Foliar Application of Marmarin on Antioxidant Activity and Storage Time of Garden Cress (*Lepidium sativum* L.)

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Summary

Two separate experiments were conducted to study the effects of algae extract ('Marmarin') foliar application on growth potential and storage life of garden cress. In the first experiment, the effect of 'Marmarin' foliar application on growth characteristics (leaf length and width, petiole length, leaves number, roots dry weight, root length and leaves dry weight) was determined. The results showed the positive effects of foliar spray on roots and leaves dry weight, root length, leaves number and petiole length. In the second experiment, the impact of foliar application of 'Marmarin' on harvested plants was assayed. The results showed significant effect of foliar spray treatment and storage time on chlorophyll b, total soluble solids and chlorotic leaves number. The highest total soluble solids were obtained by foliar application of 5 ml L^{-1} algae extract at harvest and at 4 days after storage, as well as with 10 ml L^{-1} foliar application at harvest time. Foliar application treatment with 10 mg L^{-1} at harvest and four days after storage had significant effects on chlorophyll b content. The highest chlorotic leaves number was determined without foliar application at 12 days after storage. Chlorophyll a, anthocyanin and total phenolics content were independently affected by foliar application and storage time. The highest amount of chlorophyll a was attained by foliar application of 5 and 10 ml L^{-1}. The highest amount of anthocyanin and total phenolics was determined at application of 5, 10 and 15 ml L^{-1}, and 10, 15 and 20 ml L^{-1}, respectively. The highest content of anthocyanin was determined at the harvest. Also, the highest contents of total phenolics and chlorophyll a were determined at the harvest and were statistically equal with amount determined at 4 days after harvest.

Key words

anthocyanin, plant growth characteristics, total phenolic content, total soluble solids

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Introduction

Garden cress (*Lepidium sativum* L.) belonging to *Cruciferae* is an edible and fast growing annual herb. It is recommended in the treatment of hypertension, diabetes and renal disease (Diwakar et al., 2010). Garden cress is one of the most complete and nutritional foods and provides proteins, carbohydrates, fiber, minerals, and vitamins. Although, traditionally garden cress has been considered to be antiscorbutic, depurative and a stimulant, it is also attractive food with connotations of freshness and lightness. It can be consumed as components of salads, soups, and sandwiches; adding texture, a pleasant appetizing flavor, and visual interest to the dishes (Conforti et al., 2009).

Nowadays, due to population growth, the interest in vegetable production greatly has increased. Intensive production systems, that warrant high yield and quality, require extensive use of chemical fertilizers. However, over-using these chemicals has the disadvantage of huge production casts and the environment pollution. There is an urgent need to the alternative methods to fulfill the nutritional demands of plants. Foliar application of nutrients is an easy and cheap way to meet these needs and furthermore, this method of fertilizers application has more efficiency than soil based application methods (Naeem et al., 2006). Nutrients applied to the foliage are generally absorbed more rapidly than when applied to the soil. Uses of bio-fertilizers containing beneficial microorganisms instead of synthetic chemicals are known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (Lola-Luz et al., 2014; Ramya et al., 2015). 'Marmarin' (brown marine algae that contains mineral elements, vitamins, amino acids, plant hormones and some other nutrient such as protein, mannitol and alginic acid; Table 1) is one of the important biological fertilizers used as foliar application. Beneficial effect of 'Marmarin' foliar application in mango growth and productivity was demonstrated by El-Sharony et al. (2015).

Nowdays is a growing interest in using plants with high antioxidant activity as natural antioxidants. Free radicals have one or more unpaired electrons are produced by cell metabolism. Reactive oxygen species (ROS) react easily with free molecules to become radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), as well as non-free radicals H_2O_2 and the singled oxygen (O_2). ROS can cause lipid peroxidation in foods, leading to their deterioration (Machu et al., 2015). The peroxidation products such as malondialdehyde and 4-hydroxyinonenal can react with biological substrates such as protein, amines and deoxyribonucleic acid. The absence of structural damages in the algae leads to consider that these organisms are able to generate the necessary compounds to protect themselves against oxidation (Conforti et al., 2009). In this respect, algae can be considered as an important source of antioxidant compounds that could be suitable also for protecting human bodies against the reactive oxygen species formed e.g., by human metabolism or induced by external factors (as pollution, stress, UV radiation, etc.). There are antioxidant substances of very different nature in algae, among which vitamin E (α-tocopherol) and carotenoids can be highlighted with in the fat-soluble fraction, whereas the most powerful water-soluble antioxidants found in algae are polyphenols, phyco-biliproteins and vitamins (vitamin C) (Plaza et al., 2008). As a result of this, considerable attention has been focused on the use of antioxidant compounds, especially natural antioxidants, to inhibit lipid peroxidation and to protect from damage by free radicals. There is little information available on the effect of bio-organic application as foliar treatment on garden cress. Therefore, the aim of this investigation was to evaluate the effect of 'Marmarin' foliar application on some physiological traits, morphological traits and antioxidant activity of garden cress during harvest and postharvest time.

Material and methods

This work was conducted at the research greenhouse of Azarbaijan Shahid Madani University, Tabriz, Iran. Seeds of *Lepidium sativum* were sown in plastic pots (5 L) filled with soil (soil characteristics: pH 7.9; EC 1.89 dS m^{-1}; organic matter 0.5%; 6% N; 51 mg P kg^{-1}; 374 mg K kg^{-1}, and sandy loam soil texture). The plants were treated with five levels (0, 5, 10, 15 and 20 ml L^{-1}) of 'Marmarin' (produced by Hasel Novin(20 days after planting. 'Marmarin' solution for the treatments was freshly prepared before spraying. The second treatment was applied 15 days later. After three weeks, the plants herbage was harvested by cutting over the soil surface and plant growth characteristics (plant dry weight, leaf petiole length, leaf length, leaf width, root length and leaf number) were determined.

In the second experiment, the impact of foliar application of 'Marmarin' on harvested plants was assayed. Harvested plants were kept in disposable containers in the refrigerator (at 4°C) for 4, 8 and 12 days. After storage period some post-harvest traits such as the number of chlorotic leaves, antioxidant activity, chlorophyll content, total soluble solids content, total phenolic, and anthocyanin contents were determined.

For; experimental design was Completely Randomized Design was used for the first experiment and factorial based Randomized Complete Block Design with three replications was used for the second experiment. The data obtained were subjected to standard analysis of variance. The values of LSD were calculated at 1 and 5% level of significance.

Table 1. The analysis of 'Marmarin' used in the experiment

Parameter test	Report level
Total Protein	7.13%
Total Fat	1.99%
Vitamin A	19 μg kg^{-1}
B1, B2, B3, B6 and B12	4.5 - 7 μg kg^{-1}
E	100 μg kg^{-1}
K	2 μg kg^{-1}
Gibeerilins	5.89 μg kg^{-1}
Auxin	12.7 μg kg^{-1}
Cytokinin	1.97 μg kg^{-1}
Mineral elements	varied depending on the element

Total chlorophyll content

Chlorophyll content was calculated according to Arnon (1949). Chlorophyll content was determined in acetone extract after centrifugation. The absorbance was read spectrophotometrically at 663 and 645 nm.

Antioxidant Activity

In this study, antioxidant activity was measured according to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH). First, 0.5 mM of DPPH was prepared in methanol (control solution). Then, control solution (1 mL) was added to 3 mL of the solution of each sample.

The samples were put in a dark place at room temperature for 30 minutes. The amount of absorbance was measured at 517 nm using a spectrophotometer. Antioxidant compounds were evaluated in different concentration of samples to obtain the amount of IC_{50} (Zhang and Hamauzu, 2004). The percentage of inhibition was calculated by the following formula where A_0 was the absorbance of the control reaction and A1 was the absorbance of the standard sample:

Percent of inhibition was calculated by the following formula:

$$\text{Percent inhibition} = A_0 - A_1 / A_0 \times 100$$

Total phenolic content

Total phenolics content (TPC) was determined using the Folin-Ciocalteu method. The phenolic content was expressed as mg of gallic acid equivalent per gram of dry sample (mg GA g^{-1}) using the linear equation based on the calibration curve (Kim et al., 2006).

Total anthocyanin content

Total anthocyanins were determined in fresh leaf (0.5 g). Leaves were homogenized in acidified (HCl) methanol. The absorption of anthocyanins at 550 nm was measured by a spectrophotometer according to Wagner (1979).

Total soluble solids content (°Brix)

Filtered supernatant juice was used for determination of TSS by a digital Refractometer (Erma, Tokyo, Japan).

Results and discussion

Morphological characteristics of plant

The highest leaf number was recorded in 5 and 10 ml L^{-1} 'Marmarin' foliar applications (Table 2 and 3). As the results shows, with increasing 'Marmarin' concentrations the leaf number per plant was reduced (Table 3). The results of this experiment are in agreement with the results obtained by Zodape et al. (2011) in tomato. The increase in leaf number leads to yield adding up in leafy plants. It seems that, foliar application of brown-algae extract has positive effect on growth parameters mainly due to the fact that the extract is rich in vitamins, micro and macroelements, and growth promoting hormones (auxin, cytokine and GA_3). Cytokinins have great role in cell division and also stimulate the mobilization of assimilates towards the sink centers. In that way they promote growth. Furthermore, cytokinins delay the leaf senescence in leafy vegetables (Nour et al., 2010).

The highest leaf width was observed at 5 (1.9 cm) and 10 (2.3 cm) ml L^{-1} 'Marmarin' application and the lowest leaf width was recorded at 20 ml L^{-1} (1.0 cm), (Tables 2 and 3). The results of this experiment are in agreement with the finding of Sivasangari et al. (2015) in *Solanum melongena*. El-Sharony et al. (2015) in mango tree showed that foliar application of algae and plant extract increased all growth parameters (shoot length, shoot thickness and average leaf area).

Data presented in Table 2 and 3 indicate that 'Marmarin' foliar application had significant effect on leaf petiole length. The results showed that 15 mL L^{-1} 'Marmarin' had positive effect (12.1cm) on leaf petiole length. A study on tomato revealed that using red algae extract (Kappaphycus extract) as foliar spray at suitable concentration improved plant height (Zodape et al., 2011). This might be due to the presence of macro and micro nutrients as well as growth promoting substances like auxins and cytokinin in 'Marmarin'. The results showed that all treatments had positive effects on root length except in control plants (Table 3).

Foliar application of 5 and 10 ml L^{-1} 'Marmarin' increased garden cress leaf dry weight to 2.3 and 3 g, respectively (Table 3). As Table 3 shows, high concentration of 'Marmarin' had negative effect on leaf dry weight. The results of this experiment are in agreement with the finding of Ramya et al. (2015) in *Solanum melongena* plants. They have noted that low concentration of algae extract more efficiently influenced plant yield and performance. Khan et al. (2009) reported that algae extract increased the plant yield by increase in chlorophyll content of the leaves. Increased leaf number leads to the corresponding add up in net photosynthesis rate and hence raises yield. It seems that increased yield may be attributed to the hormonal content of the extract as well; the main part is due to its

Table 2. ANOVA for the effect of 'Marmarin' foliar application on some growth characteristics of *Lepidium sativum* L

Source of variation	df	Leaf length	Leaf width	Leaf petiole length	Leaf number	Root length	Root dry weight	Leaf dry weight
Replication	2	0.9 ns	0.06 ns	5.92 ns	0.36 ns	14.1 *	0.005 ns	0.008 ns
'Marmarin' foliar application	4	1.99 ns	0.35 **	14.9 **	8.29 **	8.3 **	0.04 **	1.80 **
Error	8	0.59	0.08	1.5	1.05	0.94	0.003	0.06
Coefficient of Variation (%)		23.9	16.1	14.2	14.9	13.10	18.07	13.15

ns, *, and ** show no significant and significant at P≤0.05 and P≤0.01, respectively.

Table 3. The effect of 'Marmarin' foliar application on some growth characteristics of *Lepidium sativum* L

'Marmarin' foliar application	Root dry weight (g)	Leaf dry weight (g)	Root length (cm)	Leaf number per plant	Leaf petiole length (cm)	Leaf width (cm)
0	0.2 bc	0.9 c	4.9 b	5.6 b	7.1 c	1.4 c
5	0.32 a	1.63 bc	8.0 a	7.2 ab	9.4 b	1.9 ab
10	0.38 a	2.34 ab	9.1 a	9.5 a	6.9 c	2.3 a
15	0.39 a	3.0 a	8.4 a	6.3 b	12.1 a	1.7 b
20	0.13 c	1.6 bc	6.6 ab	5.5 b	7.2 c	1 d
LSD 1%	0.15	0.69	2.6	2.8	3.3	0.55

Similar letters in the columns are non-significant based on LSD test.

Table 4. ANOVA for the effects of 'Marmarin' foliar application and storage time on some physiological characteristics of *Lepidium sativum* L.

Source of variation	df	Chlorophyll b	Chlorophyll a	Anthocyanin content	Total phenolic content	TSS	Number of chlorotic leaf	IC50
Replication	2	0.02 ns	0.005 ns	2.3 **	82.2 **	0.13 ns	10.11 ns	0.03 ns
'Marmarin' foliar application	4	0.49 **	0.41 **	3.4 **	757.3 **	0.88 **	1848.0**	0.12 **
Storage time	3	2.1 **	2.67 **	27.1 **	21.3 **	6.08 **	2441.8 **	0.05 ns
'Marmarin' foliar application × Storage time	12	0.05 **	0.04 ns	0.31 ns	45.7 ns	0.16 *	432.1 **	0.07 ns
Error	38	0.01	0.02	0.17	83.3	0.06	7.4	0.02
Coefficient of Variation (%)		11.9	12.7	9.4	11.8	14.3	20.6	8.6

ns,* and ** show non-significant and significant at P≤0.05 and P≤0.01, respectively

highest cytokinin content. Cytokinins take part more in the nutrient mobilization and translocation, but, during the reproductive growth period, they have major role in fruit set and fruit growth and development (Nour et al., 2010).

The lowest root dry weight was recorded in control (0.2 g) and 20 ml L^{-1} (0.13 g) 'Marmarin' foliar applications (Table 3). Turan and Kose (2004) reported that in grapevine, algae extract increased the root growth due to sufficiency of Cu absorption by plant.

Chlorophylls content

Results are showing that there was a significant interaction effect of 'Marmarin' foliar application and storage time on chlorophyll b content (Table 4). The highest amount of chlorophyll b content was recorded for 10 ml L^{-1} 'Marmarin' at harvest and 4 days after storage (Table 5). Results revealed the individual effects of foliar application of 'Marmarin' and storage time on chlorophyll a content of *Lepidium sativum* L. (Table 4). Chlorophyll a content was influenced by 5 and 10 mL L^{-1} 'Marmarin' concentration (Table 5). The highest amount of chlorophyll a content was recorded at harvest (1.7 mg g^{-1} FWt) and 4 days after storage (1.6 mg g^{-1} FWt). Our findings are in line with the finding of Whapham et al. (1993) in cucumber. The positive impact of algae extract on chlorophyll content may be because of the content of Fe in extract. This microelement is a structural part of cytochromes and holdes a vital role in oxidation and reduction, and chlorphyll biosynthesis (Rezaei and Afiauni, 2000).

Total Soluble Solids (TSS)

The present study clearly showed that TSS content was influenced by the interaction of 'Marmarin' foliar application and storage time (P≤5%), (Table 3). TSS content was increased by 5 ml L^{-1} at harvest and 4 days after storage and 10 mL^{-1} at harvest time (Table 5). The results are in agreement with those obtained by El-Sharony et al. (2015) in mango plant. A research done by Noguchi and Niki (2000) revealed that 20% algae extract foliar application increased TSS content compared to control plants. They noted that TSS content in plant is depended upon the ions translocation and organic solutes movements that finally affect the sugars especially glucose content. They stated that TSS content of plants was dependent upon the iron transport and the amount of organic solutes that are converted to glucose.

Table 5. Effects of 'Marmarin' foliar application and storage time on some physiological characteristics of *Lepidium sativum* L.

'Marmarin' foliar applications level (ml L^{-1})	Storage time (Days)	TSS (^{0} Brix)	Chlorophyll b (mg g^{-1} FWt)	Chlorotic leaves number
0	0	2.1 bcd	1.1 cd	0 g
0	4	1.7 def	0.8 efg	24.6 c
0	8	1.1 ghi	0.5 hij	36.6 b
0	12	0.74 i	0.3 j	79.3 a
5	0	2.8 a	1.5 b	0 g
5	4	2.7 a	1.2 c	3 fg
5	8	1.43 efg	0.82 efg	8.6 def
5	12	0.8 hi	0.49 ij	12.3 d
10	0	2.5 ab	1.8 a	0 g
10	4	2.03 cd	1.7 a	3 fg
10	8	1.8 cde	0.98 de	12 d
10	12	1.2 gh	0.68 ghi	14.33 d
15	0	1.83 cde	1.5 b	0 g
15	4	1.7 cde	1.28 c	3.3 efg
15	8	1.4 efg	0.97 de	9.6 de
15	12	1.4 efg	0.68 ghi	13.3 d
20	0	1.8 cde	1.1 cd	0 g
20	4	1.7 def	0.9 ef	4.3 efg
20	8	1.3 fg	0.7 fghi	11 d
20	12	0.8 hi	0.74 fghi	30 c
LSD 1%		0.44	0.195	6.05

Similar letters in the column are non-significant based on LSD test

Table 6. Effects of 'Marmarin' foliar application on some physiological characteristics of *Lepidium sativum* L.

'Marmarin' foliar application (mL L^{-1})	Chlorophyll a (mg g^{-1} FWt)	Anthocyanin content (µg g^{-1} FWt)	Total phenolic content (mg GA g^{-1} DWt)	IC50 (mg mL^{-1})
0	1.0 c	3.0 c	65.8 c	2.0 b
5	1.3 ab	4.6 a	74.2 bc	2.7 a
10	1.5 a	4.5 ab	77.4 ab	1.8 c
15	1.2 b	4.8 a	85.9 a	1.5 d
20	1.1 bc	3.7 b	83.4 ab	1.8 c
LSD 1%	0.17	0.45	10.1	7.6

Similar letters in the column are non-significant based on LSD test

Table 7. Effects of storage time on some physiological characteristics of *Lepidium sativum* L.

Storage time (Days)	Chlorophyll a (mg g^{-1} FWt)	Anthocyanin content (µg g^{-1} FWt)	Total phenolic content (mg GA g^{-1} DWt)
0	1.7 a	5.9 a	90.4 a
4	1.6 a	4.8 b	82.7 a
8	1.0 c	3.9 c	73. 3b
12	0.7 d	2.7 d	60.8 c
LSD 1%	0.15	0.4	9.0

Similar letters in the column are non-significant based on LSD test

Chlorotic leaf percentage

ANOVA (Table 4) showed that the chlorotic leaf percentage was also affected by the interaction effects of 'Marmarin' foliar application and storage time; with the highest percentage (79.3) in control plants at 12 days after storage (Table 5). The results showed that in all treatments 'Marmarin' foliar application had no-significant effect on chlorotic leaf number at harvest. So, the conclusion is that 'Marmarin' foliar application had positive effects on storage time (Table 5). Beneficial effects observed in this study might be due to the possible effects of mineral elements (such as zinc, manganese, and copper), as well as, vitamins, amino acids and pseudo- hormons contained in 'Marmarin' extract (Sutharsan et al., 2014). The same idea has been reported by El-Yazied et al. (2012). Nour et al. (2010) reported that algae extract foliar application raised the N, P and K, as well as, protein content of plants, which eventually improved the yield of plants. The most possible reason for the prolonged green life of the leaves treated with algal extract may be because of the increased nutrient content and even elevated cytokinins content. More cytoknin biosynthesis and accumulation is possibly the major reason that inhibits chlorophyll breakdown.

Antioxidant activity

The lowest IC_{50} (1.5 mg mL^{-1}) was obtained by 15 ml L^{-1} 'Marmarin' foliar application (Table 6). The results from Table 6 also show that the highest amounts of anthocyanin and phenolics were obtained by 15 ml L^{-1} 'Marmarin' foliar application, which confirmed the high antioxidant effect in this treatment. Novoa et al. (2001) reported that the seaweed lyophilized extract contains 8.08 mg g^{-1} of total polyphenols and can prevent thiobarbituric acid reactive substances formation during spontaneous lipoperoxidation of rat brain homogenates with IC_{50} of 23.3 µg mL^{-1}. Nagai and Ukimoto (2003) reported that marine algae were considered to be a rich source of antioxidants. They also reported that algae inhibitions were stronger than vitamin C and E. The result from this experiment is in agreement with the finding of Khan et al. (2009) on the increased antioxidant capacity of *Phaseolus vulgar*is plants under seaweed application.

Total phenolic content

Total phenolic content was independently affected by 'Marmarin' foliar application and storage time (Table 6). The results obtained revealed that phenolic content was increased with increased 'Marmarin' concentration. The highest amount of total phenolic content was recorded at harvest (90.4 mg g^{-1} DWt) and four days after storage (82.7 mg g^{-1} DWt). Using algae extract as foliar spray is a useful method to increase quality of vegetables and also the contents of phenolics (Kocira et al., 2016). The research conducted by Lola-Luz et al. (2014) in broccoli plants showed that total phenolic and flavonoids contents were higher in all seaweed treatments compared to control plants. The reason for the increased phenolics content behind algal treatments is the elevated biosynthesis of intermediate compounds by chalcone isomerase enzyme.

Total anthocyanin content

Result showed that foliar application of 'Marmarin' and storage time independently affected anthocyanin content (Table 5). The results showed that with increasing 'Marmarin' concentration to 15 ml L^{-1}, anthocyanin content was increased, but high concentration of 'Marmarin' (20 ml L^{-1}), had negative effects on anthocyanin content (Table 6). Also, anthocyanin content was influenced by the storage time. The highest concentration of anthocyanin was recorded at harvest (5.9 µg g^{-1} FWt), and the lowest 12 days after storage (2.7 µg g^{-1} FWt) (Table 7). The results are in line with the findings of Khan et al. (2009) considering the anthocyanin content increase in *Phaseolus vulgar*is in response to algae extract application.

Conclusion

The positive effects of algae-extract 'Marmarin' on vegetative growth and some physiological effects of garden cress with the present experiment was mainly due to the compositional content of the extract for mineral nutrients (N, P, K, Ca, Mg and microelements), vitamins, growth promoters, polyamins, vitamin C, and anthocyanins. Since garden cress is a sensitive leafy vegetables and its postharvest storage time is quite short (the leaves get chlorotic soon after harvest), it seems that foliar application of algae extract would be simple and cheap method to increase the storage time and the quality of this high valued crop. This method can enhance the production systems, pioneer producers and storage facilitates.

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