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Full length Research

Effects of some red Algae on antioxidant and phytochemical contents of Maize (*Zea mays* L.) plants

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Biofertilizers have many beneficial effects for environment and agriculture especially in controlling many of plant diseases and enhancing the growth of a lot of crops. The effect of three species of red marine algae i.e., *Laurenciaobtusa*, *Corallina elongate* and *Janiarubens* was evaluated to enhance the phytochemical contents of maize (*Zea mays* L.) plants to improve the production and plant disease controlling. After 60 days from planting, application of single algae or their mixtures significantly enhanced the phytochemical contents of maize plants. The application of *Janiarubens* increased both of whole plant nitrogen content and protein content by 129.2% while application of *Janiarubens+Corallina elongate* increased the sugar contents by 32.4% and it was the superior treatment in increasing shoot polyphenolic contents and antioxidants content while the treatment of *Corallina elongate* was the superior one in increasing the shoot polyphenolic content, shoot antioxidants and root tannic acid content. Application of the three algae gave the highest amount in the root tannic acid content.

Key words: Red algae, marine algae, maize, phytochemicals, polyphenol, antioxidant capacity.

INTRODUCTION

Maize (*Zea mays* L.) is grown in many parts of the world for their economic importance. *Zea mays* is cultivated in almost all region of the world including Africa, America, Australia and Asia (Nurhanan *et al*., 2012). Maize (*Zea mays* L.) is consumed by people globally and is considered as a good nutrient (Pandey *et al*., 2013). Corn silk contains protein, vitamins, carbohydrates, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} salts, fixed and volatile oils, steroids (sitsterol and stigmasterol), alkaloids, saponins, tannins and flavonoid (Velazquez *et al.,* 2005) and also contains phytochemical components which exhibited antioxidant activities (Ebrahimzadeh *et al.*, 2008; Maksimović *et al*., 2005). The silks were observed to contain significant amount of polyphenol compounds. Polyphenols are known for its antioxidant characteristics and has been found to be rich in vegetables, fruits, herbs and other plant sources (Pandey *et al.,* 2013). They exhibit a wide Rangeof biological activities, viz., antibacterial, anti-inflammatory, anti-allergic,hepatoprotective, antithrombic, antiviral, anticarcinogenic and vasodialatory actions (Middleton *et al.,* 2000; Nardini and Ghiselli, 2003). Many polyphenolics containing plant sources were reported to exhibit antiradicals, antimutagenic, antibacterial and cytotoxic effects (De Mejía *et al.,* 1999; Kilani *et al.*, 2008;Pereira *et al.,* 2007). Hybrid maize cultivars were found to require high fertilizer rate for optimum yield (Onasanya *et al.,* 2009). Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility (Mishra *et al.,* 2013).Bio-fertilizers are likely to assume greater significance as complement and/or supplement to chemical fertilizers in improving the nutrient supplies to cereal crops because of high nutrient turn-over in cereal production system, exorbitant cost of fertilizers and greater consciousness on environmental protection (Ahmed, 2009). Strik *et al.* (2004)ported that the seaweeds extracts are effective fertilizers

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in many crops. Seaweed extracts are known to enhance seed germination, improve plant growth, and induce resistance to frost, fungal and insect attack and increase nutrient uptake from soil (Mohan *et al.,* 1994;Venkataraman *et al.,* 1993). Safinaz and Ragaa, (2013) reported that application of single marine alga or its mixtures enhanced certain growth parameters of maize plant after 60 days. *Luranciaobtuse+Aphenocapsaalbida* increased the whole plant length, dry weight, total phenol contents and nitrogen contents by 46.3%, 96.5%, 315% and 86% respectively. Lozano *et al.* (1999) stated that the application of an extract from algae to soil or foliage increased ash, protein and carbohydrate content of potatoes. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Yu *et al.,* 2002).

According to the recent reports, highly positive relationship between total Phenols and antioxidant activity was found in many plant species (Velioglu *et al.,* 1998; Nurhanan *et al.,* 2012). Flavonoids can protect against free radicals generated during photosynthesis. Terpenoids may attract pollinators or seed dispersers, or inhibit competing plants. Alkaloids usually ward off herbivore animals or insect attacks (Bernhof, 2010).

The present study aimed to assess the effect of three red algae species, i.e. *Laurenciaobtusa*, *Corallina elongate* and *Janiarubens* on the antioxidant and phytochemical contents in maize plants.

MATERIALS AND METHODS

Algae Collection and Preparation

Laurenciaobtusa was collected in May, 2012 from shallow water beside the shore of Red Sea of Safaga, *Corallina elongate* and *Janiarubens* were collected in May, 2012 from shallow water beside the shore of Mediterranean Sea at AbouQuair coast in Egypt.

After collection, algae were washed with fresh sea water to remove the epiphytes, sand and other extraneous matter then they were dried in shadow open air and completing the drying process in the oven at 60ºC for 5 hours. Then, dried algae were ground to fine powder by mechanic grinder.

The algae were applied as a soil treatment at the rate of 3 gm powdered algae/Kg soil seven days before planting and watered twice daily.

Plant Material

Seeds of maize (*Zea mays* L.) cv. K-8 were surface sterilized with ethanol 70% and washed by sterile distilled water, then dried in shadow open air. The seeds were planted in 30 cm diameter earthen pots containing mixture of 1:1 autoclaved peat and sand soil. Every pot contained 4 seeds. They watered every week.

The Treatments

1- Control, 2-*Laurencia obtusa,* 3-*Corallina elongate*, 4-*Jania rubens*, 5-*Laurencia obtusa* + *Janiarubens*, 6-*Laurencia obtusa* +*Corallina elongate*, 7-*Jania rubens* + *Corallina elongate,* 8-*Laurencia obtusa* + *Janiarubens*+*Corallina elongate*.

CHEMICAL ANALYSIS

Total Nitrogen Content

The determination of total nitrogen was carried out with Micro-Kjeldahl method (Anonymous, 1990). Half gram of dried and finely ground shoot and root sample was taken in a Kjeldahl flask. Three ml of digestion mixture (H_2SO_4 + K_2SO_4) in the ratio of 1:9 was added and followed by 20 ml of H_2SO_4. The sample was boiled in digestion apparatus for 1.5 to 2hrs until the contents became clear. The digested material was cooled and diluted up to 250 ml in a volumetric flask by adding distilled water. An aliquot 10 ml of it was transferred to the micro Kjeldahl distillation apparatus. It was mixed with 10 ml of 40 % NaOH and distilled in a receiver containing 10 ml of 2 % boric acid solution with methyl red as indicator. The contents of the distillate were titrated against standard sulfuric acid (N/10 H_2SO_4) to light pink color end point. From the volume of acid used, percentage of nitrogen was calculated based on ammonia liberated. Protein content was determined by multiplied nitrogen content by 6.25.

Lipids Extraction

Two gm of samples were soaked in 20 ml chloroform–methanol (1:1 v/v) for 48h and filtrated" the residues were repeated soaked in (the previous solvent) chloroform–methanol (1:1 v/v) for 48h and filtrated. The filtrates are taken and concentrated in vacuum until drying. The weight of the crude lipids obtained from each sample was measured using an electronic scale (Lee *et al.*, 2010).

Sugar Contents

Total carbohydrate contents were estimated with the phenol–sulphuric acid method using glucose as standard (Dubois *et a*l., 1956).

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined by the Folin Ciocalteu method (Singleton and Rossi, 1965). The color intensities were measured at wavelength 765 nm by using spectrophotometer (UV-200-RS LW Scientific) and compared to gallicacid calibration curve, TPC expressed as grams of gallicacid equivalents per 100 g plant extract

through the standard curve. The TPC was calculated by comparing the absorbance with the gallic acid calibration curve according to the formula:

TPC (mg/g) = C x V / g where;

C = concentration of the gallicacids equivalent from

standard curve (mg/mL)

V = volume of the extract used (ml)

g = weight of extract (g)

The contents were expressed as gallic acids (mg GAE/g dry wit).

Distilled water (3.16 ml) was mixed with the 40 µl of sample, and then 200 µl of FolinCiocalteu reagent was added. After 5 min, 600 µl of 20 % sodium carbonate solution was added and solutions were mixed again. The solutions were left at room temperature for 2 hrs.

Antioxidant Capacity (DPPH Assay)

The free radical scavenging activity was carried out calorimetrically using 1,1- diphenyl-2-picryl-hydrazyl (DPPH) assay. The reaction mixture contained 100 µl of test extracts and 1 ml of methanolic solution of 0.1 mM DPPH radical. The mixture was then vigorously shaken and incubated at 37 ºC for 30 min. the color intensities were measured at wavelength 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity which was calculated using the following equation:

DPPH scavenging effect (%) = 100 x (Ao- A1) /(Ao)

Where Ao is the absorbance of the control reaction and A1 is the absorbance of reaction mixture containing DPPH and extract at 517 nm.

The antioxidant activity of the extract was expressed as IC50 value which is defined as the concentration of extract that inhibits the formation of DPPH radicals by 50%. This was obtained from linear regression analysis (Gupta *et al.,* 2012).

Determination ofTannins (Vanillin - HCL Assay)

Samples (0.2 g) of ground parts (shoot and root) were extracted with 10 ml of methanol for 24 hr at 30º C. One milliliter of the resulting extract was reacted with 5 ml of vanillin reagent (50:50 mixtures of 1% vanillin / 8% HCl in methanol) for 20 min at 30º C, and absorbance was read at 500 nm. For blanks, 4% HCl in methanol instead of vanillin reagent was added to the extract, and absorbance was also read at 500 nm.

Blank values were subtracted from experimental values to give adjusted data. Tannic acid standard curve from 0.0-1.0 mg/ml was used in calculating tannin levels (Earp et al, 1981).

STATISTICAL ANALYSIS

Obtained data were subjected to analysis of variance, and the means were compared using the "Least Significant Differences (LSD)" test at the 0.05 level, as recommended by Snedecor and Cohran, (1982).

RESULTS AND DISCUSSION

Laurenciaobtusa, Corallina elongate, *Janiarubens* and their combinations were used to study their effect on the phytochemicals contents of maize plants. In Vivo studies, Table 1 showed that *Janiarubens* caused the best increasing in the whole plant nitrogen content % of maize plants (129.2%) followed by *Laurenciaobtusa* which caused (81.53%) while the application of the three algae caused the lowest value of N-content. This result is in agreement with Strik *et al.,* (2004) who reported that seaweed extracts are effective fertilizers in many crops. Also, Farfour and Hamouda, (2013) reported that application of single marine alga or its mixture enhanced certain growth parameters of maize plant after 60 days from planting.

Sugar content of maize plants was determined after 60 days from sowing and the result was expressed in Figure (1) which showed that the treatment of *Janiarubens+Cora-llina elongate* caused highest sugar content (32.42%) followed by the treatment of *Corallina elongate* (30%).This result was in agreement with Lozano *et al.,* (1999) who also reported that the application of an extract from algae to soil or foliage increased carbohydrate content of potatoes.

The results illustrated in Figure (2) cleared that both of the treatments of *Janiarubens* and *Laurenciaobtusa* gave the highest increase in lipid content of whole maize plants followed by *Corallina elongate* + *Laurenciaobtusa.*

The effect of some red marine algae on the polyphenol content of maize plants after 60 days from sowing was cleared in Figure (3) which showed that the application of *Janiarubens* + *Corallina elongate* gave the best results of the shoot polyphenol content followed by *Laurenciaobtuse*+*Janiarubens*, whereas the application of *Corallina elongate* gave the highest value of the root polyphenol content followed by *Laurencia obtuse* + *Janiarubens.* Hamouda and Farfour, 2013 stated*Luranciaobtusa*+ *Aphenocapsaalbida* increased the whole plant length, dry weight, total phenol content and nitrogen content by 46.3%, 96.5%, 315% and 86% respectively.

Figure (4) illustrate the effect of red marine algae on the content of antioxidants in the maize plants roots and shoots content after 60 days from sowing and cleared that *Janiarubens +Corallina elongate* caused the best in-Creasing in the antioxidants in the shoots followed by

Table 1.Effect of some algal treatments on the nitrogen content % of maize plant after 60 days from sowing.

Treatments	Shoots	Roots	Total plants	Increasing %
Control	0.4	0.3	0.7	-
Lo	0.9	0.3	1.2	81.5
Jr	1.1	0.4	1.5	129.2
Ce	0.8	0.3	1.1	63.1
Lo+Jr	0.8	0.3	1.1	70.8
Ce+Lo	0.6	0.4	0.9	47.7
Jr+Ce	0.8	0.3	1.1	67.7
Jr+Lo+Ce	0.6	0.4	1.0	53.8

Lo: *Laurencia***Jr**:*Jania***Ce**:*Corallina*

Table 2.Effect of algal treatments on the protein contents % of maize plants after 60 days from sowing.

Treatments	Shoots	Roots	Total plant	Increasing %
Control	2.3	1.8	4.1	-
Lo	5.4	2.0	7.3	79.8
Jr	6.8	2.6	9.3	129.3
Ce	4.8	1.8	6.6	63.0
Lo+Jr	4.9	2.0	6.9	70.6
Ce+Lo	3.8	2.2	6.0	47.1
Jr+Ce	4.8	2.0	6.8	67.7
Jr+Lo+Ce	3.5	2.8	6.3	53.9

Lo: Laurencia**Jr:**Jania**Ce:**Corallina

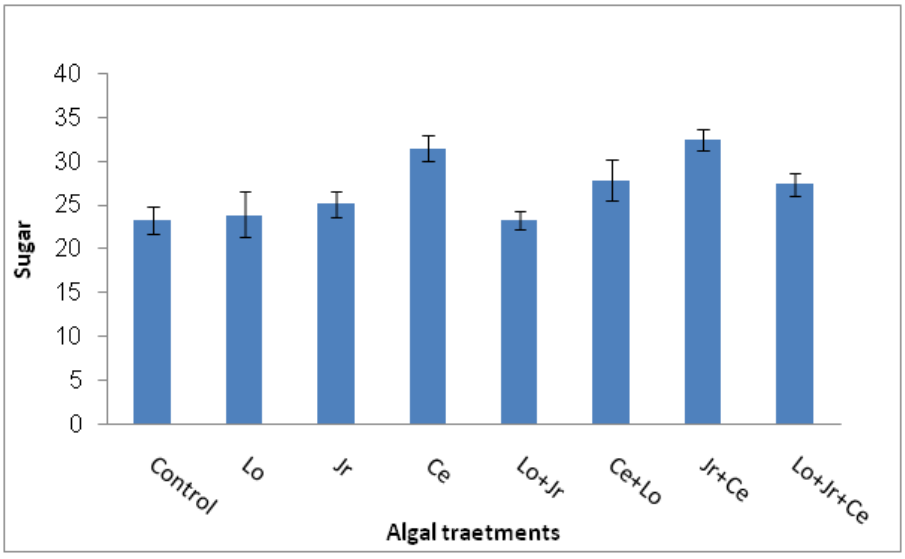

Figure 1. Effect of algal treatments on the sugar content of maize plants after 60 days from sowing.
Lo: *Laurencia***Jr:***Jan***Ce:** *Corallina*

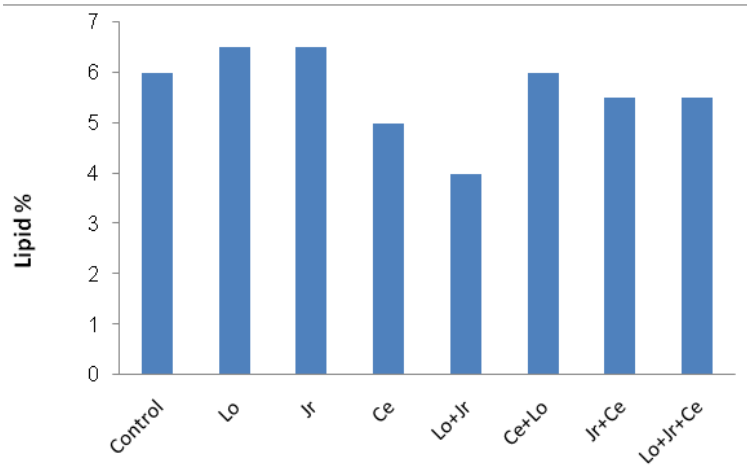

Figure 2. Effect of algal treatments on lipid contents of maize plants after 60 days from sowing.
Lo: *Laurencia***Jr:** *Jania***Ce:** *Corallina*

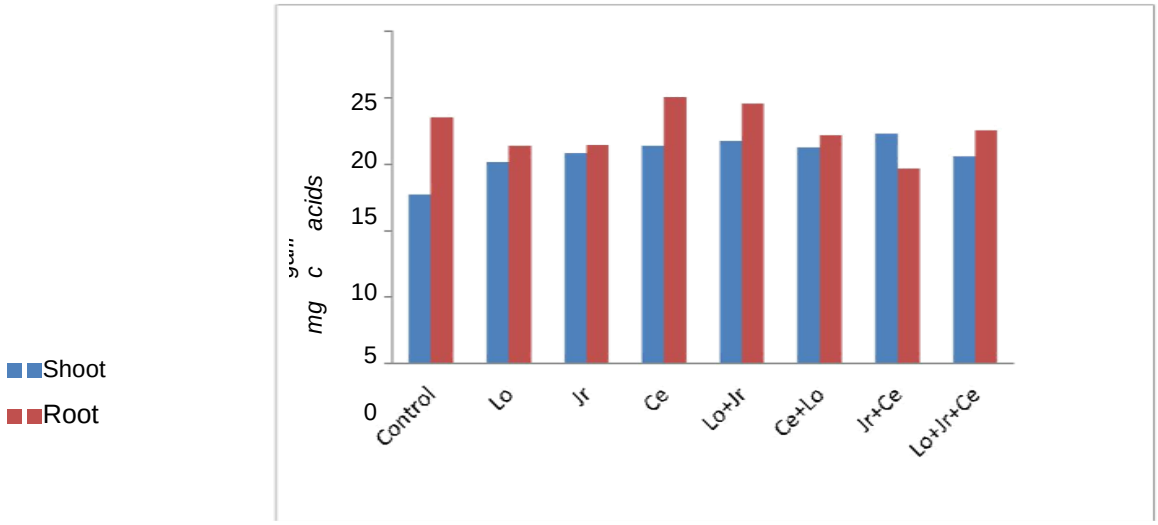

Figure 3. Effects of algal treatments on polyphenolic contents of Maize plants (Roots and Shoots) after 60 days from sowing
Lo: *Laurencia***Jr:** *Jania***Ce:** *Corallina*

Laurenciaobtusa + whereas the treatment of*Corallina elongate* gave the best results in the roots antioxidants content followed by *Laurenciaobtusa* + *Janiarubens*. These results were in agreement with Velioglu *et al*., (1998) and Nurhanan *et al*., (2012) who reported that there is highly positive relationship between total phenols and antioxidants activity in many plant species.

Figure (5) cleared that the application of *Corallina elongate* caused the highest increase in the tannic acid contents of maize plant shoots and roots followed by *Janiarubens* +*Corallina elongate,* whereas the application of the three algae gave the best results in the roots tannic acid content followed by *Corallina elongate* + *Janiarubens* .

CONCLUSION

Seaweed algae have an effective role in enhancing the phytochemical contents in maize plants. *Janiarubens* gave the best results in nitrogen, protein and lipid contents. *Corallina elongate* caused the best increase in both of root polyphenolic, root antioxidants and whole plant tannic contents while the application of *Janiarubens*+

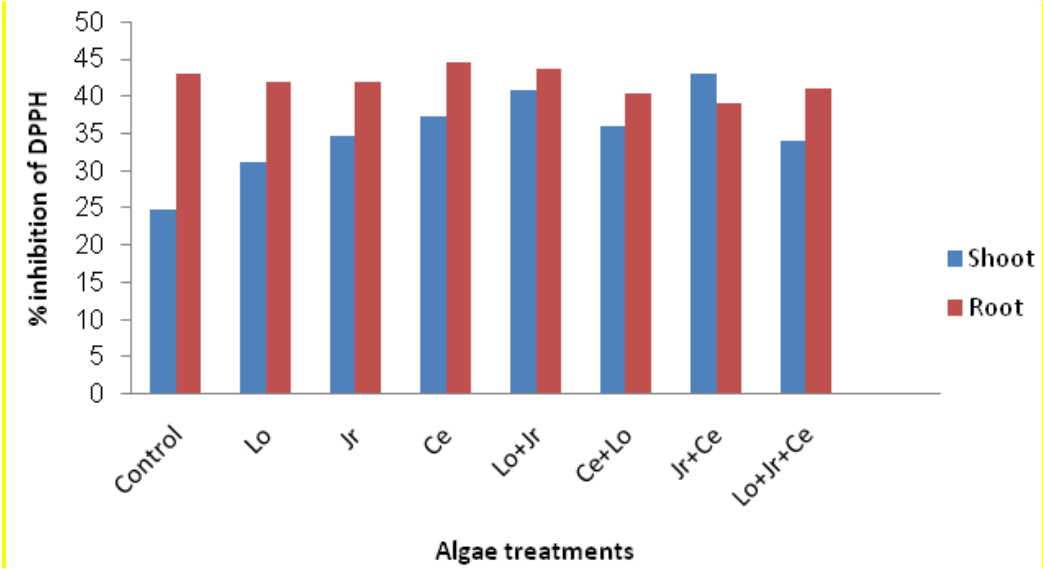

Figure 4. Effects of algal treatments on the antioxidant capacityof maize plants after 60 days from sowing.
Lo:*Laurencia***Jr:***Jania***Ce:***Corallina*

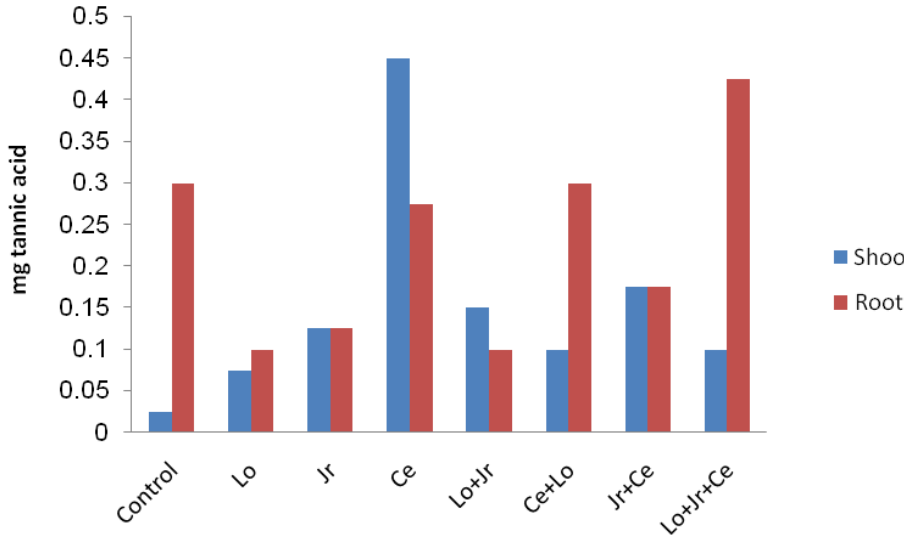

Figure 5. Effect of algal treatments on tannins contents of maize plants (Roots and Shoots) by modified vanillin hydrochloric assay
.**Lo:***Laurencia***Jr:***Jania***Ce:***Corallina*

Corallina elongate caused the highest increase in sugar and shoot antioxidants contents.

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