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Original Article

The Biochemical Properties of Zarrin-Giah *(Dracocephalum kotschyi* Boiss*)* Medicinal Plant Affected by Seaweed Extract and Amino Acid Spraying

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Abstract

The use of medicinal plants, as favorite sources of different metabolites for human health, has become more popular, worldwide. It is accordingly important to find methods, which may enhance the growth and quality of medicinal plants including the important medical Zarrin-giah *(Dracocephalum kotschyi* Boiss*)*. The plant is used for the treatment of different diseases including cancer and Alzheimer. In the presented research, the effects of different biostimulants including seaweed extracts (Wuxal Ascofol, 1 (A1), 2 (A2), and 3 (A3) g/L) and algaton amino acid (1 (J1), 2 (J2), and 3 (J3) g/L) on the biochemical properties of Zarrin-giah were investigated in the greenhouse. Different plant biochemical properties including chlorophyll contents (a, b, and total), carotenoid, flavonoid, total phenol, antioxidant activity and leaf greenness were determined. The results indicated the positive effects of the experimental treatments on the quality of Zarrin-giah by improving the biochemical properties of the plant. The algal and the amino acid treatments significantly increased the chlorophyll a content of the plant, compared with the control treatment (0.34 mg/g). This was also the case for chlorophyll b and total chlorophyll. Although there was not much difference among the algal and amino acid treatments, on the content of carotenoid, treatments A1 (0.118 mg/g) and J1 (0.116 mg/g) resulted in the higher carotenoid content, significantly different from the other treatments including control (0.102 mg/g). The highest and significantly different flavonoid contents were resulted by the A2 (0.28%) and the J2 (0.276%) treatments. Interestingly, the least flavonoid content was related to the A3 (0.22%) treatment, even significantly less than the control treatment (0.236%) The J3 treatment (6076.336 mg/kg) resulted in the highest and significantly different content of total phenol. However, the least contents of the total phenol were related to the A3 (3510.556 mg/kg) and the control (3637.984 mg/kg) treatments. Algal treatments were more effective on the increased activity of the antioxidants. Accordingly, treatment A3 (77.167%) resulted in the highest and significantly different activity of antioxidant, followed by A2 (58.974%), and the least one was resulted by control (27.473%). The highest leaf greenness (SPAD) was related to the A2 treatment (39.933), significantly higher than the other treatments including J2 (31.733), J3 (27.033) and A1 (20.067). If the proper concentrations of biostimulants including seaweed extract and amino acid treatments tested in this research are used, it is possible to enhance the quality of Zarrin-giah, because at the higher rates, they may not favorably affect the plant quality.

Keywords: Algal extract, Antioxidants, Flavonoid, Nutrients, Phenol.

Introduction

The worldwide use of medicinal plants for nutritional purposes and treating different diseases indicate their significance [1,2]. Zarrin-Giah *(Dracocephalum kotschyi* Boiss*)* is a medicinal plant with plenty of medicinal properties and is used in different parts of the world including Iran. The plant contains different kinds of essential oils, flavonoids, rosmarinic acid and monoterpene glycoside, which are useful for the treatment of different diseases such as Alzheimer, Artritis rheumatoid, multiple sclerosis, and dizziness [3].

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The other important products in Zarrin-giah are: 1) limonene (an inhibitor of angiotensin converting enzyme), which is useful for the treatment of tumors and viral diseases, and can act as a bactericide, fungicide, anti-spasm, and pain controller, 2) geraniol prohibiting the growth and synthesis of poly amines in carcinogenic cells in human, 3) spinal Z, which is also an affective compound in the cancer treatment, and 4) rosmarinic acid, which has considerable biological activities and can act as an antiviral and antioxidant [4,5].

With respect to the above-mentioned details, it is important to find methods, which may enhance the quantity and quality of Zarrin-giah. The use of chemical fertilization is not much recommendable as it results in the pollution of the environment affecting human health. The use of plant growth promoting stimulants affecting crop plant growth and quantity can be a suitable method from environmental and economical point of view [6]. Such compounds can stimulate plant metabolic activities and processes resulting in the enhanced growth efficiency. The basic compounds, used for the production of stimulants include amino acids, nutrients, hydrolysed proteins, humic acid, algal and seaweed extracts, and other metabolites [7, 8]. Amino acids are polar molecules with the formula of $C_2H_4O_2NR$ acting as biomolecules, and components of protein and peptide molecules affecting different plant enzymatic and metabolic activities. The hydrolysis of amino acids results in the production of intermediate molecules, which are eventually converted to glucose or are oxidized in the cycle of citric acid [9]. Biological fertilization including amino acids, as a source of nitrogen, enhance plant tolerance under stressful conditions by increasing plant chlorophyll content and hence the process of photosynthesis [10,11]. The benefits of using seaweed extract, as a source of fertilization in organic farming and sustainable agriculture has been indicated by research work [12,13]. It is a favorite source of nutrients because despite chemical fertilization, it is not a source of pollution, does not have negative effects on the environment, and is non-toxic. Research has indicated the positive effects of seaweed extracts, also recently tested by spraying, on the quantity and quality of different crop plants. It is because the extract contains different plant stimulating substances such as plant hormones (gibberellins, auxins, cytokinins), and nutrients including iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), molybdenum (Mo), manganese (Mn), nickel (Ni), and vitamins [14,15]. Such useful products can stimulate plant growth and yield production by affecting the cellular growth, the process of flowering, root and leaf growth, and enhancing plant tolerance under different stresses including drought, salinity, and suboptimal root temperature [16,17].

With respect to the above-mentioned details and because, there is not any data on the use of seaweed extracts and amino acids affecting the growth and quality of Zarrin-giah, this research was conducted to investigate the effects of biostimulants on the biochemical properties of Zarrin-giah.

Material and Methods

This research was conducted in the greenhouses of the Greenhouse Research Center of Islamic Azad University, Isfahan (Khorasgan) Branch, Iran (Fig. 1). The experiment was a completely randomized design with two factors of seaweed extract (Wuxal Ascofol, 1, 2, and 3 g/L) and amino acid (algaton, 1, 2 and 3 g/L) in three replicates. A control treatment was also used.

The Wuxal Ascofol fertilizer (extract) is a suspension of seaweeds with a high density extracted from the brown algae of *Ascophyllum nodosum* [18]. The seaweed extracts contained the following nutrients: boron (3%), manganese (0.8%), zinc (0.5%), copper, iron, iodine, calcium and also betaine, cytokinin, auxin, and carboxylic acid. The bulk density of the extract was 727 g/cm^3, with the pH of 6.9 and a brown color [12].

The analysis of amino acid is according to the following: total nitrogen (N) (W/W: 6%, at 75 g/L), organic N (W/W: 6%, at 75 g/L), organic carbon (C) (W/W: 20%, at 250 g/L) and organic matter (38%, with the nominal molecular weight < 50 kDa).

Each plot consisted of five pots (4-L each) with the growth medium of perlite and coco peat at an equal ratio, containing the homogenous seedlings of Zarrin-giah. The seedlings were sprayed at the complete establishment, each 15 days, until the flowering stage. Different plant biochemical properties were determined according to the following.

Fig. 1 Different stages of the experiment

Chlorophyll and Carotenoid Contents

The chlorophyll content was determined according to Arnon [19] and the carotenoid content was measured using the method of Lichtenthder [20]. Accordingly, 0.5 g of fresh tissue was treated with 0.5 mL acetone (80%, 80 mL acetone + 20 mL distilled water) and crushed using a crucible. The extract was then filtrated using Whatman filter paper #2. The extract was brought up to the volume of 20 mL using acetone (80%), and the contents of chlorophyll and carotenoid were determined at the wavelengths of 663, 645 and 470 nm, respectively using the UNICO spectrophotometer. Total chlorophyll, chlorophyll a, b and carotenoid were calculated using the following formulas:

Chl.a ($mg.g^{-1}$) = [(12.7×Abs 663) – (2.6 × Abs 645)] × V/W × 1000

Chl.b ($mg.g^{-1}$) = [(22.9 × Abs 645) – (4.68 × Abs 663)] × V/W × 1000

Chl.total ($mg.g^{-1}$) = Chl.a + Chl.b

Carotenoid = (1000 A470- 1.82 Chla – 85.02 Chlb) / 198

Abs 645 = Absorbance at 645 nm

Abs 663 = Absorbance at 663 nm

Abs 470 = Absorbance at 470 nm

V= Consumed acetone (mm)
W = Fresh weight of leaf sample (g)

Total Flavonoid

Total flavonoid was determined according to the following [21]. Leaf samples (0.5 g) were treated with acidic ethanol (ethylic alcohol at 99 mL and glacial acetic acid at 1 mL) and crushed using a crucible. The samples were then centrifuged at 4000 *g* for 10 min, and were placed in a Bain-Marie. The rate of absorbance was determined at the wavelength of 300 nm using the UNICO spectrophotometer S-2100, USA and its percentage were calculated using the following formula:
100 (V/700) fla = ABS (300 nm)
in which "V" is the volume of the extract

Total Phenol

Total phenol of the plant samples, was determined using the method of Folin–Ciocalteu [22]. The standard solution was prepared according to the following: the basic solution of galic acid (0.1 g galic aicd was brought up to the volume of 100 mL using pure methanol), folin (5 mL Folin was brought up to the volume of 50 mL using Folin) and sodium carbonate 7.5 % (using 1.5 g sodium carbonate at 20 mL distilled water). The volumes of 10, 15, 20, 25 and 50 µL of galic acid were poured into little glass containers, which were treated with 2.5 mL of Folin and 2 mL of sodium carbonate (7.5%). The absorbance of samples at the wavelength of 760 nm was determined using the UNICO spectrophotometer S-2100, USA. The rate of plant phenol was determined cording to the following: 30 µL of the extract was brought up to the volume of 1 mL and was then treated with 2.5 mL Folin; after 5 min, it was treated with 2 mL of sodium carbonate. The samples were measured after 1.5 h (at the room temperature and at the dark) at the wavelength of 760 nm. Gallic acid was used as the standard curve (Fig. 2) and the total phenol content of the extracted was determined according to the absorbance of sample and the standard solution (mg Galic acid/ L plant extract).

Antioxidant Activity

The antioxidant activity of plant was determined using the neutralizing activity of 2, 2-diphenylpicrylhydrazyl (DPPH) free radical [23]. In this method, the reduction absorbance of DPPH, after neutralization by antioxidant compounds, at the wavelength of 515 nm was determined using UNICO spectrophotometer model S-2100, USA. Accordingly, the plant extract was centrifuged at 10000 *g*, at 4 ^{o}C for 10 min; 30 µL of the centrifuged extract was brought up to the volume of 1 ml using DPPH 0.1 M containing methanol. The solution was kept under the room conditions and at the dark for 15 min to make it homogenous. The absorbance reduction at 515 nm (three times for each treatment) was determined using the UNICO spectrophotometer. The antioxidant activity of extracts as the inhibition percentage was calculated according to the following [24].
RSA (%) = (OD control – OD sample) / OD control × 100
in which OD control is the absorption of the control sample, OD sample is the absorbance of sample and RSA is the removing activity of the free radical.

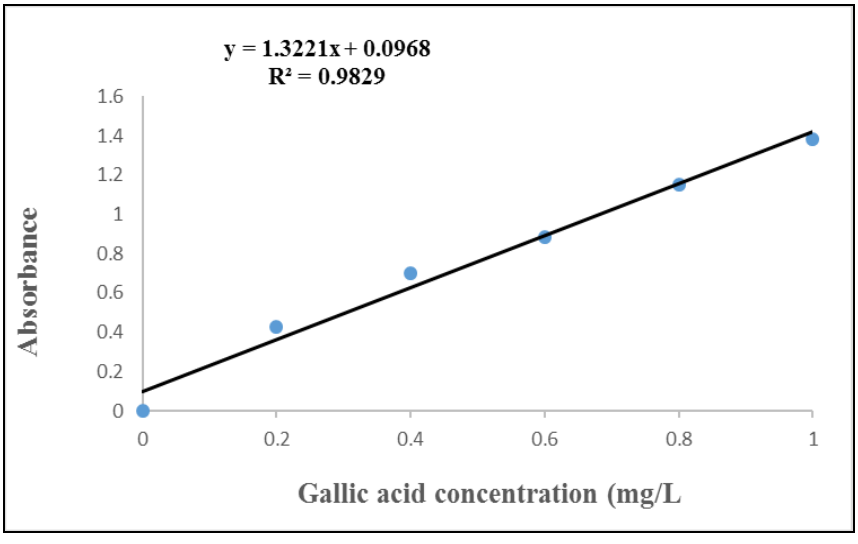
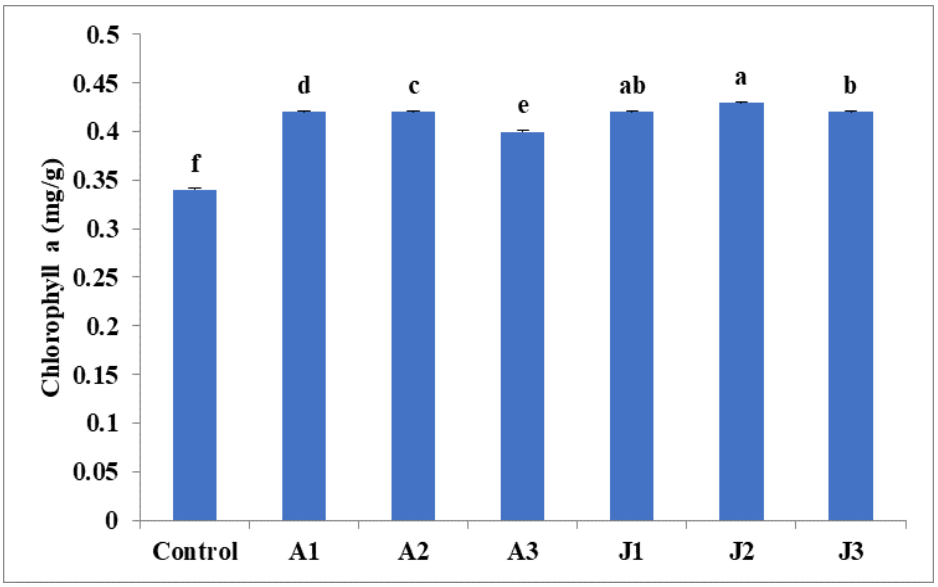


Fig. 2 Standard curve of gallic acid

A
LSD= 0.002



B
LSD= 0.0044

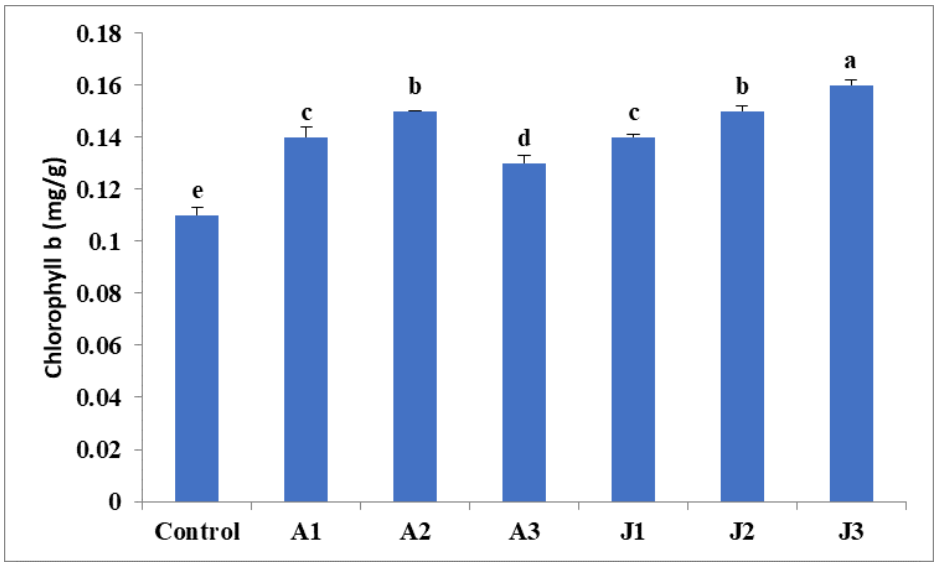


Fig. 3 Zarrin-giah chlorophyll a (A) and chlorophyll b contents (B) affected by different experimental treatments including control, seaweed extracts (Wuxal Ascofol, 1 (A1), 2 (A2), and 3 (A3) g/L) and algaton amino acid (1 (J1), 2 (J2), and 3 (J3) g/L). Columns followed by different letters are significantly different determined by LSD at P= 0.05. Columns are presented with their respective standard deviations.

Leaf Greenness

The leaf greenness was determined 10 day after the final spraying using a chlorophyll meter (Model CL-01); accordingly, chlorophyll contents were determined three times for each randomly selected leaf at five different plant parts and the average was used as the mean chlorophyll.

Statistical Analysis

Data were subjected to analysis of variance using SAS. Means were compared using least significant difference (LSD) method at P= 0.05.

Results

Chlorophyll Contents

The results indicated that the algal and the amino acid treatments significantly increased the chlorophyll a content of the plant, compared with the control treatment (0.34 mg/g); however there was not much difference among the algal and the

amino acid treatments affecting plant chlorophyll a content (Fig. 3 A). The least chlorophyll b content was resulted by the control (0.11 mg/g) and the A3 (0.13 mg/g) treatments, significantly different from the other treatments. However, J3 (0.16 mg/g), J2 (0.15 mg/g), and A2 (0.15 mg/g) resulted in the highest chlorophyll b content (Fig. 3 B). There was a significant effect of algal and amino acid treatments on the total chlorophyll content of the plant, compared with the control treatment (0.45 mg/g). The effects of the amino acid treatments (0.57-0.59 mg/g) were more evident on the total chlorophyll content of the plant than the algal treatments (0.53-0.57 mg/g) (Fig. 3 A).

Carotenoid Content

Although there was not much difference among the algal and amino acid treatments, on the content of carotenoid, treatments A1 (0.118 mg/g) and J1 (0.116 mg/g) resulted in the higher carotenoid content, significantly different from the other treatments. The least carotenoid content was resulted by the control treatment (0.102 mg/g) (Fig. 4 B).

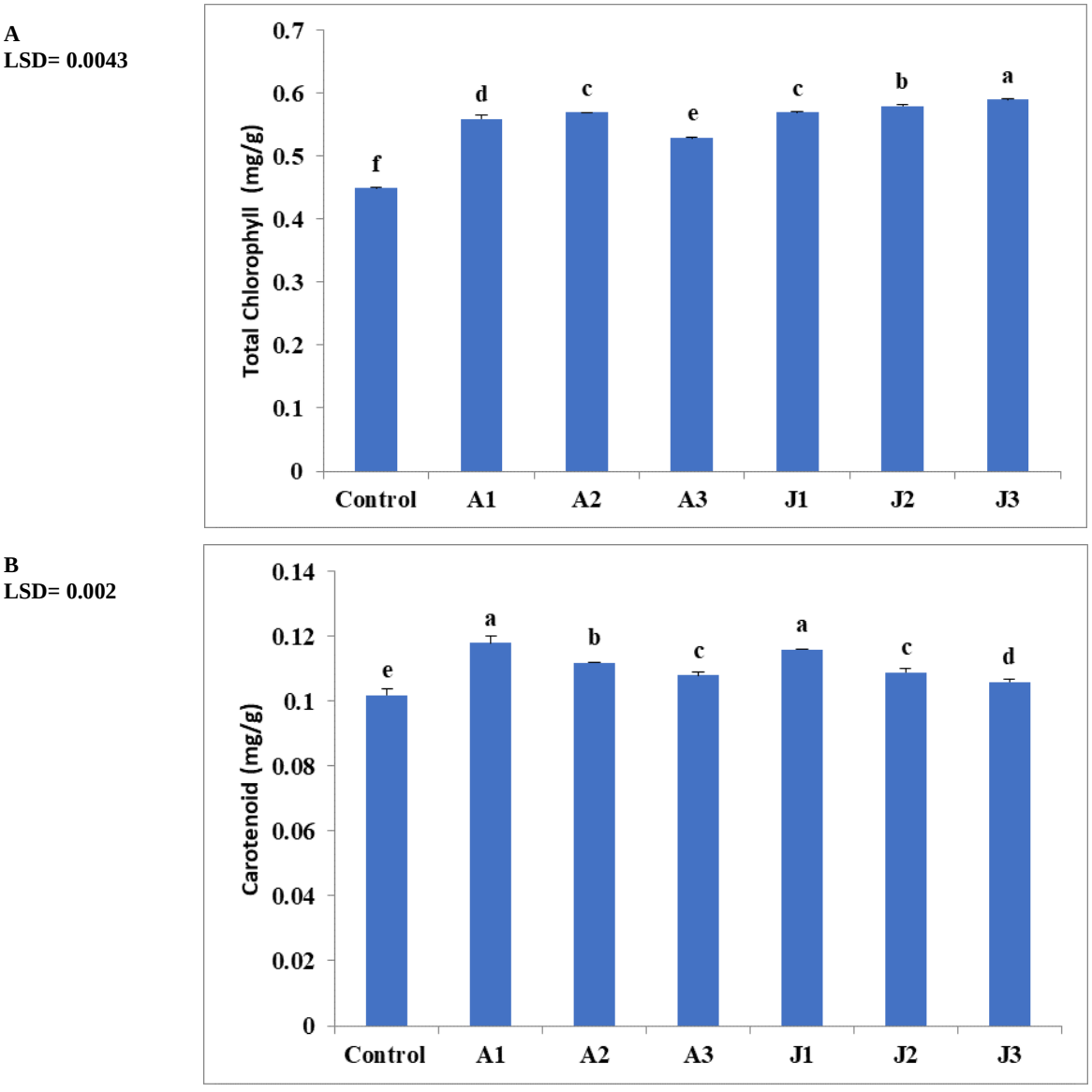


Fig. 4 Zarrin-giah total chlorophyll (A) and carotenoid contents (B) affected by different experimental treatments including control, seaweed extracts (Wuxal Ascofol, 1 (A1), 2 (A2), and 3 (A3) g/L) and algaton amino acid (1 (J1), 2 (J2), and 3 (J3) g/L). Columns followed by different letters are significantly different determined by LSD at P= 0.05. Columns are presented with their respective standard deviations.

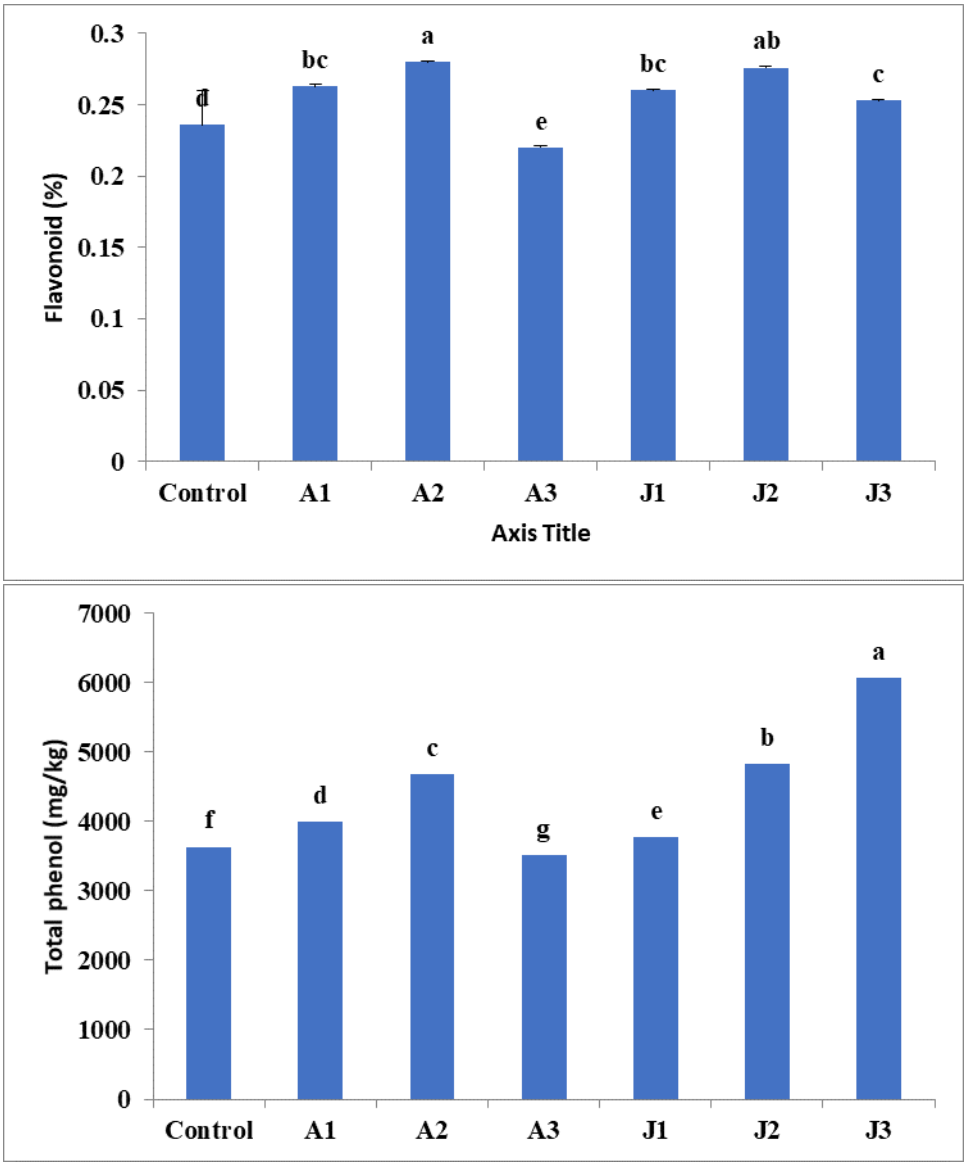


Fig. 5 Zarrin-giah flavonoid (A) and total phenol contents (B) affected by different experimental treatments including control, seaweed extracts (Wuxal Ascofol, 1 (A1), 2 (A2), and 3 (A3) g/L) and algaton amino acid (1 (J1), 2 (J2), and 3 (J3) g/L). Columns followed by different letters are significantly different determined by LSD at P= 0.05. Columns are presented with their respective standard deviations.

Flavonoid Content

According to the results, the highest flavonoid content was resulted by the A2 (0.28%) and the J2 (0.276%) treatments, significantly higher than the other treatments. Interestingly, the least flavonoid content was related to the A3 (0.22%) treatment, even significantly less than the control treatment (0.236%) (Fig. 5 A).

Total Phenol

The J3 treatment (6076.336 mg/kg) resulted in the highest content of total phenol, significantly higher than the other treatments. However, the least contents of the total phenol were related to the A3 (3510.556 mg/kg) and the control (3637.984 mg/kg) treatments (Fig. 5 B).

Antioxidant Activity

According to the results, the highest antioxidant activity was resulted by A3 (77.167%) significantly higher than the other treatments. However, A2 (58.974%) was the second most effective treatment and the least antioxidant activity was resulted by the control treatment (27.473%).

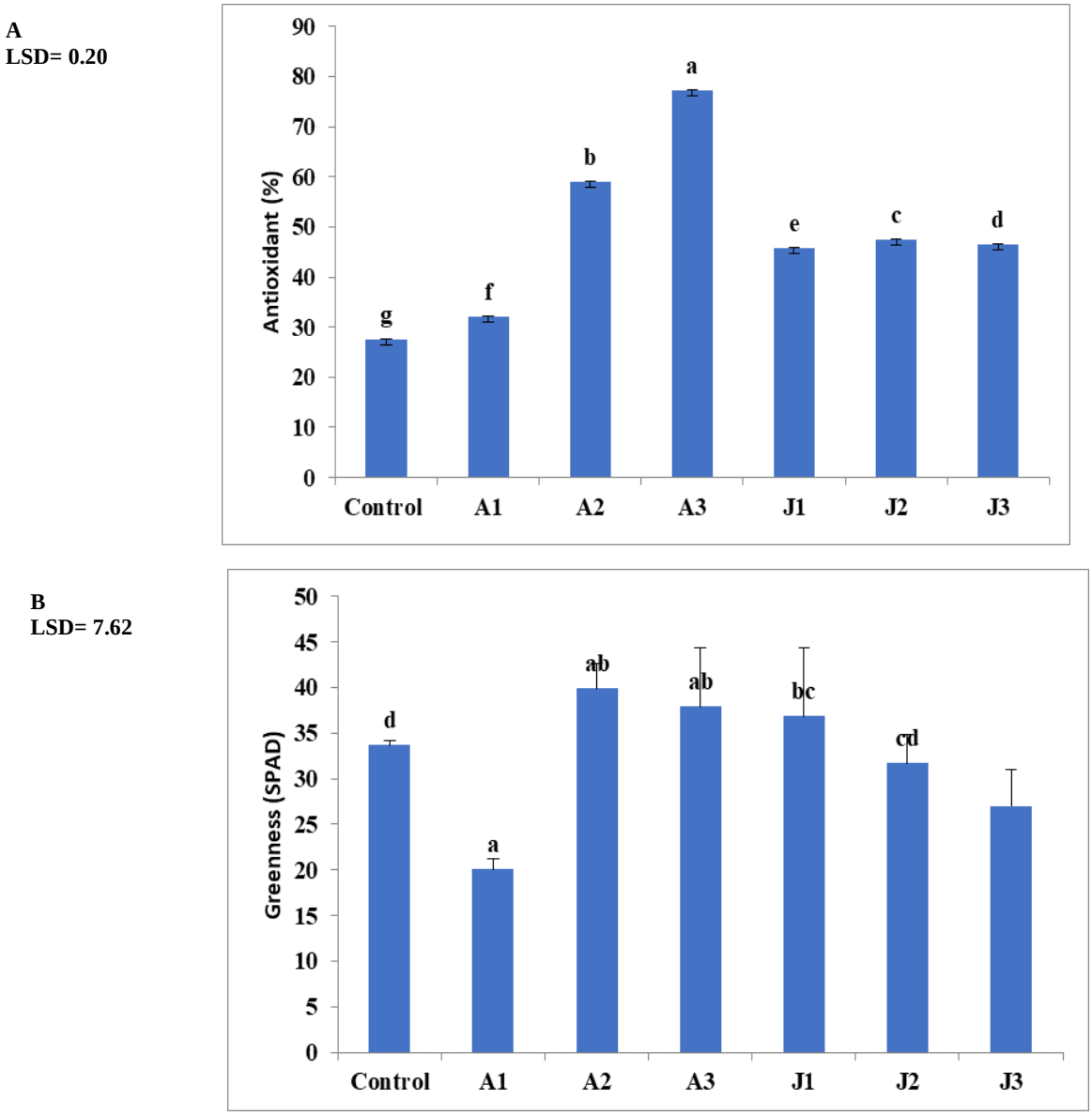


Fig. 6 Zarrin-giah antioxidant activity (A) and greenness (B) affected by different experimental treatments including control, seaweed extracts (Wuxal Ascofol, 1 (A1), 2 (A2), and 3 (A3) g/L) and algaton amino acid (1 (J1), 2 (J2), and 3 (J3) g/L). Columns followed by different letters are significantly different determined by LSD at P= 0.05. Columns are presented with their respective standard deviations.

Although the amino acid treatments also significantly increased the antioxidant activity, the effect of algal treatments was more pronounced on the increased activity of the antioxidants. The effects of the amino acid treatments were not much different compared with the effects of the algal treatments (Fig. 6 A).

Leaf Greenness (SPAD)

The highest greenness (SPAD) was related to the A2 treatment (39.933), significantly higher than the other treatments including J2 (31.733), J3 (27.033) and A1 (20.067). Interestingly, although A3, A2 and J1 resulted in the higher SPAD values than the control treatment, the difference was not significant (Fig. 6 B).

Discussion

The biochemical properties of medicinal plants, including Zarrin-giah, are considered as the determining factors affecting the quality of the plant for medical usage and health purposes. Accordingly, finding methods, which may enhance the quality of medicinal plants, is of significance. The presented research examined the effects of two different biostimulants including the seaweed

extract (Wuxal Ascofol) and the amino acid (algaton) on the biochemical properties of Zarrin-giah.

The results indicated the significant effects of the experimental treatments on the quality of Zarrin-giah. Although the two experimental treatments indicated effectiveness in the improvement of the biochemical properties of the plant, there were differences in the two treatments and in some cases the seaweed extract was more effective. Another interesting point about this research is that the highest level of algal extract (seaweed extract) negatively affected the quality of the plant, as in some cases even the quality of the plant was worse than the control treatment.

The beneficial effects of seaweed extracts on plant growth are due to the presence of: 1) plant hormones including cytokinin, gibberellins, brassinosteroids, strigolactones, and abscisic acid, 2) ammonium molecules such as betaine and proline, which can act as osmoregulators under drought and salinity stress, 3) polysaccharides and alginate affecting root growth, especially in the presence of soil microbes, plant systemic tolerance including the induction of stress plant genes, and 4) nutrients and trace elements enhancing plant growth and yield production [14,25]. The analysis of the two experimental treatments used for this research indicated their ingredients and how such treatments may enhance Zarrin-giah quality [12].

Accordingly, research has indicated the suitable effects of seaweed extracts on plant growth by establishing plant seedlings and controlling plant pathogens. The presence of fucans, ulvans and algiantes can also promote plant growth [25]. Seaweed extracts can also improve plant growth by supplying micro- and macro-nutrient for plant use [26].

According to the results seaweed extracts and amino acids favorably affected different biochemical properties of Zarrin-giah including the chlorophyll contents, carotenoid, flavonoid, phenol, antioxidant activity, and leaf greenness. This can undoubtedly contribute to the improved health properties of Zarrin-giah. Michalak *et al* [12] found that water is the most suitable solvent for the extraction of seaweed biochemical contents indicating their hydrophilic nature. The increased antioxidant activity of the plant is correlated with the increased content of the plant biochemical contents including chlorophyll, carotenoid, flavonoid and phenol. The enhancing effects of seaweed extracts on plant growth have been indicated by research [26].

Although the highest antioxidant activity was resulted by A3, the A2 treatment was the most effective treatment significantly enhancing the biochemical properties of Zarrin-giah. Higher concentrations of seaweed extracts may have negative effects on plant growth and biochemical properties. Latique *et al* [27] found that the less concentrations of seaweed extracts increased plant chlorophyll contents, which can be attributed to the increased activity of nitrate reductase affecting N metabolism and providing N required for chlorophyll production and plant growth. Another reason for the increased chlorophyll content in plant, in the presence of seaweed extracts, is the inhibition of chlorophyll degradation by glycine betaine in the extracts [27]. Similarly different research has indicated the presence of the biochemical contents examined in the presented research in the seaweed extracts. For example, Lola-Luz *et al* [28] found that the use of brown seaweed (*Ascophyllum nodosum*) extracts significantly enhanced the phenol and flavonoid contents of cabbage (*Brassica oleraceae*) under field conditions. Vasantharaja *et al* [29] indicated that foliar application of brown seaweed extracts significantly increased the phenolic and flavonoid contents as well as the antioxidant activity in cowpea (*Vigna unguiculata* L. Walp). Research has indicated the presence of carotenoids in seaweeds and their favorable effects on plant quality and human heath, which include their use for the treatment of cancer, obesity, diabetes, etc. [30]. Research has also indicated the positive effects of amino acids on the plant quality and growth, by enhancing the biochemical properties of the plant, however, it has been indicated that the seaweed extracts are more effective [31, 32]. The positive effects of the amino acid tested in the presented research are due to providing Zarrin-giah with N, C and organic carbon.

Conclusion

The enhancing effects of the two experimental treatments including seaweed extracts and amino acid on the biochemical properties of Zarrin-giah including chlorophyll contents, carotenoid, flavonoid and phenol contents, antioxidant activity and leaf greenness were determined. Although both treatments enhanced the quality of Zarrin-giah by

enhancing their biochemical properties, the seaweed extract was a more effective treatment. However, at higher concentrations the negative effects of the seaweed extract on the biochemical properties of Zarrin-giah were evident. It is possible to enhance the biochemical properties of Zarrin-giah using seaweed extracts and algaton amino acid if the proper concertation of the treatment is used. This can improve the health properties of the plant and make it more effective for the treatment of different diseases. The use of such treatments to treat the plant under stress is suggested for future research.

Conflict of Interest

The authors declare they do not have any conflict of interest.

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