JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(1), 15-30

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Enhancing mineral uptake and antioxidant enzymes activity of kiwifruit via foliar application of brown macroalga extract

Mahshid Ghafouri[1], Farhang Razavi[1]*, Masoud Arghavani[1] and Ebrahim Abedi Gheshlaghi[2]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[2]Department of Horticulture Crops Research, Guilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran

ARTICLE INFO

Original Article

Article history:
Received 4 November 2023
Revised 15 January 2024
Accepted 17 January 2024
Available online 18 February 2024

Keywords:
Catalase
Hydrogen peroxide
Malondialdehyde
Preharvest treatment
Superoxide dismutase

DOI: 10.22077/jhpr.2024.6926.1343
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

Email: razavi.farhang@znu.ac.ir

ABSTRACT

Purpose: The application of natural organic compounds without harmful environmental effects in the production of horticultural and agricultural products is considered as a new method to reduce waste before and after harvesting, increase the storage life and maintain antioxidant activity in developed agriculture. As regards, this study investigated the impact of foliar applications of brown macroalga extract on antioxidant enzyme activity and mineral uptake in 'Hayward' kiwifruits. **Research method**: Four treatment levels of brown macroalga extract (0, 1, 2, and 3 g/L) were applied at three distinct phases, occurring 110, 125, and 140 days after full bloom. Fruit samples were stored at 1±0.5°C with 95% relative humidity for 90 days, with measurements taken every 30 days. **Findings:** Results revealed that the 3 g/L extract treatment significantly increased the uptake of calcium (47.82 %), nitrogen (20.52 %), potassium (12.06 %), phosphorus (19.81 %), and iron (25.77 %) compared to the control. The extract demonstrated a substantial effect on all recorded traits. Among the applied treatments, 3 g/L of brown macroalga extract concentration had the best effect in reducing electrolyte leakage (25.10%), malondialdehyde accumulation (96.73%), hydrogen peroxide content (54.54%) and increasing activities of antioxidant enzymes including superoxide dismutase (50.42%), catalase (84.90%), ascorbate peroxidase (79.02%), and peroxidase (49.40%) compared to the control in 90 days of storage. **Research limitations:** No limitations were found. **Originality/Value:** The results suggest that the 3 g/L brown macroalga extract concentration holds promise for enhancing the quality of 'Hayward' kiwifruits.

INTRODUCTION

Kiwifruits (*Actinidia deliciosa*) are revered for their significant antioxidant content, boasting compounds such as ascorbic acid, carotenoids, and flavonoids. Their nutritional richness and potential health benefits, including anticancer properties due to their high vitamin C and mineral content, have earned them a well-deserved place in the realm of healthy dietary choices (Cassano et al., 2006).

The global production of kiwifruits, as reported by FAO (2021), now exceeds 4 million tons, with Iran ranking fifth, contributing approximately 289,000 tons. As agriculture worldwide embraces organic and sustainable farming, the reduction in chemical fertilizers and synthetic inputs becomes a pivotal goal. In pursuit of this eco-conscious approach, the use of bio stimulants, particularly seaweed extracts, has emerged as a promising avenue to enhance crop growth and development. An increasingly organic-driven agricultural sector has unfolded, fueled by the remarkable rise in global organic food consumption over the past decade (Rana & Paul, 2017).

Seaweeds, brimming with growth stimulators, vitamins, antioxidants, organic acids, organic nitrogen, phosphorous, and potassium compounds, have gained attention as a source of plant hormones derived from *Ascophyllum*. Notably, pre-harvest applications of nutrient solutions, like seaweed extracts, have demonstrated an ability to bolster both the quantity and quality of horticultural crops. Such interventions can also enhance post-harvest storage properties and marketability (Keyrouz et al., 2011).

A compelling body of evidence indicates that liquid seaweed extracts wield a positive influence on crop yield and quality across various species. Mandarins, oranges, strawberries, grapes, apples, melons, and other fruits have all exhibited improved productivity and quality with the application of these extracts. These outcomes are attributed to the presence of cell metabolism-enhancing compounds, including plant growth regulators such as auxin, gibberellin, cytokinin, as well as organic osmolytes like betaine, amino acids, minerals, polysaccharides, and vitamins (Zodape et al., 2001; Fornes et al., 2002, Masny et al., 2004, Geny et al., 2007; Lai et al., 2007, Abdel-Mawgoud et al., 2010).

Furthermore, the study of commercial brown macroalgae extracts has demonstrated their potential in enhancing the storage and nutritional quality of vegetables such as spinach. The application of these extracts not only boosts plant tolerance to various environmental stresses but also augments the activity of antioxidant enzymes, fortifying the plants' defense mechanisms against diseases (Jayaraman et al., 2011). Nikoogoftar-Sedghi et al. (2023) showed that foliar application of *Ascophyllum nodosum* (L.) seaweed extract in pistachio enhanced carbohydrate, protein, total phenol and flavonoid levels, and improved antioxidant enzymes activity.

A study by Rathore et al. (2008) further exemplified the benefits of seaweed applications, revealing an increase in nutrient uptake by rain-fed soybeans, particularly with regard to calcium. This heightened calcium content is integral in enhancing cell wall resistance in plants and fruits, further emphasizing the agricultural advantages of seaweed extracts. Building upon this background, our research embarks on an exploration of the effects of foliar applications of brown macroalgae extract to kiwifruit vines, 'Hayward' cultivar. We investigate how this application influences nutrient uptake and the activity of antioxidant enzymes in kiwifruits during post-harvest cold storage.

MATERIALS AND METHODS

Plant material and experimental setup

Ten-year-old kiwifruit vines (*Actinidia deliciosa* cv. 'Hayward') cultivated in a commercial vineyard located in Astara County, Iran, were selected for this study. The experiment adopted a factorial design based on a randomized complete block format, encompassing three replicates. The first factor pertained to the application of brown macroalga extract at varying concentrations: 0, 1, 2, and 3 g/L. The second factor involved storage duration with four levels: 0, 30, 60, and 90 days' post-storage. The foliar application of the brown macroalga extract was conducted at three distinct phases, occurring 110, 125, and 140 days after full bloom. Ripe fruits were collected, and subsequent assessments were carried out in the Postharvest Physiology Laboratory of the Department of Horticulture at the University of Zanjan. The fruits were subjected to cold storage, maintaining a temperature of 1±0.5°C and 95% relative humidity for a span of 90 days. Evaluations were performed at 30, 60, and 90 days' post-storage. Also, to replicate conventional shelf-life conditions, the fruits were stored at 25°C for 72 hours before the measurement of the traits.

Measurement of traits

Extraction of nutrients

To extract and quantify nutrients, plant samples were subjected to drying before analysis. After weighing, the fruits were sliced into 20-mm sections, encompassing both flesh and skin. These slices were subsequently oven-dried at 75°C for 72 hours, followed by grinding. The concentrations of various nutrients were analyzed separately. The dried kiwifruit samples were powdered using a Chinese mortar. Subsequently, 1 gram of the powdered sample was placed into a porcelain crucible and incinerated at temperatures ranging from 500-550°C for 5-6 hours. The resultant ash was rinsed with 11 mL of 2N hydrochloric acid until the digestion process was completed. Following this, they were transferred to a 100 mL beaker, heated for 5-10 minutes to induce a change in solution color, and subsequently filtered with filter paper. The volume was adjusted to 100 mL using distilled water (Westerman, 1990).

Nutrient measurement methods

The nitrogen content of the powdered samples was determined through the Kjeldahl method. Calcium and potassium levels were measured using a complexometric method and a flame photometer, respectively. Phosphorus content was determined through the molybdenum vanadate method and the application of a yellow reagent. Iron, manganese, and zinc levels were quantified using an atomic absorption device (Westerman, 1990). Selenium accumulation was determined in treated kiwifruits with various concentrations of brown macroalga extract by the method outlined by Zasoski and Burau (1977). All data were expressed in mg kg^{-1} dry weight.

Measurement of oxidative stress and antioxidant enzymes

Malondialdehyde (MDA)

The concentration of malondialdehyde, a marker of membrane peroxidation, was assessed following the procedure described by Heath and Packer (1968). In this method, 1 gram of fresh tissue was homogenized with 5 mL of 1% trichloroacetic acid (TCA). To this mixture, 4 mL of TCA (20%) containing 0.5% thiobarbituric acid was added to 1 mL of the supernatant. The mixture was then heated to 95°C, cooled, and the MDA concentration was determined by measuring the absorbance at 532 nm and 600 nm, utilizing an extinction coefficient of 155 $mmol^{-1}cm^{-1}$.

Electrolyte leakage (EL)

Electrolyte leakage was estimated according to the method proposed by Lim et al. (1998) and calculated using the formula: "EL (%) = (Final EL - Initial EL) / Initial EL × 100."

Hydrogen peroxide (H_2O_2)

The measurement of H_2O_2 content involved homogenizing 1 gram of fruit tissue, including the skin, with 5 mL of 1% TCA in an ice bath. The mixture was centrifuged, and 500 µL of the supernatant was combined with 500 µL of 10 mM potassium phosphate buffer (pH = 7) and 1 mL of 1 M potassium iodide (KI). The absorbance was recorded at 765 nm, and the H_2O_2 content was calculated from a standard curve and reported in nM/g fresh weight (FW) (Alexieva et al., 2001).

Antioxidant enzymes

To determine the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), 1 gram of frozen kiwifruit tissue was homogenized with 50 mM phosphate buffer (pH = 7) containing 2% PVP. The resulting extract was centrifuged at 12,000 × g at 4°C for 20 minutes, and the supernatant was employed as an enzymatic extract (Zhang et al., 2013). Enzyme activity was measured at specific wavelengths using a spectrophotometer, and specific enzyme activities were calculated in units per gram of fresh weight (U/g FW).

Experimental design and data analysis

The experimental design involved a factorial arrangement within a randomized complete block design with three replications, with each replication containing one vine. The various concentrations of nutrients were analyzed as completely randomized blocks, while the biochemical traits and antioxidant enzyme activities were treated as a factorial. Data were analyzed using the SPSS software (ver. 20). Statistical comparisons were conducted through Duncan's multiple range tests at a significance level of $P < 0.05$. Graphs and tables were generated using Excel (ver. 2016).

RESULTS AND DISCUSSION

Our analysis of variance (ANOVA) revealed significant effects of foliar application of brown macroalga extract in reducing EL, MDA and H_2O_2 accumulation and increasing activities of antioxidant enzymes including SOD, CAT, APX and POD at the $P<0.01$ level. The application had a profound impact on the uptake of nitrogen (N), potassium (K), and iron (Fe), exhibiting statistical significance at the $P < 0.01$ level. Additionally, it had significant effects, albeit at the $P < 0.05$ level, on the uptake of calcium (Ca) and phosphorus (P). In contrast, its effect was not statistically significant concerning the uptake of selenium (Se), magnesium (Mg), zinc (Zn), and manganese (Mn) (Table 1 and 2).

Table 1. Analysis of variance (ANOVA) for the effect of macroalga (*Ascophylum nodosum*) extract on nutrient contents of kiwifruits at harvest time.

Sources of variation	df	Mean of squares								
		Se	N	P	K	Ca	Mg	Fe	Zn	Mn
Block	2	2.5005	2737.583	1525	2708.333	700	75	0.32	0.006	0.481
Concentration of brown algae	3	0.001^{ns}	437589.639**	2866.667*	235719.444**	1800*	163.889^{ns}	1.001**	0.381^{ns}	1.159^{ns}
Error	30	0.001	6878.139	1291.667	5919.444	500	130.556	0.084	0.256	0.633
C.V (%)		24.32	1.73	9.45	2.07	8.60	8.96	7.33	18.45	29.35

**, *, ns: significant at the 1% and 5 % of probability level and non-significant.

Table 2. Analysis of variance (ANOVA) for the effect of macroalga (*Ascophylum nodosum*) extract on evaluated characteristics of kiwifruit cv. 'Hayward'during storage period.

Sources of variation	df	Mean of squares						
		EL	MDH	H_2O_2	CAT	POD	APX	SOD
Block	2	0.682	0.008	0.100	0.150	2.543	33.90	0.658
Concentration of brown algae (CBA)	3	136.23**	5.749**	0.51**	18.91**	362.33**	452.27**	10012.52**
Storage time (ST)	3	914.05**	6.023**	0.548**	66.585**	1439.85**	1456.13**	8281.02**
CBA × ST	9	3.58**	0.815**	0.136**	0.958**	27.47**	102.82^{*8}	171.21**
Error	30	1.077	0.011	0.001	0.158	3.25	10.9	1.929
C.V (%)		4.48	4.90	2.37	7.47	5.95	11.47	0.670

**, *, ns: significant at the 1% and 5 % of probability level and non-significant.

Effect of brown macroalga extract on nutrient contents

The comparison of means demonstrated the significance of foliar application of brown macroalga extract on the Ca content. The highest Ca content (340 mg/kg DW) was achieved with the application of 3 g/L of the brown macroalga extract, while the lowest (230 mg/kg DW) was observed in the control group (Fig. 1a). There was a clear positive relationship between Ca content and extract concentration, with higher extract rates resulting in greater Ca content.

Similarly, the K content in the fruit tissue exhibited an increase with higher extract rates. The application of the macroalga extract at a rate of 3 g/L led to the highest K content (3936.66 mg/kg DW), while the control group showed the lowest K content (3306.66 mg/kg DW) (Fig. 1b). Although different extract rates did not display significant differences, the effect was statistically significant ($P < 0.01$) compared to non-use.

The analysis of data revealed a rising trend in P content within the fruit tissue with increasing brown macroalga extract rates. The various extract treatments displayed significant differences in P content, with the highest and lowest P contents (423.33 and 353.33 mg/kg DW) observed at the 3 g/L extract rate and in the control group, respectively (Fig. 1c).

The positive effect of the extract on N content in the fruit tissue was evident through the increasing trend observed. Significant differences were identified among the various brown macroalga extract treatments. The highest N content (5109.66 mg/kg DW) was recorded in plants treated with 3 g/L of the brown macroalga extract, while the untreated plants had the lowest content (4239 mg/kg DW) (Fig. 1d).

The results also revealed an increasing trend in Fe content within the vines subjected to the foliar application of brown macroalga extract. These treatments showed significant differences, with the 3 g/L extract rate associated with the highest Fe content (4.06 mg/kg DW), and the control group with the lowest (3.23 mg/kg DW) (Fig. 2).

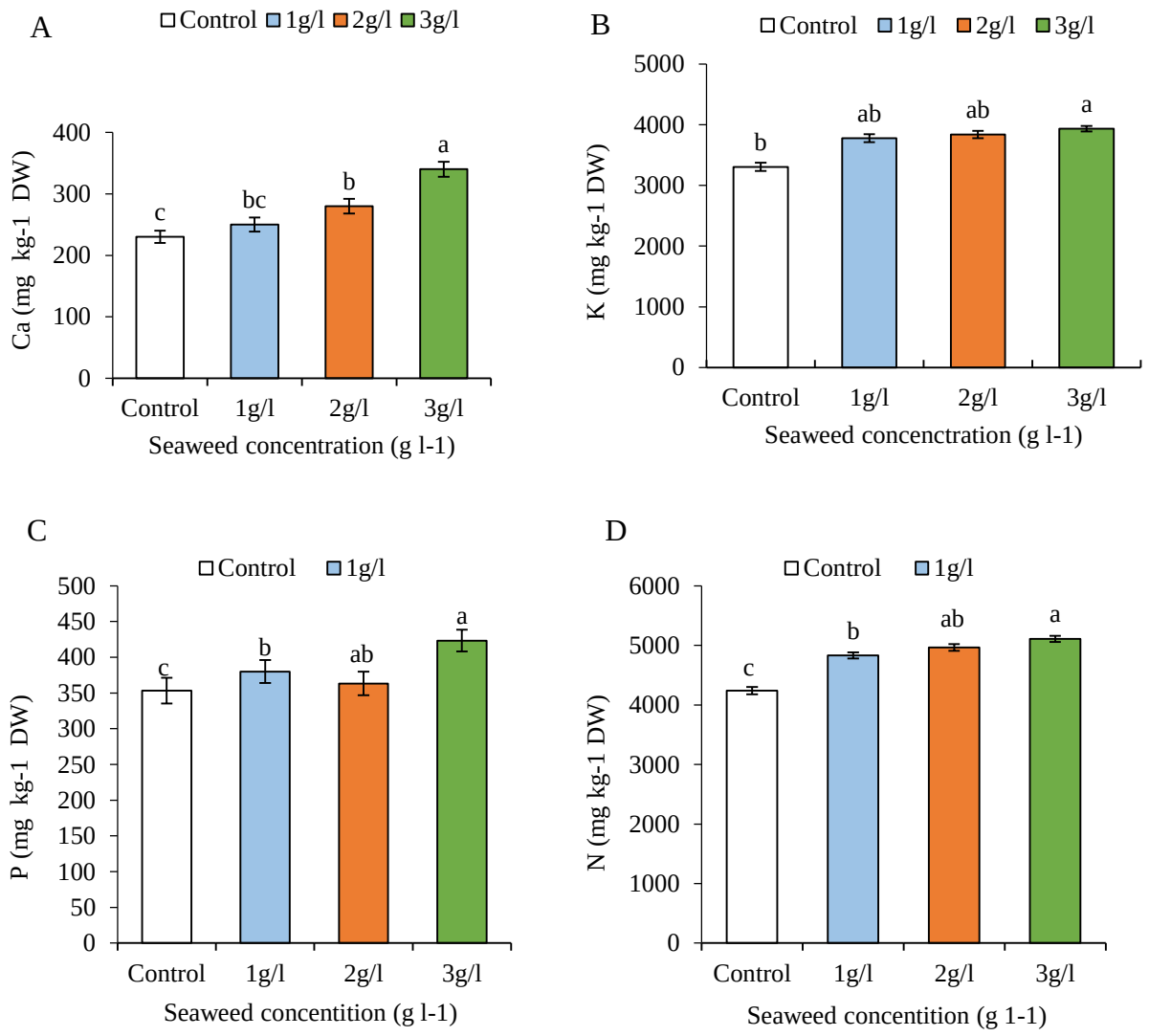

Fig. 1. The effect of *Ascophyllum nodosum* seaweed extract at various concentrations (0, 1, 2, and 3 g/L) on the content of (A) calcium, (B) potassium, (C) phosphorus, and (D) nitrogen in 'Hayward' kiwifruit at the time of harvest. Each value represents the mean ± standard error of three replications.

The nutrition of plants during the fruit growth stage significantly impacts post-harvest crop quality (Ramezanian et al., 2009). Optimal nutrition is a fundamental requirement for enhancing both crop quantity and quality. Beyond ensuring the availability of all necessary nutrients in adequate quantities, maintaining a balanced nutrient profile is of paramount importance. Nutritional imbalances can not only fail to enhance yields but can also disrupt plant growth, ultimately leading to reduced crop yields. Effective nutrient management that ensures the availability of all required nutrients is essential. Even a short-term nutrient deficiency during the growing season can diminish potential crop yields. Mismanagement of nutrients, even at trace levels (ppm) found in fruit trees, can result in adverse economic consequences. In contrast, proper nutrient management represents an efficient strategy in horticulture (Malkouti & Homai, 2005).

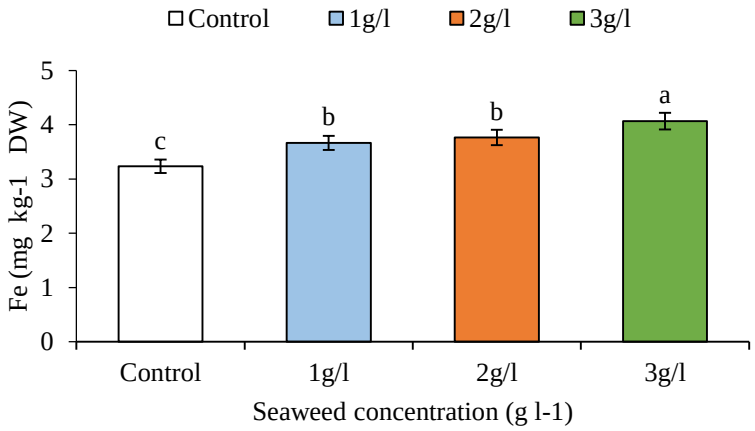

Fig. 2. The effect of *Ascophyllum nodosum* seaweed extract at various concentrations (0, 1, 2, and 3 g/L) on iron content in 'Hayward' kiwifruit at the time of harvest. Each value represents the mean ± standard error of three replications.

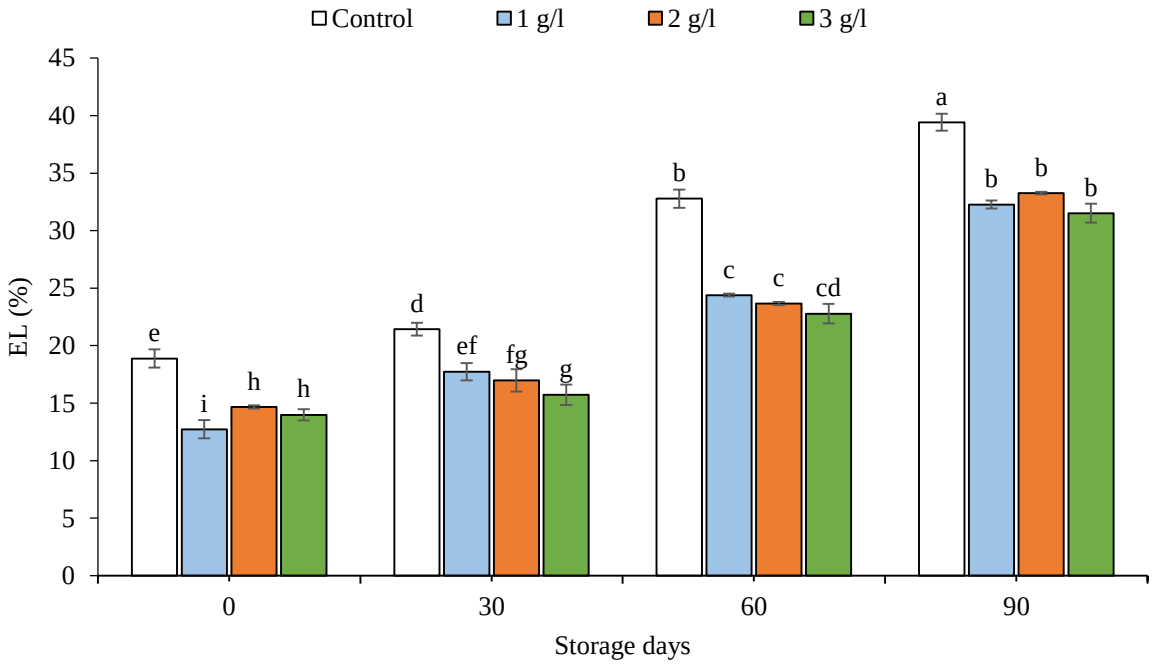

Fig. 3. The effect of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the electrolyte leakage of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

The macroalgal extract from *Ascophyllum nodosum* contains minerals such as N, P, K, Ca, Fe, Mg, Zn, Na, and S (Rayorath et al., 2009). These minerals are readily absorbed through the stomata and hydrophilic pores of cuticles on the leaves. The uptake of these minerals from the leaf surface is influenced by environmental factors including temperature, humidity, and light intensity, which, in turn, affect stomatal opening and the permeability of cuticles and cell walls.

Bio-stimulators, in general, are compounds that induce and optimize plant metabolism and metabolic processes to enhance plant efficiency (Starck, 2005). Seaweed extracts serve as bio-stimulators and are officially recognized in agriculture for their ability to improve plant

growth. The focus of our research was the foliar application of seaweed extract. Foliar application is a method that efficiently delivers nutrients to higher plants by spraying a solution containing these nutrients onto the plant foliage. This method allows plants to acquire nutrients much more rapidly than they would through root uptake from the soil, though it is not without its drawbacks. Despite these limitations, foliar application remains the preferred practice in specific conditions (Marschener, 2022). Seaweeds contain growth stimulators, organic macro- and micro-nutrients, vitamins, antioxidants, organic acids, organic NPK, organic acids of seaweed origin, and plant hormones extracted from *Ascophyllum* algae (Hurtado et al., 2009).

Among the various nutrients, calcium (Ca) has garnered considerable attention in recent years with respect to nutrient management. Calcium application is particularly beneficial during the storage phase, as it contributes to prolonging crop shelf-life and reducing physiological disorders and rot (Rathore et al., 2008). Achieving an optimal balance in nutrient solutions is a vital goal for all agricultural systems (Dong et al., 2005). A study investigating the effect of seaweed extract on nutrient uptake in rainfed soybeans reported an increase in nutrients such as Ca, further highlighting the significance of calcium in plant nutrition (Rathore et al., 2008). Increased calcium content in plants enhances cell wall resistance and the pectin content in both plants and fruits (Rathore et al., 2008).

Electrolyte leakage (EL) and malondialdehyde (MDA)

The comparison of means unveiled an upward trend in EL over the 90-day storage period. The lowest EL (12.72%) was associated with the treatment involving 1 g/L of brown macroalga extract recorded at the time of harvest, whereas the highest EL (39.42%) was observed in the control group, recorded 90 days after storage.

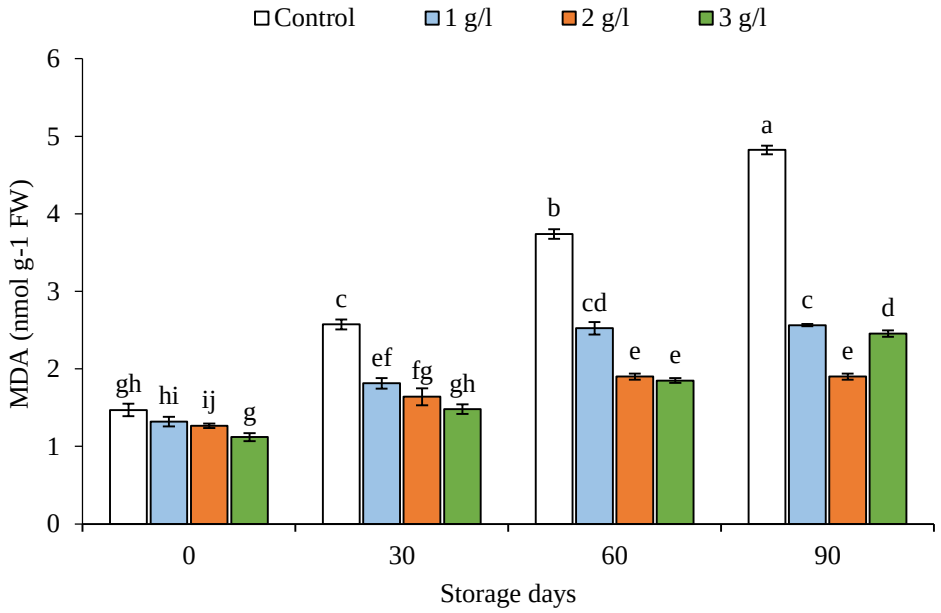

Fig. 4. The impact of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the malondialdehyde (MDA) content of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

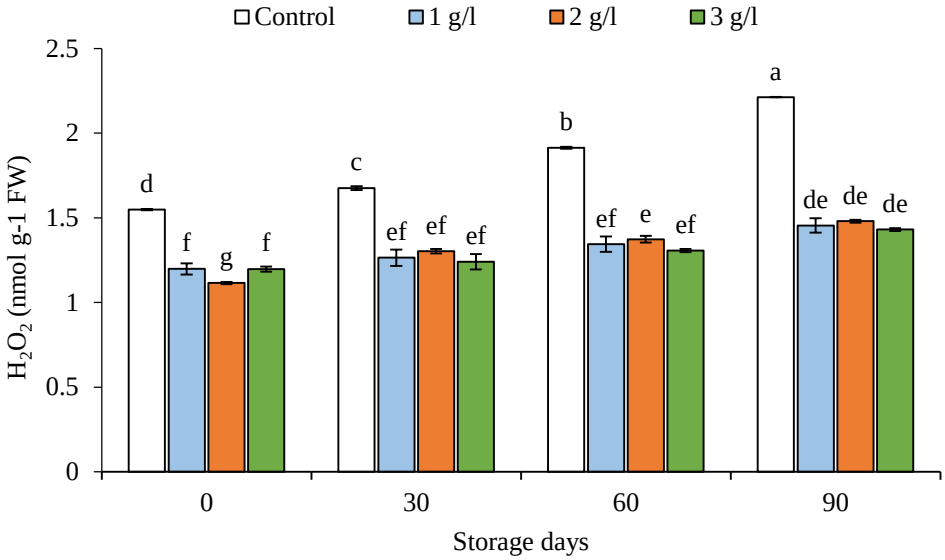

Fig. 5. Influence of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the hydrogen peroxide (H_2O_2) content of 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

Furthermore, the comparison of means demonstrated a consistent increase in MDA levels in all fruits throughout the storage period. This trend was more pronounced in the control samples, while MDA accumulation was notably suppressed in the treated fruits over the three months of storage. At the conclusion of the storage period (90 days) plus an additional three days of shelf-life at 25°C, the highest MDA accumulation (4.82 nmol/g FW) was attributed to the control group, while the lowest (1.12 nmol/g FW) was recorded in fruits treated with 3 g/L of seaweed extract at the time of harvest.

The results clearly indicate that the brown macroalga extract has a beneficial impact on reducing EL and inhibiting MDA accumulation. Cell membrane integrity, as the foremost cellular structure influenced by chilling (Rui et al., 2010), is of paramount importance. Chilling induces the phase transition of the cell membrane from a flexible crystalline liquid to a solid gel-like structure, leading to a loss of membrane permeability (Aghdam and Bodbodak, 2013). Prolonged exposure to chilling stress results in the rupture of cell membranes, leading to the leakage of intracellular water, ions, and metabolites, a phenomenon that can be accessed via EL (Sharom et al., 1994).

EL is an effective indicator for measuring cell membrane integrity and thus serves as a reliable indicator of the same. Moreover, lipid peroxidation, a factor that diminishes cell membrane integrity, can be gauged through the assessment of MDA synthesis. The accumulation of MDA, the final product of lipid peroxidation, is indicative of lipid peroxidation and cell membrane damage (Luo et al., 2012; Cheng et al., 2011). Free radicals, when present in excess, can target the double bonds of fatty acids, resulting in MDA production. They can also replace the methyl groups of fatty acids, thereby increasing membrane peroxidation (Radotic et al., 2000).

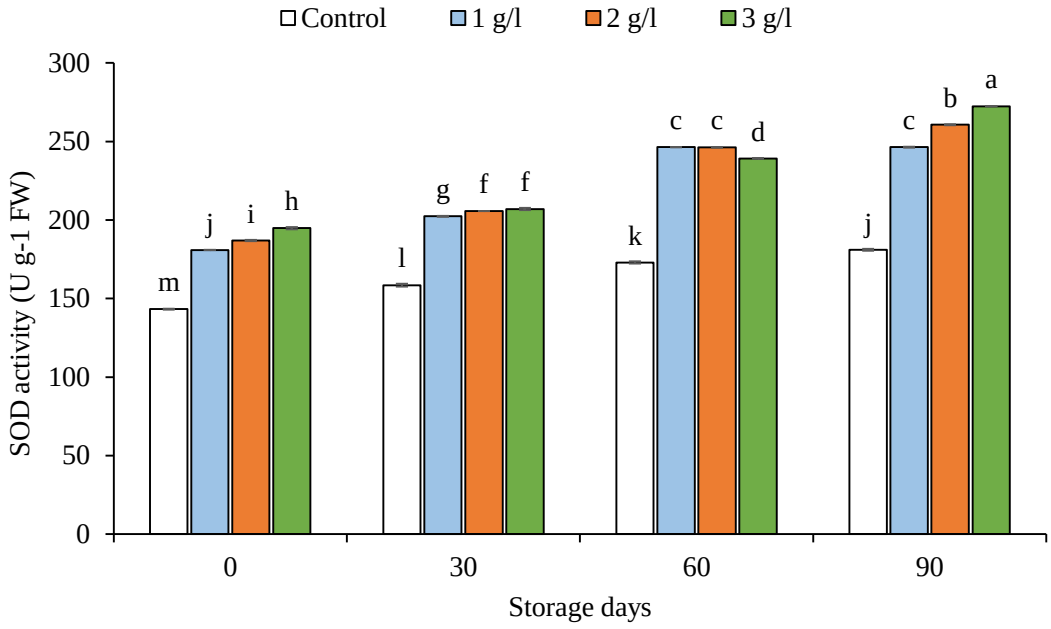

Fig. 6. The effect of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the superoxide dismutase (SOD) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

In fruits, chilling alters membrane structure through peroxidation, and MDA content is an indicator of declining cell membrane integrity and the onset of chilling-induced damage (Shewfelt & Purvis, 1995). As observed in the EL trend, MDA content increased in control fruits as chilling stress intensified and membrane damage accumulated during storage. However, EL and MDA content in kiwifruits treated with the brown macroalga extract decreased, owing to the positive influence of the extract on enhancing the activity of antioxidant enzymes. The key mechanisms for scavenging reactive oxygen species (ROS) in plants involve enzymatic elements such as ascorbate peroxidase, peroxidase, and catalase, which can neutralize ROS.

Higher membrane peroxidation may result from the inability of the plants enzymatic or non-enzymatic antioxidant systems to effectively neutralize ROS (Allen & Ort, 2001). Similar results regarding the impact of brown macroalga extract on EL and MDA accumulation have been reported in different crops. The application of 15% seaweed liquid fertilizer to wheat reduced MDA content compared to the control over an eight-day storage period at 25°C (GhaffariZadeh et al., 2015).

Hydrogen peroxide (H_2O_2)

Maximum velocity (Vmax) and constant of Michaelis-menton (Km) were estimated by Sigma plot software. Results of analysis showed that Vmax and Km in the *in vivo* and *in vitro* experiment were 18.75; 38.65 and 68.03 109.3 respectively.

The comparison of means revealed an increase in H_2O_2 content during storage. However, the brown macroalga extract treatments effectively restrained the rise in H_2O_2 levels during storage. The lowest amount of H_2O_2 (1.115 nmol/g FW) was related to the treatment with 2g/L of the extract at harvest, while the highest (2.212 nmol/g FW) was observed in the control group during the third month of storage (Fig. 5). H_2O_2 accumulation in fruits typically increases during the post-harvest period due to various stresses, including chilling. Cold temperatures induce a phase transition from a liquid to a rigid solid gel, leading to reduced

cell membrane selectivity. This transformation results in an over synthesis of H_2O_2 radicals and superoxide, leading to membrane damage and membrane lipid peroxidation (Zhang et al., 2015). H_2O_2 is generated through the beta-oxidation of fatty acids in glyoxysomes and/or by photorespiration in peroxisomes (Foyer & Noctor, 2000). Free radicals, including H_2O_2, have a dual role in plants. At low concentrations, they function as signaling molecules in the plant's defense system against stress (Zhou et al., 2012) and can enhance the plant's antioxidant capacity by increasing the activity of antioxidant enzymes. However, at higher concentrations, they become detrimental to plants, prompting the antioxidant system to scavenge excess radicals (Hu et al., 2012). Seaweed extracts can reduce ROS synthesis due to their composition (Farvin & Jacobsen, 2013). A. nodosum, in particular, plays a crucial role in suppressing the formation of free radicals at the initiation of oxidation and in expanding free radical chain reactions as an electron donor (Kindleysides et al., 2012). Similar results have been reported regarding the decrease in H_2O_2 levels as influenced by the application of brown macroalga extract (marine) at rates of 1000 and 2000 mg/L (ppm) in grapes cv. 'Rasheh' under drought stress (Amani et al., 2018), corroborating our findings.

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activity

Based on the comparison of means, SOD, CAT, APX, and POD activities increased in all fruits during the storage period. The rate of increase was more pronounced in the treated fruits, while it was less rapid in the control group, becoming even more marginal in the final 30 days. The highest SOD activity (272.39 units/g FW) was recorded in fruits treated with 3 mg/L of the brown macroalga extract after 90 days of storage, while the lowest (143.28 units/g FW) was observed in the control group at the time of harvest (Fig. 6). The comparison of means indicated an increase in CAT activity throughout the 90-day storage period in all fruits. However, the increase was less prominent in the control group. The highest and lowest CAT activities were observed in the fruits treated with 3 g/L of the extract after three months of storage and the control fruits at the time of harvest (9.8 and 1.8 units/g FW, respectively) (Fig. 7).

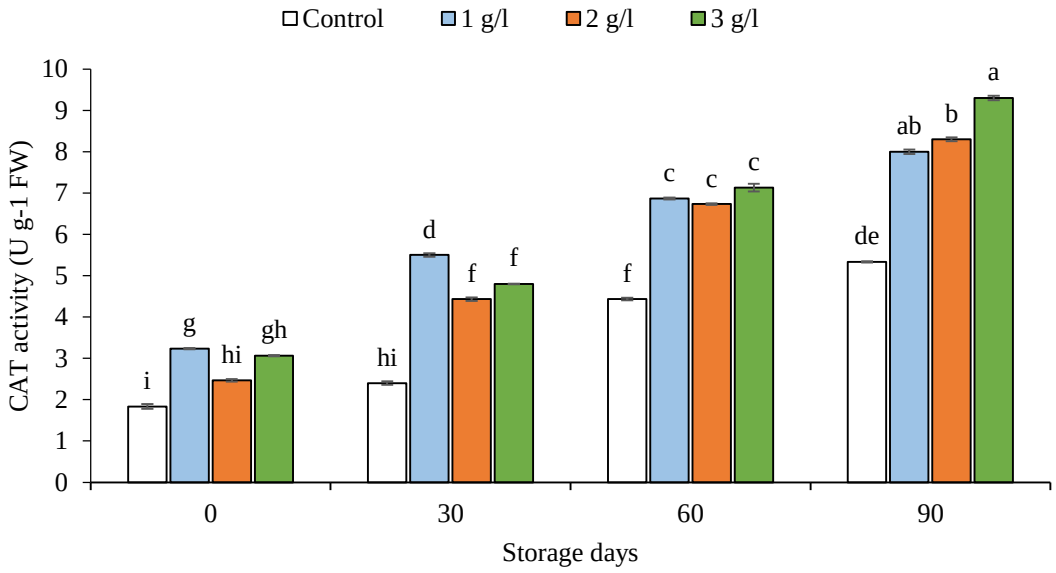

Fig. 7. The impact of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the catalase (CAT) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

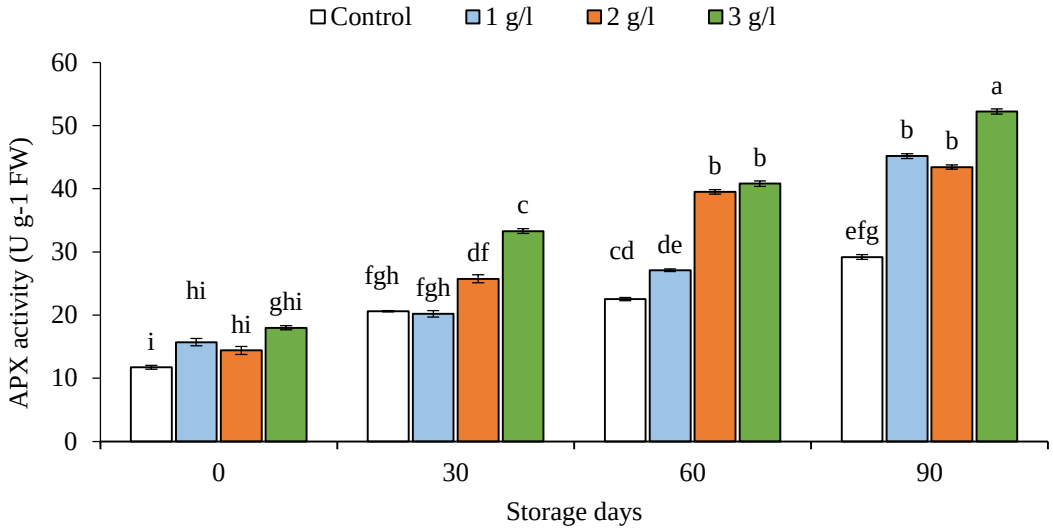

Fig. 8. Influence of *Ascophyllum nodosum* seaweed extract at varying concentrations (0, 1, 2, and 3 g/L) on the ascorbate peroxidase (APX) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

Our research also revealed a steady increase in APX activity during the storage period in all fruit samples. However, the treated fruits exhibited higher APX activity than the control group. The highest APX activity (52.24 units/g FW) was recorded in fruits treated with 3 g/L of brown macroalga extract after 90 days of storage, while the lowest (11.75 units/g FW) was associated with the control fruits at the time of harvest (Fig. 8). Furthermore, the comparison of means indicated a consistent increase in POD activity during storage in all fruit samples. Once again, the treated fruits exhibited notably higher POD activity compared to the control group. The highest POD activity (49.69 units/g FW) was recorded in fruits treated with 3 g/L of the brown macroalga extract after 90 days of storage, while the control group displayed the lowest POD activity (14.97 units/g FW) at the time of harvest (Fig. 9).

The upsurge in SOD, CAT, APX, and POD activities is indicative of enhanced antioxidant enzyme systems in fruits treated with brown macroalga extract. These enzymes play a crucial role in scavenging reactive oxygen species (ROS) and preventing oxidative damage to cellular structures. SOD catalyzes the dismutation of superoxide radicals into oxygen and H_2O_2, which is further detoxified by CAT and APX. The coordinated action of these enzymes helps in maintaining cellular redox homeostasis. Similarly, POD catalyzes the breakdown of H_2O_2, reducing its potential to cause cellular damage (Rui et al., 2010; Zhang et al., 2015).

The significant increase in antioxidant enzyme activities in treated fruits during storage is a testament to the extract's ability to enhance the fruit's defense mechanisms against oxidative stress and maintain the integrity of cellular structures. The importance of these antioxidant enzymes in preventing chilling injury and maintaining fruit quality has been well-established in previous studies. For example, in bananas, increased SOD, CAT, and POD activities were associated with reduced chilling injury (Chen et al., 2011). Similar findings were reported in studies on peaches, where higher SOD, CAT, and POD activities were linked to enhance chilling tolerance and reduced membrane damage (Wang et al., 2019). Therefore, our findings underscore that increased antioxidant enzyme activity reduces ROS levels, ultimately contributing to the preservation of cell wall structure and the extension of fruit longevity.

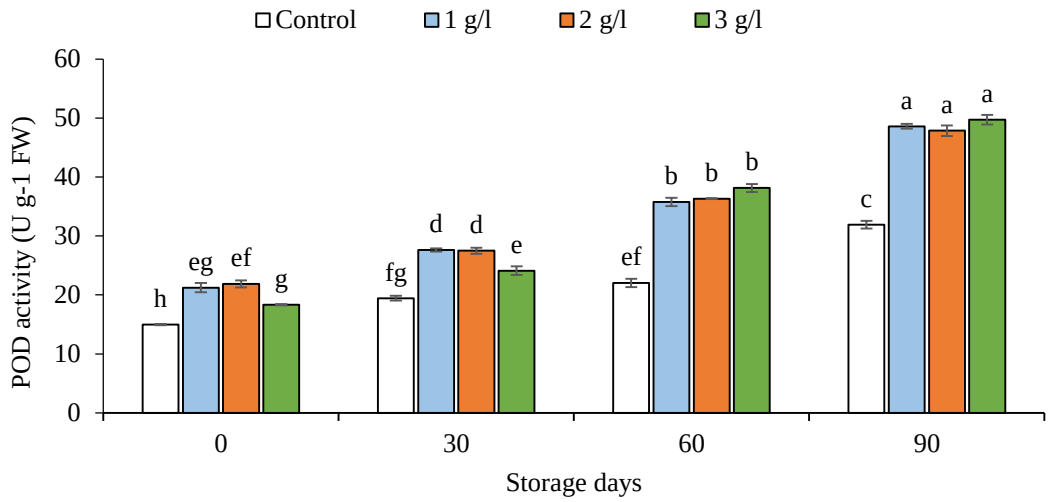

Fig. 9. The effect of *Ascophyllum nodosum* seaweed extract at different concentrations (0, 1, 2, and 3 g/L) on the peroxidase (POD) activity in 'Hayward' kiwifruit during storage (0, 30, 60, and 90 days after storage). Each value represents the mean ± standard error of three replications.

CONCLUSION

In conclusion, the foliar application of brown macroalga extract resulted in significant improvements in nutrient uptake, reduced chilling injury, and delayed ripening during the storage of kiwifruits. Notably, it contributed to the preservation of cell membrane integrity, as evidenced by reduced electrolyte leakage, lower malondialdehyde levels, and enhanced antioxidant enzyme activities, including superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase. The results of this study provide insights into the potential applications of brown macroalga extract in fruit preservation and contribute to our understanding of the mechanisms underlying its effects on nutrient uptake and post-harvest quality. Among the various extract rates studied in this research, the application of 3 g/L was notably the most effective treatment. Hence, we recommend this concentration as the optimal choice for achieving the desired improvements in kiwifruit quality and longevity.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Abdel-Mawgoud, A. M. R., Tantaway, A. S., Magda, M. H., & Hoda, A. M. (2010). Seaweed extract improves growth, yield and quality of different watermelon hybrids. *Research Journal of Agriculture and Biological Sciences*, *6*(2), 161–168.

Aghdam, M. S., & Bodbodak, S. (2013). Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments. *Scientia Horticulturae, 156,* 73-85. https://doi.org/10.1016/j.scienta.2013.03.028.

Alexieva, V., Sergiev, I., Mapelli, S. & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell and Environment*, *24*(12), 1337-1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Allen, D. J., & Ort, D. R. (2001). Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Science*, *6*(1), 36-42. https://doi.org/10.1016/S1360-1385(00)01808-2.

Amani, P., Javadi, T. & Ghaderi. N. (2018). *The effect of Ascophyllum nodosum (marmarine) on the quality and quantity of grape cv. Rasheh in drought and not drought.* University of Kurdistan. Kurdistan.

Cassano, A., Figoli, A., Tagarelli, A., Sindona, G., & Drioli, E. (2006). Integrated membrane process for the production of highly nutritional kiwifruit juice. *Desalination*, *189*(1-3), 21–30. https://doi.org/10.1016/j.desal.2005.06.009.

Chen, T. H., & Murata. N. (2011). Glycine betaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant, Cell & Environment*, *34*(1), 1-20. https://doi.org/10.1111/j.1365-3040.2010.02232.x.

Dong, S., cheng, L., Scagel., C & Fuchigami, L. (2005). Timing of urea application affects leaf and root N uptake in young Fuji/M9 apple trees. *The Journal of Horticultural Science and Biotechnology*, *80*(1), 116-120. https://doi.org/ 10.1080/14620316.2005.11511901.

FAO. org. (2021). Statistical Database/ faostat/collections. Production crop.

Farvin, K. S. & Jacobsen, C. (2013). Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chemistry*, *138*(2-3), 1670-1681. https://doi.org/10.1016/j.foodchem.2012.10.078.

Fornes, F., Sanchez-Perales, M. & Guardiola, J. L. (2002). Effect of a seaweed extract on the productivity of 'de Nules' Clementine mandarin and Navelina orange. *Botanica Marina*, *45*(5), 486-489. https://doi.org/10.1515/BOT.2002.051.

Foyer, C. H. & Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signalling. *New Phytologist*, *146*(3), 359-388. https://doi.org/10.1046/j.1469-8137.2000.00667.x.

Geny, L., Bernardon Mery, A., & Larrive, G. (2007). A physiological pathway source of agronomic progress: The activation of flowering hormones. An algae filtrate acts on grapevines and apple trees. *Physio Activator Technology*, 609, 37-40.

GhaffariZadeh, A., Seyed Nejad, S. M., & Gilani, A. (2015). The effect of different levels of urea fertilizer and brown seaweed extract on physiological traits and grain yield of wheat. *Crop Physiology Journal*, *7*(27), 69-83.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. *Archives Biochemistry Biophysics*, *125*, 850-857. https://doi.org/10.1016/0003-9861(68)90654-1.

Hu, L. Y., Hu, S. L., Wu, J., Li, Y. H., Zheng, J. L, Wei, Z. J., Liu, J., Wang, H. L., Liu, Y. S., & Zhang, H. (2012). Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *Journal of Agricultural and Food Chemistry*, *60*, 8684-8693. https://doi.org/10.1021/jf300728h.

Hurtado, A. Q., Yunque, D. A., Tibubos, K., & Critchley, A. T. (2009). Use of Acadian marine plant extracts powder from *Ascophyllum nodosum* in tissue culture of Kappaphycus varieties. *Journal of Applied Phycology*, *21*(6), 633-639. https://doi.org/ 10.1007/s10811-008-9395-4.

Jayaraman, J., Norrie, J., & Punja, Z. (2011). Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *Journal of Applied Phycology*, *23*(3), 353-361. https://doi.org/10.1007/s10811-010-9547-1.

Keyrouz, R., Abasq, M. L., Le Bourvellec, C., Blanc, N., Audibert, L., ArGall, E., & Hauchard, D. (2011). Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chemistry*, *126*, 831–836. https://doi.org/10.1016/j.foodchem.2010.10.061.

Kindleysides, S., Quek, S. Y., & Miller, M. R. (2012). Inhibition of fish oil oxidation and the radical scavenging activity of New Zealand seaweed extracts. *Food Chemistry*, *133*(4), 1624-1631. https://doi.org/10.1016/j.foodchem.2012.02.068.

Lai, A., Santangelo, E., Soressi, G. P., & Fantoni, R. (2007). Analysis of the main secondary metabolites produced in tomato (*Lycopersicon esculentum*, Mill) epicarp tissue during fruit ripening using fluorescence techniques. *Postharvest Biology and Technology*, *43*(3), 355-342. https://doi.org/10.1016/j.postharvbio.2006.09.016.

Lim, C. C., Arora, R., & Townssenal, E. C. (1998). Comparing comports and richards function to estimate freezing injury in rhododendron using electrolyte leakage. *Journal of the American Society for Horticultural Science*, *123*(2), 246-252. https://doi.org/10.21273/JASHS.123.2.246.

Luo, Z. S., Wu, X., Xie, Y., & Chen, C. (2012). Alleviation of chilling injury and browning of postharvest bamboo shoot by salicylic acid treatment. *Food Chemistry*, *131*(2), 456-61. https://doi.org/10.1016/j.foodchem.2011.09.007.

Malkouti, M. J., & Homai, M. (2005). Fertility of soils in arid and semi-arid regions (problems and solutions) Tehran: Tarbiat Modares University, Scientific Publication Office. (In Persian).

Marschener, P. (2022). *Marschner's mineral nutrition of higher plants*. Third Edition, Academic Press is an imprint of Elsevier.

Masny, A., Basak, A., & Zurawicz, E. (2004). Effect of foliar application of Kelpak and Goemar BM 86 preparations on yield and fruit quality in two strawberry cultivars. *Journal of Fruit and Ornamental Plant Research*, *12*, 23-27.

Nikoogoftar-Sedghi, M., Rabiei, V., Razavi, F., Molaei, S., & Khadivi, A. (2023). The effect of foliar application of *Ascophyllum nodosum* (L.) Le Jol. seaweed extract on biochemical traits related to abiotic stresses in pistachio (*Pistacia vera* L. cv. Kaleh-Ghoochi). *BMC Plant Biology*, *23*(1), 635. https://doi.org/10.1186/s12870-023-04654-5.

Radotic, K., Ducic, T., & Mutavdzic, D. (2000). Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. *Environmental and Experimental Botany*, *44*(2), 105-113. https://doi.org/10.1016/s0098-8472(00)00059-9.

Ramezanian, A., Rahemi, M., & vazifehshenas, M. R. (2009). Effects of foliar application of calcium chloride and urea on quantitative and qualitative characteristics of pomegranate fruits. *Scientia Horticulturae*, *121*(2), 171-175. https://doi.org/10.1016/j.scienta.2009.01.039.

Rana, J., & Paul, J. (2017). Consumer behavior and purchase intention for organic food: A review and research agenda. *Journal of Retailing and Consumer Services*, *38*, 157-165. https://doi.org/10.1016/j.jretconser.2017.06.004.

Rathore, S. S., Chaudhary, D. R., Boricha, A., Ghosh, B. P., Bhatt, S. T., & Zodape, J. S. (2008). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *South African Journal of Botany*, *75*, 351–355. https://doi.org/10.1016/j.jretconser.2017.06.004.

Rayorath, P., Benkel, B., Hodges, D. M., Allan-Wojtas, P., MacKinnon, S., Critchley, A. T., & Prithiviraj, B. (2009). Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in Arabidopsis thaliana. *Planta*, *230*, 135–147. https://doi.org/ 10.1007/s00425-009-0920-8.

Rui, H., Cao, S., Shang, H., Jin, P., Wang, K., & Zheng, Y. (2010). Effects of heat treatment on internal browning and membrane fatty acid in loquat fruit in response to chilling stress. *Journal of the Science of Food and Agriculture*, *90*(9), 1557-1561. https://doi.org/10.1002/jsfa.3993.

Sharom, M., Willemot, C., & Thompson, J. E. (1994). Chilling injury induces lipid phase changes in membranes of tomato fruit. *Plant Physiology*, *105*(1), 305-308. https://doi.org/10.1104/pp.105.1.305.

Shewfelt, R. L. & Purvis, A. C. (1995). Toward a comprehensive model for lipid peroxidation in plant tissue disorders. *Horticulture Science*, *30*(2), 213-218.

Starck, Z. (2005). Application of growth regulators and biostimulators in modern plant cultivation (in Polish). Rolnik Dzierawca. *Growing Assistant*, 2, 74-86.

Wang, L., Bokhary, S. U. F., Xie, B., Hu, S., Jin, P., & Zheng, Y. (2019). Biochemical and molecular effects of glycine betaine treatment on membrane fatty acid metabolism in cold stored peaches. *Postharvest Biology and Technology*, *154*, 58–69. https://doi.org/10.1016/j.postharvbio.2019.04.007.

Westerman, R. L. (1990). *Soil testing and plant analysis*. 3rd Edition, Soil Science Society of America Book Series, Number 3, Madison, Wisconsin, USA.

Zasoski, R. J., & Burau, R. G. (1977). A rapid nitric perchloric acid digestion method for multielement tissue analysis. *Communications in Soil Science and Plant Analysis*, *8*(5), 425-436. https://doi.org/10.1080/00103627709366735.

Zhang, Z., Huber, D. J., & Rao, J. (2013). Antioxidant systems of ripening avocado (*Persea americana* Mill.) fruit following treatment at the pre climacteric stage with aqueous 1-methylcyclopropene. *Postharvest Biology and Technology*, *76*, 58–64. https://doi.org/10.1016/j.postharvbio.2012.09.003.

Zhang, Z., Huber, D. J., Qu, H., Yun, Z., Wang, H., Huang, Z., Huang, H., & Jiang, Y. (2015). Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. *Food Chemistry*, *171*, 191-199. https://doi.org/10.1016/j.foodchem.2014.09.001.

Zhou, J., Wang, K., Shi, X., Xia, Y., J. Zhouand, J., & Yu. Q. (2012). Hydrogen peroxide is involved in the cold acclimation-induced chilling tolerance of tomato plants. *Plant Physiology and Biochemistry*, *60*, 141-149. https://doi.org/10.1016/j.plaphy.2012.07.010.

Zodape, S.T. (2001). Seaweeds as a bio fertilizer. *Journal of Scientific and Industrial Research*, *60*, 378–389.