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MARINE ALGAE EXTRACT IN INTEGRATION WITH HUMAX EFFECTS ON THE BIOCHEMICAL COMPOSITION OF THE POMEGRANATE

N.K.A. AL-MAYAHI*, S.M. AL-RUBAEI, and M.A.F. HASSAN

Department of Horticulture and Landscape Engineering, University of Kerbala, Kerbala, Iraq
*Corresponding author's email: sabraoassociateeditors@gmail.com, alialinesreen9@gmail.com
Email addresses of co-authors: Suzan.mohammed@uokerbala.edu.iq, manar.a@coagri.uobaghdad.edu.iq

SUMMARY

The presented study determined the effects of foliar application of marine algae extract and Humax on the pomegranate (*Punica granatum* L.) seedlings beneath the plant canopy, carried out in 2023 at the University of Kerbala, Kerbala, Iraq. The experiment layout was in a randomized complete block design (RCBD) with factorial arrangement, three factors, and three replications. The first factor was the two pomegranate cultivars (Wonderful and Slimi), the second factor was marine algal extract concentrations (0, 1.5, and 3 ml L^{-1}), while the third was the Humax levels (0.25, 0.25, and 0.50 g L^{-1}). Pomegranate cultivar Wonderful showed the highest content of chlorophyll, carbohydrates, nitrogen, phosphate, and potassium, compared with the cultivar Slimi. Marine algal extract (3 ml L^{-1}) and humax (0.50 g L^{-1}) also performed better and improved the biochemical composition of the pomegranate. Cultivars in interaction with marine algae extract also performed well and improved the traits. However, cultivar Wonderful with marine algal extract (3 ml L^{-1}) and humax (0.50 g L^{-1}) had the highest averages for the chlorophyll, carbohydrates, nitrogen, phosphate, and potassium. The marine algae extract and Humax binary interaction considerably affected the study variables in the pomegranate.

Keywords: Pomegranate (*P granatum* L.), marine algae extract, Humax, interaction, physiological and biochemical traits

Key findings: The pomegranate (*P. granatum* L.) cultivar Wonderful, in integration with marine algae (3 ml L^{-1}) and humax (0.50 g L^{-1}), achieved the highest averages for physiological and biochemical traits.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae, one of the oldest deciduous fruits. Past studies claimed pomegranate originated in Central Asia and Persia (Hooks *et al*., 2021). Approximately 6,495,705 fruit trees produced a total of 242,671 tons of fruits per year, with 2.37 kg per tree in Iraq (CSO, 2020). Pomegranate economic relevance stems from its extended market display, since the fruits mature from late summer to mid-winter (Jumaa and Karumi, 2005).

Pomegranate cultivars contain varied ratios of vitamins, nutritional elements, anthocyanin, fats, sugars, organic acids, fibers, protein, pectin, and tannin in their roots, stems, leaves, flowers, and fruits (Yan-hui *et al.,* 2022). The foliar feeding of the nutrients with different plant extracts served to boost the seedling growth, essential for fruit culture. Foliar nutrition provides key nutrients, including macro- and microelements for plant growth and development. These necessary nutrient elements also generate carbohydrates, proteins, amino acids, and other growth chemicals; therefore, their shortage can cause hindrances in plant development and even mortality (Al-Marsoumi, 2015).

Foliar application of fertilizer solutions on the seedlings ensures the leaves directly absorb the macro-nutrients like nitrogen, phosphate, and potassium. As it includes minerals, growth regulators, vitamins, and organic acids, it makes it easy for the plants to absorb. Foliar application of marine algae extract has also become a popular physiological stimulant. Thus, it decreases the use of chemical fertilizers and the health risks and pollutants (Khudair *et al*., 2015).

Organic fertilizer promotes cell membrane permeability and mineral mobility owing to water and nutrient absorption in tiny amounts. It also activates plant enzymes and improves soil physical and chemical properties by reducing pH and increasing the availability of essential elements in soil due to organic acids (humic and fulvic acids), improving biochemical composition of the pomegranate seedling (Stion *et al*., 2009).

Past studies enunciated sweet cherry seedlings sprayed with marine algae extract (2 g L^{-1}) showed the highest content of carbohydrates, nitrogen, phosphorus, and potassium (Yassin and Al-Zubaidi, 2019). Al-Hajaimi and Al-Khafaji's (2016) findings disclosed peach seedlings with foliar application of marine algae (4 ml L^{-1}) also provided the enhanced ratios of nitrogen, phosphorus, potassium, and carbs. The pomegranate cultivar Salimi gave the maximum leaf nitrogen, phosphorus, potassium, chlorophyll, and carbohydrates by adding humic acid (80 g $plant^{-1}$) (Al-Hathal, 2020).

In persimmon, applying humic and fulvic acid boosted the nitrogen, phosphorus, and potassium content in plant leaves (Abd-Al-Rheem *et al*., 2017). The study sought to determine the best type and concentration of marine algae extract and Humax. It also aimed to improve the biochemical composition of the pomegranate seedlings by knowing the effects of foliar application of marine algae extract and Humax with different concentrations on the pomegranate (*P. granatum* L.).

MATERIALS AND METHODS

The conducted study on pomegranate (*Punica granatum* L.) seedlings with foliar application of marine algae extract and Humax beneath the plant canopy sought to determine their effects on the biochemical composition of pomegranate cultivars. The research happened in 2023 at the University of Kerbala, Kerbala, Iraq. The experiment layout had a randomized complete block design (RCBD) with factorial arrangement, three factors, and three replications. The first factor was the two pomegranate cultivars (Wonderful and Slimi), the second factor was marine algal extract concentrations (0, 1.5, and 3 ml L^{-1}), while the third was the Humax levels (0.25, 0.25, and 0.50 g L^{-1}).

Homogeneous in vegetative growth and size, the 270 ten-month-old pomegranate seedlings, picked from the sandy soil and placed in polyethylene black plastic bags with 1.25 kg, had final transfer to 10-kg-soil pots. With three replicates of 90 seedlings each, five seedlings were samples used per experimental unit. The seedlings received irrigation four times a month with a 2-liter hand sprayer. Instead of Tween-20, another 1 ml detergent became a diffuser with each concentration to minimize water molecule surface tension and wet vegetative plant portions. The fertilization of seedlings with foliar application of marine algae extract Algazone ensued in the morning. Watering of the control treatment was a day before spraying and sprayed with distilled water and detergent to improve plant absorption. Watering the plants before spraying reduces leaf cell solute concentration, and thus, increases spray solution ions' penetration into leaf cells (Al-Sahhaf, 1989). Humax injection transpired five times at three concentrations, with a month difference between applications. The field practices, including irrigation and weeding, were of equal applications, as needed.

Data recorded

The data recording comprised the following physiological and biochemical traits in the different treatments of the two pomegranate cultivars.

Leaf chlorophyll content (mg g^{-1} fresh weight)

The leaf chlorophyll content estimated in the fresh leaves of pomegranate used the methodology of Chappelle *et al*. (1992). Using the fourth leaf of five seedlings in each experimental unit consisted of rinsing it to remove dust, then, drying and taking 0.1 g from each, cutting them into small pieces with scissors. Afterward, soaking them in 80% acetone continued until the dye's extraction in a dark place at room temperature. Then, using a spectrophotometer at 645 and 663 nm wavelengths, calculated the chlorophyll content as mg g^{-1} of fresh plant tissue.

$$Chlorophyll\ \ Total = \frac{\{20.2(D\ 645) + 8.02\ (D\ 663)\} \times V}{1000 * W}$$

D: Chlorophyll optical density read of the extract

V: Final volume of the filtrate (10 ml) after the separation process completed by centrifuging
W: fresh weight (0.1 g)

Leaf carbohydrates (%)

According to Joslyn (1970), the application of 8.8 g of 80% ethyl alcohol to 0.2 g of dry powder sample helped measure pomegranate leaf carbohydrates. After 30 min in a 60 °C water bath, the mixture received centrifugation for 15 min at 3000 rpm. After adding 10 ml of 80% perchloric acid to the sediment, it sustained heating to 60 °C for 60 min. The mixture underwent another centrifugation at 3000 rpm, then added with 10 cc of perchloric acid to the sediment and centrifuged twice. After gathering 50 ml of filtrate, the removal of 1 ml continued, transferring to a test tube with 1 ml of 5% phenol solution. Employing cold mixture, assessed total carbs content (%) using a Spectrophotometer at 490 nm after calibrating with 80% perchloric acid solution and multiplying by 50.

Nitrogen concentration (%)

Based on Page *et al.* (1982), engaging a Micro Kjeldahl device helped estimate the nitrogen percentage in digested pomegranate plant samples by taking 10 ml of the sample and adding 10 ml of 40% sodium hydroxide (NaOH). The distilling of the mixture released the liberated ammonia collected in a glass beaker containing 10 ml of 2% boric acid with two indicators, i.e., Methyl Red and Bromocresol Green. The following equation calculated the nitrogen percentage.

$$\text{Nitrogen (\%)} = \frac{\text{acid volume used through titration x acid normality x 14 x dilution volume}}{\text{distilled sample volume x digested sample volume x 1000}} \text{x100}$$

Phosphorus concentration (%)

In a 50 ml flask, 10 ml of the digested pomegranate sample gained mixing with ammonium molybdate and ascorbic acid to quantify phosphorus (Al-Sahhaf, 1989). After filling the capacity with distilled water, continued to transfer 10 ml of the prior solution in a 100 ml Erlenmeyer flask. Next, adding 0.1 g ascorbic acid and 4 ml ammonium molybdate (made by dissolving 10 g in 400 ml distilled water, adding 150 ml of strong sulfuric acid, and filling a 1 L beaker with distilled water) followed. Afterward, the mixture was hotplate-heated for a minute to turn blue. After quantifying the beaker's contents, adding distilled water ensued to the 100 ml beaker. Finally, a UV-visible spectrophotometer recorded the read at 620 nm.

Potassium concentration (%)

Potassium percentage estimation in the digested pomegranate sample comprised taking 0.2 g of it, adding 1 ml of $CuSO_4$, and then adding 5 ml of sulfuric acid 98%. With the obtained clear solution, the sample remained still to cool; then, followed with adding 50 ml of water. Next, the recorded reading used a flame photometer according to the method given by Hayness (1980).

Statistical analysis

All the recorded data assessment underwent the analysis of variance (ANOVA), as per randomized complete block design with split-plot arrangement (Gomez and Gomez, 1984). The least significant difference ($LSD_{0.05}$) test aided to compare and separate the mean differences for all the parameters of the pomegranate. The statistics software GenStat12 was the program used for the analysis.

RESULTS

Leaf chlorophyll content

The pomegranate cultivars, marine algae extract, Humax, and their interaction have a significant effect on the chlorophyll content in the pomegranate leaves (Table 1). Cultivar Wonderful was superior by 15.295% in the chlorophyll content (1.54500 mg g^{-1} fresh weight) compared with the other cultivar Slimi (1.34004 mg g^{-1}). Results also showed the increased concentration of marine algae extract (3 ml L^{-1}) also raised chlorophyll content (1.72894 mg g^{-1}). However, the control treatment appeared with the lowest average (1.13406 mg g^{-1}), indicating the marine algae treatment increased this average by 52.455%. Moreover, adding Humax affected the chlorophyll content in the pomegranate leaves, and its increased concentration (0.50 g L^{-1}) provided the highest chlorophyll content (1.55850 mg g^{-1}), and outperforming by 16.339% the lowest average obtained through the control treatment of the pomegranate (1.33961 mg g^{-1}).

The binary interactions between the study factors also displayed a significant impact, and the cultivar Wonderful treated with marine algae extract (3 ml L^{-1}), showed the topmost chlorophyll content (1.80600 mg g^{-1}) (Table 1). In contrast, the lowest concentration came from the cultivar Slimi by treating with the control treatment (0.99411 mg g^{-1}), referring to an increase of 81.670% from cultivar Wonderful results. Regarding the interaction between the pomegranate cultivars and Humax, cultivar Wonderful treated with Humax (0.50 g L^{-1} of) gave the utmost leaf chlorophyll content (1.62633 mg L^{-1}), compared with the lowest content (1.22289 mg L^{-1}) given by the interaction treatment of cultivar Slimi with no Humax added. The first treatment exhibited an increase of 32.990%.

In the interaction between marine algae extract and Humax, on average, the marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) gave the maximum chlorophyll content (1.81033 mg g^{-1}) and excelled the lowest average obtained through control treatments (1.00000 mg g^{-1}) with 81.033% (Table 1). The triple interaction showed a significant effect in increasing the average of chlorophyll content, and the cultivar Wonderful with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}), provided the highest average (1.86700 mg g^{-1}). In contrast, cultivar Slimi without adding marine algae extract and Humax, exhibited the lowest average (0.82800 mg g^{-1}), and revealing an escalation for the first triple interaction by 125.483%.

Table 1. Effect of the cultivars, marine algae extract, Humax, and their interactions on the leaf chlorophyll.

Cultivars	Marine algae extract concentration (ml L^{-1})	Humax concentration (g L^{-1}) 0	0.25	0.50	Cultivar × marine algae extract (mg g^{-1} fresh weight)
Wonderful	0	1.17200	1.29500	1.35500	1.27400
	1.5	1.43300	1.57500	1.65700	1.55500
	3	1.76400	1.78700	1.86700	1.80600
Slimi	0	0.82800	0.91800	1.23633	0.99411
	1.5	1.27633	1.36400	1.48200	1.37411
	3	1.56433	1.63767	1.75367	1.65189
$LSD_{0.05}$	0.001728				0.000997
Humax means (mg g^{-1} fresh weight)		1.33961	1.42944	1.55850	Cultivars means (mg g^{-1} fresh weight)
$LSD_{0.05}$	0.000705				
Cultivar × Humax	Wonderful	1.45633	1.55233	1.62633	1.54500
	Slimi	1.22289	1.30656	1.49067	1.34004
$LSD_{0.05}$	0.000997				0.000576
Marine algae × Humax interactions (mg g^{-1} fresh weight)					MA extract means (mg g^{-1} fresh weight)
	0	1.00000	1.10650	1.29567	1.13406
	1.5	1.35467	1.46950	1.56950	1.46456
	3	1.66417	1.71233	1.81033	1.72894
$LSD_{0.05}$	0.001222				0.000705

Table 2. Effect of the cultivars, marine algae extract, Humax, and their interactions on the leaf carbohydrate concentration.

Cultivars	Marine algae extract Concentration (ml L^{-1})	Humax concentration (g L^{-1}) 0	0.25	0.50	Cultivars × marine algae extract (%)
Wonderful	0	21.981	25.348	26.604	24.644
	1.5	27.870	29.278	29.553	28.901
	3	30.288	32.193	34.506	32.329
Slimi	0	20.167	24.894	25.455	23.506
	1.5	26.688	27.808	27.984	27.493
	3	28.580	28.993	29.599	29.057
$LSD_{0.05}$	0.7806				0.4507
Humax means (%)		25.929	28.086	28.950	Cultivars means (%)
$LSD_{0.05}$	0.3187				
Cultivars × Humax	Wonderful	26.713	28.940	30.221	28.625
	Slimi	25.145	27.232	27.679	26.685
$LSD_{0.05}$	0.4507				0.2602
Marine algae × Humax interactions (%)					Marine algae extract means
	0	21.074	25.121	26.029	24.075
	1.5	27.279	28.543	28.768	28.197
	3	29.434	30.593	32.052	30.693
$LSD_{0.05}$	0.5520				0.3187

Leaf carbohydrates

For leaf carbohydrates, the pomegranate cultivars, marine algae extract and Humax concentrations, and their interactions revealed significant differences for total carbohydrates in the leaves (Table 2). Cultivar Wonderful, producing 28.625% total carbohydrates in the leaves, excelled the other cultivar Slimi (26.685%) by 7.270%. Marine algae extract with increased concentration (3 ml L^{-1}) also enhanced the leaf carbohydrate content to 30.693%, which were considerably higher than its control treatment (24.075%) by 27.489%. The higher Humax concentration (0.50 g L^{-1}) added to the pomegranate plants also boosted the concentration of carbohydrates (28.950%) in plant leaves over the control treatment (25.929%) by 11.651%.

The binary interactions notably affected the said trait. The cultivar Wonderful treated with marine algae extract (3 ml L^{-1}) gave the highest average of carbohydrate in plant leaves (32.329%) versus the lowest carbohydrate concentration from cultivar Slimi with the control treatment (23.506%), showing an increase of 37.535% (Table 2). For the interaction between cultivars and Humax, the foremost carbohydrate concentration (30.221%) resulted in the cultivar Wonderful with Humax (0.50 g L^{-1}) compared with the lowest concentration (25.145%) given by the cultivar Slimi with the control treatment (20.186%).

The interaction between the marine algae (3 ml L^{-1}) extract and the Humax (0.50 g L^{-1}) also influenced the said trait and exhibited the highest carbohydrate concentration (32.052%). It exceeded the lowest carbohydrate concentration with the control treatment (21.074%) with an increase of 52.092% (Table 2). The triple interaction of the studied factors significantly affected the carbohydrate content. The interaction of the pomegranate cultivar Wonderful treated with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) produced the ultimate carbohydrate concentration (34.506%) compared with the lowest concentration (20.167%) recorded in cultivar Slimi with the control treatment, providing an enhancement of 71.101%.

Nitrogen concentration

The cultivars, marine algae extracts, Humax, and their interaction revealed substantial differences in nitrogen concentration in the pomegranate leaves (Table 3). Cultivar Wonderful, on average, was superior for nitrogen concentration (2.581037%) than the cultivar Slimi (2.434333%), indicating an increase of 6.026%. Higher marine algae (3 ml L^{-1}) showed the maximum percentage of nitrogen (2.836444%) and outperformed the lowest nitrogen concentration given by the control treatment (2.195444%), with an increase of 29.196%. The Humax (0.50 g L^{-1}), on average, displayed an elevated nitrogen percentage in the leaves (2.648611%) versus the control treatment (2.383500%), with an upsurge by 11.122%.

The binary interaction between cultivar Wonderful and the marine algae (3 ml L^{-1}) gave the highest nitrogen percentage (2.946889%) compared with the lowest value (2.155667%) obtained from the cultivar Slimi without marine algae. It showed an increase of 36.704% in pomegranate leaves (Table 3). Cultivar Wonderful with Humax (0.50 g L^{-1}) gave the topmost average nitrogen (2.755889%). In comparison, the lowest average occurred with the cultivar Slimi without adding Humax (2.311556%), denoting an increase of 19.222%. The interaction between marine algae extract and Humax, on average, also affected the said trait. The treatment with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) gave the maximum average (3.049000%), while the lowest nitrogen average was evident with the control treatment (2.071000%), with an increase of 47.223%.

The triple interaction had a remarkable effect on raising the average nitrogen concentration in pomegranate leaves (Table 3). Cultivar Wonderful with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) demonstrated the highest average, amounting to 3.276000%. In contrast, the cultivar Slimi without the marine algae extract and Humax gave the lowest average (1.994000%), with an increasing percentage of 64.292%.

Phosphorus concentration

The cultivars, the marine algae extract and Humax different concentrations, and their interactions significantly affected the phosphorus concentration in the pomegranate leaves (Table 4). Cultivar Wonderful was superior by giving the highest phosphorus concentration (0.43089%) than the cultivar Slimi recorded with the lowest phosphorus concentration (0.42685%), with an increase of 0.946%. The marine algae extract (3 ml L^{-1}) also showed the foremost average for this trait (0.45183%) compared with the lowest average obtained in the control treatment (0.40711%). It recorded an elevated percentage of 10.984%. Furthermore, the Humax (0.50 g L^{-1})

Table 3. Effect of the cultivars, marine algae extract, Humax, and their interactions on the nitrogen concentration.

Cultivars	Marine algae extract Concentration (ml L^{-1})	Humax concentration (g L^{-1}) 0	0.25	0.50	Cultivars × marine algae extract (%)
Wonderful	0	2.148000	2.253000	2.304667	2.235222
	1.5	2.462000	2.534000	2.687000	2.561000
	3	2.756333	2.808333	3.276000	2.946889
Slimi	0	1.994000	2.185000	2.288000	2.155667
	1.5	2.323000	2.427000	2.514000	2.421333
	3	2.617667	2.738333	2.822000	2.726000
$LSD_{0.05}$	0.0011170				0.0006449
Humax means (%)		2.383500	2.490944	2.648611	Cultivars means (%)
$LSD_{0.05}$	0.0004560				
Cultivars × Humax	Wonderful	2.455444	2.531778	2.755889	2.581037
	Slimi	2.311556	2.450111	2.541333	2.434333
$LSD_{0.05}$	0.0006449				0.0003723
Marine algae × Humax interactions (%)					Marine algae extract means
	0	2.071000	2.219000	2.296333	2.195444
	1.5	2.392500	2.480500	2.600500	2.491167
	3	2.687000	2.773333	3.049000	2.836444
$LSD_{0.05}$	0.0007898				0.0004560

Table 4. Effect of the cultivars, marine algae extract, Humax, and their interactions on the phosphorus concentration.

Cultivars	Marine algae extract Concentration (ml L^{-1})	Humax concentration (g L^{-1}) 0	0.25	0.50	Cultivars × marine algae extract (%)
Wonderful	0	0.39700	0.41233	0.41867	0.40933
	1.5	0.42300	0.42600	0.43767	0.42889
	3	0.44533	0.45500	0.46300	0.45444
Slimi	0	0.38700	0.41100	0.41667	0.40489
	1.5	0.42267	0.42533	0.43133	0.42644
	3	0.44200	0.44800	0.45767	0.44922
$LSD_{0.05}$	0.001250				0.000722
Humax means (%)		0.41950	0.42961	0.43750	Cultivars means (%)
$LSD_{0.05}$	0.000510				
Cultivars × Humax (%)	Wonderful	0.42178	0.43111	0.43978	0.43089
	Slimi	0.41722	0.42811	0.43522	0.42685
$LSD_{0.05}$	0.000722				0.000417
Marine algae extract concentration × Humax (%)					Marine algae extract means
	0	0.39200	0.41167	0.41767	0.40711
	1.5	0.42283	0.42567	0.43450	0.42767
	3	0.44367	0.45150	0.46033	0.45183
$LSD_{0.05}$	0.000884				0.000510

gave the optimum average of phosphorus concentration (0.43750%) compared with the control treatment (0.41950%), with an increase of 4.290%.

Results also indicated a considerable effect of the binary interactions between the study factors on the phosphorus concentration in pomegranate leaves (Table 4). Cultivar Wonderful with marine algae extract (3 ml L^{-1}) showed the highest average phosphorus (0.45444%), while the lowest average resulted in the cultivar Slimi without marine algae extract (0.40489%), with an increase of 12.237%. Likewise, the cultivar Wonderful with Humax (0.50 g L^{-1}) displayed the paramount phosphorus concentration (0.43978%) compared with the obtained value from the interaction of the cultivar Slimi without adding Humax (0.41722%), with an enhancement of 5.407%.

Table 5. Effect of the cultivars, marine algae extract, Humax, and their interactions on the potassium concentration.

Cultivars	Marine algae extract Concentration (ml L^{-1})	Humax concentration (g L^{-1})			Cultivars × marine algae extract (%)
		0	0.25	0.50	
Wonderful	0	0.86433	0.97700	1.05400	0.96511
	1.5	1.08333	1.10400	1.10667	1.09800
	3	1.15300	1.16200	1.16767	1.16089
Slimi	0	0.84300	0.95300	1.00000	0.93200
	1.5	1.01400	1.06700	1.08900	1.05667
	3	1.10300	1.10700	1.13767	1.11589
$LSD_{0.05}$	0.001462				0.000844
Humax means (%)		1.01011	1.06167	1.09250	Cultivars means (%)
$LSD_{0.05}$	0.000597				
Cultivars × Humax	Wonderful	1.03356	1.08100	1.10944	1.07467
	Slimi	0.98667	1.04233	1.07556	1.03485
$LSD_{0.05}$	0.000844				0.000487
Marine algae extract concentration × Humax (%)					Marine algae extract means
	0	0.85367	0.96500	1.02700	0.94856
	1.5	1.04867	1.08550	1.09783	1.07733
	3	1.12800	1.13450	1.15267	1.13839
$LSD_{0.05}$	0.001034				0.000597

The interaction between marine algae extract and Humax affected the average of phosphorus concentration in pomegranate leaves (Table 4). The marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) treatments gave the supreme average (0.46033%) compared with the lowest of the control treatment (0.39200%), showing an escalation of 17.431%. The triple interaction also significantly influenced in increasing the average phosphorus concentration. Cultivar Wonderful with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) exhibited the highest average of the said trait (0.46300%), outperforming the cultivar Slimi without the marine algae extract and Humax. It had the lowest average (0.38700%), with an increase of 19.638%.

Potassium concentration

The outcomes showed a significant effect of the cultivars, concentration of marine algae and Humax, and their interactions on the potassium percentage in pomegranate leaves (Table 5). Cultivar Wonderful outperformed and recorded the highest potassium percentage (1.07467%) compared with cultivar Slimi, recording the lowest percentage (1.03485%), with an increase of 3.847%. Regarding the marine algae (3 ml L^{-1}), it enhanced the potassium concentration in the leaves (1.13839%) versus the low percentage of the potassium recorded with the control treatment (0.94856%), with an increase of 20.012%. The higher Humax concentration (0.50 g L^{-1}) also boosted the potassium percentage (1.09250%) compared with the lowest percentage obtained in the control treatment (1.01011%), showing an increase of 8.156%.

The binary interaction between the studied factors affected the said trait significantly (Table 5). The pomegranate cultivar Wonderful with marine algae extract (3 ml L^{-1}) gave the highest potassium concentration (1.16089%) compared with the lowest average recorded from the cultivar Slimi with the control treatment (0.98667%), indicating an increase of 12.442%. The interaction between the marine algae (3 ml L^{-1}) and Humax (0.50 g L^{-1}) produced the maximum potassium percentage in the leaves (1.15267%) compared with the lowest percentage recorded with the control treatment (0.85367%), and providing an increase of 35.025%.

The effect of the triple interaction was noteworthy, and the cultivar Wonderful treated with marine algae extract (3 ml L^{-1}) and Humax (0.50 g L^{-1}) gave the greatest potassium concentration in the pomegranate leaves (1.16767%) (Table 5). However, the cultivar Slimi without any treatment of the marine algae and Humax provided the lowest potassium percentage (0.84300%), revealing an increase of 38.513%.

DISCUSSION

The presented results showed the pomegranate cultivar Wonderful was superior to the other cultivar Slimi in the studied physiological and biochemical traits. This may be due to the genetic variance among the cultivars and their ability in absorbing more nutrients, leading to increased concentration of different biochemical contents within the plants. Marine algae extract has a significant effect on all the studied traits, which could refer to the natural compounds and hormones it contains, especially cytokinins. These have a positive role in cell division and the activation of photosynthesis enzymes increasing the leaf area, as reflected in increased chloroplasts and chlorophyll in various crop plants (Al-Tamimi, 2009).

In addition to the role of the marine algae extract in supplying the pomegranate plants with necessary mineral elements, it also contains nitrogen, phosphorus, and potassium. These affect increasing the leaf area, and thus, increasing photosynthesis, which, in turn, boosting the vegetative growth and enhancing the manufactured carbohydrates. The increase in nitrogen concentration might be due to the amino acids and betaine in the marine algae extract, which is a potential source of nitrogen in plants (Abdel-Amir *et al.,* 2011). The rise in phosphorus concentration could also be due to the marine algae extract containing potassium, stimulating the energy compound ATP, involved in the phosphorus constitution. This leads to an increased photosynthesis rates and generation of energy compounds and phosphorus in crop plants (Zhang *et al.,* 2023).

The amplified potassium concentration was due to the marine algae containing zinc and NPK, which help absorb and accumulate more potassium in the plant leaves. Similarly, the presence of plant growth regulators and amino acids in the extract boosted the vegetative growth, as reflected in the absorption of macro-elements and increasing their concentration in the leaves of the crop plants (Jan *et al.,* 2014). These findings were also consistent with past observations in the seedlings of peach (Al-Hajaimi and Al-Khafaji, 2016) and sweet cherry (Yassin and Al-Zubaidi, 2019).

The relevant results also authenticated the addition of Humax had a significant positive impact on the studied biochemical traits and the increase in the concentration of chlorophyll due to the acids. It enhanced the nitrogen and its direct role in building the chlorophyll pigment, as it is one of the components of porphyrins involved in the green pigment synthesis in peach trees (Kandil *et al.,* 2010). Humic acid also has a remarkable role in photosynthesis through its ability to increase membrane permeability and facilitate the absorption of nitrogen and iron, and thus, enhancing the processes of plastid formation and photosynthesis and carbohydrate contents in pomegranate leaves.

The increased concentration of nitrogen, phosphorus, and potassium was because of the humic and fulvic acids content in Humax. Their positive role enhanced the availability of these elements in the soil and their absorption by the crop plants, thus, increasing their concentration in the leaves. Humax also positively build the chlorophyll content and increase the synthesis of carbohydrates, which eventually reflected in the plant's biochemical traits in persimmon trees (Abd-AlRheem *et al.*, 2017) and pomegranate (Zarei, 2017; Al-Mayahi, 2018; Al-Hathal, 2020; Al-Mayahi *et al*., 2024).

CONCLUSIONS

The marine algae extract has a positive role in improving the biochemical traits due to its physical and chemical components the extract contains. It is also an environment-friendly product. Similarly, Humax is a local product to improve the physical and chemical properties of soil, aiding the readiness to absorb more nutrients and improve the nutritional status of crop plants.

REFERENCES

Abd-AlRheem KhM, Mohammed KAS, El-Damarawy YA (2017). Effect of humic and fulvic acid on growth, yield and nutrients balance of -Costata‖ persimmon trees. *J. Agric. Food Technol*. 7(4): 1-5.

Abdel-Amir MK, Abdel Majeed QJ, Mahmoud WS (2011). Effect of spraying with foliar fertilizer, prosol, and garlic infusion on orange seedlings (*Citrus aurantium* L.). *Al-Furat J. Agric. Sci.* 3(4): 54- 65.

Al-Hajaimi SHJ, Al-Khafaji MA (2016). Effect of CO_2 enrichment and foliar spraying with Agroleaf and Kelpak on the leaf content of N, P, K, protein and carbohydrates of smooth peach seedlings. *Iraqi J. Agric. Sci*. 47: 106-111.

Al-Hathal NM (2020). Effect of humic acid, spray of Chitosan and anti-transpiration agents on some vegetative and flowering indicators, yield, and fruit cracking in pomegranate plant, Slimmi cultivars. Ph.D. Thesis. Ministry of Higher Education and Scientific Research. College of Agriculture. University of Diyala. pp. 211.

Al-Marsoumi SAZ (2015). Effect of some nutrients on the growth of olive seedlings grown under desert conditions. *Anbar J. Agric. Sci.* 13(1): 3-9.

Al-Mayahi FHR (2018). Study of genetic variation and the effect of some physiological parameters on the growth and yield of pomegranates and their content of medically active compounds. Ph.D. Thesis. College of Agriculture, University of Baghdad, Iraq. pp. 314.

Al-Mayahi NKA, Al-Rubaei SM, Hassan MAF (2024). Pomegranate (*Punica granatum* L.) response to marine algae extract in interaction with Humax acid for growth traits. *SABRAO J. Breed. Genet.* 56(6): 2561-2570. http://doi.org/10.54910/sabrao2024.56.6.38.

Al-Sahhaf FH (1989). Applied Plant Nutrition. Dar Al-Kutub for Publishing and Printing. Ministry of Higher Education and Scientific Research. University of Baghdad, Iraq. pp. 456.

Al-Tamimi JYA (2009). Effect of humic acid and marine algae extracts on the growth, chemical properties and oil qualities of the rosemary plant *Rosemarinus officinalis* L. Proceedings of the Sixth Scientific Conference. Department of Biology, College of Education, University of Tikrit, Iraq. 7: 5-14.

Chappelle EW, Kim MS, McMurtrey III JE (1992). Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sensing Environ*. 39(3): 239-247.

Chen Y-H, Gao H-F, Wang S, Liu X-Y, Hu Q-X, Jian Z-H, Shi J-L (2022). Comprehensive evaluation of 20 pomegranate (*Punica granatum* L.) cultivars in China. *J. Integ. Agric*. 21(2): 434-445.

CSO (2020). Central Statistical Organization, the Ministry of Planning, Iraq. pp: 1-17.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. John Wiley and Sons, Inc., 2nd ed. New York, USA. pp. 157.

Hayness RJ (1980). A comparison of two modified Kjeldahl digestion technique for multi elements plant analysis with conventional wet and dry washing methods. *Commun. Soil Sci. Plant Anal.* 11(5): 459-467.

Hooks T, Niu G, Masabni J, Sun Y, Ganjegunte G (2021). Performance and phytochemical content of 22 pomegranate (*Punica granatum* L.) varieties. *Hortic. Sci*. 56(2): 217-225.

Jan K, Rather AM, Boswal MV, Ganie AH (2014). Effect of biofertilizer and organic fertilizer on morpho- physiological parameters associated with grain yield with emphasis for further improvement in wheat yield production (*Triticum aestivum* L.). *Int. J. Agric. Crop Sci.* 7(4): 178-184.

Joslyn MA (1970). Method in food analysis physical, chemical and instrumental method. *IOP Conf. Ser.: Earth Environ. Sci.* pp. 412.

Jumaa FF, Karumi MF (2005). Effect of growth regulators on the sex ratio and some natural traits of the pomegranate varieties Slimi and seedless Rawa. *J. Agric. Sci.* 2(3): 410- 416.

Kandil EA, Fawzi MIF, Shahin MFM (2010). Effect of some slow release nitrogen fertilizers on growth, nutrient status and fruiting of Mit Ghamr peach trees. *J. Am. Sci*. 6(12): 195-201.

Khudair SM, Aziz HM, Kamel YS (2015). Effect of spraying with garlic and whey extract on some vegetative growth characteristics of pomegranate seedlings, *Punica granatum* L. *Kerbala Univ. Sci. J.* 13(3): 8-13.

Page AL, Miller RH, Keeney DR (1982). Method of Soil Analysis. Part 2. 2nd ed. Agron. Publisher, Madison, Wisconsin, USA. pp. 124.

Stion RG, Mohsen AT, Maksoud MA, Abd El-Migeed MMM, Gomaa AM, Ibrahim AY (2009). Bio–organic fertilization and its impact on apricot young trees in newly reclaimed soil. *Am. Eurasian J. Agric. Environ. Sci.* 6: 62-69.

Yassin SA, Al-Zubaidi SR (2019). Effect of ammonium sulfate and marine extract (Alga21st) as a foliar fertilizer on the nutritional content of two varieties of sweet cherry seedlings, Ivy Lully and Champion. *Iraqi J. Agric. Sci.* 50(5): 1269-1280.

Zarei A (2017). Biochemical and pomological characterization of pomegranate accessions in fars province of Iran. *SABRAO J. Breed. Genet*. 49(2): 155–167.

Zhang J, Sun C, Wen Q, Miao R, Zhang B, Yan Z, Wang J (2023). Rational design of a novel halotolerant ATP regeneration system for biocatalytic CTP production. *J. Chem. Technol. Biotechnol.* 98(*4):* 1025-1031.