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USE OF SOME BIOSTIMULANTS TO IMPROVE THE GROWTH AND CHEMICAL CONSTITUENTS OF SWEET PEPPER

Rabab Maraei, Noha Eliwa, Amina Aly

ABSTRACT
The experiment was conducted during two successive seasons 2016 and 2017 on sweet pepper plants to study the effect of foliar application of some natural extracts (fulvic acid at 2, 4 and 6% or algae at 1, 2 and 4 g.L^{-1}) were applied three times along each season (after 2, 4 and 6 weeks of planting). The influence was evaluated through the response of vegetative growth, and some physical and chemical characteristics of sweet pepper fruits. The results obtained showed that the algae extract at 1 g.L^{-1} in most cases was better than the other spray treatments investigated to improve most fruit characteristics (length, diameter and yield of fruits), vegetative growth, and chemical properties followed by 6% fulvic acid. With regard to organic acids, malic and citric acids are the main organic acids found in sweet pepper. Malic, succinic and glutaric acids were higher in 1 g.L^{-1} algae extract treatment, but the concentration of citric acid was higher in 6% fulvic acid treatment. Therefore, algae extract and fulvic acid could be safely recommended as a natural biostimulants application for improving most desirable characteristics of sweet pepper grown under the same experimental condition.

Keywords: sweet pepper; fulvic acid; algae; vegetative growth; fruit characteristics

INTRODUCTION
Sweet pepper is one of the most important vegetable crops as well as important exportable crops in Egypt and it is considered an excellent source of bioactive nutrients. Vitamin C, carotenoids and phenolic compounds are the main antioxidant components **(Marín et al., 2004)**. The levels of these compounds in peppers and other vegetables depend on several factors, such as cultivar, type of agriculture (organic or non-organic), maturity and storage conditions **(Lee and Kader, 2000)**. Sweet pepper or its hot varieties (*Capsicum annuum* L.) are important vegetable crops that can be used to produce seasonings such as paprika spices. Green pepper is an important condiment containing phenolic compounds such as 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(N-ethylamino) benzamide and phenolic acid glycosides, suggesting a high radical scavenging activity of them. Addition of paprika spices (dried fruits of the pepper family) and pepper spices (mainly black pepper, white or green peppers) could change foods sensory quality and also, due to the antioxidant's presence, lower oxidation in the foods. Antioxidants help to control oxidation in foods effectively, therefore usage of spices such as paprika or pepper spices are also of great economical interest. Polyphenols are often responsible for the antioxidant capacity **(Škrovánková et al., 2017)**. Plant biostimulants, can be considered as substances that can be added to the environment around the plant and encourage growth and have positive effects on nutrition. They also increase plant tolerance and protective effects against various types of environmental stress such as water deficit, salinity and exposure to temperatures unsuitable for growth **(Du Jardin, 2015)**. Biostimulants are not nutrients but they facilitate nutrient uptake or contribute beneficially to promote growth and stress resistance **(Brown and Saa, 2015)**. The role of biostimulants, specifically in regard to growth promotion and nutrient availability, has been reviewed **(Du Jardin, 2015)**. Many categories of specific biostimulants have been extensively reviewed such as protein hydrolysates **(Colla et al., 2015)**, seaweed extracts **(Battacharyya et al., 2015)**, silicon **(Savvas and Ntatsi, 2015)**, chitosan **(Pichyangkura and Chadchawan, 2015)**, humic and fulvic acids **(Canellas et al., 2015)** plant growth-promoting rhizobacteria **(Ruzzi and Aroca, 2015)**. Seaweed extracts (SWE) as biostimulants are emerging as commercial formulations for use as plant growth promoting factors, and a method to improve tolerance to salinity, heat, and drought. Seaweeds are red, green, and brown macroalgae that represent 10% of marine productivity. Macroalgae have been used as organic fertilizers for thousands of years and are still in use **(Craigie, 2011)**. Currently, there are over 47 companies producing and marketing various algal extracts for agricultural use; the majority of the formulations are from

the brown algae, *Ascophyllum nodosum* **(Sharma et al., 2014)**. Fulvic acid is an important fraction of soil organic matter, an important portion of the dissolved organic C pool in soils **(van Hees, Lundström and Giesler, 2000)**. It is humic acids with a higher oxygen content and lower molecular weight **(Bulgari et al., 2015)**. Fulvic acid plays an important role in the acid – base buffering capacity of soil, and transfer of metal ions and organic compounds into soil **(Senesi and Miano, 1995)**. Foliar application of marine bioactive substances to grape plants (*Vitis vinifera* L.) increased leaf water potential and stomatal conductance under drought stress **(Mancuso et al., 2006)**. Humic and fulvic acids are produced by the biodegradation of organic matter resulting in a mixture of acids containing phenolate and carboxyl groups.

Scientific hypothesis

Evaluation of the effect of foliar application of some natural extracts (fulvic acid and algae) on vegetative growth, and some fruit physical and chemical characteristics of sweet pepper.

MATERIAL AND METHODOLOGY

The experiment was conducted during the two seasons 2016 and 2017 where the sweet pepper seeds (*Capsicum annuum* L.) were sown in trays in the mid-March in both growing seasons in the experimental farm of Natural products Department, National Center of Radiation Research and Technology. After 45 days from seeds sowing, transplants were transplanted into soil. They planted on both sides of lines 70 cm apart and 40 cm between plants. Plants were grown at 29 and 18 ºC day/night temperature conditions. All agricultural needs were done. Soil mechanical and chemical analysis of the experimental site is shown in Table 1. Fulvic acid (2, 4 and 6%) and algae extract (1, 2 and 4 $g.L^{-1}$) were sprayed after two, four and six weeks of transplanting. A complete randomized block design (CRBD) with three replicates was used.

Table 1 Physical and chemical properties of experimental soil in 2016 and 2017 seasons.

Soil properties	Experimental year	
	2016	2017
pH (1:1)	7.32	7.31
EC (1:1) $dS.m^{-1}$	2.2	1.8
Soluble anions ($meq.L^{-1}$)		
$CO_3^{=}$	1.5	1.3
HCO_3^{-}	4.5	3.6
Cl^{-}	8.0	5.9
$SO_4^{=}$	11.7	9.8
Soluble cations ($meq.L^{-1}$)		
Ca^{++}	10.5	9
Mg^{++}	3.5	2
K^{+}	0.50	0.4
Na^{+}	11.2	9.2
Sand (%)	57.3	58.4
Silt (%)	21.2	18.2
Clay (%)	21.5	23.4
Texture class	Sandy clay loam	Sandy clay loam

Measurements and analysis

The effect of the differential investigated spray treatments was evaluated through the response of the following measurements.

Vegetative growth parameters

After 90 days from transplanting, four plants per replicate were randomly chosen to measure, plant height (cm), root length (cm) and branches/plant.

Physical characteristics of fruit

Mature pepper fruits were harvested manually at full colour stage. Fruit length, fruit diameter and fruit yield/plant (kg) were determined.

Chemical characteristics

Photosynthetic Pigments

Chlorophyll a, chlorophyll b and carotenoids were determined in leaves **(Vernon and Seely, 1966)**. The results were calculated as $mg.g^{-1}$ fresh weight.

Ascorbic acid content

Ascorbic acid was measured as described by **(Jagota and Dani, 1982)**. Fresh weight (0.2 g) sample was homogenized in 1.5 mL 10% (w/v) trichloroacetic acid (TCA) at 4 °C. After centrifugation at 3000 × g for 5 min, 0.3 mL of the supernatant was made up to 2 mL volume with distilled water. A 0.2 mL 10% (v/v) Folin phenol reagent (Sigma Chemical Co.) was then added to the mixture, and vigorously shaken. After 10 min reaction time, maximum absorbance was measured at 760 nm. A result of the reaction between ascorbic acid and Folin phenol reagent, L (+) – ascorbic acid (Hamburg chemistry, Germany) was used as a standard.

Enzymes extraction

Sample (0.5 g) from each treatment were homogenized in 100 mM pre-chilled sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone (PVP) (w/v) at 4 ºC. The extraction ratio was 4.0 mL buffer for each one gram of sample. The homogenate was centrifuged at 15.000 x g for 15 min at 4 ºC. Supernatant was used to estimate the activities of peroxidase and polyphenol oxidase. Proteins content was determined in the enzymes extract **(Bradford, 1976)**.

The activity of peroxidase (POD)

Peroxidase was assayed following method the method of **Hammerschmidt et al. (1982)**. The reaction mixture (2.9 mL) consisted of 0.25% (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM hydrogen peroxide). Volume of 100 µL of the enzyme extract was added to launch the reaction which was determined spectrophotometrically (Jasco V-530, Jappan) at 470 nm per min. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001 min^{-1} mL^{-1}. The POD activity expressed as unit min^{-1} mg^{-1} protein.

The activity of polyphenol oxidase (PPO)

Polyphenol oxidase was assayed following method the method of **Oktay et al. (1995)**. The reaction mixture consisted of 600 µL catechol (0.1 M) and 100 µL enzyme extract was completed to 3.0 mL with 0.1 M phosphate buffer pH 7. The absorbance was registered at 420 nm by spectrophotometer. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001 min^{-1} mL^{-1}. The enzyme activity was expressed as unit min^{-1} mg^{-1} protein.

Preparation of ethanolic extract

The fruits of sweet pepper were freeze dried (Virtis model 10-324) and ground. The ground samples were extracted using ethanol 80% and used in chemical analyses. Phenolic content was determined **(Shahidi and Naczk, 1995)** using the Folin-Denis reagent. The results were expressed as $mg.g^{-1}$ of gallic (GAE) equivalent of the dry weight.

Antioxidant activity by

Reducing power

Power reduction was determined by **Oyaizu (1986)** technique. Each 0.5 mL sample was mixed with 0.2 M sodium phosphate buffer 2.5 mL and 1% potassium ferricyanide 2.5 mL and incubated at 50 °C for 20 min. Following the addition of 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged for 10 min at 200 g. The upper layer (5.0 mL) was blended with 5.0 mL of deionized water and the test sample was read against the blank at 700 nm.

Metal chelating

Metal chelating activity was consequently evaluated by 0.1 mM $FeSO_4$ (0.2 mL) and 0.25 mM ferrozine (0.4 mL) in 0.2 mL of samples **(Chew et al., 2009)**. After 10 min of incubating at room temperature, absorbance of the blend was recorded against a void reagent was registered at 562 nm. The lower absorption of the test sample suggested greater chelating capacity of ferrous ion. The control contained all the reagents except test sample.

Organic acids analysis

Organic acids were separated by high performance liquid chromatography (HPLC) Knauer, Germany, flow rate was set at 0.6 $mL.min^{-1}$, UV detector set at $\lambda = 214$ nm, column oven temperature kept constant at 65 °C. The column used was Rezex@ column for organic acids analysis, mobile phase was 0.005 M H_2SO_4, data integration by clarity-chrom software.

Statistic analysis

All the statistical analyses were performed using an ANOVA, and Duncan's multiple range tests **(Duncan, 1955)** were applied to compare the results of the experiments ($p \leq 0.05$). A three replicates was used.

RESULTS AND DISCUSSION

Vegetative growth parameters

Table 2 shows the effect of spraying with fulvic acid and algae extracts on vegetative growth parameters (plant height, root length and branches/plant). All treatments have a positive effect on vegetative growth parameters and the best treatment is algae extract at 1 $g.L^{-1}$, resulting in improved growth parameters followed by fulvic acid 6% treatment in both seasons. It was clear that the growth parameters increased through the application of these extracts. The spraying of humic acid increased growth compared with control in *Thuja orientalis* plants because of its direct effect on solubility and transport of nutrients **(Zaghloul et al., 2009)**. Also, correspond, the increase in shoots characteristics may also be due to the auxins content in the seaweed extracts that have an effective role in cell division and enlargement, resulting in increased the shoot growth **(Arancon et al., 2004; Gollan and Wright, 2006)**. On the other hand, the least increase over control in all evaluated growth measurements was always associated with foliar application of algae extract at 4 $g.L^{-1}$.

Physical characteristics of fruit

Data presented in Table 3, shows that all spraying treatments led to increase the physical characteristics of fruits (length, diameter and yield of fruit) compared to control in both seasons. The results indicated that treatment of algae extract at 1 $g.L^{-1}$ gave the highest value of these characteristics, followed by the treatment of fulvic acid at 6% in both seasons. It is clear that fulvic acid and algae extract improved the quality of sweet pepper fruits. The use of humic substances gave the highest grain yield of winter wheat compared to control **(Bezuglova et al., 2017)**. Also, the application of humic acid and calcium nitrate alone caused a significant increase in growth parameters, photosynthetic pigments of pepper plants under normal and salt stress conditions **(Akladious and Mohamed, 2018)**. Fulvic acid as an organic fertilizer chelating the minerals (non-toxic) and water binder and thus maximizes uptake through the leaves, which increases the productivity of the plant **(Malan, 2015)**.

The stimulatory effects of fulvic acid are directly related to increase uptake of nutrient such as nitrogen, phosphorus, potassium and micronutrients **(Silva et al., 2016)**, vitamins, some growth regulators and polyamines as algae extract. Also, these extracts, such as fulvic acid, contain phenols that are antioxidants.

Therefore, their use on plants will improve the vegetative growth of plants in addition to the physical and chemical properties of fruits, which will be reflected in increased productivity. Fulvic acid and humic acid when used with a concentration of 40 $mg.kg^{-1}$ increased flowering and growth parameters such as shoots number, plant diameter, flowers number and root length compared to control plants in impatiens walleriana **(Esringu et al., 2016)**. Spraying mango trees with algae extracts alone or combined with natural or plant extracts was very effective in improving fruit set, fruit retention, yield and enhanced fruit quality **(Ahmed et al., 2014; Abed El Hamied, 2014)**. Also, the application of humic substances, especially at suitable concentrations, may alleviate the damages induced by the stress, probably via the enhanced nutrient uptakes and induced physiological changes **(Hamideh et al., 2013)**.

Chemical characteristics

Photosynthetic Pigments

Fulvic acids and algae extracts applications affected fruit chlorophyll content during both two seasons (Figure 1).

Table 2 Effect of fulvic acid and algae extracts spraying on growth parameters of sweet pepper plants during the first and second seasons.

Treatments		Plant height (cm ±*SD*)	Root length (cm ±*SD*)	Branches/plant ±*SD*
First season				
Fulvic acid	Cont (Zero)	101.0 ±0.954 ^{e}	11.67 ±0.268 ^{g}	26.00 ±0.306 ^{f}
	2%	105.0 ±0.814 ^{d}	18.47 ±0.277 ^{d}	30.33 ±0.493 ^{d}
	4%	113.7 ±1.26 ^{c}	20.00 ±0.178 ^{c}	34.33 ±0.794 ^{c}
	6%	124.7 ±0.252 ^{b}	22.07 ±0.378 ^{b}	38.00 ±0.517 ^{b}
Algae extract	1 g.L^{-1}	132.6 ±0.458 ^{a}	25.70 ±0.265 ^{a}	43.00 ±0.379 ^{a}
	2 g.L^{-1}	104.7 ±0.802 ^{d}	15.00 ±0.225 ^{e}	27.00 ±0.755 ^{e}
	4 g.L^{-1}	103.7 ±0.208 ^{d}	13.00 ±0.187 ^{f}	26.67 ±0.631 ^{ef}
Second season				
Fulvic acid	Cont (Zero)	102.0 ±2.08 ^{f}	11.67 ±0.764 ^{f}	25.33 ±1.02 ^{e}
	2%	107.3 ±1.80 ^{d}	18.67 ±0.697 ^{c}	29.67 ±0.862 ^{d}
	4%	115.4 ±1.16 ^{c}	21.67 ±0.289 ^{b}	33.33 ±0.603 ^{c}
	6%	126.5 ±1.89 ^{b}	22.70 ±0.289 ^{b}	36.67 ±0.751 ^{b}
Algae extract	1 g.L^{-1}	133.8 ±0.764 ^{a}	24.50 ±0.904 ^{a}	44.33 ±0.854 ^{a}
	2 g.L^{-1}	106.7 ±0.289 ^{d}	16.00 ±0.297 ^{d}	28.67 ±0.802 ^{d}
	4 g.L^{-1}	104.7 ±0.812 ^{e}	14.33 ±0.404 ^{e}	26.67 ±1.01 ^{e}

Note: Values are mean of three replications ±standard deviation. Different letters indicate statistically significant differences at $p \leq 0.05$.

Table 3 Effect of fulvic acid and algae extracts spraying on physical characteristics of sweet pepper fruits during the first and second seasons.

Treatments		Fruit length (cm ±*SD*)	Fruit diameter (cm ±*SD*)	Fruit yield/plant (kg ±*SD*)
First season				
Fulvic acid	Cont (Zero)	9.00 ±0.577 ^{b}	8.83 ±0.305 ^{b}	0.8950 ±0.021 ^{f}
	2%	10.17 ±0.631 ^{ab}	9.83 ±0.681 ^{ab}	1.700 ±0.011 ^{d}
	4%	10.50 ±0.584 ^{ab}	10.17 ±0.513 ^{ab}	1.900 ±0.01 ^{c}
	6%	11.67 ±0.543 ^{ab}	11.00 ±0.608 ^{a}	2.183 ±0.013 ^{b}
Algae extract	1 g.L^{-1}	12.17 ±0.601 ^{a}	11.50 ±0.304 ^{a}	2.550 ±0.21 ^{a}
	2 g.L^{-1}	9.67 ±0.325 ^{ab}	9.17 ±0.255 ^{b}	1.583 ±0.10 ^{d}
	4 g.L^{-1}	9.17 ±0.275 ^{b}	8.93 ±0.413 ^{b}	1.200 ±0.009 ^{e}
Second season				
Fulvic acid	Cont (Zero)	8.37 ±0.231 ^{d}	8.00 ±0.152 ^{e}	0.8467 ±0.031 ^{f}
	2%	10.33 ±0.0.312 ^{ab}	9.50 ±0.254 ^{bcd}	1.500 ±0.044 ^{d}
	4%	10.90 ±0.415 ^{ab}	9.87 ±0.274 ^{abc}	1.800 ±0.032 ^{c}
	6%	11.50 ±0.502 ^{a}	10.67 ±0.159 ^{ab}	2.000 ±0.0521 ^{b}
Algae extract	1 g.L^{-1}	11.67 ±0.344 ^{a}	11.00 ±0.312 ^{a}	2.467 ±0.0458 ^{a}
	2 g.L^{-1}	9.60 ±0.218 ^{bc}	9.33 ±0.321 ^{cd}	1.417 ±0.021 ^{d}
	4 g.L^{-1}	8.50 ±0.140 ^{cd}	8.33 ±0.173 ^{de}	1.167 ±0.0252 ^{e}

Note: Values are mean of three replications ±standard deviation. Different letters indicate statistically significant differences at $p \leq 0.05$

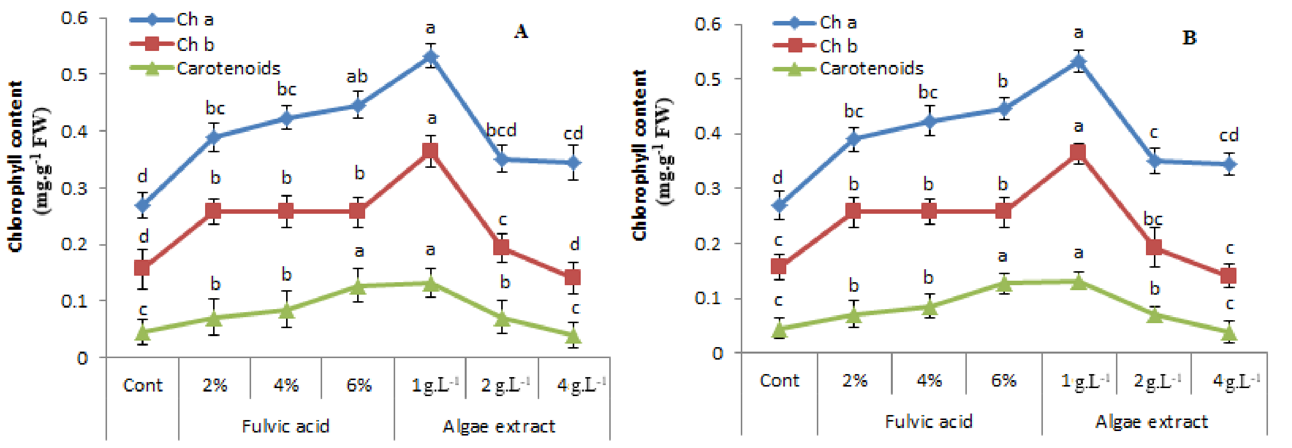

Figure 1 Effect of fulvic acid and algae extracts spraying on chlorophyll content (mg.g^{-1} FW) of sweet pepper fruits during the first (A) and second (B) seasons. Note: Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$.

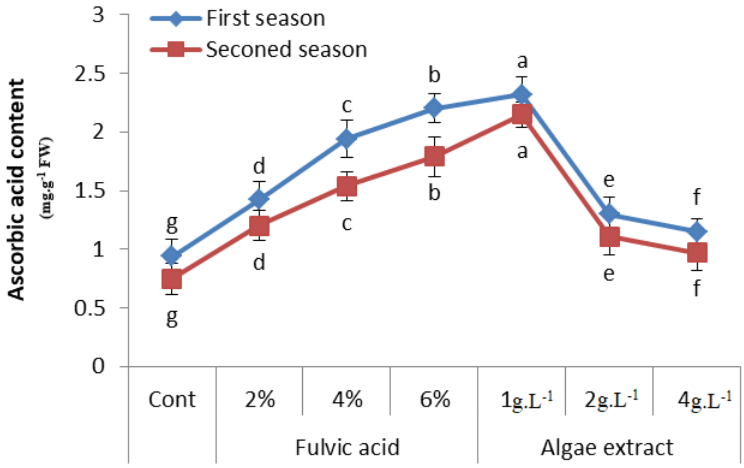

Figure 2 Effect of fulvic acid and algae extracts spraying on ascorbic acid content (mg.g^{-1} FW)) of sweet pepper fruits during the first and second seasons. Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$.

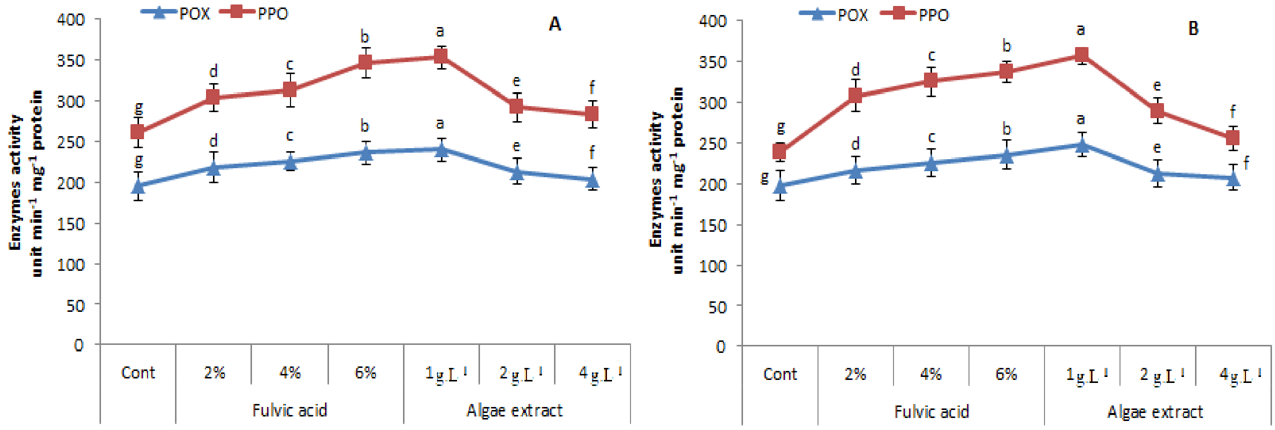

Figure 3 Effect of fulvic acid and algae extracts spraying on peroxidase (POX) and polyphenol oxidase (PPO) activities (unit min^{-1} mg^{-1} protein) of sweet pepper fruits during the first (A) and second (B) seasons. Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$.

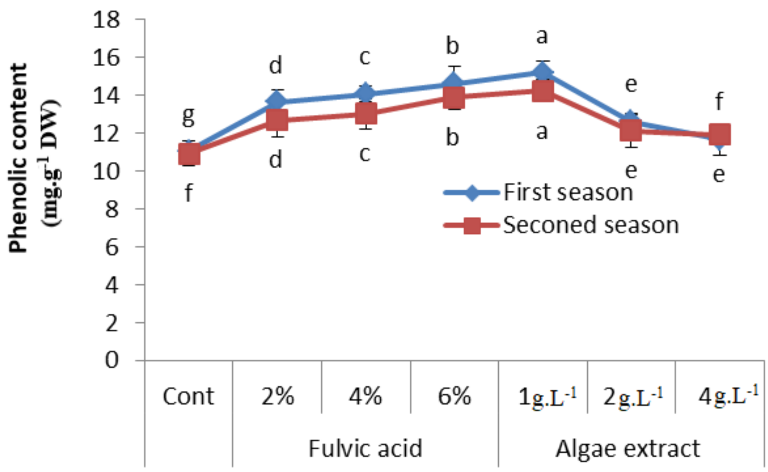

Figure 4 Effect of fulvic acid and algae extracts spraying on phenolic content (mg.g^{-1} DW) of sweet pepper fruits during the first and second seasons. Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$.

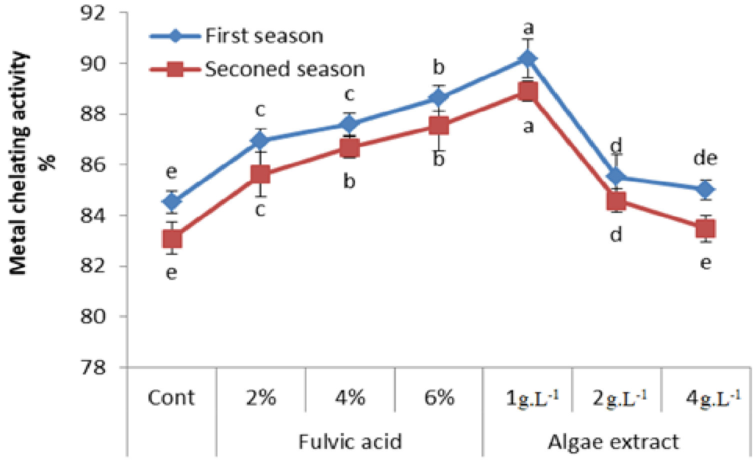

Figure 5 Effect of fulvic acid and algae extracts spraying on metal chelating activity % of sweet pepper fruits during the first and second seasons. Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$

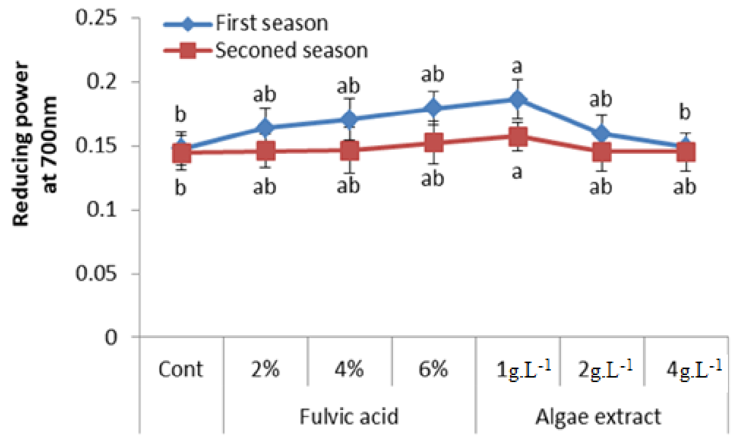

Figure 6 Effect of fulvic acid and algae extracts spraying on reducing power (at 700 nm) of sweet pepper fruits during the first and second seasons. Vertical bars show standard deviation (n = 3). Different letters indicate statistically significant differences at $p \leq 0.05$.

Table 4 Effect of fulvic acid and algae extracts spraying on organic acids (μg.mg^{-1} DW) of sweet pepper fruits during the first and second seasons.

Organic acid (μg.mg^{-1} DW)	Control	Fulvic acid			Algae extract		
		2%	4%	6%	1 g.L^{-1}	2 g.L^{-1}	4 g.L^{-1}
First season							
Oxalic acid	7.15	8.61	8.66	6.75	6.66	7.02	5.32
Citric acid	15.64	20.64	24.33	29.54	28.97	27.64	22.30
Malic acid	50.31	4764	52.64	60.31	66.43	67.5	51.97
Succinic acid	13.23	18.34	20.33	18.88	21.70	18.42	17.02
Glutaric acid	2.35	2.13	2.50	2.11	3.65	3.89	2.75
Propionic acid	0.02	0.005	Nd	Nd	Nd	Nd	Nd
Second season							
Oxalic acid	6.01	7.03	7.27	5.50	5.03	5.66	3.27
Citric acid	13.67	19.55	26.75	38.05	36.04	30.31	23.23
Malic acid	45.60	41.20	45.83	52.94	73.70	52.82	45.49
Succinic acid	11.99	15.55	19.14	17.35	19.29	15.47	13.05
Glutaric acid	3.17	2.46	3.21	1.88	5.68	3.90	3.41
Propionic acid	0.013	0.02	0.014	0.014	0.013	0.01	Nd

Note: Nd = Not detected.

Results indicate that fruits chlorophyll content increased with most of treatments. The highest content of fruit chlorophyll was obtained from algae extract (1 g.L^{-1}), followed by fulvic acid (6%).

Seaweed extracts contain cytokinins that stimulate physiological activities (such as activation of some enzymes that involved in photosynthesis), increased total chlorophyll in the plant and photosynthesis activity that will be positively reflected on shoot characteristics **(Thomas, 1996)**.

Ascorbic acid content

The effect of spray treatments with fulvic acids and algae extracts on ascorbic acid content, depends on the concentration used (Figure 2). All extracts stimulated ascorbic acid over control. Spraying with the algae extract (1 g.L^{-1}) gave the highest content of ascorbic acid (5.38 and 4.75 mg.g^{-1} FW in the first and second seasons, respectively) followed by spraying fulvic acid 6% (4.48 and 3.86 in both seasons, respectively). Humic substance application at different concentrations improved tomato vitamin C **(Dorais et al., 2008; Mauromicale, Longo and Lo Monaco, 2011)**.

Enzymes activity

The results in Figure 3 showed that spraying of algae extract at 1 g.L^{-1} resulted in peroxidase and polyphenol oxidase activities 240.08 and 353.64 unit min^{-1} mg^{-1} protein, respectively while fulvic acid (6%) gave 236.00 and 346.44 unit min^{-1} mg^{-1} protein, respectively compared with control (195.22 and 261.70 unit min^{-1} mg^{-1} protein, respectively) in the first season. The same trend was observed in the second season. Treatment with algae extract (1 g.L^{-1}) gave the highest value for the activity of each enzyme. In general, the best results were obtained from algae extract (1 g.L^{-1}) followed by fulvic acid extract (6%).

Phenolic content

It is clear from Figure 4 that all sprayed concentrations of fulvic acid and algae extracts increased the phenolic content of sweet pepper fruits compared to the control. The highest values in this respect were recorded when plants were sprayed with algae extract at 1 g.L^{-1} (15.21 and 14.24 mg.g^{-1} DW in the first and second seasons, respectively) followed by fulvic acid at 6% (14.62 and 13.89 mg.g^{-1} DW in the first and second seasons, respectively) compared to control (11.06 and 10.93 mg.g^{-1} DW in the first and second seasons, respectively).

The organic fertilizer applications significantly affected total phenolic content. Plants cannot allocate resources for growth and defence at the same time, and there is competition between proteins and phenols in plants for common precursors involved in their biosynthesis **(Aminifard et al., 2012; Riipi et al., 2002)**. The use of humic acid and calcium nitrate individually improves the quality of fruits, total phenols and antioxidant activity in pepper fruits under normal and saline conditions **(Akladious and Mohamed, 2018)**. These results led us assume that pepper plants may benefit from fulvic acid fertilizer for protein synthesis and growth development. On the other hand, organic acids and amino acids act as precursors or stimulants of plant hormones and growth substances, as well as secondary compounds in plants **(Vernieri et al., 2006)**. Also, when the light is sufficient for regular photosynthetic rates, additional carbon (C) may be allocated for the synthesis of C-based secondary compounds such as phenols in plants treated with organic fertilizers **(Toor et al., 2006)**.

Antioxidant activity

Figures 5 and 6 show the effect of spray treatments with the extracts of fulvic acid and algae on the antioxidant activity. This indicates a significant difference between all treatments and control in metal chelating activity. The best results were obtained from algae extract at 1 g.L^{-1} followed by 6% fulvic acid in both seasons. However, there were no significant differences between all treatments and the control in reducing power in both seasons.

Organic fertilizers increased the antioxidant activity **(Aminifard et al., 2012)**. There are a number of factors that can affect the total antioxidant capacity in plant tissues such as light intensity, temperature and cultivar as well as soil type and soil content of humic compounds (humic acid and fulvic acid) where the high content of humic compounds in the soil led to increase antioxidant activity **(Rimmer, 2006)**. Another view that explains the increase of antioxidant compounds in organic foods is that as the use of insecticide, fungicide and herbicide is limited in organic agriculture, the plants allocate more resources to fight pathogenic attacks, which include the generation of antioxidant compounds **(Winter and Davis, 2006)**. Thus, these findings reveal that fulvic acid had a positive influence on the antioxidant activity of sweet pepper fruits.

Organic acids

Organic acids have sour and fresh taste that impart unique flavour to the food **(Williams, 2001)**. Citric, malic, and succinic acids are the major organic acids in peppers **(Luning et al., 1995)**. The current study reports the determination of oxalic, citric, malic, succinic, glutaric and propionic acids in sweet pepper by HPLC. The results which summarized in Table 4, showed that the organic acids in the pepper fruits decreased in the order of malic >citric > succinic >oxalic >glutaric >propionic. Malic and citric acids are the principal organic acids found in peppers **(Jensen, 2007)**. While, the concentrations of glutaric and propionic acids in the sweet pepper were low. The concentrations of malic, succinic and glutaric acids were higher in 1 g.L^{-1} algae extract treatment, but the concentration of citric acid was higher in 6% fulvic acid treatment **(Luning et al., 1995)**.

CONCLUSION

The use of plant biostimulants has positive effects on growth and bioactive compounds in sweet pepper plants, especially when used at appropriate concentrations. Fulvic acid and algae extracts improved most fruit characteristics (length and diameter of fruits), vegetative growth, and chemical properties. With regard to organic acid, malic and citric acids are the main organic acids found in sweet peppers malic, succinic and glutaric acids were higher in 1 g.L^{-1} algae extract treatment, but the concentration of citric acid was higher in 6% fulvic acid treatment. The results of the current study showed that the best treatment is algae extract at 1 g.L^{-1} which gave the best value in most results followed by 6% fulvic acid so that it can be safely recommended as a natural biostimulants.

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Contact address:

Rabab Maraei, Natural Products Dept., National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. 29, Nasr City, Cairo- Egypt, Tel.: + 202-22749298,
E-mail: alrahman_27israa@yahoo.com
ORCID: https://orcid.org/0000-0003-3295-8806

*Noha Eliwa, Natural Products Dept., National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. 29, Nasr City, Cairo- Egypt, Tel.: + 202-22749298,
E-mail: nohaeliwa@hotmail.com
ORCID: https://orcid.org/0000-0002-9897-318X

Amina Aly, Natural Products Dept., National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. 29, Nasr City, Cairo- Egypt, Tel.: + 202-22749298,
E-mail: aly_amina@yahoo.co.uk
ORCID: https://orcid.org/000-0003-0756-731x

Corresponding author: *