BIOACTIVE SUBSTANCES EXTRACTED FROM SEAWEEDS AS A BIOCONTROL AGENTS, EFFECTS AND IDENTIFICATION

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ABSTRACT

Marine bioactive substances extracted from marine algae as a biocontrol agents are used in agricultural and horticultural crops as a biofertilizers to improve their yield and quality and moreover to reduce the negative environmental impact.

This study was planned to estimate the allelopathic effect of three seaweeds from different algae classes commonly dispersed in Alexandria beaches on certain (narrow and broad leaves) weeds and crops which grown in both summer and winter seasons as pre-emergence application. In addition qualitative and quantitative determined of the allelochemicals, phenol derivatives in algae using HPLC. The oxidative enzymes and total phenols were also detected. Moreover, to asses its effects as a foliar application on these plants as a post-emergence application by determined the algae phytotoxicity on chlorophyll a, b, carotinoids and total protein.

The tested plants and weeds showed different response to the allelopathic effects of the water soluble extract of the tested algae. *Ulva lactuca*, the green alga was the most effective one followed by *Pterocladia pinnate*, the red alga. The seaweed *Leathesia difformis*, the brown alga came at the last on this manner. Concerning summer plants, tomato was the most affected plant. *Leathesia difformis* by all concentration had no inhibitory effect on the winter weeds and crops germination.. *Ulva lactuca*, had no effect on plant germination only on lower two concentrations but the higher concentrations gave significant reduction to the germination reach to 100% at lettuce, canary grass and black mustered when treated with 100% from this alga. *Pterocladia pinnat* had no effect on the germination of barley and bean but this alga not only prevent completely shoot growth but also significantly reduce root growth in these two plants, also the lowest concentrations had the same effect on the rest of the plants. Concerning the acetone extracts of the seaweeds, *Pterocladia pinnate* had no effect on all plants germination. Tomato plants were the most affected one followed by lettuce, whereas, the highest concentrations of *Ulva lactuca* and *Leathesia difformis* prevent tomato germination.

The aqueous extracts of the three algae when applied as post-emergence Canary-grass, lettuce, and common perslane were caused died after 10 days from the treatment. The algae reduced the soluble protein contents, total chlorophyll and carotienoids. On the other hand all algae treatments gave significant increase in these parameters especially in the important crops, bean, barley and tomato.

HPLC analysis reflected that, Vanillin and p-coumaric acid were highly concentration in *Ulva lactuca*, *Pterocladia pinnate* and *Leathesia difformis* and the corresponding concentration in water extract were (189.64,

186.61 and 98.81 µg g^{-1} Dry weight) and (153.57, 173.34 and 76.53) respectively whereas from acetone extract were (227.1, 88.91 and 91.30 µg g^{-1} Dry weight) and (196.63, 72.81 and 77.22) respectively, followed by Pyrogallol in *Ulva lactuca*, Chlorogenic acid in *Pterocladia pinnate* and Resorcinol in *Leathesia diffomnis* in all extracts. Contrary, Salicylic acid and Ferulic acid had the lowest concentration in all tested seaweed.

INTRODUCTION

At any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. One such approach is the use of biocontrol agents. Marine bioactive substances extracted from marine algae are used in agricultural and horticultural crops as biofertilizers, and many beneficial effects, in the terms of enhancement of yield and quality have been reported (Blunden, 1991; Crouch and Van Staden, 1994).

During the last two decades, marine chemical ecology has matured from a science where natural products chemists discovered secondary metabolites and assumed ecological functions, or where biologists observed ecological interactions and assumed the underlying chemical mechanisms, into a stronger field where chemical and biological aspects are simultaneously investigated using ecologically realistic conditions (Hay, 1996). Seaweeds or marine macroalgae are potential renewable resource in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes viz., green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae. The total global seaweed production of the world in the year 2004 was >15 million metric tones of which nearly 15–20% is contributed by Indian Ocean region (FAO, 2006).

Liquid extracts obtained from seaweeds have recently gained importance as foliar sprays for many crops including various grasses, cereals, flowers and vegetable species. These extracted chemicals from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereira, 2005). Seaweeds provide for an excellent source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals (Bhaskar and Miyashita, 2005), also it contains major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances and have been reported to stimulate the growth and yield of plants (Rama Rao, 1991), develop tolerance to environment stress (Zhang and Schmidt, 2000; Zhang *et al.,* 2003), increase nutrient uptake from soil (Verkleij, 1992; Turan and Köse, 2004) and enhance antioxidant properties.

The beneficial effect of seaweed extract application is as a result of many components that may work synergistically at different concentrations, although the mode of action still remains unknown

(Fornes *et al.*, 2002). In recent years, the usage of seaweed extracts has gained in popularity due to their potential use in organic and sustainable agriculture.

In the same time most macro-algae in particular, have to release bioactive secondary allelochemicals interfering with competitors in their vicinity (Gross *et al.*, 2003). Allelopathy as the influence of one plant on another by displacement under natural conditions and exerted by means of chemical rather than nutritional agents (Fang *et al.* 2009). Plant phenolic compounds are among the major allelochemicals implicated in Allelopathy ,a series of polyphenolic compounds, flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae (Yoshie *et al.*, 2000, Santoso *et al.*, 2002, and Yoshie *et al.*, 2003). Some free phenolic compounds as 4-hydroxyphenylacetic and 4-hydroxybenzoic acid were identified in *U. lactuca* (Flodin and Whitfeld 1999).

Although a lot of study on seaweed has been reported on their taxonomy, distribution, morphoecolgical studies, photochemistry and antibacterial activity, but insufficient data are available in literature about its toxicity on the crop plant and weeds. Therefore this study was planned to estimate the allelopathic effect of three seaweeds from different classes against certain arable weeds in agriculture crops as a pre-emergence application. In addition qualitative and quantitative determined of the allelochemicals, phenol derivatives in algae using HPLC and oxidative enzymes and total phenol. Moreover, to asses its effects as a foliar application on this pants as post-emergence application by determined the algae phytotoxicity on chlorophyll a, b, carotinoids and total protein.

MATERIALS AND METHODS

Preparation of aqueous extracts:

The three seaweeds *Ulva lactuca* as a Chlorophyta (green alga), *Leathesia difformis* as a Phaeophyta (brown alga) and *Pterocladia pinnate* as a Rhodophyta (red alga) were used in this study. These seaweeds were collected from sea beaches of Alexandria city; where the algae handpicked from the coastal area of Abo-Kir bay during March to May 2010. The collected samples were washed with seawater to remove unwanted impurities and transferred to the lab, and then washed using tap water. The fresh seaweed samples were homogenized by grinder with stainless steel blades at ambient temperature, filtered and stored (Eswaran *et al.*, 2005). The liquid filtrate was taken as 100% concentration (One kg of seaweed was cut into small pieces and boiled separately with 1 l of distilled water for an hour and filtered. From the later extract, the different concentrations (20, 40, 60, 80 and 100%) were prepared using distilled water (Sivasankari et al. 2006).

Preparation of acetone extracts:

According to Hassan and Ghareib (2009), the collected seaweeds was air dried under shade for 2 weeks, the dried algal material was ground. The powder (100 g) was extracted successively with acetone using Soxhlet apparatus. After 6 h. of extraction the solvents were evaporated from crude extract by rotary evaporator. The tested concentrated were (100, 300, 500 and 1000 ppm).

Bioassay the extracts on seed germination and growth of plant:

The aqueous and acetone extracts were tested as pre-emergence application via germination of the following weeds and crops, (*Vicia faba*) bean, (*Brassica niger*) black mustard, *(Hordeum Vulgare* L) barley, *(phalaris minor*) canary grass, (*Lactuca sativa* L.) lettuce, (*Echinochloa crass-galli*) barnyard grass, (*portulaca oleracea*) common perslane , (*Oryza Sativa)* rice, and (*Lycopersicon esculentum*) tomato . According to the Nober's technique, seeds were placed on sterilized 9cm. petri dishes and 10 ml. of each extract solution were added to each. After, 7 days, %germination, shoot height and root length of all survival plants was determined.

Foliar experiment:

Greenhouse experiments were designed to evaluate the effect of the aqueous extracts of three seaweeds as post-emergence application at 20, 40, 60 and 80% on the previous plants at 3-5 leave stage. Two weeks after the foliar application of the different concentrations, the plant were harvested to determine plant pigments and total soluble protein to the survival and died plants.

Chlorophyll and carotinoids content assay:

Chlorophyll content was extracted with 80% acetone and determined according to Grodzinisky and Grodzinisky (1973) and modified by Sabra (1993).

Total soluble protein assay:

According to Coolev and Foy (1992), the treated plants were homogenized with 0.1 M Tris/HCl buffer (pH 7.5) and centrifuged for 30 min at 6000 rpm at 4^0C, total soluble protein was determined according to the method described by Bradford, 1976 using Bio- Rad assay dye and the developed color was measured at 595nm.

Total soluble phenol content assay:

Total soluble phenol content in the tested algae was extracted according to Hsu *et al.* (2003). Sample of 5 gm was mixed with 80 mL methanol and kept overnight. The suspension was filtered and the filtrate was diluted to 100mL. This solution served as stock solution for subsequent analysis. According to Slinkard and Singleton (1997), two hundred microlitre of the stock solution was mixed with 1.4 mL distilled water, and 0.1mL of 50% (1N) Folin-Ciocalteu phenol reagent. After at least 30 seconds, 0.3 mL of 20% (w/v) sodium carbonate was added. The reaction mixture was allowed to stand for 2 hours, the absorbance at 765 nm was determined. Total soluble phenol content was

standardized against tannic acid and absorbance values were converted to mg of phenols per gram of fresh weight tissue. Each value reported is the average of three replicates.

Polyphenol oxidase (PPO) activity assay:

Polyphenol oxidase activity was determined according to the method of Broesch (1954), 0.25 gm of the tested algae were homogenized in borate buffer, pH 9, centrifuged at 4000 rpm for 15 min. one mL of the supernatant mixed with 2 mL of borate buffer, 1 mL P-aminobenzoic acid 1% and 1 ml 1% catechol. The reaction mixture was incubated for 1h. at 40°C. The absorbance at 575 nm was determined. The activity was expressed as A_{575}/ min/ gm of fresh weight.

Peroxidase (POD) activity assay:

According to Fehn-naum and Diamond (1967), a sample 0.25 of the tested algae were homogenized with phosphate buffer pH 6, centrifugation for 15 min at 4000 rpm was made. The enzyme activity was assayed by mixing 1 mL of the supernatant, H_2O_2 (20%) and catechol. The enzyme activity was expressed as A_{470}/ min/ gm of fresh weight. Each value reported is the average of three replicates.

HPLC analysis:

Free phenolic compounds in acetone and water extract of the seaweeds were identified by using HPLC (Shimadzu class-LC 10 AD chromatograph supplied with shimadzu SPD-10 AUV-VIS (shimadzu corporation, Japan) phenomenex C18 (25cm*4.6mm i.d, 5Mm particle size) column was used as a stationary phase for HPLC eterminations (USA). The extract was evaporated under reduced pressure by rotary evaporator at 45°C and the residue was dissolved in HPLC grade MeOH 20 µl of methanol dissolved sample was injected into HPLC column, according to Hassan and Ghareib (2009).

Statistical analysis:

The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Student–Newman–Keuls (SNK) test and differences at $P < 0.05$ were considered as significant (Cohort software Inc, 1986).

RESULTS AND DISSUASION

1-The effect of algae Water soluble extracts as a pre-emergence allelopathic compound on plant growth (germination, shoot height and root length):

1-a: The effect on winter weeds and crops:

Many studies have revealed that *Ulva lactuca* and *Ulva linza* exhibits negative allelopathic effects on microalgae (Jin and Dong, 2003; Nan *et al.*, 2004& 2008 and Jin *et al.*, 2005). Correspondingly, also approved the allelopathic effects of *U. lactuca*. Our data in table (1) showed that, *Leathesia difformis*, at all concentration had no inhibitory effect on the winter weeds and crops germination. These germinated seed showed either significant stimulatory effects on the

shoot of the tested plants or without significant deferent with the untreated plants. *Ulva lactuca*, had no effect on plant germination only on lower two concentrations but the higher concentrations gave significant reduction to the germination reach to 100% at lettuce, canary grass and black mustered when treated with 100% from this alga. In addition, at the highest concentrations 80 and 100% (w/v), caused significantly reduction to shoot and root length of the tested winter weeds and crops Table (2 and 3). On contrary, the lowest concentrations 20 and 40% caused 48.72 and 12.82% increase to barley shoot and 75.17% increase to bean. Concerning *Pterocladia pinnat*, all its concentrations had no effect on the germination of barley and bean but this alga not only prevent completely shoot growth but also significantly reduce root growth in this two plants , also, the lowest concentrations had the same effect on the rest of the plants. But the other concentrations gave significant reduction to the rest of these winter plants and caused reduction in both shoot and root height for example the concentration 80%, gave 23.81, 11.43 and 64.71% reduction in lettuce , canary grass and black mustered shoot and by 75, 80 and 64.71 to root respectively.

Table (1):Allelopathic effect of water aqueous extract (w/v) from tested algae on seeds germination (mean) of some weeds and crops

Treatments	*H. Vulgare*	*V. faba*	*L. Sativa*	*p. minor*	*B. niger*	*O. sativa*	*E. Crass-galli*	*P. Oleracea*	*L. esculentum*	Total mean
water	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ulva lactuca										
20%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10
40%	10.00	9.33	10.00	10.00	10.00	10.00	6.00	10.00	0.00	8.37
60%	10.00	9.33	3.33	1.33	9.67	10.00	6.00	10.00	0.00	6.63
80%	10.00	8.67	0.00	2.67	2.33	8.33	5.00	5.00	0.00	8.37
100%	5.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22
Pterocladia pinnate										
20%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
40%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	0.00	10.00	8.89
60%	10.00	10.00	7.33	7.33	9.33	10.00	10.00	0.00	5.33	7.70
80%	10.00	10.00	5.00	2.00	7.33	7.00	7.67	0.00	0.00	5.44
100%	10.00	10.00	3.33	2.00	6.67	6.67	3.33	0.00	0.00	4.67
Leathesia difformis										
20%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
40%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	7.33	9.70
60%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	6.00	9.56
80%	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	0.00	8.89
100%	10.00	10.00	10.00	10.00	10.00	10.00	3.33	10.00	0.00	8.15
Total mean	9.69	9.58	7.44	7.21	8.46	8.88	7.58	6.56	4.29	

L.S.D.for alga=0.2232, L.S.D.for plant=0.167

1-b: The effect on summer weeds and crops:

The tested plant and weeds showed different response to the allelopathic effects of the water soluble extract of the tested algae. *Ulva lactuca*, the green alga was the most effective one followed by *Pterocladia pinnate*, the red alga. The seaweed *Leathesia difformis*, the brown alga came at the last in this respect. As shown in table (1), tomato was the most affected plant, the all concentration of *Ulva lactuca* prevent compliantly the germination of the seed except 20% (w/v) followed by the other two algae at concentration 80 and 100% which prevent the tomato germination. The second plant in this respect was common perslane, *Pterocladia pinnate was* prevent its germination at all concentration except at 20%. From tables 2 and 3, the seaweed *Leathesia difformis* at the lowest concentration 20 and 40% (w/v) significantly stimulated tomato shoot height and root length by 28, 24, 90 and 70% respectively, compared with the untreated seeds

Table (2):Allelopathic effect of water aqueous extract (w/v) from tested algae on shoot height (cm.) of some weeds and crops

Treatments	*H. Vulgare*	*V. faba*	*L. Sativa*	*p. minor*	*B. niger*	*O. sativa*	*E. crass-galli*	*P. Oleracea*	*L. esculentum*
water	13.00	2.33	3.50	5.83	5.67	5.50	7.33	0.63	4.17
Ulva lactuca									
20%	19.33	3.67	5.00	5.00	7.67	6.00	9.00	2.17	4.23
40%	14.67	3.67	5.00	4.33	4.33	3.83	8.67	1.67	0.00
60%	0.00	1.67	4.00	4.00	2.67	2.67	5.00	1.00	0.00
80%	0.00	0.00	0.00	1.67	2.00	0.00	4.67	1.00	0.00
100%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pterocladia pinnate									
20%	19.67	5.00	3.00	6.67	6.83	7.33	6.67	1.00	5.67
40%	19.33	4.33	3.00	6.00	5.83	4.67	6.00	0.00	1.00
60%	17.33	4.67	2.67	5.33	5.33	4.33	3.67	0.00	0.00
80%	11.33	3.33	2.67	5.17	2.00	3.00	3.50	0.00	0.00
100%	8.33	2.00	2.33	3.33	1.83	2.33	3.00	0.00	0.00
Leathesia difformis									
20%	19.17	3.67	5.67	6.50	7.67	7.50	10.67	3.00	5.33
40%	18.33	4.00	5.33	5.83	7.17	7.00	7.83	2.50	5.17
60%	15.50	1.83	5.00	5.67	5.83	6.00	7.00	2.33	2.83
80%	13.83	1.50	4.17	5.50	5.83	5.33	5.33	2.00	0.00
100%	14.00	2.00	4.17	4.17	5.00	2.00	5.00	2.00	0.00
L.S.D(0.05)	0.90	0.967	0.60	1.73	0.882	0.668	0.697	0.398	0.57

Table (3):Allelopathic effect of water aqueous extract (w/v) from tested algae on root length (cm.) of some weeds and crops

Treatments	*H. Vulgare*	*V. faba*	*L. Sativa*	*p. minor*	*B. niger*	*O. sativa*	*E. crass-galli*	*P. Oleracea*	*L. esculentum*
water	5.50	2.83	2.00	6.67	1.67	6.67	5.17	0.20	3.33
Ulva lactuca									
20%	5.00	2.50	1.50	5.33	1.00	8.33	3.33	0.43	5.33
40%	3.33	2.50	1.50	3.83	1.00	5.33	2.50	0.30	0.00
60%	1.50	1.67	1.00	3.50	1.00	0.20	2.00	0.23	0.00
80%	1.00	2.00	0.00	1.67	0.50	0.13	1.00	0.20	0.00
100%	1.17	2.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
Pterocladia pinnate									
20%	5.00	5.00	0.50	3.00	1.00	3.00	3.00	0.10	2.83
40%	6.33	5.00	0.50	2.83	0.93	2.17	3.00	0.00	2.00
60%	8.00	5.00	0.30	2.50	0.63	2.33	2.33	0.00	0.50
80%	6.83	4.00	0.50	1.33	0.57	0.00	2.33	0.00	0.00
100%	3.00	2.67	0.23	1.17	0.50	0.00	2.00	0.00	0.00
Leathesia difformis									
20%	9.17	4.00	2.00	6.00	3.00	7.33	3.33	1.17	6.33
40%	9.33	3.67	2.00	5.83	2.33	6.00	3.00	1.00	5.67
60%	5.83	5.50	1.33	5.33	2.33	5.50	3.00	1.00	4.00
80%	5.83	4.00	1.33	4.67	1.83	4.00	3.00	0.53	0.00
100%	5.67	2.00	1.67	4.00	1.83	2.17	2.17	0.20	0.00
L.S.D(0.05)	0.464	1.004	0.295	0.73	0.50	0.523	0.97	0.133	0.494

Leathesia difformis had no effect on the rice and barnyard grass germination except the concentration 100% (w/v) which caused significant reduction of barnyardgrass germination by 66.67%. In addition, the other two algae at concentration of 20, 40, 60% (w/v) had no effect on plant germination (Table 1) but they caused significant reduction to rice and barnyard grass shoot and root length. On the other hand, the lower concentration of *Ulva lactuca* caused stimulation effect of rice shoots and roots by 9 and 25% respectively, (tables 2 and 3).

Table (4):Allelopathic effect of acetone extracts from tested algae on seeds germination (mean) of certain weeds and crops

Treatments	*H. Vulgare*	*V. faba*	*L. Sativa*	*p. minor*	*B. niger*	*O. sativa*	*E. crass-galli*	*P. Oleracea*	*L. esculentum*	Total mean
water	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
Ulva lactuca										
100 ppm	10.00	10.00	7.67	10.00	10.00	10.00	10.00	10.00	7.33	9.44e
300 ppm	10.00	10.00	4.67	10.00	10.00	10.00	10.00	10.00	4.33	8.78d
500 ppm	10.00	10.00	0.00	10.00	10.00	10.00	10.00	10.00	2.33	8.04b
1000 ppm	10.00	10.00	0.00	10.00	10.00	10.00	10.00	10.00	0.00	7.78a
Pterocladia pinnate										
100 ppm	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
300 ppm	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
500 ppm	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
1000 ppm	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
Leathesia difformis										
100 ppm	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00f
300 ppm	10.00	10.00	8.67	10.00	10.00	10.00	10.00	10.00	6.67	9.48e
-500 ppm	10.00	10.00	7.33	10.00	10.00	10.00	10.00	10.00	0.00	8.59c
1000 ppm	10.00	10.00	6.33	10.00	10.00	10.00	10.00	10.00	0.00	8.48c
Total mean	10.00c	10.00c	7.28b	10.00c	10.00c	10.00 c	10.00c	10.00c	6.21a	

2-The effect of algae acetone extracts as a pre-emergence allelopathic compound on plant growth (germination, shoot length and root hight):

Acetone extracts from different algae and growth responses of summer and winter plants were tested to find an appropriate extract showing the maximum varietal difference of allelopathic potential. The data were presented in tables 4, 5 and 6. In general, the acetone extracts of *Pterocladia pinnate* had no effect on all plants germination. Tomato plants were the most affected one within all three algae followed by lettuce. The three algae had no effect on the rest of the plants as shown in table (4).

2-a: The effect on winter weeds and crops:

Lettuce was the most affected winter plant whereas 500 and 1000 ppm from *Ulva lactuca* completely prevented its germination. The other alga, *Leathesia difformis* at 300, 500 and 1000ppm reduced the germination rate by 13.33, 26.67 and 36.67% respectively Table (4). On the other hand, 300, 500 and 1000 ppm acetone extracts from *Ulva lactuca* has a simulative effect on barley shoot, they gave 17.95, 38.46 and 15.38% increase respectively. These alga lower concentrations (100 and 300 ppm) gave 24.29 and 21.43% increase in bean shoot height.

In addition, *Ulva lactuca* at all concentration gave significant increase in root length of black mustard. On the other hand, all its concentrations gave significant reduction in root and shoot height of canary grass and lettuce. Although, *Pterocladia pinnate* had no effect on plant germination but it significantly reduced the root and shoot length of canary grass, for example 1000 ppm reduced shoot and root by 28.57 and 37.37% respectively. On contrast, this alga significantly stimulated the growth barley and bean, for example, 500 ppm stimulated barley shoot by 71.79 and 40.63 % increase in root length. Also, 100 and 300 ppm gave 28.57 % increased in bean shoot. On the other hand, the lowest concentrations of *Leathesia difformis*, gave stimulative of barley and bean length by 18.75 and 67.47% and for lettuce, it gave 20 and 50% increased in both shoot and root respectively, (tables 5 and 6). This alga significantly increased root length of black mustared but significantly decreased its shoot height; 500 ppm gave reduction of shoot height. by 29.41% and increased root by 90%.

2-b: The effect on summer weeds and crops:

Tomato plant was the most affected plant in this situation, its germination was completely prevented by 100 ppm from *Ulva lactuca* and *Leathesia difformis* at 500 and 1000 ppm (table 4).

Table (5):Allelopathic effect of acetone extracts from tested algae on shoot height (cm.) of certain weeds and crops.

Treatments	H. Vulgare	V. faba	L. Sativa	p. minor	B. niger	O. sativa	E. crass-galli	P. Oleracea	L. esculentum
water	5.33	2.83	2.00	6.33	1.67	6.67	5.17	0.20	3.33
Ulva lactuca									
100 ppm	5.67	2.00	1.00	2.17	4.00	1.00	2.00	0.30	3.00
300 ppm	5.67	2.50	0.50	2.00	4.67	5.50	2.00	0.23	3.00
500 ppm	5.00	1.00	0.00	5.17	3.00	7.50	2.50	0.43	4.00
1000 ppm	6.00	0.50	0.00	5.00	2.67	7.17	1.33	0.00	0.00
Pterocladia pinnate									
100 ppm	3.33	2.00	2.00	3.50	2.33	7.33	2.00	0.20	2.00
300 ppm	5.67	2.83	2.00	1.83	2.00	8.33	2.83	0.20	4.67
500 ppm	7.50	1.33	1.00	5.00	2.67	9.50	2.67	0.20	5.67
1000 ppm	7.67	5.00	1.00	3.00	3.33	7.00	2.00	0.20	3.00
Leathesia difformis									
100 ppm	6.33	5.00	3.00	5.67	3.67	8.33	1.00	0.20	2.00
300 ppm	5.00	1.33	2.33	1.83	3.33	8.00	1.00	0.20	1.67
500 ppm	5.00	2.83	1.33	5.33	4.83	4.67	1.67	0.20	0.00
1000 ppm	4.00	1.70	1.00	4.33	1.50	5.00	1.00	0.20	0.00
$L.S.D_{(0.05)}$	1.49	0.359	0.537	1.12	0.748	0.76	0.671	0.089	0.806

The three algae at all concentrations had no effect on barnyard grass germination but they significantly reduced root length for example, the highest concentrations of three algae gave 74.19, 64.52 and 80.65% reduced in root length for *Ulva lactuca, Pterocladia pinnate and Leathesia difformis*, respectively. On the other hand, most of the three algae concentrations gave significantly increased both rice's shoot and root length , for example 500 ppm of *Ulva lactuca and Pterocladia pinnate* gave 21.21 and 14% increased in shoot and root length respectively. The same concentration (500 ppm of *Pterocladia pinnate*) gave 33.33 and 42.5% increase in both shoot and root length. The lowest concentration of *Leathesia difformis* gave significantly increasing effect against growth of shoot and root by 39.39 and 25% at 100 ppm.

Table (6):Allelopathic effect of acetone extracts from tested algae on root length (cm.) of certain weeds and crops.

Treatments	*H. Vulgare*	*V. faba*	*L. Sativa*	*p. minor*	*B. niger*	*O. sativa*	*E. crass-galli*	*P. Oleracea*	*L. esculentum*
water	13.00	2.33	3.50	5.83	5.67	5.50	7.33	0.63	4 17
Ulva lactuca									
100 ppm	11.83	2.00	3.00	6.00	5.83	7.50	6.00	0.87	2.00
300 ppm	15.33	2.83	1.00	4.67	5.67	6.83	5.33	1.00	1.00
500 ppm	18.00	0.63	0.00	5.33	6.17	6.67	5.00	0.53	2.00
1000 ppm	15.00	0.50	0.00	4.33	4.50	6.83	5.00	0.50	0.00
Pterocladia pinnate									
100 ppm	9.67	3.00	4.33	3.67	3.83	8.00	5.83	0.50	4.00
300 ppm	18.00	3.00	3.33	4.67	3.00	7.83	6.00	0.57	4.00
500 ppm	22.33	2.17	3.33	6.50	5.50	7.33	5.83	0.57	5.33
1000 ppm	18.67	0.00	2.00	4.17	7.00	6.33	4.83	0.57	2.00
Leathesia difformis									
100 ppm	12.33	1.00	4.00	7.67	4.83	7.67	7.00	0.53	1.67
300 ppm	18.17	1.67	3.00	5.17	5.00	7.00	6.00	0.50	1.50
500 ppm	8.83	0.50	2.83	6.00	4.00	7.00	6.67	0.53	0.00
1000 ppm	6.67	0.50	2.00	5.67	2.83	6.67	6.17	0.57	0.00
$L.S.D_{(0.05)}$	0.849	0.502	0.268	0.748	0.839	0.931	0.644	0.23	0.537

3-The effect of foliar application of seaweeds as a post-emergence allelopathic compound on plant components:

3-a: The effect on total soluble protein contents:

In general, from table (7), all algae treatments with all concentrations, significantly reduce the contents of total soluble protein, this decreasing effects was ranged between 2.7 to 14.45%, except *Ulva lactuca* at 20% which caused an increase in protein reach to 20%. Canary grass, lettuce, and common perslane were died after 10 days from the foliar application of the three algae with all concentrations. Canary grass was the most affected plants followed by lettuce, and common perslane. On the other hand and considering the rest of the plants, all algae treatments gave significant increase in total soluble protein. For example, considering *Ulva lactuca* at 20%, it increased the soluble protein in bean and barley (the winter crops) by 65.02 and 32.41% but this concentration significantly decreases the soluble protein of Canary grass (the winter grass) by 83.94%.

Table (7): The effect of seaweed foliar application (w/v) on total soluble protein as (mg. protein/gm.D.W)

Treatments	H. Vulgare	V. faba	L. Sativa	p. minor	B. niger	O. sativa	E. crass-galli	P. Oleracea	L. esculentum	Total mean
water	367.66	538.72	403.40	388.09	203.40	266.38	320.85	504.68	195.74	354.32bc
Ulva lactuca										
20%	534.47	633.19	365.96	348.94	350.64	308.94	605.11	205.11	520.85	430.35d
40%	551.49	555.74	85.11	42.55	397.45	332.77	627.23	106.38	573.62	363.59c
60%	486.81	573.62	63.83	63.83	399.15	363.40	569.36	80.85	502.13	344.77abc
80%	605.11	593.19	45.11	45.11	384.68	366.81	530.21	71.49	474.89	346.28abc
Pterocladia pinnate										
20%	485.11	623.32	29.79	22.98	416.17	301.28	401.70	79.15	509.36	318.76ab
40%	391.49	628.26	39.15	22.13	421.28	382.98	371.91	80.00	488.51	313 96ab
60%	375.32	633.19	38.30	21.28	415.32	395.74	310.64	65.53	534.04	309.93a
80%	389.79	638.13	28.09	19.57	430.40	484.26	206.81	49.36	481.70	303.12a
Leathesia difformis										
20%	642.55	598.64	62.13	36.60	535.32	279.15	350.64	52.77	444.60	333.5abc
40%	472.34	603.57	68.09	42.55	581.19	330.21	355.74	70.64	423.66	327.55abc
60%	472.34	608.51	63.83	46.81	573.62	349.79	386.38	70.64	487.83	339.97abc
80%	451.06	613.45	21.28	21.28	547.23	371.06	573.62	60.43	466.81	347.35abc
Total mean	478.88 e	603.2 f	101.1 ab	86.28 a	435.06 d	348.7 c	431.55 d	115.15b	469.51e	

L.S.D.for alga=26.57 , L.S.D.for plant=22.11

The other two algae have the same behavior, *Leathesia difformis* and *Pterocladia pinnate* significantly increase the total soluble protein in bean by 65.51 and 72.22% , and for barley, the percentage of increasing were 28.47 and 2.08%. On the contrary, these two algae caused significant decrease in soluble protein extracted from barnyard-grass (the died grass) by 87.94 and 94.53%. Considering the summer crop tomato, the three algae, significantly increased its total soluble protein content, *Ulva lactuca* at the concentration of 20 to 80% caused increased between 29.17 and 56.02% but these concentrations caused significante decrease total soluble protein content of common perslane between 85.83 and 59.36%. Also the other two algae at the same concentrations caused significant increase in total soluble protein by 15.23 and 32.69% for *Leathesia difformis* and from 31.02 to 45.25 for *Pterocladia pinnate*. The total soluble protein content from common perslane had been decreased by *Leathesia difformis* by 89.54 to 86.00% and for *Pterocladia pinnate*, the percentages of increasing were 90.22 to

84.32. These results were contrary with the finding of other workers; the highest protein content was recorded at 20% concentration of seaweed liquid fertilizers (SLF) soaked treatment in *Vgna sinensis*. The increase in the protein content at lower concentration of SLF might be due to absorption of most of the necessary elements by the seedlings (Kannan and Tamilselvan, 1990; Anantharaj and Venkatesalu, 2001& 2002).

3-B- the effect on plant pigments:

3-b-1 the effect on chlorophyll:

Data in Table (8) indicated that, Canary grass, lettuce, and common perslane were the most affected plants in reducing total chlorophyll. This reduction is due to the effect of the algae extracts on cha and chb. As a total means, all algae concentration caused either significant increased in total chlorophyll or caused slight reduction without significant deferent with untreated plants. Considering *Ulva lactuca*, as a total means 40, 60 and 80% significantly caused decrease in total chlorophyll contents in Canary grass, lettuce, for example, 80% caused 90.14 and 62.07% decreases in these two plants respectively. On the other hand, this alga had no effect on chlorophyll in bean and moreover it caused significant increase in barley total chlorophyll as a total means. In addition this concentration caused significant decrease in common perslane reach to 68.14%, but it increased tomato total chlorophyll reach to 49.80%. *Leathesia difformis* caused significant reduction in both Canary grass, lettuce total chlorophyll whereas it caused significant increase in the total chlorophyll of barley. The reduction in Canary grass caused by 80% *Leathesia difformis* reach to 75.66% and in , lettuce and bean equal to 59.76 and 39.07% respectively and this alga caused 69.1% increased in barley. Concerning to the summer crop and weed, this alga at 60% caused 31.75% reduction in common perslane but it increase total chlorophyll in tomato by 16.7%. The third alga, *Pterocladia pinnate* caused significant reduction in the total chlorophyll extracted from Barnyard-grass, lettuce and barley by 93.33, 54.69 and 21.11% respectively, but the total chlorophyll extracted from bean increased by 68.35%. In addition, this concentration caused 96.25 and 26% reduction in common perslane and tomato, total chlorophyll. The results reported before by many workers using seaweeds as the liquid fertilizer were as follows, the lower concentrations of the two seaweed liquid fertilizers (*Sargassum wightii* and *Caulerpa chemnitzia*) promoted the chlorophyll content of *Vigna. sinensis* up to 20% when compared to control. Higher concentrations were decreased the chlorophyll content. A similar observation was made in *Scytonema* sp. (Venkataraman and Mohan, 1997a) and in *Vigna mungo* (Venkataraman and Mohan, 1997b). The seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants (Blunden *et al.*, 1996). In other search study, the foliar spray of alga *Kappaphycus*

alvarezii which was applied twice at seven concentrations (0, 2.5, 5, 7.5, 10, 12.5 and 15% v/v) of seaweed extract, significantly enhanced soybean yield parameters. The highest grain yield was recorded with applications of 15% seaweed extract, followed by 12.5% seaweed extract that resulted in 57% and 46% increases respectively compared to the control (Rathore *et al.* 2009). On contrast, the alga *Cladonia verticillaris* extracts and fumarprotocetraric acid showed inhibitory effect on the germination rate of *Alllum Cepa (*Yano-Melo *et.al,* 1999).

Table (8): The effect of seaweed foliar application on chlorophyll contents as mg./g.F.W.

	Hordeum Vulgarea			*Vicia fab*			*Lactuca Sativa*			*phalaris minor*			*Brassica niger*		
Treatments	cha	chb	Total	cha	chb	total	cha	chb	total	cha	chb	total	cha	chb	total
Water	0.572	0.165	0.742	0.305	0.117	0.504	0.628	0.439	1.294	0.603	0.046	0.647	0.653	0.359	1.000
Ulva lactuca															
20%	0.594	0.371	0.964	0.504	0.198	0.730	0.525	0.022	0.548	0.746	0.047	0.793	0.682	0.440	1.122
40%	0.639	0.332	0.971	0.542	0.179	0.722	0.536	0.015	0.551	0.245	0.056	0.301	0.816	0.411	1.227
60%	0.896	0.192	1.088	0.571	0.140	0.711	0.594	0.027	0.639	0.059	0.071	0.130	0.894	0.558	1.452
80%	1.062	0.193	1.254	0.577	0.149	0.726	0.418	0.071	0.491	0.021	0.043	0.064	1.010	0.550	1.560
Pterocladia pinnate															
20%	0.522	0.244	0.766	0.466	0.393	0.859	0.500	0.027	0.535	0.690	0.045	0.735	1.466	0.138	1.604
40%	0.451	0.160	0.611	0.519	0.444	0.963	0.499	0.046	0.543	0.626	0.061	0.687	1.433	0.136	1.569
60%	0.434	0.159	0.593	0.518	0.406	0.925	0.565	0.029	0.571	0.054	0.035	0.089	1.466	0.246	1.712
80%	0.431	0.154	0.585	0.919	0.310	1.228	0.539	0.045	0.586	0.029	0.014	0.043	1.349	0.163	1.511
Leathesia difformis															
20%	0.599	0.332	0.931	0.466	0.129	0.595	0.540	0.024	0.566	0.190	0.140	0.330	1.051	0.523	1.575
40%	0.611	0.307	0.918	0.443	0.124	0.567	0.445	0.036	0.486	0.193	0.091	0.284	1.079	0.446	1.526
60%	0.643	0.315	0.959	0.437	0.061	0.498	0.465	0.041	0.493	0.207	0.082	0.289	0.706	0.541	1.247
80%	0.683	0.321	1.004	0.409	0.036	0.445	0.488	0.046	0.520	0.096	0.062	0.157	0.622	0.654	1.277
Total mean			0.88e			0.73d			0.60c			0.35a			1.41i

Table (8): Continued

Treatments	*Oryza Sativa*			*Echinochloa crass-galli*			*Portulaca Oleracea*			*Lycopersicon esculentum*			Total mean
	cha	chb	Total	cha	chb	total	cha	chb	total	cha	chb	total	
Water	0.357	0.193	0.535	0.621	0.156	0.727	0.504	0.198	0.730	0.653	0.350	1.025	0.662 a
Ulva lactuca													
20%	0.748	0.439	1.188	0.885	0.485	1.370	0.529	0.237	0.766	0.746	0.424	1.170	0.802 ef
40%	0.970	0.447	1.417	0.767	0.515	1.282	0.053	0.272	0.325	0.773	0.422	1.195	0.77 bcd
60%	1.031	0.426	1.457	0.691	0.669	1.360	0.049	0.284	0.332	0.860	0.567	1.427	0.849 def
80%	1.129	0.606	1.735	0.513	0.703	1.217	0.045	0.188	0.232	0.976	0.559	1.535	0 864 f
Pterocladia pinnate													
20%	0.840	0.400	1.240	0.507	0.328	0.835	0.466	0.129	0.595	0.984	0.541	1.525	0.772 bcde
40%	0.830	0.413	1.243	0.900	0.293	1.193	0.443	0.124	0.567	1.012	0.464	1.476	0.776 cde
60%	0.980	0.499	1.479	0.971	0.485	1.456	0.437	0.061	0.498	0.709	0.487	1.196	0.8bcde
80%	1.215	0.509	1.724	1.306	0.534	1.840	0.409	0.036	0.445	0.508	0.091	0.599	0.81bc
Leathesia difformis													
20%	0.436	0.239	0.675	0.997	0.435	1.432	0.466	0.393	0.859	0.456	0.395	0.851	0.684 cdef
40%	0.452	0.245	0.698	0.796	0.804	1.599	0.062	0.130	0.191	0.423	0.393	0.816	0.624 b
60%	0.453	0.229	0.682	0.791	0.827	1.617	0.055	0.083	0.138	0.462	0.404	0.867	0.63 a
80%	0.643	0.267	0.910	0.857	0.987	1.844	0.058	0.044	0.102	0.339	0.420	0.758	0.687 a
Total mean			1.15g			1.37h			0.45b			1.11f	

3-b-2- the effect on carotenoids:

As shown in table (9), Canary-grass and lettuce were the most affected plants without any significant deferments followed by common perslane. On the other hand the differences between the total means of all algae extracts gave significant increase in carotenoids contents, whereas *Ulva lactuca* at 60% ranked first in this manner, it gave 53.748% increase in total carotenoids followed by the rest of the same alga concentration in order.

Table (9): The effect of seaweed foliar application on total carotenoids as mg./g.F.Wt.

Treatments	H. Vulgare	V. faba	L. Sativa	p. minor	B. niger	O. sativa	E. crass-gall	P. Olerace a	L. esculentu m	Total mean
Water	0.038	0.027	0.103	0.008	0.085	0.045	0.035	0.046	0.082	0.052 a
Ulva lactuca										
20%	0.088	0.046	0.003	0.008	0.104	0.104	0.114	0.055	0.100	0.069 bc
40%	0.078	0.041	0.002	0.013	0.096	0.104	0.122	0.067	0.099	0.069 bc
60%	0.042	0.032	0.004	0.018	0.132	0.099	0.160	0.069	0.134	0.076 cd
80%	0.042	0.034	0.015	0.011	0.129	0.142	0.169	0.047	0.132	0.08 d
Pterocladia pinnate										
20%	0.057	0.094	0.004	0.007	0.026	0.056	0.101	0.094	0.094	0.059 ab
40%	0.037	0.107	0.009	0.012	0.026	0.058	0.193	0.032	0.094	0.063 b
60%	0.037	0.097	0.004	0.008	0.052	0.054	0.198	0.020	0.097	0.063 b
80%	0.035	0.071	0.008	0.003	0.032	0.062	0.237	0.011	0.101	0.063 b
Leathesia diffformis										
20%	0.078	0.029	0.003	0.033	0.123	0.093	0.078	0.029	0.127	0.066 b
40%	0.073	0.028	0.007	0.021	0.104	0.097	0.067	0.028	0.108	0.059 ab
60%	0.074	0.013	0.008	0.019	0.129	0.117	0.114	0.013	0.116	0.066 b
80%	0.075	0.007	0.009	0.015	0.157	0.118	0.124	0.007	0.020	0.058 ab
Total mean	0.057 d	0.047 c	0.014 a	0.014 a	0.091 e	0.088 e	0.131 g	0.039 b	0.1 f	

L.S.D. for alga=0.00726, L.S.D. for plant=0.00604

4- Identification and determination of total phenol, polyphenol oxidase (PPO), peroxidase (POD) and ten phenolic compounds.

Many studies have demonstrated that seaweeds are an important source of bioactive secondary metabolites (Hornsey and Hide, 1976; Reichelt and Borowitzka, 1983; Hay, 1996; Harder *et al.*, 2004; Steinberg, 2001; and Engel *et al.*, 2006). Other studies indicated that, Macroalgae suppressed phytoplankton growth through the excretion of chemical substances (Elakovich and Wooten, 1995; Gross, 1999; and Gross *et al.*, 2003). The interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during the last years (Celikler, *et al* 2009). Although fresh and dried seaweeds are extensively consumed, particularly by coastal peoples in several countries, they are considered as under-exploited resources (Fayaz *et al.*, 2005).

Phenolic compounds are secondary metabolites that have diverse biological activities, such as in defense mechanisms of plants under different environmental stress conditions like wounding, infection, excessive light or UV irradiation (Muchuweti *et al.*, 2007).The phenolic compounds in the aqueous and acetone extract, in addition total phenol and two enzymes (polyphenol oxidase (PPO) and peroxidase (POD)) were determined in three seaweed *U. lactuca*, *L. difformis* and *P. pinnate*.

The data in table 11 consists of the retention times, molecular formula and concentration of ten phenolic compounds (Pyrogallol, Resorcinol, Protocatechuic acid, Chlorogenic acid, Caffeic acid, *p*-coumaric acid, Vanillin, Ferulic acid, Salicylic acid and Benzoic acid) which determined in *U. lactuca*, *L. difformis* and *P. pinnate* extracts, were identified by the reverse-phase (HPLC) high performance liquid chromatography. These compounds identification was based on comparison of their relative retention time with those obtained from the different standard compounds. The concentration range of detected phenolic compounds in water extract was 189.64-14.26 µg g^{-1} Dry weight for *Ulva lactuca*, 186.61-16.85for *Pterocladia pinnate* and 98.81-5.46 for *Leathesia difformis*. In acetone extract, the concentration range was between 227.10 to14.16 µg g^{-1} Dry weight for *Ulva lactuca*, *Pterocladia pinnate* (88.91-37.13) and *Leathesia difformis* (91.30-14.01).

The results concerning the concentration of total phenol were 0.403, 0.139 and 0.116 mg/g F.W. for *U. lactuca*, *P. pinnate* and *L. difformis* respectively, (Table 10). These results are agreement with the total concentration of phenolic compounds in table 11. The level were 805.93, 718.91 and 324.193 in water extract and 781.15, 556.85 and 407.26 in acetone extract for *U. lactuca*, *P. pinnate* and *L. difformis* respectively. In addition, the concentration levels of polyphenol oxidase (PPO) were 0.025, 0.014 and 0.014 A470/min/g F.W. and peroxidase (POD) were 0.245, 0.249 and 0.248 A575/min/g F.W from *U. lactuca*, *P. pinnate* and *L. difformis* respectively.

The result reflected that, the highly constituents compounds in the three algae were Vanillin and p-coumaric acid and the corresponding concentration in water extract were (189.64, 186.61 and 98.81 µg g^{-1} Dry weight) and (153.57, 173.34 and 76.53) for *U. lactuca*, *P. pinnate* and *L. difformis* respectively whereas from acetone extract were (227.1, 88.91 and 91.30 µg g^{-1} Dry weight) and (196.63, 72.81 and 77.22) respectively, followed by Pyrogallol in *Ulva lactuca*, Chlorogenic acid in *Pterocladia pinnate* and Resorcinol in *Leathesia difformis* in all extractes (table 11).

On the other hand, Salicylic acid and Ferulic acid had the lowest concentration in all tested seaweed. These results are in agreement with the results of Hassan and Ghareib (2009), they reported that, Vanillin and *p*- coumaric acid in the *Ulva lactuca*

acetone extract was recorded as the abundant compounds while ferulic acid and salicylic acid existed in less amounts.

In conclusion, the water extracts of the seaweeds had stimulatory effect on the growth of different tested plants. The stimulation effect could be attributed to the possible synergistic effect between the different types of phenolic compounds as mentioned by Gerig and Blum (1991). Such a stimulatory effect comes in agreement with that research done by Reigosa *et al.*, (1999) and Hegab (2005). They reported that, free phenolic compounds (such as, vanillin) stimulating the germination and seedling growth of different plants. Also, Monerri *et al.*, (1986) indicated that, the increasing of amylase activity during germination of the seeds treated with the extracts contained similar phenolic compounds.

On the other hand, phenolic acids of acetone extract inhibited the germination at high concentrations. Similarly, Souto et al. (1995), revealed that germination of *Trifolium repens* seeds was inhibited at high concentrations of p-vanillin, gallic, vanillic, ferulic, *p*-hydroxybenzoic and *p*-coumaric acids. The observed reduction in radicle growth by treatment with phenolic acids agreed with the results stated by Ng *et al.* (2003) and Chung *et al.* (2002), they reported that, many alterations in root growth of different plant species were recorded in the plants treated with phenolic acids (p-hydroxybenzoic and p-coumaric acids).

We can conclude that, algae as a pre- emergence application were toxic to the crops and weeds, although it did not prevent the germination of certain crops but it had reduced the growth. On the other hand, algae as foliar application were safe to be post-emergence application on winter crops, such as barley and bean to control canary- grass, and also in tomato to control common perslane.

Table (10):Total phenols, polyphenoloxidase (PPO) and peroxidase (PPO) activity extracted from tested algae.

algae	total phenol mg/g F.W	POD A575/min/g F.W	PPO A470/min/g F.W.
U. lactuca	0.403	0.245	0.025
P. pinnate	0.139	0.249	0.014
L. difformis	0.116	0.248	0.014

Table (11): HPLC analysis of free phenolic compounds in water and acetone extracts

Standard phenolic compounds	molecular formula	Retention Time (min.)	Concentration in water (µg g^{-1} Dry weight)		
			U	P	L
Pyrogallol	$C_6H_6O_3$	9.69	135.24	51.96	23.93
Resorcinol	$C_6H_6O_2$	13.74	54.84	33.48	32.103
Protocatechuic acid	$C_7H_6O_4$	14.02	71.78	62.24	19.46
Chlorogenic acid	$C_{16}H_{18}O_9$	15.93	78.96	80.47	29.23
Caffeic acid	$C_9H_8O_4$	19.31	52.85	56.43	12.44
p- coumaric acid	$C_9H_8O_3$	21.21	153.57	173.34	76.53
Vanillin	$C_8H_8O_3$	22.41	189.64	186.61	98.81
Ferulic acid	$C_{10}H_{10}O_4$	24.55	14.26	16.85	5.46
Salicylic acid	$C_7H_6O_3$	31.11	22.63	21.52	11.57
Benzoic acid	C_6H_5COOH	42.6	32.16	36.01	14.66
Total concentration			805.93	718.91	324.193
Standard phenolic compounds	molecular formula	Retention Time (min.)	Concentration in aceton (µg g^{-1} Dry weight)		
Pyrogallol	$C_6H_6O_3$	9.69	166.31	45.81	69.62
Resorcinol	$C_6H_6O_2$	13.74	44.20	44.21	51.76
Protocatechuic acid	$C_7H_6O_4$	14.02	30.80	51.23	23.48
Chlorogenic acid	$C_{16}H_{18}O_9$	15.93	29.69	62.09	29.69
Caffeic acid	$C_9H_8O_4$	19.31	34.25	55.60	21.09
p- coumaric acid	$C_9H_8O_3$	21.21	196.63	72.81	77.22
Vanillin	$C_8H_8O_3$	22.41	227.1	88.91	91.30
Ferulic acid	$C_{10}H_{10}O_4$	24.55	14.16	37.13	14.01
Salicylic acid	$C_7H_6O_3$	31.11	19.83	43.46	15.91
Benzoic acid	C_6H_5COOH	42.6	18.18	55.60	13.18
Total concentration			781.15	556.85	407.26

U= *Ulva lactuca*, P= *Pterocladia pinnate* and L= *Leathesia difformis*

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الملخص العربي

التعرف على المكونات النشطة المستخلصة من الأعشاب البحرية وتقدير كفائتها البيولوجية

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تعد المركبات المستخلصه من الطحالب البحريه من المركبات التى لها نشاط طبيعى فى المكافحه البيولوجيه حيث انه من المعروف ان الطحالب تستخدم فى الزراعه كمنشطات طبيعيه لزياده انتاج وتحسين محاصيل الحقل والخضر وتقلل من الآثار الضارة على البيئة.

وقد خططت هذه الدراسه لتقدير التأثير الاليلوباثى لثلاثه من اكثر الطحالب انتشارا فى شواطىء الاسكندريه والتى تنتمى الى ثلاث مجاميع مختلفه من مجموعات الأعشاب البحرية وذلك على عدد محدد من المحاصيل والحشائش سواء الشتويه أوالصيفيه سواء كانت عريضه او رفيعه الأوراق. قد تم تقدير كفاءة المستخلصات المائيه ومستخلصات الاسيتون لمعرفه تأثيرهما كمركبات قبل الانبثاق على عمليه الانبات والنمو للمحاصيل والحشائش وكذلك تم اختبارسميتها النباتيه بالرش كمركبات بعد الانبثاق . كذلك تم تقدير محتوى النباتات المعاملة بهذه المستخلصات من البروتين

والصبغات النباتيه. بالاضافه الى تقدير كمى ونوعى للمشتقات الفينوليه فى مستخلصات الطحالب البحرية موضع الدراسة.

ومن النتائج المتحصل عليها اتضح ان النباتات والمحاصيل أختلفت فى استجاباتها لمستخلصات الطحالب، فالطحلب الأخضر (اولفا) كان اكثر الطحالب تأثيرا على الانبات يليه الطحلب الأحمر (كولودينا) وفى النهايه يأتى الطحلب البنى (لاتيسيا). وأتضح أن بذور الطماطم تعتبر من أكثر النباتات الصيفيه تأثرا وأما بالنسبه للمحاصيل والحشائش الشتويه لم يكن للطحلب البنى اى تأثير بكل تركيزاته. اما الطحلب الاخضر فلم يكن له تأثير بتركيزاته المنخفضه ولكن أعلى تركيزاته (١٠٠%) احدثت خفض معنوى لأنبات كل النباتات موضع الدراسة، حيث أدى الى منع الأنبات نهائيا فى الخس وحشيشه الكنارى والكبر. أما عن الطحلب الاحمرلم يكن له تأثير على إنبات المحاصيل الشتويه كالشعير والفول وبعض التركيزات منعت نهائيا نمو المجموع الخضرى واحدثت خفض معنوى للمجموع الجذرى.

أما عن مستخلصات الاسيتون وكمتوسط عام للمعاملات، لم يكن للطحلب الاحمر اى تأثير على أنبات كل النباتات وكانت الطماطم الصيفيه الاكثر تأثرا يليها الخس الشتوى حيث منعت التركيزات المرتفعه فقط من الطحلب الاخضر والبنى أنبات بذور الطماطم نهائيا.

أدى رش المستخلص المائى للطحالب الى موت حشيشه الكنارى الشتويه ونبات الخس وكذلك الى موت الرجله الصيفيه بعد ١٠ أيام من الرش ولم يؤثر ذلك على محصول الشعير أو الفول الشتويين أو الطماطم الصيفى. وقد أدت هذه الطريقة من الرش الى زياده البروتين الذائب الكلى والصبغات النباتيه سواء الكلوروفيل وكاروتينويد فى محتوى النباتات المعاملة، مما يدلل على أمكانية أستخدام المستخلصات المائيه للطحالب كمركبات تعمل بعد الانبثاق فى هذه المحاصيل لمكافحه تلك الحشائش.

وقد أوضح تحليل الكروماتوجرافى السائل للمستخلصات المائيه للطحالب موضع الدراسة أن حمض الفانيليك والبارا كيوماريك كانا اكثر تركيزا فى الطحلب الأخضر يليه الأحمر ثم البنى ولكن فى مستخلصات الاسيتون كان البيروجالول اكثر تركيزا فى الطحلب الاخضر و حامض الكلوروجينك فى الطحلب الاحمر والروزوريسينول فى البنى. وعلى النقيض من ذلك كان حمض الساليسيلك والفيروليك اقل المركبات تركيزا فى الثلاث طحالب.