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Full Length Research Paper

Bioactivity of *Ulva lactuca* L. acetone extract on germination and growth of lettuce and tomato plants

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Ethanol, butanol and acetone extracts of *Ulva lactuca* L. (Chlorophyta) at concentrations 10, 30, 100 and 300 ppm for each were applied to determine their biological activity under laboratory conditions using etiolated wheat's coleoptile growth bioassay. Acetone extract was the most active one and was selected to investigate the allelopathic potential on lettuce and tomato's seed germination and seedling growth. Nine free phenolic compounds were detected in the Ulva acetone extract by using HPLC analysis. Vanillin and *p*- coumaric acid was recorded as the abundant compounds while ferulic acid and salicylic acid existed in less amounts. Compared with the control, the germination of lettuce and tomato were stimulated by 5.8 and 21.5%, respectively, when treated with low concentration of acetone (30 ppm). In contrast, high concentration (300 ppm) retarded the germination and recorded high inhibition effect on lettuce (44.6%). Acetone extract, at 30 ppm, stimulated the radicle and plumule growth of lettuce by 10.6 and 27%, respectively, and tomato by 22.5 and 35%, respectively. Meanwhile, at 300 ppm, radicle and plumule growth of lettuce was greatly inhibited by 40.6 and 30%, respectively, whereas tomato was slightly inhibited. Moreover, roots appeared more sensitive to allelopathic effect than shoots.

Key words: *Ulva lactuca*, allelopathy, phenolic compounds, wheat coleoptile, lettuce, tomato.

INTRODUCTION

Marine algae are bloom forming over coastal graces due to their physiological characteristics, such as their rapid nutrient uptake, high growth rate and broad environmental tolerance (Smayda, 1997; Taylor et al., 2001). Some marine macro-algae species have caused disastrous damage to fisheries, marine culture and are dangerous to human health (Horner et al., 1997; Huo et al., 2001). Some of these species have negatively allelopathic actions on the growth of the other organisms by the production and releasing secondary metabolites (Graneli and Hansen, 2006).

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). In many studies, crude-extracts of entire algal material were directly screened for antimicrobial activities with a small number of laboratory-cultivable bacteria, diatom and fungal isolates (Hellio et al., 2001, 2002; Engel et al., 2006; Paul et al., 2006; Bushmann and Ailstock, 2006; Chinnadurai et al., 2008).

Allelopathy is defined by Evenari (1961) as the influence of one plant on another by displacement under natural conditions and exerted by means of chemical rather than nutritional agents. Plant phenolic compounds are among the major allelochemicals implicated in allelopathy (Inderjit and Mallik, 2002). A series of polyphenolic compounds, flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae (Santoso et al., 2002, Yoshie et al., 2000; Yoshie-Stark et al., 2003). Some free phenolic compounds as 4-hydroxyphenylacetic and 4-hydroxybenzoic acid were identified in *U. lactuca* (Flodin and Whitfeld 1999).

Johnson and Welsh (1985) found that extracts prepared from fresh *U. lactuca* L. inhibited the development of crab larvae. *U. lactuca* is a common species of macroalgae found in green tides (Valiela et al., 1997; Hernandez et al., 1997; Bhang and Kim, 2000). Many studies have revealed that *U. pertusa* and *U. linza* exhibits negative allelopathic effects on microalgae (Jin and Dong, 2003; Nan et al., 2004; Jin et al., 2005). Correspondingly, Nan et al. (2008) also proved that the allelopathic effects of *U. lactuca* on the red tide microalgae and

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presented evidence for the potential feasibility of utilization of bloom-forming green macroalgae against red tide microalgae.

This study aimed to investigate the allelopathic potential of most active organic extract of *U. lactuca* on germination and growth of selected agricultural crops (lettuce and tomato), to perform the identification and quantification of free phenolic compounds in the most active solvent extract by HPLC amalysis.

MATERIALS AND METHODS

Collection of algal material, sample preparation and extraction

The green sea weed *U. lactuca* were collected in April, 2007 during low tide at Red Sea beach of Al-Quser province at latitude (26° 07` N) and (34° 13` E), Egypt. The collected sea weed was washed succes-sively with tap water, distilled water and air dried under shade for 2 weeks. The dried algal material was ground to 2 mm or smaller par-ticle size. The powder (100 g) was extracted successively with ace-tone, butanol and ethanol using Soxhlet apparatus. After 6 h of ex-traction the solvents were evaporated from crude extract by rotary evaporator to yield 3.15, 4.14 and 5.1 g, respectively (Solomon et al., 2005). After a preliminary test for the three extracts using etio-lated wheat coleoptiles bioassay, the obtained data indicated that acetone had a good stimulatory effect at low concentrations com-pared with the other two solvent extracts. So acetone extract was used for the bioassay of lettuce and tomato's seed germination and growth.

Bioassay for growth of wheat coleoptiles

Fifty wheat grains (*Triticum aestivum* L. cv. Sides 1) (five replicates with a total of 250 grains) were sown in Petri dishes and incubated darkly at 22 ± 1°C for 4 days (Hancock et al., 1964). The apical 2 mm of the shoots were cut off and discarded and then the next 4 mm of the coleoptiles were taken under a green safelight for bioassay. Four different concentrations of each solvent extract were diluted in a phosphate–citrate buffer solution containing 2% sucrose at pH 5.6 (Nitsch and Nitsch, 1956) to prepare 10, 30, 100 and 300 ppm (the experimental solutions). Five wheat coleoptiles were placed in test tube containing 4 ml of the experimental solution (three replicates of each experiment) and control tubes were filled with 4 ml of the buffer solution. All test tubes rotated slowly for 24 h at 22 ± 1°C in dark condition. The coleoptiles lengths were measured and the data expressed as percentage of differences as compared with the control in order to see whether the extracts had an additive or negative effect on the growth.

Bioassay for germination and growth of lettuce and tomato

The algal acetone extract was dissolved in very small amount of DMSO and then diluted with MES buffer (10–2M 2-[Nmorpholino] ethanesulphonic acid) and addition of NaOH 1M solution to adjust pH at 6.0 to prepare the experimental solutions (10, 30, 100 and 300 ppm). One hundred of lettuce seeds (*Lactuca sativa* L. Giza113, Egypt) and tomato seeds (*Lycopersicon esculentum*), were placed on Petri dishes (three replicates of each experiment) fitted with two sheets of Whatman no.1 filter paper. Then 10 ml of each solution was added to the corresponding dish and control dishes were watered with MES buffer containing 1% DMSO. The dishes were put into the culture chamber with suitable temperature (25 ± 1°C). Germination rate, radicle length and plumule length were recorded after ten days.

HPLC analysis

Free phenolic compounds in acetone extract 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 determinations (USA). The extract was evaporated under reduced pressure by rotary evaporator at 45°C and the residue was dissolved in HPLC grade MeOH to give 1000 ppm then 20 µl of methanol dissolved sample was injected into HPLC column. The high purity twenty standard phenolic compounds were used for HPLC analysis.

Statistical analysis

The experimental design was completely randomized with three replications. The results of bioassay experiments with one-way analysis of variance and the mean values were separated at $P< 0.01$ and $P<0.05$. The statistical analysis was done using the SPSS® / PC computer software package ver. 11.1 (2001).

RESULTS

Bioassay for growth of wheat coleoptiles

Bioassay results of the different *U. lactuca* extracts on etiolated wheat coleoptiles growth is represented in Figure 1. Acetone solutions recorded the highest activity level with stimulation values near to 5% at 10 ppm as compared with the control. Wheat coleoptiles were stimulated slightly when treated with 30 ppm of acetone extract near to 1%, meanwhile ethanol and butanol extracts (30 ppm) inhibited growth by about 9 and 22%, respectively. The obtained data indicated that acetone extract had the most stimulatory effect in comparing with butanol and ethanol extracts. So it was chosen to investigate his potential on the germination and growth of lettuce and tomato seedlings.

Bioassay for germination and growth of lettuce and tomato

Low concentrations of acetone extract (10 and 30 ppm) had significant stimulation effects on both tomato and lettuce germination and growth, while 100 ppm enhanced tomato growth, but suppressed those of lettuce. The most stimulatory dose (30 ppm) enhanced germination percentage, radicle length and plumule length of tomato by values 5.8 (Figure 2), 10.6 (Figure 4) and 27% (Figure 4), respectively. Similarly, it enhanced the germination and growth of lettuce but with low rate (Figures 2 and 3). Acetone extract solutions at 100 and 300 ppm had significant inhibitory effect on the germination and growth parameters, where, the germination percentage declined progressively for higher concentration solution (300 ppm) to reach 44.6 and 16.21% for lettuce and tomato respectively. The results indicated that radicles of both target plants were more sensitive to allelochemicals comparing

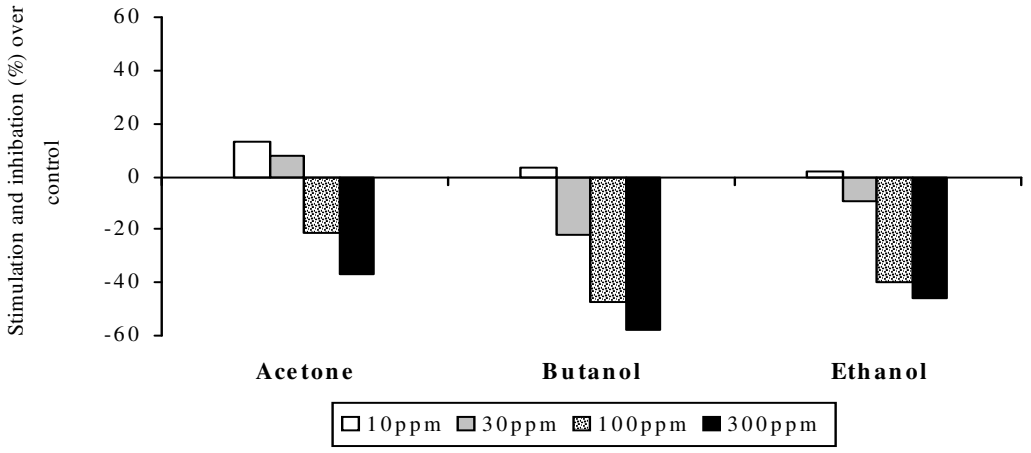

Figure 1. Effect of *Ulva* extracts at different concentrations (ppm) on the wheat's coleoptile growth (% control). Values are the mean of three replicates.

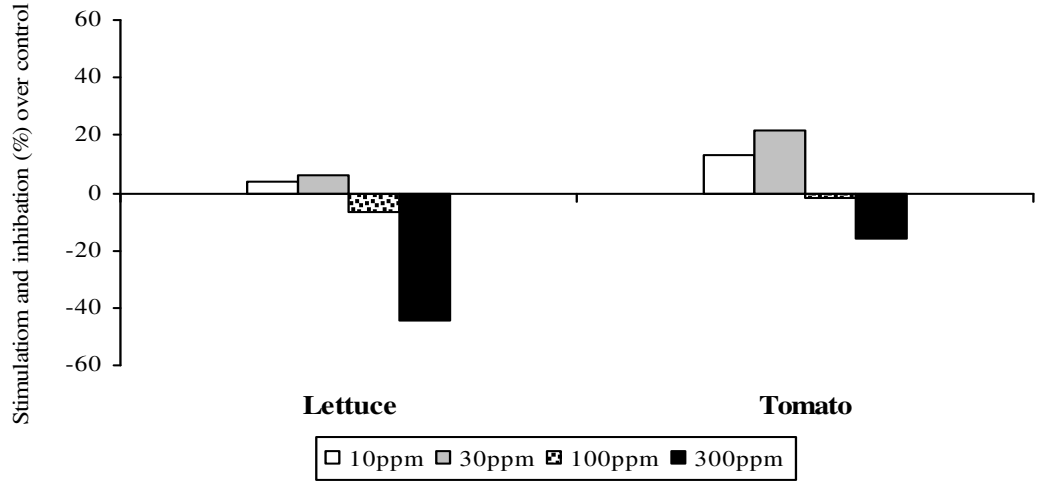

Figure 2. Effect of *U. lactuca* L. acetone extract at different concentrations (ppm) on the lettuce and tomato germination percentage as compared with the control Values are the mean of three replicates.

with plumules and the degree of inhibition increased with increasing extract concentration.

Free phenolic compounds analysis

Nine free phenolic compounds were identified by the reverse-phase (HPLC) high performance liquid chromatography (Table 1). These compounds identification was based on comparison of their relative retention time with those obtained from the different standard compounds. Vanillin and p-coumaric acid constituted the major components content which recorded about 27.6 and 21%, respectively. Whereas, ferulic and salicylic acids were detected at relative low percentages of 2.06 and 3%, respectively.

DISCUSSION

Allelopathy has been studied in aquatic habitats (marine and freshwater) and that all primary producing organisms (cyanobacteria, micro- and macro-algae as well as angiosperms) are capable of producing and releasing allelopathically active compounds (Gopal and Goel, 1993; Korner and Nicklisch, 2002; Gross et al., 2003). The production and excretion of allelochemicals by aquatic macroalgae could be an effective defense strategy against other phototrophic organisms competing for light and nutrients, like epiphyton and phytoplankton (Jeong et al., 2000; van Donk and Van de Bunk, 2002; Mulderij et al., 2005). It was also observed that marine organisms collected from the Southeast coast of India have been shown to possess a number of biological activities (Ely et

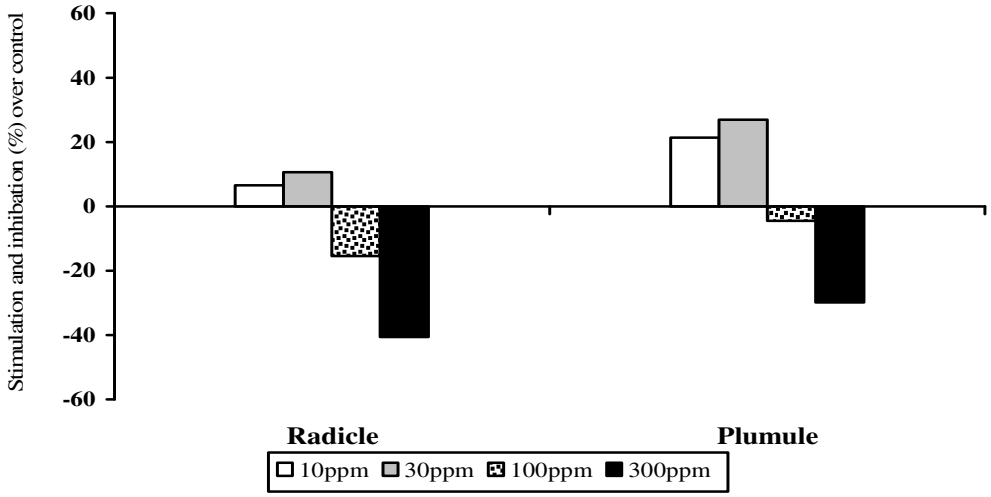

Figure 3. Effect of *Ulva* extracts at different concentrations (ppm) on the radicle and plumule length of lettuce (% control). Values are the mean of three replicates.

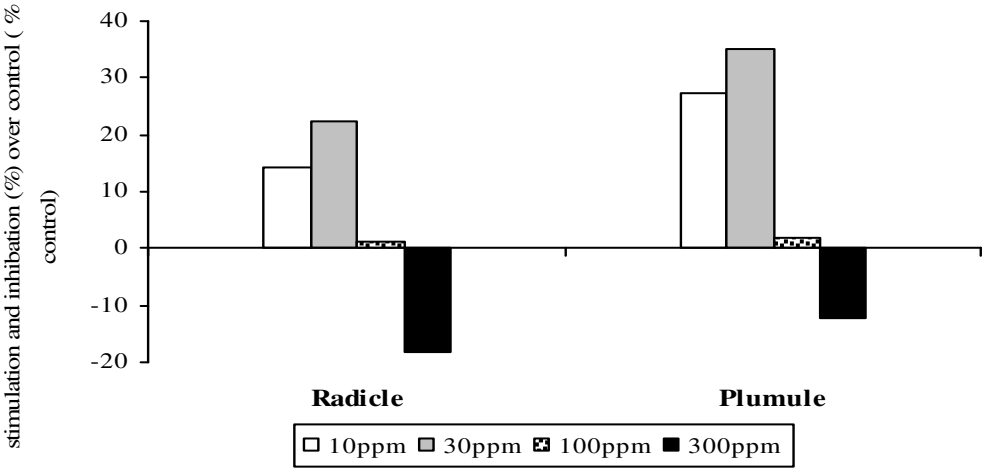

Figure 4. Effect of *Ulva* extracts at different concentrations (ppm) on the radicle and plumule length of tomato (% control). Values are the mean of three replicates.

al., 2004).

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; Engel et al., 2006). Other studies indicated that, Macroalgae suppressed phytoplankton growth through the excretion of chemical substances (Elakovich and Wooten, 1995; Gross, 1999; Gross et al., 2003). Different organic solvents extracts of our species *U. lactuca* have different allelopathic effects and that agreed with previous works which shown that the yields of secondary metabolites can be hanged by extraction solvent (Carlson et al., 1989; Muzika et al., 1990; Cork and Krockenberger, 1991), drying procedure (Lindroth and Pajutee, 1987; Cork and Krockenberger, 1991) and duration of extraction (Lindroth and Pajutee, 1987; Zobel and Brown, 1988).

The obtained data indicated that acetone extract had stimulatory effect on the germination and growth of lettuce and tomato and such a stimulatory effect could be attributed to the possible synergistic effect between the tested phenols as mentioned by Gerig and Blum (1991). Such a stimulatory effect comes in agreement with that reported from Reigosa et al., (1999) and Hegab (2005) who reported that, free phenolic compounds (such as, vanillin) stimulated the germination and seedling growth of different plants. Also, Monerri et al. (1986) indicated the increasing of amylase activity during germination of the seeds treated with the extracts contained similar phenolic compounds.

Table 1. HPLC analysis of free phenolic compounds *in U. lactuca* Lacetone extract.

Standard phenolic compounds	Retention Time (min.)		Concentration (µg g^{-1} Dry weight)
	Standard	Sample	
Caffeic acid	19.310	19.01	108.2
Chlorogenic acid	15.931	15.85	54.32
Ferulic acid	24.551	24.32	18.52
p- coumaric acid	21.208	21.46	186.52
Protocatechuic acid	14.019	13.76	111.83
Pyrogallic acid	9.698	9.35	88.90
Resorcinol	13.739	13.36	54.25
Salicylic acid	31.109	30.80	26.98
Vanillin	22.411	22.30	247.52
Total concentration			891.04

In our experiment, plumule length was stimulated at low concentrations and the same results were reported by Hegab (2005) who stated that, vanillin as well as gallic, p-coumaric, p-hydroxybenzoic, and ferulic acids showed stimulatory effects on plumule length of soybean seedlings. Vanillin and p-coumaric acid were found to exist in high contents and they could play an important role in the growth enhancement through their stimulation for metabolic processes as the biosynthesis of both nucleic acids (Baziramakenga et al., 1997), and chlorophyll (Yang et al., 2004) and increasing the antioxidant activities (Shahrzad and Bitsch, 1996; Floridi et al., 2003).

On the other hand, phenolic acids of acetone extract inhibited the germination of lettuce and tomato 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), who reported that many alterations in root growth of different plant species treated with phenolic acids (p-hydroxybenzoic and p-coumaric acids). Using the same phenolic compounds, other results reported a significant reduction in nitrogen content of 3-week old tomato leaves (Mersie and Singh, 1988) as well as a reduction in the fresh biomass of the aerial part of soybean seedlings (Mitiku, 1991).

The disturbance in the tested plants growth at high concentrations of phenol extracts may be due to the interfere of phenols, as ferulic acid, with many metabolic activities, such as photosynthesis and protein synthesis (Mersie and Singh, 1993) and growth hormone synthesis (Devi and Prasad, 1992). Furthermore, it was reported that the phenolic acids suppress absorption of phosphate, potassium, nitrate and magnesium ions, as well as, overall changes in tissue (Alsaadawi et al., 1986).

In our study, roots appeared more sensitive to the allelopathic extracts than shoots. The stronger inhibitory effects of *U. lactuca* extracts had on roots might have been caused by the fact that roots were in direct contact with the extract and subsequently with inhibitory chemicals as described in earlier works with various crops and weeds (Bhowmik and Doll, 1984; Qasem, 1995). The inhibitory effect was a function of the extract concentration value and consequently greatest inhibition observed under the application by high concentration value (van Aller 1985; Batish et al., 2007).

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