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Original article

Effect of Some Seaweed Extracts on Germination and Growth of Barley (*Hordeum Vulgare L*)

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ABSTRACT

Aims*. The present investigation aims to study the algae-based biofertilizers' effect of different concentrations (0.1, 0.5 and 1%) of Cystoseira barbata (CBAE) and Laurencia papillosa(LPAE).* ***Methods****. Aqueous Extracts of both donor species on seed germination and some growth physiological parameters (germination percentage (GP), seed germination index (SGI), energy of germination (GE) and plumule and radicle (PL and RL) lengths, seedling fresh and dry weight, of Hordeum vulgarea (crop species) and photosynthetic pigments,* ***Results.*** *In general, the measured all growth parameters, there was a significant increase in (GP, SGI, GE) under effect of both donor species as well as in (PL, RL) lengths, fresh and dry weight.Furthermore, the increase in total photosynthetic pigment contents of H. vulgarea seedlings upon treatment with (CBAE, LPAE) could be attributed to the increase in both Chl.a and Chl.b.* ***Conclusion.*** *The species with the strong fertilizer potential of low concentrations, Cystoseira barbata and Laurencia papillosa must be examined for its selective action on other field conditions.*

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INTRODUCTION

Food security is a critical issue in the current global scenario. The most critical key factors preventing the current demand for sustainable food supply are the exponential increase in global human population, depletion of cultivable agricultural landscape, and massive depletion of natural resources. Implementing algae-based biofertilizers to control food supply is one of the most sustainable and green revolutionary approaches. The seaweed extracts contain plant growth hormones, regulators, promoters, carbohydrates, amino acids, antibiotics, auxins, gibberellins and vitamins which enhance the yield and quality which are induce the yield of crops, seed germination, resistance to frost, fungal and insect attacks [1]. An adequate amount of potassium, nitrogen, growth promoting hormones, micronutrients, humic acid etc. present in seaweeds make it an excellent fertilizer. Fertilizers derived from seaweeds (*Fucus*, *Laminaria*, *Ascophyllum* and *Sargassum*) are biodegradable, nontoxic, nonpolluting and non-hazardous to human, animals and birds. Seaweed manure besides increasing the soil fertility increases the moisture holding capacity and supplies adequate trace elements thereby improving the soil structure [2]. On the other hand, chemical fertilizers have degraded the fertility of the soil by making it acidic, rendering it unsuitable for raising crops they are not environmentally friendly.

Algal biofertilizers can replace traditional chemical biofertilizers. To that end, the aforementioned naive characteristics of algae, economic feasibility, technical acceptability, and environmental benefits denote algal biomass as a most promising and in-demand bioresource for sustainable green agricultural [3]. Barley is mainly used in most countries of the world as animal feed or as a feed from green or its grains in a mixture of concentrated rations, and it is also used as a narrow band in human nutrition, especially in developing countries, by mixing flour with flour wheat in making bread [4]. Barley is considered the main cereal crop in Libya. The volume of production of barley increased from 71 thousand tons in 1980 to 264 thousand tons in 2000 and 299 tons in 2003, then declined to 260 thousand tons in 2005, productivity per hectare has increased from 0.253 tons per hectare in 1980 to 1.3 tons per hectare in 2005. Barley is most commonly used in the rural areas, and it is traditionally consumed by the Libyan people on a regular basis, where wheat is less readily available for

bread making but it is also used, less regularly, by the urban population and is always a feature of meals on special occasions [5]. As a result, this study concentrated on the progress made in algae-based biofertilizers on the germination and growth of the barley plant.

METHODS

Study settings

The study work was achieved during year 2021-2022. Two species of algae *Cystoseira barbata; C. Agardh.* (Sargassaceae), *Laurencia papillosa; J.V. Lamouroux* (Rhodomelaceae) (Donor species) were used in this investigation to study their biological activities on seed germination, some growth physiological parameters on *Hordeum vulgare* cv. alhsad 176(Poaceae) as (crop species).

Sample macro algae collection and preparation

Manual harvesting of seaweed has been practiced for centuries and it is still common for species naturally growing in coastal areas [6].

Macro algae samples were collected *Cystoseira barbata*; C. Agardh (Phaeophyta, Fam. Sargassaceae), *Laurencia papillosa*; J.V. Lamouroux, (Rhodophyta, Fam. Rhodomelaceae), from Al-Hamama coast located in the northeastern Mediterranean coast of north of the city of Al-Bayda city, Libya, during June 2021. All samples were brought to the laboratory in plastic bags containing seawater to prevent evaporation. In laboratory, seaweeds were cleaned from epiphytes and rock debris and given a quick fresh water rinse to remove surface salts. Seaweeds were then air dried in the shade at room temperature (25–30 °C) on absorbent paper for estimation of moisture content. Then, they were pulverized in a cereal grinder for 5 min and sieved, using a 100 mesh sieve, to obtain a fine and homogeneous powder that was stored in hermetic sealed plastic bags and stored at –20 °C until for further chemical analysis. All seaweeds were identified taxonomically following the methods of [7-10]. The names of the species were used according to Guiry, [11] and were confirmed using algae base website. The collected samples were identified in Botany Department, Faculty of Science, Omar Al Mokhtar University.

Preparation of Donor Species Aqueous Extracts:

Dried powders of the two donor species (75 g for each) were extracted with 1000 ml distilled water. The extract was conducted in dark for 24 h at 25oC. The supernatant was taken and centrifuged at 3000rpm for 15 minutes; this would be full strength concentration (100%). The extracts were prepared no more than 48 h in advance and were kept in a refrigerator at 5^{o}C until used and the purified extract was adjusted to pH 6.8 with 1M HCl. Series of dilutions were prepared from the stock solutions (0.1, 0.5 and 1% besides the control) for *Cystoseira barbata* (CBAE) and *Laurencia papillosa* (LPAE) were tested for their effects on germination parameters, and *Hordeum vulgare* seeds were obtained from the Department of Plant Crops, College of Agriculture, Omar Al-Mukhtar University, Libya.

Germination Bioassay

Petri-dish experiment was applied to investigate the bio fertilizer of the donor species aqueous extracts on germination percentage (GP), seed germination index (SGI), energy of germination (GE) and plumule (PL) and radicle (RL) lengths of *Hordeum vulgare* (crop species).

To achieve this experiment, ten seeds were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 19-22^{o}C and night temperature from 12-14^{o}C. About 10 ml of the respective donor species aqueous extracts (0.1, 0.5 and 1%) or distilled water as control were added daily to two replicates in a randomized complete block design. Before sowing, the seeds were immersed in 2% Chlorex for 2 minutes then rinsed four times with distilled water. Finally, the seeds were soaked in aerated distilled water for 24 hours.

Germination percentages (GP) and plumule (PL) and radicle (RL) lengths were recorded after seven days.

Calculations

1. Germination percentage (GP) was calculated according to the following equation

$$GP = Ni / S*100$$

Ni= is the number of seeds germinated on day i

S= is the total number of seeds planted.Seed germination index (SGI) was calculated according to the following equation [12].

2. SGI= Σ Ti Ni/S
Where,
Ti= is the number of days after sowing
Ni= is the number of seeds germinated on day i
S= is the total number of seeds planted
3. Energy of germination (GE) was recorded according to [13] at the 4th day after sowing. It is the percentage of germinating seeds (GP) four days after sowing relative to the total number of seeds tested (TNST).
GE= GP (4th day)/TNST

Fresh and Dry Weight
The determination of seedling fresh weight. Other samples were dried at 65ºC till constant weight to determine the seedling dry weight.

Photosynthetic pigments
The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically according to Metzner [14]. A known fresh weight of leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged and the supernatant was made up to known volume with 85% acetone and measured against a blank of pure 85% aqueous acetone at 3 wavelengths of 452.5, 644 and 663 nm. Taking into consideration the dilution made, it was possible to determine the concentrations of the pigment fractions (chlorophyll a, chlorophyll b and carotenoids) as g / ml using the following equations:

Chlorophyll a = 10.3 E 663 - 0.918 E 644
Chlorophyll b = 19.7 E 644 - 3.87 E 663
Carotenoids = 4.2 E 452.5 - (0.0264 chlorophyll a + 0.426 chl. b)
The values were then expressed as (mg/g fresh wt.)

Statistical Analysis
Statistical analysis was performed using a computer run program (Minitab software). By ANOVA followed by Turkey's test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard Error Mean (SEM). P-value below 0.05 was considered to be statistically significant.

RESULTS
Germination parameters
Bioassays were carried out to test the fertilizer effects of *Cystoseira barbata* and *Laurencia papillosa* aqueous extract (CBAE and LPAE) on germination percentage (GP), seed germination index (SGI) and energy of germination (GE) of (*Hordeum vulgare* L. Family: Poaceae).
The germination percentage (GP) of *H. vulgare* seeds was effectively increasing with increasing the concentrations of CBAE (Figure 1). The percentage increased from 50% at the control to 57% at 1% concentration level after seven days from sowing. On the other hand, the percentage was greatly increased with increasing the concentrations of LPAE (Figure 1). It increased from 50% at the control to 83% at 1% concentration level after seven days from sowing. Data indicated that LPAE exerted a high significant effect on the germination of the seeds of the test species and the effect was prominent in LPAE compared to CBAE.

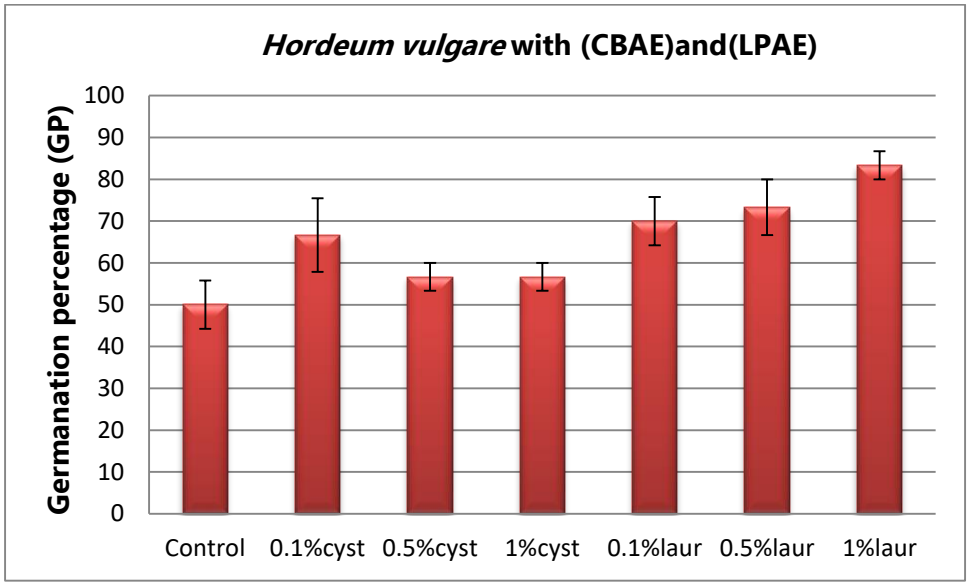


Figure 1. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on germination percentage of Hordeum vulgare.

Seed germination index (SGI) and Energy of germination (GE) of *H. volgare* are illustrated in figures 2, 3. With regard to SGI, the value significantly increased as CBAE concentration increased in samples. In control level, a value of about 3.5 was increased to 4.5 at 1% concentration level of extracts while in LPAE a value of about 3.5 was increased to 6.5 at 1% concentration level of extracts. Concerning GE, the value increased moderately as the extract concentration increased. GE started with a value of about 30 at control level which increased to 50 at 1% concentration level. In LPAE, GE started with a value of about 30 at control level which increased to 66.6 at 1% concentration level.

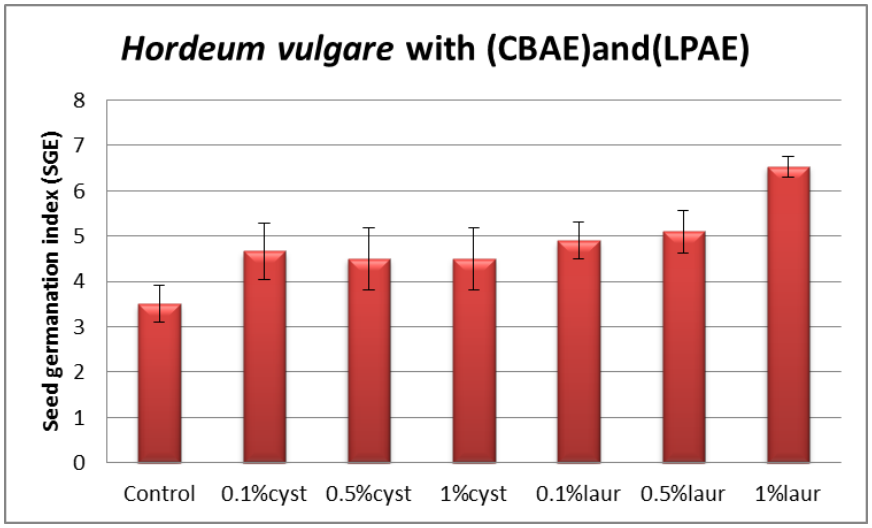


Figure 2. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on seed germination index of Hordeum vulgare.

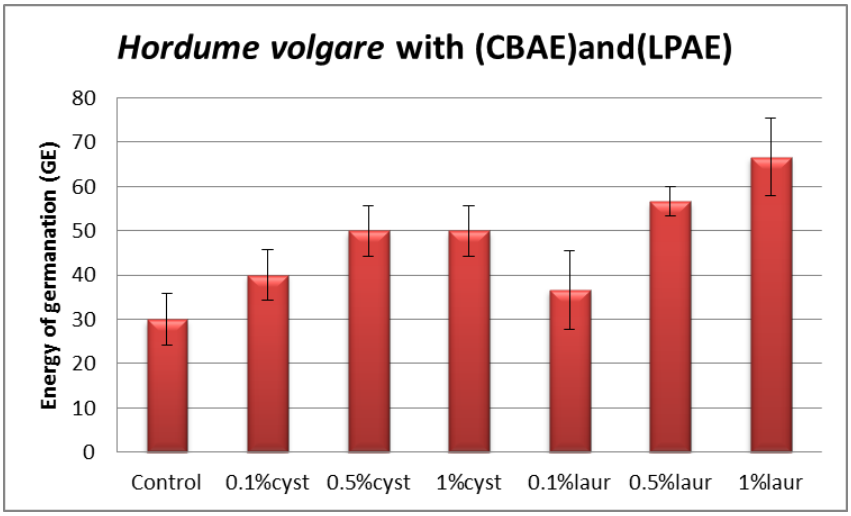


Figure3. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on energy of germination of Hordeum vulgare.

The fertilizers of the different concentrations of CBAE and LPAE on plumule and radicle lengths (PL), (RL) are presented in Figures 4 and 5 respectively. Generally, all concentrations of the applied extract increased PL. The length was visibly heightened about 101.82% and 115.45% at 1% concentration for CBAE and LPAE after seven days from the beginning of the experiment respectively (Figure 4).

While data demonstrated that in both extracts the RL increased significantly upon applying different concentrations of the extract. In CBAE the length increased about50% at 1% concentration level. Figure 5 confirmed the different effects of the extract on RL through the application LPAE increasing comparatively, in LPAE the length increased about 78% at 1% concentration level.

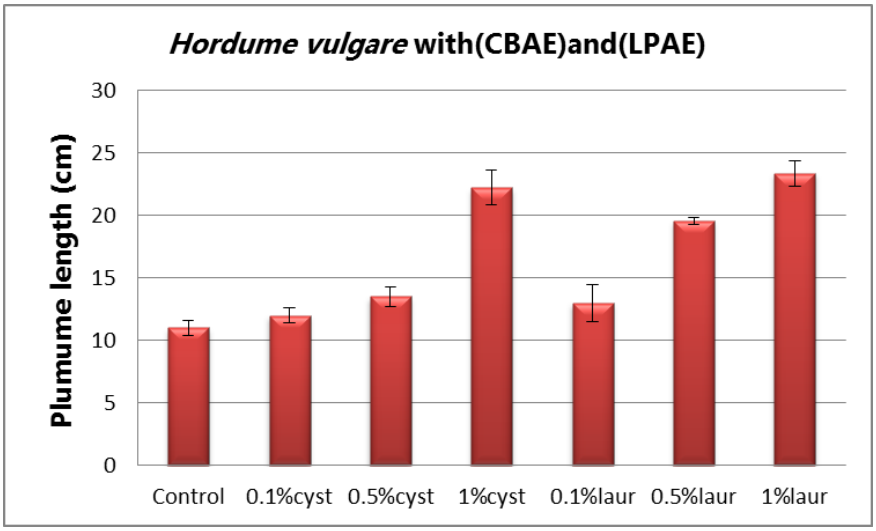


Figure 4. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on plumule length of Hordeum vulgare.

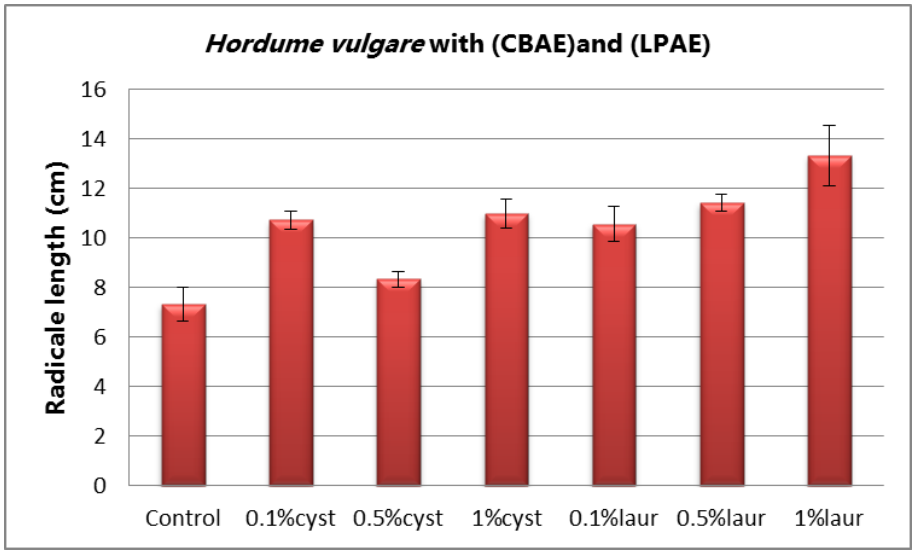


Figure 5. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on radicale length of Hordeum vulgare

Seedlings fresh and dry weight (g)

Generally, all concentrations of the applied extracts increased seedlings fresh weight. There was a steady increase in the fresh weight of *H. vulgare* seedlings with increase in the concentrations of CBAE. The fresh weight of the control seedlings grown was 0.63g increase to 0.78g in 0.5%. On the other hand, seedlings grown in LPAE had relatively higher fresh weights than those grown in CBAE with an increasing percentage of about 34.62% in the same concentration.

The fertilization effect of CBAE on seedling dry weight of *H. vulgare* was recorded. There was a slight difference in *H. vulgare* seedling dry weight of the control plants grown in 0.5% (0.17g) concentration. At control was (0.13g) in seedlings dry weight, the percent increase was 30.77% in seedlings grown in CBAE 0.5% and 130.77% in LPAE 1% concentration respectively.

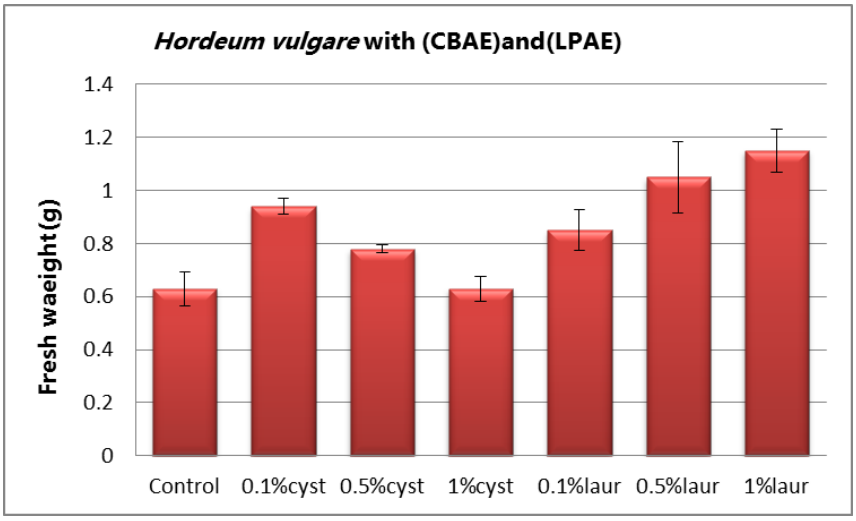


Figure 6. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on fresh weight of Hordeum vulgare

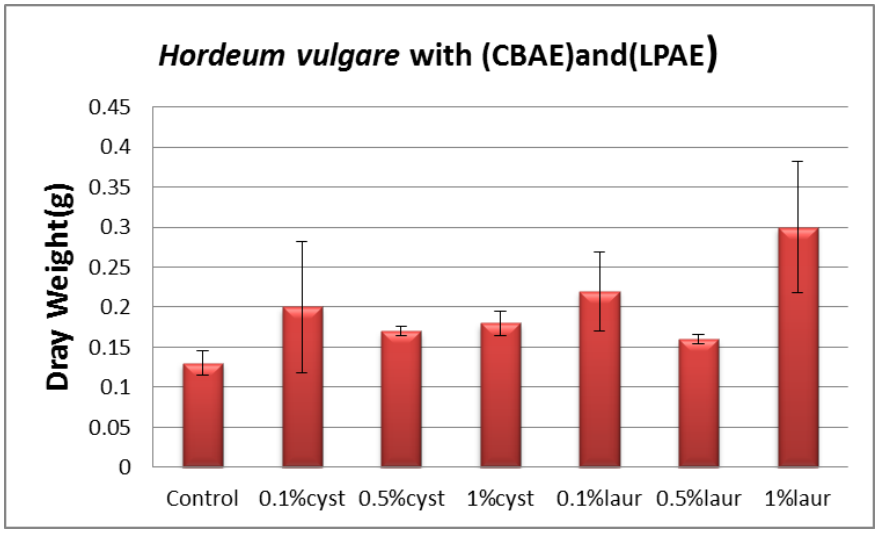


Figure 7. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on dry weight of Hordeum vulgare.

Variation in contents of the photosynthetic pigments

The total content of the photosynthetic pigments of the recipient species upon applying CBAE, LPAE in figure 8. The content in *H. vulgare* was increased to 147.47% with respect to 1% LPAE which may be ascribed to the increase in both Chl.a and Chl.b, the increasing percentage in Chl.a, it accounted to 52.61% ,35.97% in 0.1% CBAE, LPAE respectively compared to the corresponding control values figure 9. While the percent increase in Chl.b accounted to 55.31%,32.97% in 0.1% CBAE, LPAE respectively compared to the corresponding control values, figure10. The percent increase in carotenes, however, was less than that of chlorophylls A and B, it accounted to 41.40% ,35.49% in 0.1% CBAE, LPAE respectively compared to the corresponding control values figure11

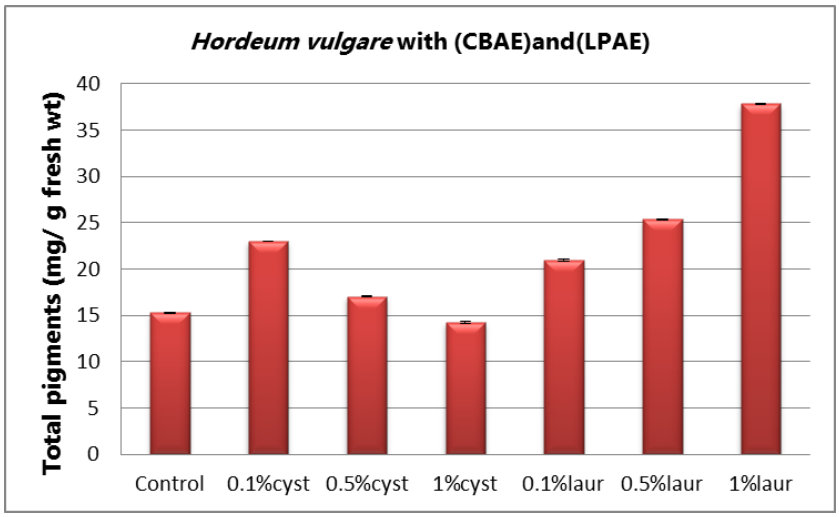


Figure 8. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on Total pigments of Hordeum vulgare.

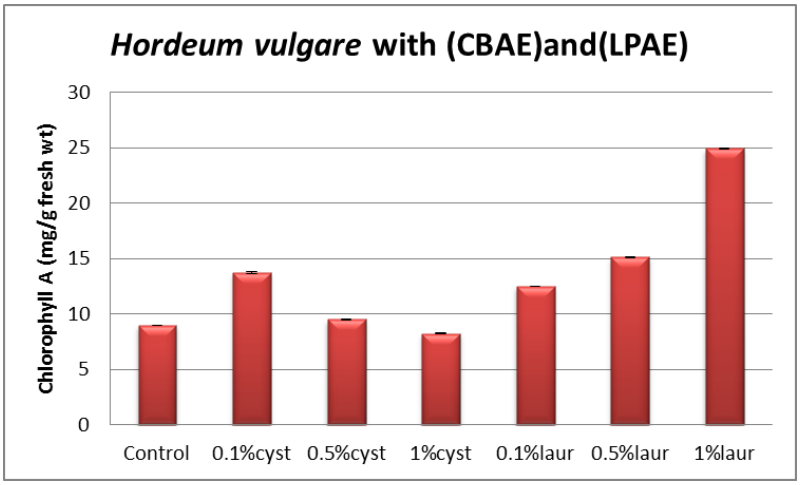


Figure 9. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on chlorophyll A of Hordeum vulgare.

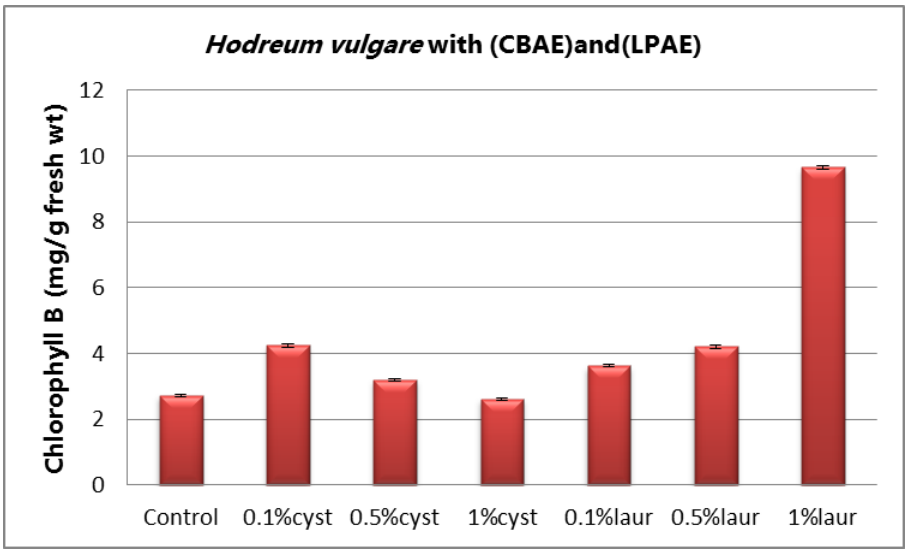


Figure 10. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on chlorophyll B of Hordeum vulgare.

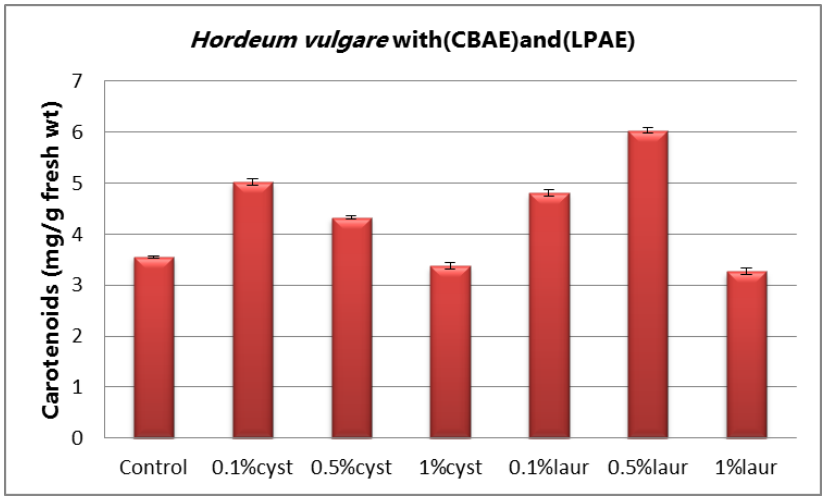


Figure 11. Fertilizer effect of different concentrations of Cystoseira barbata (CBAE) and Laurencia papillosa (LPAE) aqueous extract on Carotenoids of Hordeum vulgare.

DISCUSSION

Seaweeds are used in agriculture for ages and well acknowledged for their plant growth enhancing properties. Extracts of seaweed are rich in phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene, as well as amino acids, vitamins, betains, polyamines, carrageenans, polysaccharides and sterols which play significant role as a biostimulant, elicitor for abiotic stress and increase the productivity of plants. Besides being stimulant for growth, seaweed saps are known for their antimicrobial potency and ability to provide protection to plants against their natural invaders. In general, extracts of seaweed can induce changes in the physiological/biochemical process associated with plant nutrient uptake and growth in agriculture [15]. Thus, the application of *Cystoseira barbata*, *Laurencia papillosa*, increased the seed germination rate at lower concentrations. This enhanced growth effect is thought to be due to various organic compounds present in the seaweed extract. More specifically it is thought to be due to the presence of phytohormones, mainly cytokinins in the seaweed extracts [16]. Our findings coincide with those of earlier studies in *Vigna catajung*[17], *Dolichos biflorus*[18], *Vigna sinensis*[19], *Cajanus cajan*[1], *Brassica nigra*[20], *Oryza sativa*[21], *Abelmoscus esculentus*[22], *Lycopersicon esculentum*[23, 24]*Triticumaestivum*[25] and *Hordeum vulgare*[26].

High levels of cytokinins and auxins amino acids, and a number of major mineral elements and the smaller ones that stimulate the division of the cell and its expansion rather than any. It leads to a balance in physiological and biological processes affecting root growth and increasing their ability to absorb water and nutrients soluble in it, which reflects positively on growth. The vegetative system, especially the leaves, this result agrees with what, reported by Jabar-Abdul and others [27]. Gollan and Wright [28] who confirmed the increase in the leaf area of plants wheat when sprinkled with seaweed extract. Marginatum at low concentration promoted the growth of from the brown alga *Rosenvingea intricata*, applied to *Sorghum vulgare*[29] and *Vigna sinensis*[30, 31] crop plant showed better results in all aspects of growth.

CONCLUSION

The present study suggests that the presence of various micro and macronutrients in seaweed liquid extract of *Cystoseira barbata* and *Laurencia papillosa*. The practices of application of have increased the plant germination and growth and make the seaweed liquid extract as an excellent choice of organic fertilizer. Ecofriendly seaweed liquid extracts to crops is recommended to the growers for attaining better yield.

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Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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