presents results on the study of the effect of three brown algae *C. gibraltarica, B. bifurcata* and *F. spiralis* on the biochemical and phytochemical parameters of two vegetable plants (tomato and pepper) pots grown in greenhouse. In general, fertilization with the three brown algae improved the biochemical and phytochemical parameters of tomato and pepper. In particular, the two algae *B. bifurcata* and *F. spiralis* showed high efficacy on all parameters studied. Our results showed that treatment with aqueous extracts had a higher positive effect than treatment with amendments. It can also be noted that the algal species affects the various parameters studied. These three algae proved to be effective and good candidates for the development of biostimulants to improve the parameters studied.

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