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The Bio-Stimulating Effects of Invasive *Caulerpa* Var. *cylindracea* Extract on *Vignia sinensis* and *Phaseolus vulgaris* (Fabaceae)

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Abstract

Caulerpa racemosa var. *cylindracea* is a biological pollution in the Mediterranean Sea because of its invasive character since 1991. No published eradication method exists in the scientific literature on this potential invader so far. The coastlines of 13 Mediterranean countries are under thread of *Caulerpa racemosa* var. *cylindracea* because of its impact on the biodiversity. The present study proposes an alternative evaluation of over produced biomass of *Caulerpa racemosa* var. *cylindracea* as seaweed fertilizer. The effect of seaweed extract on the growth and antioxidant system of *Vignia sinensis* and *Phaseolus vulgaris* seedlings were studied. Higher root and shoot lengths of *V. sinensis* were observed in extract supplemented groups (5%, 10% and 20%, $p<0.05$) than that of control group. Increased catalase (CAT), ascorbate peroxidase (APX), α-amylase activities and chlorophyll-a, b levels were observed in extract soaked-water treated (ESWT) *V. sinensis* seedlings ($p<0.05$). Water soaked-extract treated (WSET) induced the increase in the root and shoot lengths of *P. vulgaris* seedlings. In the shoots of *P. vulgaris,* SOD, CAT and APX activities increased, whereas MDA levels decreased. ($p<0.05$). Lower MDA contents indicate that the seaweed extracts from C. racemosa var. cylindracea may stimulate the antioxidant enzyme activities and protect the cell membrane structure in *V. sinensis*. Therefore, *Caulerpa racemosa* var. *cylindracea* extract might be used as an antioxidant supplement for seedlings which are exposed to oxidative stress caused by chilling, salt stress, UV radiation and heavy metals etc.

INTRODUCTION

The coastlines of Turkey which are surrounded by Mediterranean, Aegean and Black Sea are quite rich in terms of biological diversity. *Caulerpa racemosa* (Forsskål) J. Agardh (Caulerpales, Chlorophyta) is a green marine alga, which was observed first time by Hamel (1926) in Sousse Harbour, Tunisia [1]. Up to 1990, the spread of *Caulerpa racemosa* was not thought as an invasive species, therefore it was considered as Lessepsian migrant. After 1990, the unknown form of *Caulerpa racemosa* which showed invasive character was observed in 13 Mediterranean countries including Albania, Algeria, Croatia, Cyprus, France, Greece, Italy, Libya, Malta,

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Monaco, Spain, Tunisia and Turkey [2]. According to Verlaque et al.'s DNA analysis, invasive version of *Caulerpa racemosa* was identified as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque [3]. The one of the important reasons for the invasive success of *Caulerpa racemosa* var.*cylindracea* is caused by its sesquiterpenoid secondary metabolite, caulerpenyne (CPN). So far, cytotoxic, antiproliferative, antiviral and apoptotic effects of this metabolite on some animals and cell lines have been proved in previous reports [4-6]. Unfortunately, no published and validated eradication method exists in the scientific literature on invasive *Caulerpa racemosa* var. *cylindracea* from the Mediterranean Sea. The lack of eradication method for this species motivated our research group to perform some alternative experimental approaches to the use of this over-produced biomass of *Caulerpa racemosa* var. *cylindracea* in Turkish coastlines. In our previous scientific reports, *Caulerpa racemosa* var. *cylindracea* biomass was proposed to be used for methylene blue adsorption [7], malachite green adsorption [8], bovine serum albumin immobilization [9]. In the present study, wet biomass of *Caulerpa racemosa* var. *cylindracea* was aimed to be used as a seaweed fertilizer in organic agriculture. Although seaweeds have so many benefits to people, these species have not been used enough in Turkey. Because of rising population, urbanization and industrialization, water sources have been decreasing rapidly all over the world. At the same time, the world temperature is increasing due to the global warming. This poses a threat to our food supply. In Turkey, the use of chemicals and synthetic growth promoters for raising the crop productivity are very high. Plants consume chemicals and synthetic fertilizers to their structure from soil but the residuals disperse into the waters (ground water, marines etc.). The negative effects of these fertilizers find out the requirement of new alternative approaches to provide sufficient and healthy food demand in the world [10]. In recent years, organic agriculture is very popular among the developing countries for protecting the balance of natural life and increasing the food quality. Organic biostimulants have beneficial effects on plant growth. Seaweeds have been used as sources of biological products for many years. According to the reports, seaweed fertilizers used due to its vitamin, protein and amino acid contents. The effect of seaweed *Spirulina platensis* as feed additive was used by Grinstead et al. (2000) on the growth of weanling pigs [11]. Beside the side effects, seaweeds are started to use as a liquid fertilizer in agriculture. There are many countries like Australia, France, Great Britain, India, Japan, New Zealand, Scotland, Spain and USA are using seaweed liquid fertilizers commercially [12]. These are the names of some well-known liquid fertilizers; Maxicrop which is used in United Kingdom, Kelpak 66 in South Africa, Algifert in Norway, Seagro in New Zealand and Seasol in Tasmania [13]. Seaweed extracts have positive effects to plant growth because of the presence of growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids [14]. The use of seaweed extracts induced increasing the crop quality, reducing the insect attacks and resistance to stress conditions [15]. There are two types of environmental stresses which are biotic and abiotic. Both biotic stresses (infection and/or competition by other organisms) and abiotic stresses (chilling, drougth, UV, salts and heavy metals etc.) induce plants to produce reactive oxygen species (ROS) [16]. ROS cause major damages to the plant tissues. Plants have several antioxidant enzymes and metabolites in their cell compartments to prevent the negative effects of ROS [17]. ROS which include superoxide radical anion, hydrogen peroxide, hydroxyl radical are the most reactive compounds known to be produced during oxidative stress [18]. ROS affect lipids, proteins, DNA, carbohydrates and enzymes of cells.

Therefore, determination of enzymatic activities of antioxidant system (superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and lipid peroxidation (LPO)) could be important parameters for the understanding of plant stress. SOD catalyzes the dismutation of superoxide radicals to hydrogen peroxide [19]. CAT and APX detoxify H_2O_2 to water and oxygen. These defence systems protect the plant cells against LPO. Carotenoids play an important role in the protection of chlorophyll pigments under stress conditions [20]. α- Amylase is an important enzyme for germination period of seeds. This enzyme breaks the α-1→ 4 glycosidic bonds in the polysaccharides and release six-carbon contained monomers for energetic purposes.

The present study reports the growth parameters and antioxidant system of *Vigna sinensis* and *Phaseolus vulgaris* (Fabaceae) cultivated with seaweed extract obtained from *Caulerpa racemosa* var. *cylindracea*. To the best of our knowledge, this is the first biochemical investigation on the potential use of invasive *Caulerpa racemosa* var. *cylindracea* as a liquid fertilizer.

MATERIALS AND METHODS

Collection of seaweeds

C. racemosa var. *cylindracea* was collected by hand-picking from Seferihisar-İzmir in September, 2008. The geographical coordinates are 38° 07' 58.61'' N, 26° 50' 07.71'' E. The seaweeds were transported to the laboratory immediately and rinsed with tap water then distilled water to remove salt and epiphytes. After this treatment seaweeds were left onto the filter paper to remove excess water then separated to the polyethylene bags. The samples were stored at -20°C until used.

Preparation of seaweed fertilizer extract

Sivasankari *et al*. (2006) method was used to prepare the seaweed fertilizer [13]. One kg of fresh seaweed was homogenized first with mortar and pestle then homogenizator. The homogenized seaweed sample was heated with distilled water for an hour at 95°C then filtered. The filtered extract was named as stock solution. By using serial dilutions, different concentrations (5%, 10%, 15% and 20%) of seaweed extracts were prepared using distilled water. The extracts were kept in refrigerator at + 4°C.

Preparation of seeds to germination

Due to the high consumption in Turkey, *Vigna sinensis* and *Phaseolus vulgaris* seeds were selected and obtained from Ege Tarımsal Araştırma Enstitüsü (Ege Agricultural Research Institute) İzmir, Turkey. For sterilization, each type of seeds was soaked in 1% H_2O_2 for 5 min. After 5 min seeds were washed with tap water then distilled water. The experimental studies were separated as two groups.

The abbreviations of the groups were defined below:
ESWT: Extract soaked-water treated (Experiment 1)
WSET: Water soaked- extract treated (Experiment 2)

For experiment 1 (ESWT), 200 seeds were soaked in different concentration of seaweed extracts (5%, 10%, 15% and 20%) in sterile falcon tubes for 24 h in dark at growth chamber (Nuve ID 501). For the control group, seeds were soaked in distilled water. After soaking, the undamaged seeds were selected and placed in petri plates with filter paper. The seeds were watered with 10 ml distilled water every 24 h for 15 days. For the experiment 2 (WSET), 200 seeds were soaked in distilled water in sterile falcon tubes for 24 h in dark at growth chamber (Nuve ID 501). For the control group, seeds were soaked in distilled water. After soaking, the undamaged seeds

were selected and placed in petri plates with filter paper. The seeds were watered with 10 ml different concentrations of seaweed extract every 24 h for 15 days. All the experiments were done in a growth chamber (ID NUVE 501). The conditions of growth parameters were set as temperature 25°C, humidity 55% and photo-period 16 h light/dark. Growth parameters including germination percentage, root and shoot length were measured in *V. sinensis* and *P. vulgaris* (Table 1-4).

Preparation of supernatants for enzyme activities

50 mg of wet root, shoot and leaf were homogenized with pre-cooled mortar and pestle by adding 50 mM 1 ml phosphate buffer (pH 7.0). The homogenates were centrifuged in a refrigerated centrifuge (Universal 32R, Hettich Zentrifuggen) at +4°C for 10 min at 12000 rpm**.** The supernatants were used in determination of enzyme activities and protein levels.

Protein Determination

Protein concentration in the supernatant was measured according to the method of Bradford (1976) [21]. Bovine serum albumin (BSA, Sigma) was used to obtain the standard curve of protein contents.

Catalase (CAT) assay

CAT activity was measured according to Aebi's method (1985) at 25°C [22]. CAT activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm using with UV-VIS Spectrophotometer. The reaction mixture (1 ml) contained 50 mM phosphate buffer (pH 7.0), 50 µl sample and 950 µl 10 mM H_2O_2. One unit will decompose 10 mM of H_2O_2 per minute at pH 7.0 and 25°C.

Superoxide dismutase (SOD) assay

For the measurement of SOD activity superoxide dismutase kit (RANDOX, SD 125) was used. According to the kit manual, formazan dye was formed in the presence of superoxide radical formed by xanthine-xanthine oxidase system and standard SOD inhibited the reaction. SOD activity in the samples was estimated by using a calibration curve which was obtained from standard SOD. The absorbance at 505 nm was measured with spectrophotometer at 37°C against air as mentioned in its manual. One unit of SOD causes a 50% inhibition of the rate of reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT).

Ascorbate peroxidase (APX) assay

The activity of APX was determined according to the oxidation of ascorbate in the presence of hydrogen peroxide [23-34]. The reaction of ascorbate was obtained using 50 µl supernatant, 950 µl 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.5 mM ascorbate and 0.1 mM H_2O_2. The decreases of absorbance at 290 nm were measured with UV-VIS 1601 Shimadzu Spectrophotometer (Tokyo, Japan) at 20°C. 1 IU APX was defined as oxidation of 1 mmol ascorbate in reaction mixture per min.

LPO determination

LPO was measured according to Zhu et al. (2004) with small modifications [25]. 0.2 g fresh root, shoot and leaf were homogenized in 2 ml of 1.15% KCl with glass homogenizer. 1 ml of homogenate was taken in falcon tubes containing 3 ml of 2% TBA (w/v) made in 20% TCA. The mixture was heated at 95 °C for 30 min. After cooling samples, they were centrifuged at 12000 rpm at 10 min. Specific absorbance was measured at 532 nm and non-specific absorbance at 600 nm by using UV-VIS 1601 Schimadzu Spectrophotometer.

Chlorophyll a-b and total carotenoid determinations

Chlorophyll a-b and total carotenoid levels were

determined according to Lichtenthaler and Welburn (1985) and Dere et al. (1998)'s methods [26,27]. For each seed, 1 cm^2 leaf samples were homogenized by using glass homogenizator. Well-homogenized samples were placed in a falcon tube and then 5 ml 100% acetone was added. The homogenates were centrifuged in 2500 rpm at 20°C in a refrigerated centrifugator for 5 min (Universal 32R, Hettich Zentrifuggen). The absorbances at 470, 645 and 662 nm were used to estimate the total carotenoids, chlorophyll a and chlorophyll b levels of the leaf samples. The formulas below were used:

Chlorophyll a = 11.75 A_{662} - 2.350 A_{645}
Chlorophyll b = 18.61 A_{645} - 3.960 A_{662}
Total Carotenoids = (1000 A_{470} - 2.270 Chlorophyll a - 81.4 Chlorophyll b) / 227

Determination of α-amylase

The reducing groups liberated from starch were measured by the reduction of 3, 5 dinitrosalicylic acid according to Bernfeld (1951) method [28]. 0.02 M phosphate buffer (pH 6.9) containing 0.006 M NaCl was used to prepare 1% starch solution. 1 g dinitrosalicylic acid color reagent prepared in 50 mL distilled water then added slowly 30 g sodium potassium tartarate tetrahydrate and 20 mL of 2 M NaOH. The final volume was diluted with distilled water. Standard curve was obtained using different concentrated maltose solutions. For the measurement 250 µl homogenate were put in a sterile falcon tube. 250 µl starch solution was added then incubated for 3 min at 25°C. After incubation, 500 µl dinitrosalicylic acid color reagent was added to the each tube. Then, all tubes were incubated in a boiling water bath for 5 min. After cooled to room temperature 10 ml distilled water was added then mixed well. The absorbance was measured at 540 nm spectrophotometrically.

Statistical Analysis

MiniTAB (13.0) was used for statistical analysis. One way ANOVA and Tukey test were used to see the differences between seaweed fertilizer and other parameters. For the correlation Pearson correlation test was used to evaluate the data. The results are the means of three different experiments. The error bars in the figures show the standard deviations. The statistical significance was considered as $p<0.05$.

RESULTS

In the present study, the effects of *C. racemosa* var. *cylindracea* extract in both experiments (water soaked and extract soaked) on growth parameters of *V. sinensis* and *P. vulgaris* seedlings were shown in Tables 1-4. According to the Tables 1 and 2, WSET and ESWT *V. sinensis* seedlings, the maximum root and shoot lengths were found at 10% group when compared to control group. 5%

Table 1. Germination and growth parameters of *V. sinensis* seedlings (WSET). Different letters indicate significant differences ($p<0.05$).

Extract Concentration	Germination percentage	Root length (cm/seedling)	Shoot length (cm/seedling)
Control (0)	73	1.1 ± 0.7 [a]	1.4 ± 1.0 [a]
5%	100	1.4 ± 0.9 [a]	2.5 ± 2.1 [a]
10%	80	2.9 ± 1.8 [a]	3.5 ± 2.0 [a]
15%	67	1.0 ± 0.5 [b]	1.8 ± 1.0 [a]
20%	80	0.7 ± 1.1 [a]	0.9 ± 0.7 [a]

Table 2. Germination and growth parameters of *V. sinensis* seedlings (ESWT). Different letters indicate significant differences ($p<0.05$).

Extract Concentration	Germination percentage	Root length (cm/seedling)	Shoot length (cm/seedling)
Control (0)	73	1.1 ± 0.7 [a]	1.4 ± 1.0 [a]
5%	100	1.4 ± 0.9 [a]	2.5 ± 2.0 [a]
10%	80	2.9 ± 1.7 [a]	3.5 ± 2.0 [a]
15%	67	1.0 ± 0.5 [b]	1.8 ± 1.0 [a]
20%	80	0.9 ± 0.3 [a]	1.1 ± 0.4 [a]

seaweed extract affected positively the germination percentage of *V. sinensis* (Tables 1,2). According to statistical results there was statistical difference between 15% WSET and control group in the root length of *V. sinensis* ($p<0.05$).

Table 3 shows the effect of different extract concentrations of WSET *P. vulgaris* seedlings. 5% concentration of seaweed extract was affected positively the growth parameters of *P. vulgaris* compared to control group ($p<0.05$).

Although there was no statistically difference among the control group, maximum shoot length was observed at 15% WSET (3.7 ± 2.8 cm/seedling, Table 3). When compared to Tables 3 and 4, growth parameters of WSET *P. vulgaris* seedlings were higher than ESWT seedlings.

Table 3. Germination and growth parameters of *P. vulgaris* seedlings (WSET). Different letters indicate significant differences ($p<0.05$).

Extract Concentration	Germination percentage	Root length (cm/seedling)	Shoot length (cm/seedling)
Control (0)	93	1.5 ± 1.0 [a]	1.7 ± 1.3 [a]
5%	100	3.4 ± 1.6 [b]	3.0 ± 2.2 [b]
10%	53	2.0 ± 1.4 [a]	2.8 ± 2.3 [a]
15%	100	2.5 ± 2.0 [a]	3.7 ± 2.8 [a]
20%	100	2.6 ± 1.8 [a]	2.6 ± 1.9 [a]

Table 4. Germination and growth parameters of *P. vulgaris* seedlings (ESWT). Different letters indicate significant differences ($p<0.05$).

Extract Concentration	Germination percentage	Root length (cm/seedling)	Shoot length (cm/seedling)
Control (0)	93	1.5 ± 1.0 [a]	1.7 ± 1.3 [a]
5%	93	1.6 ± 1.4 [a]	1.4 ± 0.9 [a]
10%	67	1.3 ± 0.7 [a]	2.0 ± 1.0 [a]
15%	73	1.4 ± 0.7 [a]	1.9 ± 1.0 [a]
20%	60	0.9 ± 0.5 [a]	1.3 ± 0.5 [a]

This situation might be indicated that treatment with seaweed extract might stimulate the growth rate of *P. vulgaris* seedlings.

The chemical parameters of *C. racemosa* var. *cylindracea* were analysed by Hıfzısıhha Enstitüsü and were presented in Table 5. The seaweed extract contained high levels of potassium, magnesium, sodium, phosphorus, iron, chloride, sulphate, and silica.

Table 5. Chemical parameters of seaweed fertilizer of *Caulerpa racemosa* var. *cylindracea.*

Parameters	Results	
Cu	0.06	mg/kg
Zn	0.40	mg/kg
Fe	4.90	mg/kg
P	16.12	mg/kg
K	57.71	mg/kg
Si	43.28	mg/kg
Na	856.7	ppm
Mg	26.03	ppm
SO_4	41.74	mg/kg
NO_3	7.70	mg/kg
PO_4	30.40	mg/kg
Cl	1300	mg/kg

The protein contents of *V. sinensis* root, shoot and leaf were presented in Figure 1A-B. For WSET *V. sinensis* leaf maximum protein content was observed at 5 and 20% concentrations of extract (0.83 ± 0.04 mg /ml supernatant and 0.84 ± 0.03 mg/ml supernatant, $p<0.05$, Figure 1A). In ESWT *V. sinensis* roots, maximum protein content was found at 10% concentration of seaweed extract (0.59 ± 0.02 mg/ml supernatant, $p<0.05$, Figure 1B).

The protein content was raised with increased concentration of seaweed extract in WSET *P. vulgaris* leaves (Figure 1C-D). Among WSET

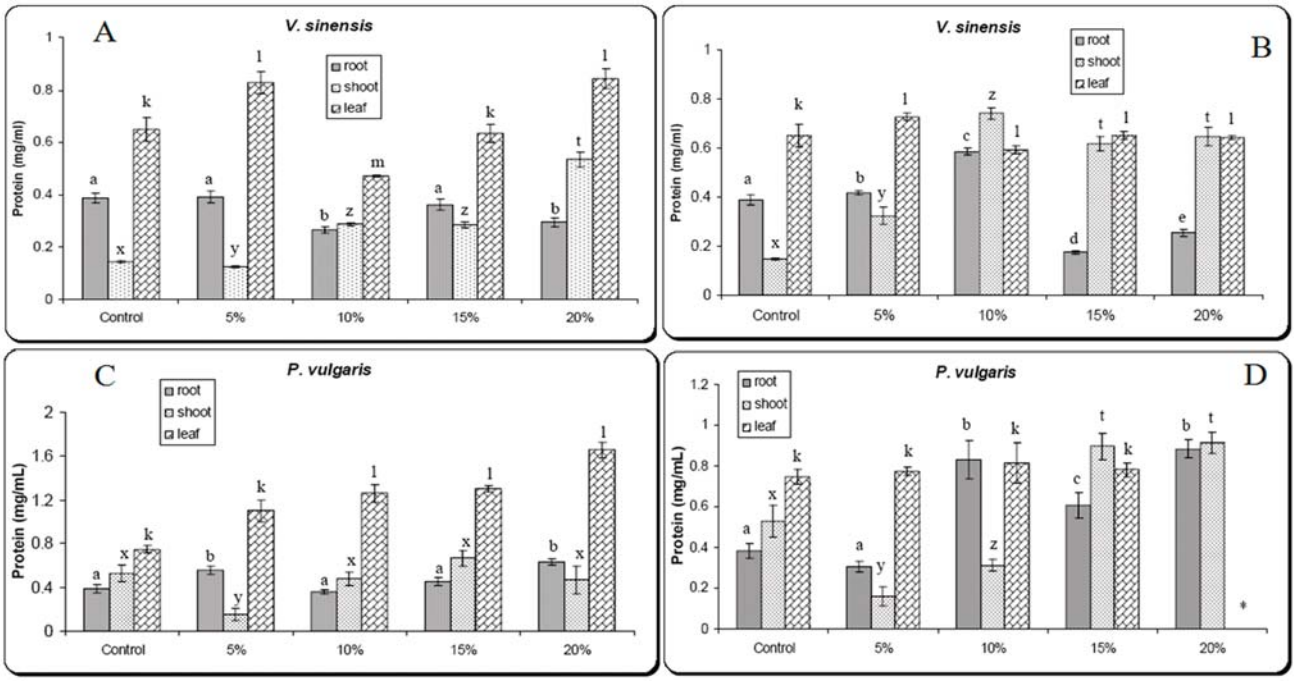

Figure 1. A-B. The protein contents of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The protein contents of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at $p<0.05$. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

groups, maximum protein content was observed as 1.65 ± 0.07 mg/ml supernatant ($p<0.05$) at 20% concentration of extract in the leaf of *P. vulgaris* (Figure 1C). In the root and shoot of ESWT *P. vulgaris* seedlings the highest protein values were found at 20% concentration of extract (0.89 ± 0.09 mg/ml; 0.92 ± 0.04 mg/ml, respectively; $p<0.05$, Figure 1D). In Figure 2A, the highest catalase activity was observed at 10 and 20% WSET *V. sinensis* leaves (285 ± 21.4 IU/mg protein and 295 ± 32.2 IU / mg protein, $p<0.05$).

Pearson correlation test was used to understand the significant differences between parameters. WSET *V. sinensis* roots and leaves showed positive correlations between extract and CAT activity. ($r= 0.918$, $r= 0.879$, respectively, $p<0.05$).

According to Figure 2B, maximum catalase activity was found at 10% concentration of extract in *V. sinensis* leaves (400.9 ± 3.5 IU/mg protein, $p<0.05$, Fig 2B), and thereafter it declined. Maximum catalase activities were observed as 153.7 ± 15.1 IU/mg protein in the roots and 227.1 ± 6.5 IU/mg protein in the shoots of *V. sinensis* at 5% ESWT (Figure 2B, $p<0.05$).

Among WSET groups, the highest catalase activity was observed at 5% extract of *C. racemosa* var. *cylindracea* in *P. vulgaris* roots and shoots (215.9 ± 14.9 IU/mg protein, 618.2 ± 35.2 IU/mg, $p<0.05$; Figure 2C).

The highest catalase activity (365.4 ± 21.4 IU/mg protein) was observed at 5% ESWT *P. vulgaris* leaves ($p<0.05$; Figure 2D). According to Figure 8, catalase activities increased with different concentrations of seaweed extract in the leaves of *P. vulgaris* (Figure 2D).

Figures 3A and B indicated the ascorbate peroxidase activities of *V. sinensis* seedlings. Higher APX activity was found in the control group of WSET *V. sinensis* shoot as 31.4 ± 2.8 IU/mg protein, $p<0.05$ (Fig 3A). APX activity was observed higher than control group at 10% WSET *V. sinensis* leaves (9.7 ± 0.8 IU/mg protein, $p<0.05$, Figure 3B). Among ESWT groups, maximum APX activity was observed

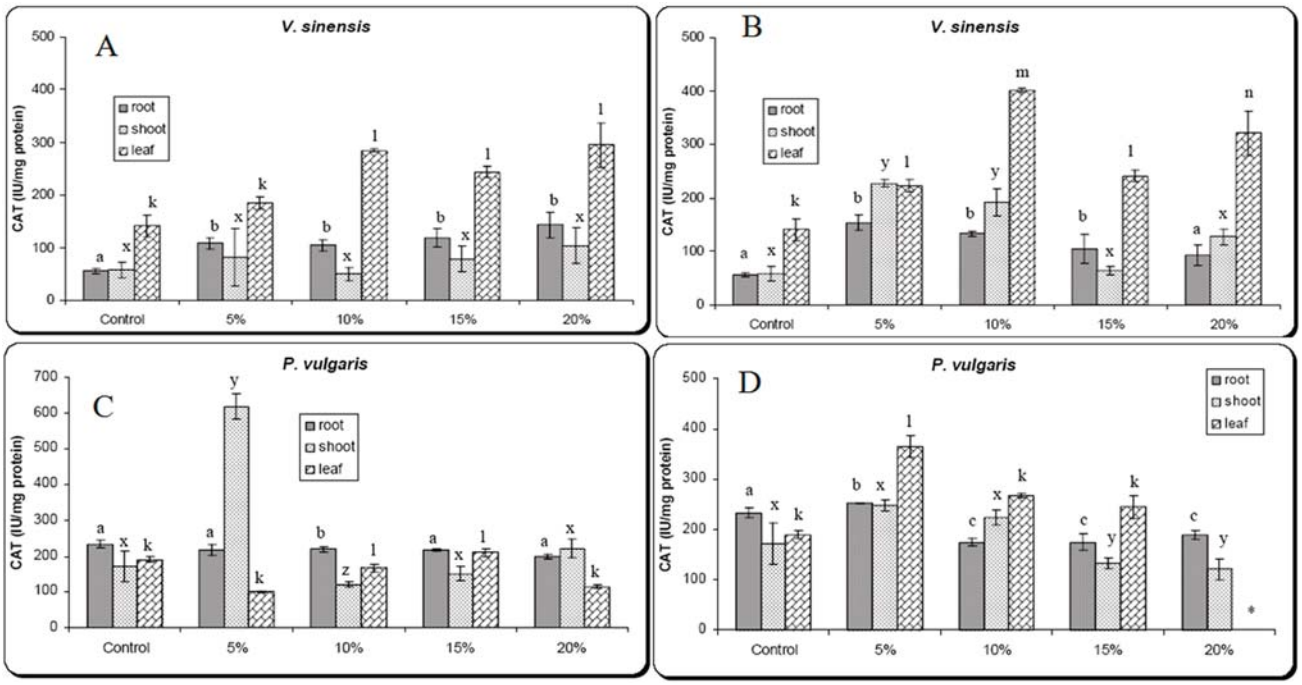

Figure 2. A-B. The CAT activities of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The CAT activites of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at $p<0.05$. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

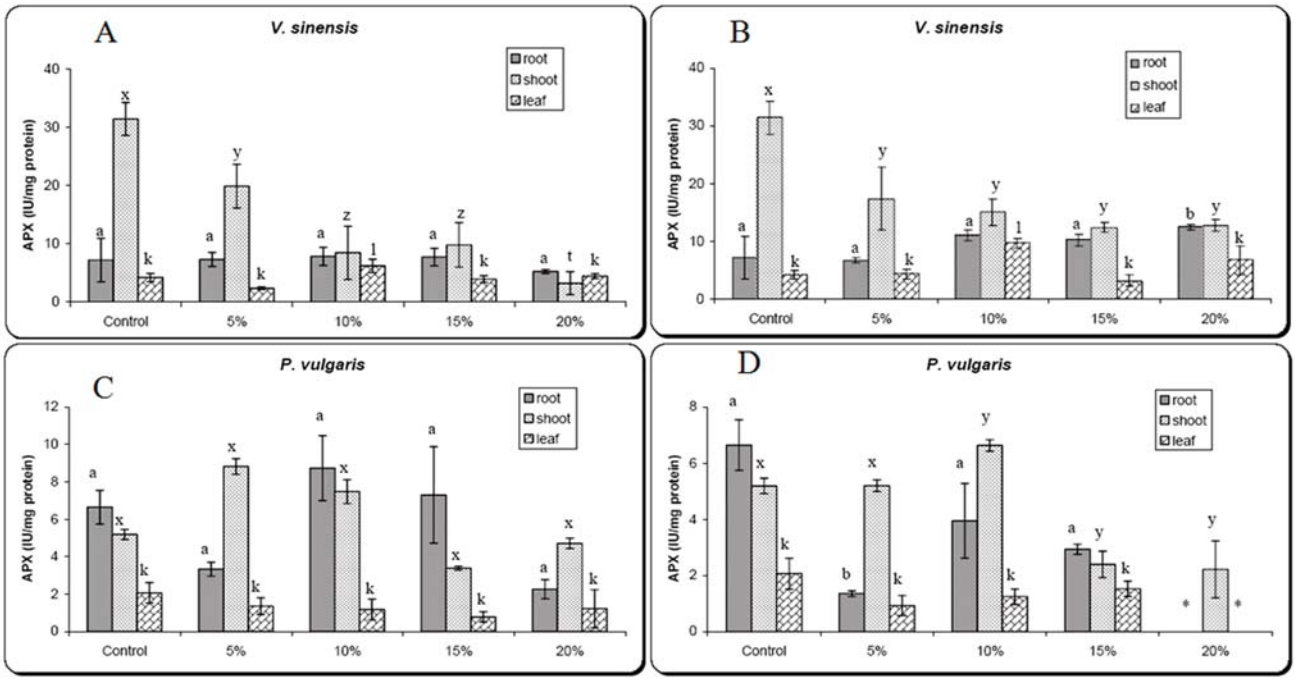

Figure 3. A-B. The APX activities of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The APX activites of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at $p<0.05$. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

at 20% concentration of extract in the roots of *V. sinensis* (Figure 3B). According to Figures 3A and 3B, APX activities in the shoots of *V. sinensis* were decreased when treated with seaweed extract.

Although maximum APX activity was found at 5% WSET *P. vulgaris* shoot (8.82 ± 0.4 IU/mg protein), there was no statistical significance between control group and 5% concentration of extract ($p>0.05$, Fig 3C).

The negative effect of seaweed extract was

observed in ESWT *P. vulgaris* roots (Fig 3D). Higher APX activities were found at 10% ESWT *P. vulgaris* shoots as 6.62 ± 0.2 IU/mg protein ($p<0.05$). However, the negative effect of the seaweed extract was observed in APX activities of ESWT *V. sinensis* roots ($p<0.05$).

For WSET *V. sinensis* roots and leaves, seaweed extract significantly increased SOD activities compared to control group at 10% concentration of extract ($p<0.05$, Figure 4A).

It can be seen from Figure 4A that WSET mostly increased the SOD activities in roots of *V. sinensis* when compared to control group. Among the ESWT groups, SOD activity was observed higher than control group at 15% ESWT *V. sinensis* root as 14.14 ± 0.1 IU/mg protein ($p<0.05$, Figure 4B).

In the root and leaf of *P. vulgaris*, SOD activity was found high at control group. Maximum SOD activity was observed as 10.95 ± 0.0 IU/mg protein at 5% WSET *P. vulgaris* shoot ($p<0.05$, Figure 4C).

SOD activities were found higher than control group at 5% ESWT *P. vulgaris* roots and shoots (7.37 ± 0.1 IU/mg protein, 14.16 ± 0.1 IU/mg protein, respectively, Fig 4D).

The maximum effect of seaweed extract was observed in *V. sinensis* roots and leaves. 20% WSET was affected positively the α-amylase activities of *V. sinensis* leaf (0.99 ± 0.0 μmol maltose/mg protein.min).

In the shoot of *V. sinensis* maximum α-amylase activity was found at 5% concentration of extract as 2.68 ± 0.0 μmol maltose/mg protein.min (Figure 5A). In the leaves of *V. sinensis* the increasing trend was observed with raising extract concentrations (Figure 5B).

The increased α-amylase activities were observed in the root of WSET *P. vulgaris* compared to control group. Maximum α-amylase activity was found at 5% concentration of extract in WSET *P. vulgaris* shoot (6.25 ± 0.0 μmol maltose/mg protein, Figure 5C). The seaweed extract was affected positively

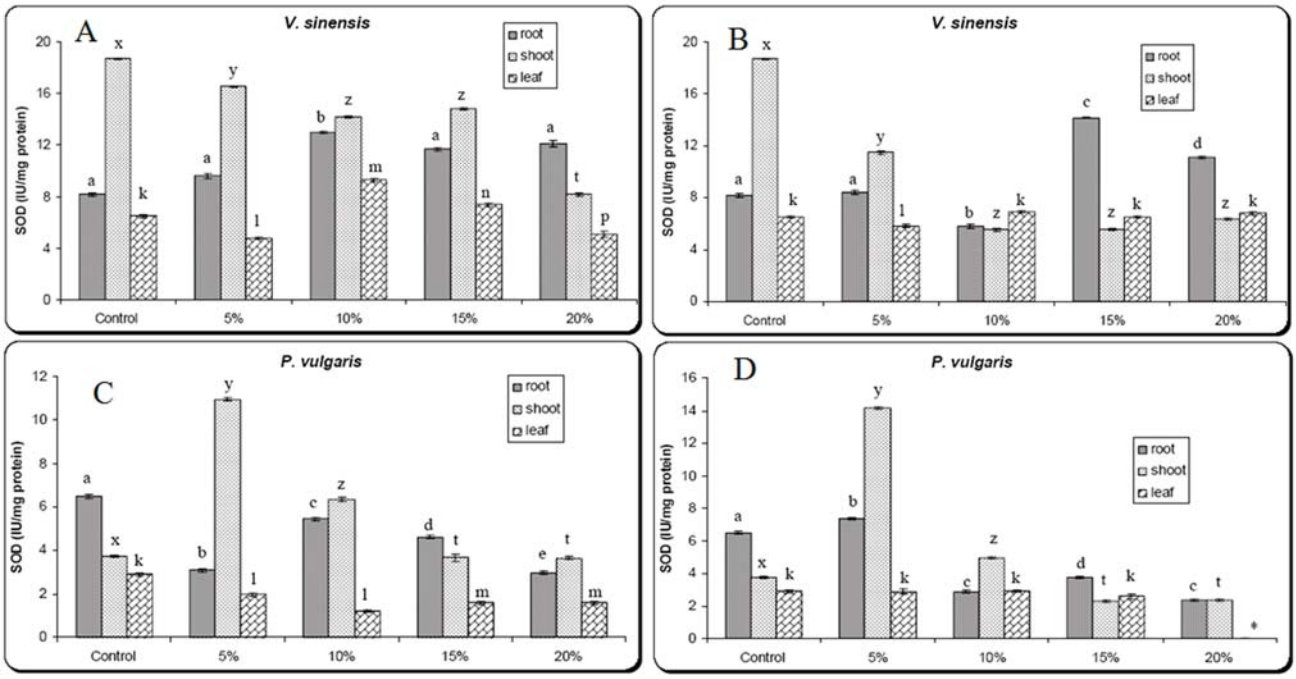

Figure 4. A-B. The SOD activities of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The SOD activites of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at $p<0.05$. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

the α-amylase activities of the ESWT *V. sinensis* leaves (r= 0.960, p<0.05). The α-amylase activities increased with concentration levels upto 20% in leaves of ESWT *P. vulgaris* (Figure 5D).

The effect of *C. racemosa* var. *cylindracea* extract on LPO levels of *V. sinensis* and *P. vulgaris* were presented in Figure 6A-D. As can be seen in Figure 6A, the LPO levels of WSET *V. sinensis* root was observed higher than control group (Control: 0.5 ± 0.01 µmol MDA/g wet weight, 5%: 0.9 ± 0.01 µmol MDA/g wet weight, 10%: 1.4 ± 0.02 µmol MDA/g wet

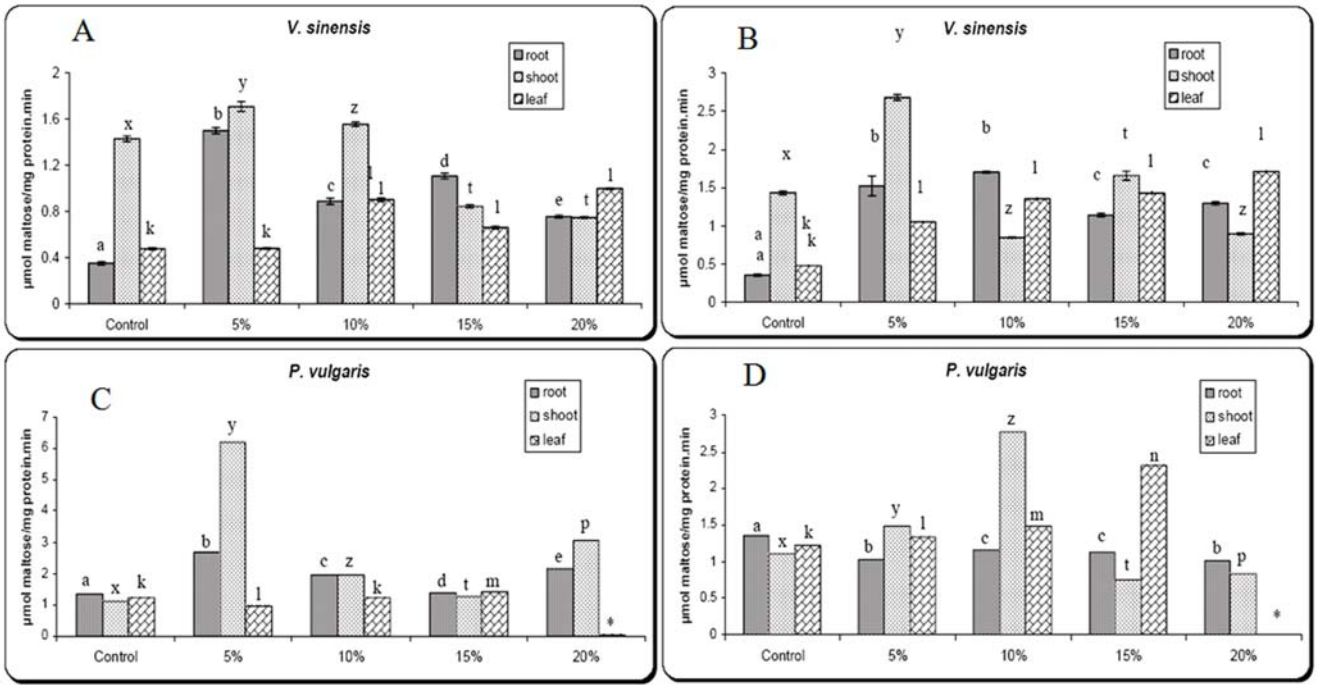

Figure 5. A-B. The α-amylase activities of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The α-amylase activites of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at p<0.05. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

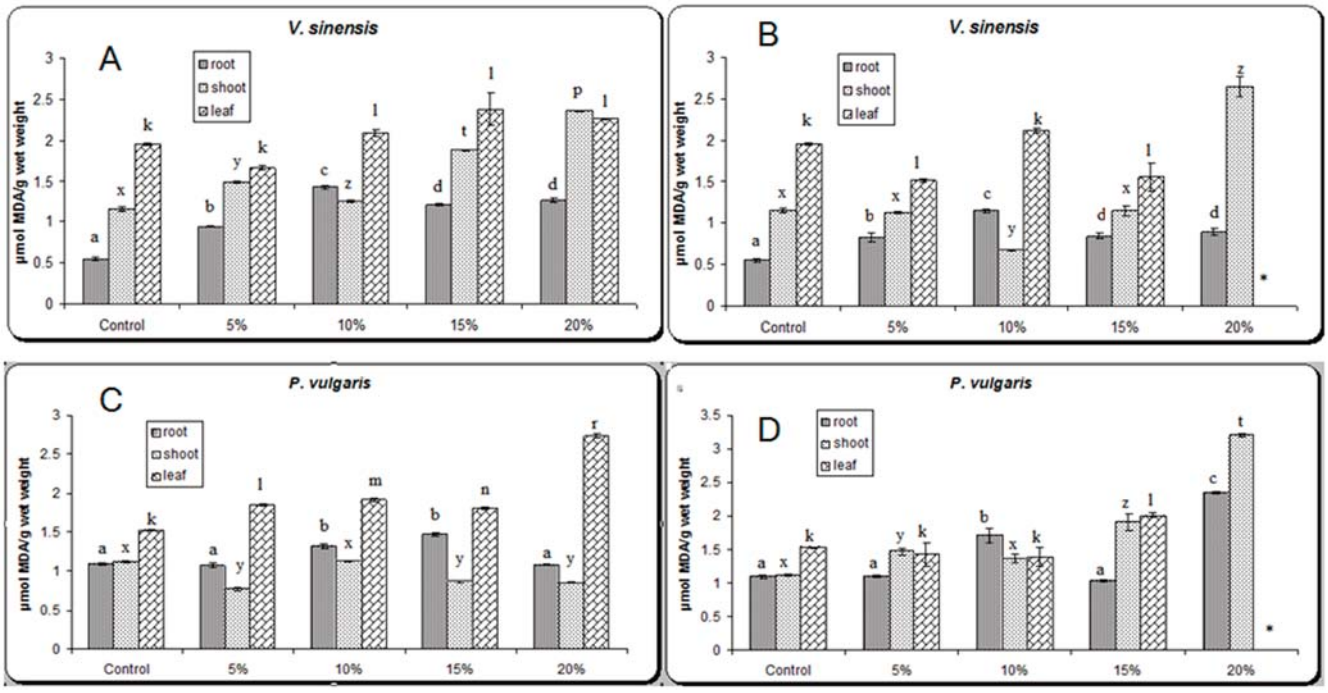

Figure 6. A-B. The LPO levels of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The LPO levels of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at p<0.05. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

weight, 15%: 1.2 ± 0.01 µmol MDA/g wet weight, 20%: 1.3 ± 0.03 µmol MDA/g wet weight, p<0.05, Figure 6A). Maximum LPO level was found at 15% concentration of extract in WSET *V. sinensis* leaf (2.4 ± 0.2 µmol MDA/g wet weight, p<0.05, Figure 6B). The highest LPO level was observed at 20% ESWT *V. sinensis* shoot as 2.65 ± 0. 1 µmol MDA / g wet weight (p<0.05, Fig 6B). When compared to control group, the different concentrations of seaweed extract induced to chance in LPO levels of *V. sinensis* leaf. There were negative correlations in *V. Sinensis* shoot of SOD and APX activities (r= -0.924, r= -0.939, p<0.05), positive correlation were observed in protein and LPO levels (r= -0.907, r= -0.896, p<0.05). On the other hand, in the concentrations of 5% and %15 ESWT *V. sinensis* leaves lower MDA contents were observed (Control: 1.9 ± 0.01 µmol MDA/g wet weight, 5%: 1.5 ± 0.02 µmol MDA/g wet weight, 10%: 2.1 ± 0.03 µmol MDA/g wet weight, 15%: 1.5 ± 0.02 µmol MDA/g wet weight, p<0.05, Figure 6B). The detractive effect of seaweed extract to the LPO levels was observed in *P. vulgaris* shoots (Control: 1.1 ± 0.01 µmol MDA/g wet weight, 5%: 0.8 ± 0.02 µmol MDA/g wet weight, 15%: 0.9 ± 0.02 µmol MDA/g wet weight, 20%: 0.8 ± 0.0 µmol MDA/g wet weight, p<0.05, Figure 6C). In the root of *P. vulgaris*, LPO level at 20% WSET group was found as 2.3 ± 0.01 µmol MDA/g wet weight, p<0.05, Fig 6C). Higher LPO level was found at 15% extract of *C. racemosa* var. *cylindracea* in the leaf of *P. vulgaris* (2.0 ± 0.04 µmol MDA/g wet weight, p<0.05, Figure 6D).

Increased chlorophyll-a values were observed at the concentrations of 5% and 10% (Figure 7A). Among WSET groups high chlorophyll-b values were found at 5% concentration of extract in *V. sinensis* leaves. However, the raising concentrations seaweed extract induced to decrease in total carotenoid levels (p<0.05, Figure 7B). The effect of seaweed fertilizer (10% and 15%) was affected positively the chlorophyll- a, b and total caretenoid levels at ESWT *V. sinensis* leaves (Control: 6.1 ± 0.01 µg/cm^{2}wet weight, %10: 7.3 ± 0.01µg/cm^{2}wet weight, 6.4± 0.0 µg/cm^{2}wet weight, p<0.05, Figure 7B). The greatest chlorophyll-a, b and total carotenoid levels were

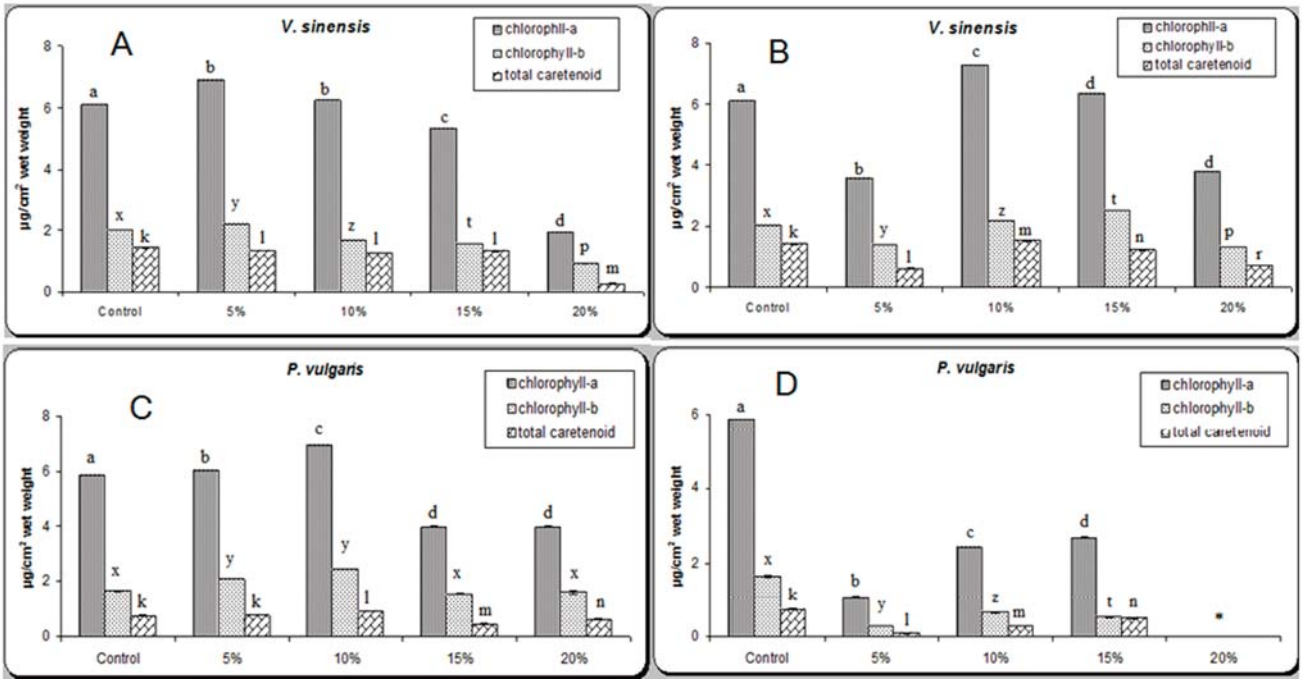

Figure 7. A-B. The chlorophyll-a,b and total carotenoid levels of *V. sinensis* root, shoot and leaf (WSET- ESWT). C-D. The chlorophll-a,b and total carotenoid levels of *P.vulgaris* root, shoot and leaf (WSET- ESWT). Different letters above the error bars indicate significant differences at p<0.05. The results are the means of three different experiments.

*There were not enough leaf grown to analyze.

found at 10% concentration of extract (7± 0.0 µg/cm^{2}wet weight, 2.4± 0.01 µg/cm^{2}wet weight, 0.9 ± 0.0 µg/cm^2 wet weight, respectively, $p<0.05$, Figure 7C). ESWT *P. vulgaris* leaves affected negatively from seaweed extract (Figure 7D). Maximum chlorophyll-a,b and total caretenoid levels were observed at control group (Figure 7D). The highest chlorophyll-a level was observed as 5.9 ± 0.0 µg/cm^2 wet weight at control group of *P. vulgaris* leaves (Fig 7D).

DISCUSSION

Under normal conditions, there is a balance between reactive oxygen species (oxidants) and antioxidants in aerobic cell metabolism [29,30]. ROS are used to prevent plants to external damages such as pathogen attacks, epiphytic organism [31]. On the other hand, disorders in oxidant-antioxidant balance damages to lipids, proteins, DNA, carbohydrates and other compounds of the plant cells. In these cases, plants benefit from antioxidants to reduce to vital effects of ROS [17]. Increased ROS due to the environmental factors (UV, high temperature, chilling, heavy metal, drought etc.) decrease the growth parameters, quality and yield of plants [32]. Agricultural areas are shortened with raising population, industrialization and global warming in the world. Turkey is affected significantly from global warming and as a result of this, abiotic stress factors such as drought and dehydration are raising day by day. All around the world, alternative production methods (organic agriculture etc.) have been developed for encouraging the natural life balance and cultivating more healthy crops. In Turkey, organic agriculture is not executing enough due to the insufficiency of organic fertilizers, organic pesticides, work force and agricultural areas [33-35]. A wide range of beneficial effects including vitamin, mineral, amino acid and trace element contents, seaweeds are started to use as a plant additive in crop improvement [10,13]. In the literature, there are many reports on the effects of seaweed extracts to the plant growth. Crouch and Staden analysed the growth of tomato seedlings when treated with *Ecklonia maxima* [10]. According to their findings, maturation and fruition ratio raised with application of seaweed fertilizer to tomato seedlings. Sivasankari et al. studied the effect of seaweed fertilizers -*Sargassum wightii* and *Caulerpa chemnitzia*- on the growth and biochemical constituents of *V. sinensis* seeds. Their results indicated *S. wightii* extract was more effective than *C. chemnitzia* on the crop improvement. Moreover, Sivasankari et al. reported the treatment of seaweed fertilizer to *V. sinensis* caused to increase in protein content [13]. This situation was explained by authors that the seedlings might have taken the major elements from seaweed fertilizer. In another research, brown seaweed *Ascophyllum nodosum* extract used as a bio-stimulant to promote growth and productivity in barley seed halves. *A. nodosum* extract induced amylase activity independent of GA_3 and enhanced germination and seedling vigor in barley [36]. Turkey is surrounded by the Mediterranean Sea, Aegean Sea, and the Black Sea and includes wide range of seaweeds. *Caulerpa racemosa* var. *cylindracea* is a well-known invasive green alga in the Mediterranean Sea. In the present study, we aimed to investigate the growth parameters and antioxidant system of *Vigna sinensis* and *Phaseolus vulgaris* which were treated with seaweed extract obtained from *Caulerpa racemosa* var. *cylindracea.* In *V. sinensis* seedlings, root and shoot lengths were observed higher than control group when applied seaweed extracts (5%, 10% and 20%). Our findings are not in agreement with Sivasankari et al. (2006) that they reported the higher concentrations of *S. wightii* and *C. chemnitzia* extracts inhibited the germination of *V. sinensis* [13]. According to our biochemical analysis, increased CAT, APX, α-amylase activities and chlorophyll-a, b levels were observed at extract soaked *V. sinensis*

seedlings. The high rate of germination percentage in *V. sinensis* seedlings may have been due to the presence of growth promoting substances such as gibberellins, cytokinins, micronutrients, vitamins and amino acids of *C. racemosa* var. *cylindracea* extract [13]. Therefore, we strongly recommended a comprehensive research on the biostimulating agents in this invasive species. Water soaked treated with seaweed extract *V. sinensis* seedlings were induced to enhance the SOD and CAT activities as well as MDA contents in *V. sinensis* roots and leaves. Over-production of superoxide radicals are converted to H_2O_2 by SOD. CAT and APX detoxify H_2O_2 to water and oxygen. Increased SOD and CAT activities might have been produced for the inhibition H_2O_2. The results revealed that antioxidant enzyme activities were not enough levels to prevent oxidative destruction in seedlings [16]. In chlorophyll-a, b levels positive effect was observed at extract soaked *V. sinensis* leaves compared to control group. For understanding the environmental stress factors in plants, chlorophyll contents are great deal of importance.

Soils which contain heavy metals affect plant metabolism negatively and reduce the plant growth. In a previous research, soils which are generous with heavy metals (Ni-Cu) induced to decrease in chlorophyll contents of *Empetrum nigrum* [37]. In the present study, we determined the chemical parameters of *C. racemosa* var. *cylindracea.* The results demonstrated that *C. racemosa* var. *cylindracea* was not exposed to heavy metal pollution due to the existence of low copper and zinc contents [38].

The use of seaweed extract in water soaked *P. vulgaris* seedlings induced to increase in the root and shoot lengths. In the shoots of *P. vulgaris* SOD, CAT and APX activities increased, on the other hand, MDA levels decreased. CAT and APX enzymes reduce H_2O_2 to molecular oxygen and water in peroxisomes of plants [18]. Low MDA contents indicated that the seaweed extracts might have been stimulated the antioxidant enzyme activities and prevented the membrane permeability in *P. vulgaris*. Although silicon (Si) is not an essential element in plants, it plays important roles in healthy plant growth and development. Previous studies reported that the addition of Si increased the activities of antioxidant enzymes exposed to salinity and freezing in cucumber and wheat [25,39]. According to our experimental results, Si which present in *C. racemosa* var. *cylindracea* extract might have a protective role against ROS in *P. vulgaris* seedlings. The addition of the extract to water soaked *P. vulgaris* seedlings, increased chlorophyll-a, b and total carotenoid levels were observed. In the literature, there are many reports on the plant growth and various oxidative stress parameters. Posmyk et al. investigated the chilling effect of the *Glycine max* (L.) *Merr.* seedlings. According to their findings, chilling effect induced to enhance the SOD and CAT activities as well as MDA contents in *G. max* seedlings [16]. In a report of Zhu *et al.*, the addition of silicon (Si) to salt stressed cucumber leaves significantly increased the antioxidant enzymes of cucumber leaves [25]. Fernandez-Orozco et al. investigated the antioxidant compounds and capacities during the germination of *Vigna radiata* cv. *emmerald, Glycine max* cv. *jutro* and *Glycine max* cv. *Merit* [40]. Chaoui et al. investigated the cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.) [41]. According to their results, lipid peroxidation raised and CAT activity decreased in both roots and leaves of *Phaseolus vulgaris* L. Li et al. reported the effect of sound wave stress on antioxidant enzyme activities and lipid peroxidation of *Dendrobium candidum* [42]. Experimental results showed that *Dendrobium candidum* exposed to sound wave stress gave positive responses to SOD, CAT, peroxidase (POD) and APX contents. In the report of

Sairam et al. drought induced raising H_2O_2 accumulation, SOD, CAT, APX and lipid peroxidation and decreasing the ascorbic acid concentration in wheat cultivars [43]. Reddy et al. studied the effects of lead in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.) [44]. Plants exposed to lead showed high lipid peroxidation, SOD, CAT, peroxidase (POD), glutathione reductase (GR) and glutathione S-transferase (GST) activities compared to untreated plants. Lead toxicity caused to oxidative destruction in plants due to the high rate of peroxidation. Consequently, the parameters mentioned above cause oxidative damage in plants [44]. According to a published paper on the antioxidant power of marine macroalgae collected from the coasts of Yucatan and Quintana Roo (Mexico), extracts of these marine algae is proposed to be used in medicine, cosmetic industry and food production [45]. Our results support latter report for food production.

In conclusion, the effect of seaweed extract from *C. racemosa* var. *cylindracea* was investigated in *V. sinensis* and *P. vulgaris* seedlings. The results mentioned above showed that the seaweed extract from *C. racemosa* var. *cylindracea* could be used as an antioxidant supplement for the seeds which are exposed to oxidative stress caused by chilling [16], salt stress [46], UV-B radiation [47], heavy metals [44] etc. to obtain more healthy and high quality crops [48].

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