In vitro effect of *Ulva rigida* extract on the growth of *Lepidium sativum* and *Allium cepa*

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Abstract

The extract of the green macroalgae *Ulva rigida* C. Agardh (*Chlorophyta*), which is collected from the Azla coast, was investigated *in vitro* to test its effect on the growth of garden cress (*Lepidium sativum* L.) and onion (*Allium cepa* L.). Effects of four aqueous concentrations of the seaweed (0.5, 1, 2 and 4 mg of dried seaweeds per ml of distilled water) were tested on the growth of seedling roots and stems of 1488 plants of garden cress and onion during two weeks. The comparison tests were carried out with an agar medium and Murashige and Skoog mineral salt medium (1962). The results indicated that extract of *U. rigida* is suitable for plants development.

Keywords: *Ulva rigida* extract, Azla coast (North of Morocco), *in vitro* growth, roots, stems, *Lepidium sativum, Allium cepa*

Introduction

The marine environment is an exceptional reservoir of bioactive compounds produced by animal or plant species. Many of which exhibit structural and chemical features not found in terrestrial natural products.

Algae are organisms that resulted from photosynthesis process. There are two major categories of algae namely Macroalgae and Microalgae. They grow in an aquatic environment, and contain secondary metabolites which are synthesized at the end of the growth phase and/or due to metabolic alterations induced by environmental stress conditions (Shalaby, 2011). Some of these compounds such as sulfated polysaccharides, novel amino acids and mineral compounds, growth hormones and colloidal elements are used in a variety of applications in agri- and horticulture (Anisimov *et al.,* 2013; Alves *et al.,* 2013), pharmaceutical industry (Pujol *et al.,* 2007; Khanavi *et al*., 2012), and recently for production of biofuel (Woertz, 2007; Eshaq *et al.,* 2010).

The beneficial impacts of seaweed or macroalgae products on the cultured plants are well cited. They improve seeds germination, seedlings development, increase plant tolerance to abiotic stresses (Jolivet *et al.,* 1991; Zhang and Ervin, 2004; 2008), and enhance plant growth and yield (Hong and Hien, 2007; Kumari *et al.,* 2011). Moreover, seaweeds are used as soil amendment (Gandhiyappan and Perumal, 2001) and also used for properties that reduce the growth of plant pathogens or decrease the injury severity of plant foliar tissues following pathogen infection (Jayaraj *et al.,* 2008; Jimenéz *et al.,* 2011).

Macroalgae are a type of multicellular plants that grow in saltwater medium. It can grow rapidly until it reaches 60 meters in length (Demirbas and Demirbas, 2010). Macroalgae are classified into three groups based on their pigments. They are Brown Algae (*Phaeophyceae*), Red Algae (*Rhodophyceae*), and Green Algae (*Chlorophyceae*). *Ulva rigida* belongs to the macroalgae family and it is widespread locally and dominant at the Mediterranean areas. Indeed, *U. rigida* is common and abundant at mid and low shore levels, and their abundance and easy access can guarantee their quantity for further biotechnological exploitation in the future.

Ulva species are an important source of ulvan, a natural sulfated polysaccharide. The latter has been extensively investigated as a novel drug and functional foods (Toskas *et al.,* 2011), and for the search of new plant biostimulants.

Recently, the beneficial effect of the ultra-low doses of bioactive compounds on various types of organisms particularly on the growth of seedlings of agricultural plants was reported (Anisimov *et al.,* 2012).

The study reported herein aimed to exploit regional macroalgae potential in agri- and horticulture because they are not used extensively in Morocco. Therefore, current research was undertaken to investigate and identify the stimulatory effects of the solution extract of *Ulva rigida* on germination and early seedling growth of two test plant species under control laboratory conditions.

Materials and methods

Seaweeds collection

The green algae (*Ulva rigida* C. Agardh) was collected in an intertidal rocky shore in Azla (Tétouan, Mediterranean Sea, Northern Morocco- 35° 32.2 N and 05° 14.7 W) at depth 0.5-1 meter during July and August 2013.

Algae were washed with seawater and brushed extensively to remove macroscopic epiphytes and sand particles. The resulting clean algae were washed with tap water to remove adhering salt. Samples were air-dried at 25°C up to four days followed by thermostat dry at 60°C for 12 hours.

Characterization of the algae extract

The nitrogen content was measured by the Kjeldahl method (Seal Analytical, 2008). Mineral matter and total protein were measured by both Analytical Chemists method (AOAC, 1990) and Bradford method (1976).

Preparation of seaweed liquid extracts (SLE)

Dried seaweeds were hand crushed and powdered with coffee-grinder. Algae were heated with boiled distilled water in a ratio of 10:100 (w/v) for 2 min into a water bath. Then the extracts were filtered through a filter paper and sterile Millipore (0.45 µm). The resulting extract was stored at 4°C for further experimental studies. Different concentrations were prepared (0.5, 1, 2 and 4 mg of dried seaweeds per ml of distillated water).

Garden cress and onion assay

Commercial seeds of garden cress *(Lepidium sativum* L.*)* and onion *(Allium cepa* L.*)* variety Valenciana (semillas Fito) were used in this study.

The seeds were immersed in 0.01% mercuric chloride with drops of Tween for 30 min and washed three times by distilled sterile water in a laminar air-flow cabinet. In all experiments, two basic mediums were tested: salts of Murashige & Skoog (1962) medium (MS) and distilled water (MG) solidified both with 7.0 $g.l^{-1}$ (BIOKAR, Agar type E) at pH 5.7. To these two basic mediums, various concentrations of extracts were added. Seeds were maintained in a growth room at 26±2°C and 2,000 lux during 16 h light provided by white fluorescent lamps (Philips TL MRS 40 W/54-765) / 8 h.

For all plantlets from *in vitro* germinated seeds cultured into plastic Petri dish, 40 at 60 seeds were tested for each assay (15 seeds per dish).

Following four weeks of culture, the significant development of *Lepidium sativum* and *Allium cepa* was indicated by the growth of roots and stems. It was visible by the brightness of the green color, with very long roots attached to the Petri. The length of the main root and stem of the plantlet was measured 10 days later.

Data were analyzed using IBM SPSS Statistics 20.0. Student t-test was used for the analysis of significant differences of root length between control groups. P-values less than 0.05 were considered statistically significant.

Results and discussion

Chemical characteristics of algae extract

The chemical characteristics of the *U. rigida* dry matter were summarized in Table 1. The protein total content of the dry matter and the algae extract is of 7.4 % and 5.13 %, respectively.

Table 1. Chemical properties of *Ulva rigida* dry matter and extract

Property	% on dry matter
Mineral matter	28.76
Total protein	7.4*
N (total nitrogen)	1.53
Total protein in extract	(5.13)

*The measured protein rate varies according to the various seasons of the year.

A total protein content of 5.13% for *U. rigida* extract indicated that the method used to breakdown the algae extract resulted in incomplete digestion. Temperature and reaction time have been found to be critical parameters in optimizing the autolysis properties of *Chlamydomonas* (Kightlinger *et al.*, 2014) and yeast (Tanguler and Erten, 2009).

Several methods are used for the preparation of the algae aqueous extracts (Alves *et al.*, 2013; Anisimov *et al.*, 2013; Kightlinger *et al.*, 2014). Further studies are therefore needed to determine the optimum digestion parameters for algae in order to maximize the nutritional content and yield of algae extract. Besides changing the temperature and/or reaction time, another option is to treat the algae with exogenous hydrolytic enzymes.

Effects of algae extract on the growth of roots and stems

The Petri dishes and the extracts were prepared as described above. For all the experiments, only the seeds germinated on medium MG0 and then transferred on the medium containing the four extracts. 1,489 plants were tested.

The resulting data showed that the four extract of *U. rigida* (0.5, 1, 2 and 4 mg of dried seaweeds per ml of distillated water) did not reduce germination potential and plants development of *L. sativum* and *A. cepa* (Figures 1 and 2).

The germination and the development of roots and stems of *L. sativum* and *A. cepa* were observed using all tested mediums in presence of *Ulva rigida* extracts. The plants continued to develop beyond four weeks. This result showed that extracts of *Ulva rigida* did not cause any phytotoxic activity.

The extracts of all examined seaweeds significantly affected growth of seedling roots and stems of *L. sativum* and *A. cepa*. The highest stimulatory effect of *U. rigida* extract was observed by using the concentration of 1 mg/ml on both mediums agar and MS, whereas the concentration of 4 mg/ml showed lower stimulation of roots and stems growth (Figures 1 and 2).

The dose-effect curves of most extracts were bimodal on agar and MS mediums. Statistical analysis showed that the concentration of 4 mg/ml had a significant inhibitory effect on the growth of the stems and roots of *L. sativum*.

The beneficial effect of the use of natural molecules in sterile conditions, *in vitro*, was shown by Macías *et al.*, (2005) and El Mtili *et al.*, (2009). The latter authors reported that the release of secondary metabolites and their assimilation was much more important in sterile conditions.

To know whether the extract products were mineral or organic, a comparison study using MS and agar mediums was performed. Generally, two strategies were used to define the balance between the organic elements and minerals

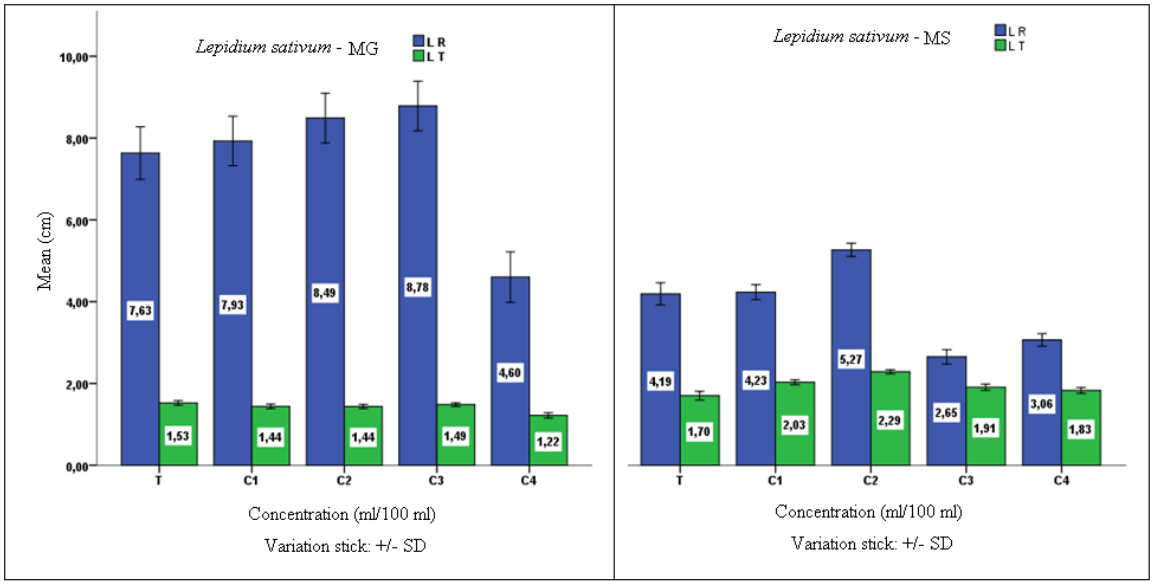

Figure 1. *In vitro* effect of *U. rigida* extract on the growth of *L. sativum.* Mean values of stems and roots length under four different concentrations of seaweed liquid extracts (C1: 0.5, C2: 1, C3: 2 and C4: 4 mg of dried seaweeds per ml of distillated water). LR: Length of roots, LT: Length of stems. MG: distilled water solidified with7.0 g/l agar, MS: salts of Murashige & Skoog (1962).

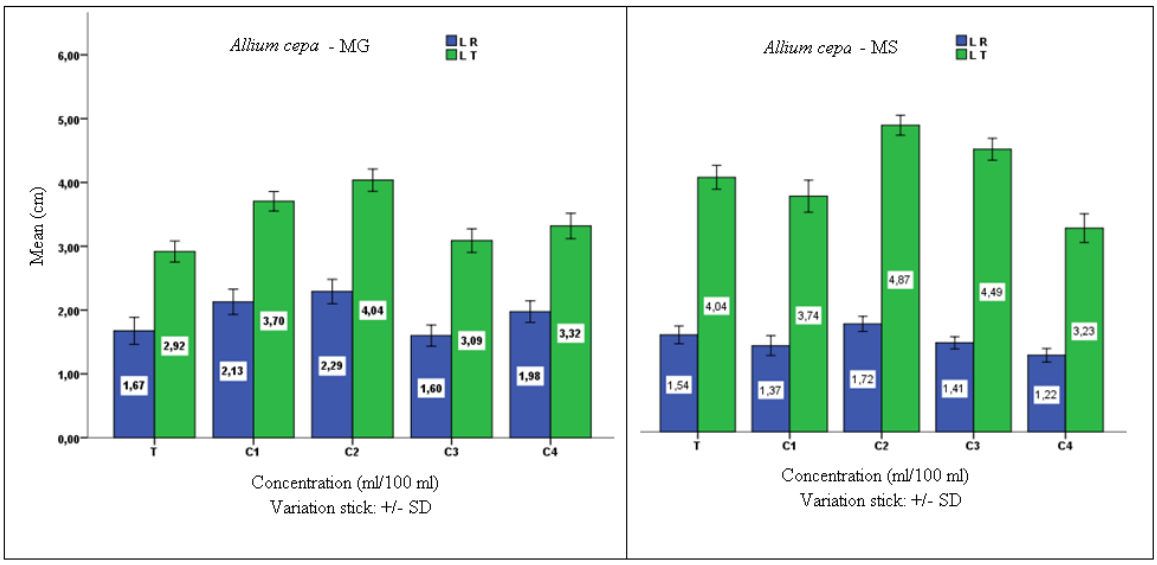

Figure 2. *In vitro* effect of *U. rigida* extract on the growth of *A. cepa*. Mean values of stems and roots length under four different concentrations of seaweed liquid extracts (C1: 0.5, C2: 1, C3: 2 and C4: 4 mg of dried seaweeds per ml of distillated water). LR: Length of roots, LT: Length of stems. MG: distilled water solidified with7.0 g/l agar, MS: salts of Murashige & Skoog (1962).

to use for optimal growth of explants. One of the well known examples was the work reported by Murashige and Skoog (1962). They established an optimal nutrient composition of the culture medium for rapid growth with tobacco callus cultures. Another strategy did adapt the mineral content of the medium according to an elemental analysis of healthy explants (El Badaoui *et al.*, 1996).

Effect of seaweed extracts at different ratio on root and stem lengths depended on basal medium tested. On agar medium, the effect of extracts on growth of the two species tested was more visible

comparatively to MS medium (Figures 1 and 2).

The *U. rigida* extract enhanced the growth of the roots of *L. sativum* and the stems of the *A. cepa* in the case of the two basic mediums tested. In our case, the impact of this extract could be allotted to organic effects and not to minerals. Analyses are necessary to determine the chemical nature of these compounds.

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