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RESEARCH

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Ecofriendly treatments to improve pomegranate trees production and tolerance to climate change

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Abstract

Background Pomegranate is capable of growing in different agro-climatic conditions ranging from tropical to subtropical. Where vegetative growth, nutritional and physiological status of pomegranate plants oscillate at high temperatures waves in the summer season. While we can overcome the cracking and sunburn of fruits, which are the main problems confronting pomegranate cultivars due to their significant impact on the quality of fruits.

Methods The present research was conducted over the course of two consecutive seasons in 2021 and 2022. The study focused on ten-year-old trees that were cultivated in sandy soil inside a private orchard situated 70 km away from Cairo on the Cairo-Alexandria desert road in Egypt (30°13′31.4″N30°39′09″E). The aim of the present work was to evaluate the effect of foliar spraying of Wonderful pomegranate trees with some nutrients (B, Zn, Fe, Mn, Ca and Cu), amino acids and irradiated licorice on vegetative growth, fruit physical, quality and chemical and leaf chemical parameters. A randomized full-block design with three duplicates was employed in the study. Using the recently computed least significant difference values at the 5% level, averages were compared.

Results The results indicated that foliar spraying with nutrients, amino acids and licorice extract (irradiated or not) alone or in combination significantly increased the vegetative growth and leaf total chlorophyll content compared to the control. Also, they significantly increased fruit length, diameter, weight, and number/tree and thus yield. As compared to the control, all treatments significantly reduced the number of sunburned and cracked fruits leading to decrease the percentage of un-marketable fruits. The juice content of anthocyanin, total sugars and total soluble solids increased significantly in response to all treatments compared to the control while the acidity% greatly reduced. When these treatments were used instead of the control, the content of N, P, K, Fe, Zn, and Mn in the leaves was also positively increased.

Conclusions In conclusion, foliar spraying of "Wonderful" pomegranate trees with a mixture of nutrients (B, Zn, Fe, Mn, Ca, Cu) and amino acids or irradiated licorice extract can be considered as a suitable management of the problem of sunburned or cracked fruits while improving the physical and chemical fruit quality with increased productivity.

Keywords Irradiated licorice, Amino acid, Nutrients, Pomegranate, Sunburn, Cracking

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Background

A significant obstacle to food security and sustainability in light of population expansion is the optimization of food production via the integrated control of root and vegetative development, while simultaneously boosting output and decreasing production losses. Pomegranate, scientifically known as *Punica granatum* L., is a popular fruit cultivated in tropical and subtropical areas. It belongs to the Punicaceae family. This plant is indigenous to central Asia and is widely grown in Mediterranean nations such as Spain, Morocco, Egypt, Iran, and Afghanistan. Evaluating pomegranate fruit quality relies on significant exterior characteristics, including size, shape, and color. Nevertheless, the color of fruit skin alone does not provide a reliable indication of ripeness or suitability for eating. Therefore, interior characteristics such as color, total soluble solids (TSS), and acidity are also considered when determining the readiness for harvest to fulfill market standards (Kader 2006; Holland et al. 2009).

The pomegranate variety has several significant challenges, including infections caused by various bacteria, fungi, pests, and insects, as well as disorders such as fruit breaking, sunburn, inadequate pigmentation of the peel and pulp, and below-average output. One of the primary obstacles faced is fruit cracking, which leads to substantial losses of around 40–60% of the total yield. Additionally, it significantly diminishes the quality of the fruit, making it difficult to sell and reducing its shelf life after harvest. Consequently, this results in large economic losses (Ikram et al. 2020). Possible causes of fruit cracking include increased evapotranspiration, decreased relative air humidity (RH), water imbalance, and rapid temperature fluctuations between day and night during the fruit's growth and maturation (Abdelrahman 2010). Moreover, the probability of fruit cracking increases in semi-arid areas, and the percentage of fruits showing signs of cracking may reach 10–35% (Khattab et al. 2012). Another important problem facing pomegranate production is sunburn. Fruits grown in regions with a snug climate are more susceptible to sunburn due to excess heat and increased duration of exposure to solar radiation. Physiologically, the most likely cause of sunburn is increased generation of reactive oxygen species due to the inability of the fruits to cope with stress (Munné-Bosch and Vincent 2019). Several practices have been used to alleviate these problems, including spraying of nutrients (Bakeer 2016), hormones (Hosein-Beigi et al. 2019) and organic and bio fertilizers (Torshiz et al. 2017), that showed variable promising effect.

So, the aim of the present work was to evaluate the effect of foliar spraying of Wonderful pomegranate trees with some nutrients (B, Zn, Fe, Mn, Ca and Cu), amino acids and irradiated licorice on vegetative growth, fruit physical, quality and chemical and leaf chemical parameters.

Methods

In a private orchard located 70 km from Cairo on Cairo Alexandria Dessert Road, Egypt (30o13′31.4°N30o39′09°E), this study was conducted over the course of two consecutive seasons in 2021 and 2022 on ten-year-old "Wonderful" pomegranate trees grown in sandy soil with a line spacing of 3.0 × 3.5 m. A drip irrigation system was used to cultivate the trees. The trees were uniform in vigor and received common horticultural practices as recommended by Ministry of Agriculture, Egypt. The tested trees were sprayed with some nutrients (B, Zn, Fe, Mn, Ca and Cu), amino acids (amino acids were as a compound contains all the amino acids as free amino acids) and irradiated licorice at 15 April, 15 May, 15 June, 15 July and 15 August for the two seasons. The nutrients solution was made up of 0.4% B (H_3BO_3), 0.4% Zn ($ZnSO_4$), 0.4% Fe ($FeSO_4$), 0.4% Mn ($MnSO_4$), 2% Ca ($CaCl_2$) and 0.2% Cu ($CuSO_4$). The extraction of licorice root was performed according to Ahmed et al. (2023). Irradiation of licorice extract with a dose of 10 KGy (dose rate of 0.86, 0.85, 0.84, 0.83 and 0.82 KGy h-1 for April, May, June, July and August 2021 and 0.76, 0.75, 0.74, 0.73 and 0.72 KGy h-1 for April, May, June, July and August 2022, respectively) was held at the National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt, using Gamma Cell (60Co).

The spraying treatments were as following:

T1: Control (Only water was sprayed on the trees).

T2: Nutrients (B, Zn, Fe, Ca, Mn, Cu).

T3: Amino acids at 1%

T4: Amino acids at 2%

T5: Non-irradiated licorice at 8mg/L.

T6: Irradiated licorice at 8mg/L.

T7: Nutrients + Amino acids at 1%

T8: Nutrients + Amino acids at 2%

T9: Nutrients + Non-irradiated licorice at 8mg/L.

T10: Nutrients + Irradiated licorice at 8mg/L.

This experiment used a full randomized block design, consisting of 10 treatments. Each treatment was replicated three times, with three trees per replicate. Each tree received 3 L of the applied solution plus 5cm per liter of Tween 20 to avoid the surface tension, except those of the control treatment, which was sprayed with water and 5cm per liter of Tween 20. The soil's physical

Table 1 Physical and chemical properties of the used soil

Soil	Soil texture	Sand (%)	Clay (%)	Silt (%)	pH	Cl^-	SO_4	Ca	Mg	Na	K	HCO_3 (%)	EC
2022	Sandy loam	41.6	3.5	13.5	9.01	95.1	10.3	25.20	18.3	43.2	1.2	1.1	9.85
2023	Sandy loam	42.1	3.8	15.00	8.30	81.3	11.1	21.4	13.5	52.3	1.6	1.3	8.51

Table 2 Chemical profile of licorice root (*Glycyrrhiza glabra*) extracts

Compounds	Non-irradiated licorice extract	Irradiated licorice extract
Total Phenols %	14.7	23.2
Total Flavonoids %	11.1	15.7
Amino acid %	5.6	9.3
N %	1.3	2.7
K %	0.85	1.89
P %	0.8	2.5
Fe ppm	36	50
Zn ppm	50	63
Mn ppm	5	7

and chemical parameters were assessed and shown in Tables 1 and 2.

Measurements

Vegetative growth parameters

The shoot length (cm) was measured for twenty shoots per tree during the growth period, with measurements taken at the conclusion of each season. Number of leaves/ shoots was recorded on the chosen twenty shoots per tree at cessation of growth in each season. Leaf area (cm^2) was measured by using leaf area meter, Model CI 203, U.S.A.

Fruit physical parameters

At harvest, samples of fifteen fruits of each tree in the replicate were used to determine the following fruit characteristics: fruit height (cm), fruit diameter (cm), fruit shape index, fruit weight (g), and fruit number /tree. Yield was recorded as kg/tree on 5th October for each season.

Fruit quality parameters

Fruit cracking: Percentage of fruit cracking / tree was calculated at harvest time.

$$\text{Fruit cracking} = \text{No. of cracked fruits/ Total No. of fruits} \times 100$$

Sunburn: During the harvest period, we determined the number of fruits per tree in each treatment. Additionally, each fruit was visually assessed to determine the extent of sunburn damage and expressed as a percentage proportional to the total number of fruits on the tree using the following equation

$$\text{Sunburn (\%)} = \text{No. of Sunburn fruits/ Total No. of fruits} \times 100$$

Marketable and Unmarketable fruits: According to Hegazi et al. (2014)

$$\text{Marketable fruits \%} = \text{Total No. of fruits} - (\text{No. of cracked} + \text{sunburn fruits}) \times 100.$$

$$\text{Unmarketable \%} = (\text{No. of cracked} + \text{sunburn fruits})/(\text{Total No. of fruits}) \times 100$$

Fruit chemical parameters

Total juice anthocyanin was determined according to Connor et al. (2002). Total juice sugars (g/100g FW) were determined by the method given in A.O.A.C. (2006). TSS % was estimated by the digital refractometer according to A.O.A.C. (2006). Total acidity percentage was determined as citric acid (g/100 ml of juice) by titration with a solution of 0.1 N., Na OH and using phenolphthalein as an indicator according to A.O.A.C. (2006). Total soluble solids/ Acid ratio were determined as the following equation: Total soluble solids/Acid ratio = TSS (%)/Total acidity (%).

Leaves chemical parameters

The quantification of chlorophyll content in leaves was conducted using a non-destructive Minolta chlorophyll meter, model SPAD 502. The name SPAD stands for soil plant analysis and development (Wood 1993). Leaf content of N% was determined using Kjeldhal method while K% was determined by flame photometer and P% analyzed by the molybdovanadate yellow color method by spectrophotometer. The content of Zn, Mn, Fe were measured using an atomic absorption spectrophotometer.

Statistical analysis

This research used a randomized full-block design with three duplicates. Using the Snedecor and Cochran

(Snedecor and Cochran 1980) methodology, the current data underwent statistical analysis. Data were subjected to one way-analysis of variance (ANOVA). Comparisons were made between averages using the newly calculated least significant difference (L.S.D.) values at the 5% level. The obtained data were calculated using (MSTAT) program according to McLain (McLain 1993).

Results

Vegetative growth parameters

The results presented in Table 3 demonstrate that the application of individual or combined sprays of various nutrients (B, Zn, Fe, Mn, Ca, and Cu), amino acids (1% and 2%), and licorice extract (8mg/L), whether exposed to radiation or not, significantly increased the shoot length, leaf number, and leaf area of Wonderful pomegranate in comparison to the control treatment for the two seasons under study. Amino acids had the most significant beneficial impact on the measures under study, with licorice extract coming next, which surpassed or occasionally matched the effects of the nutrients. Among sole treatments, the highest values of the above-mentioned parameters were obtained with amino acids at 2% while, nutrients + amino acids at 2% gave the highest values as compared to all treatments. Concerning licorice extract, irradiated form had a more enhancing effect on the studied vegetative parameters compared to non-irradiated one. Statistics indicate that the various combination sprays used have a significantly positive impact on all factors when compared to spraying each component separately.

Fruit physical parameters

Data in Table 4 showed the effect of spraying some nutrients, amino acids and irradiated licorice extract on average fruit height, diameter, shape index, weight

Table 3 Effect of different sprays on some vegetative growth parameters of Wonderful pomegranate

	Shoot length(cm)		Leaves number/shoot		Leaf area(cm)	
	2021	2022	2021	2022	2021	2022
T1	18.17^{i}	17.30^{h}	12.33^{f}	14.00^{f}	3.83^{g}	4.40^{h}
T2	24.57^{h}	25.50^{g}	19.33^{e}	21.33^{e}	5.60^{f}	5.80^{g}
T3	33.57^{e}	32.83^{d}	27.67^{d}	28.00^{d}	8.27^{d}	9.30^{d}
T4	38.53^{c}	37.30^{c}	31.33^{c}	32.33^{c}	8.63^{d}	9.70^{d}
T5	26.97^{g}	27.50^{f}	20.00^{e}	22.33^{e}	6.37^{f}	6.90^{f}
T6	29.07^{f}	30.00^{e}	20.33^{e}	22.67^{e}	7.47^{e}	7.50^{e}
T7	40.50^{b}	40.00^{b}	34.67^{b}	36.33^{b}	10.47^{b}	10.73^{b}
T8	42.97^{a}	42.33^{a}	39.33^{a}	40.00^{a}	11.63^{a}	12.00^{a}
T9	36.23^{d}	37.00^{c}	31.67^{c}	32.67^{c}	9.50^{c}	9.63^{d}
T10	37.93^{c}	38.07^{c}	34.33^{b}	36.00^{b}	9.60^{c}	10.23^{c}

Means with different letters are significantly different at $p < 0.05$

Table 4 Effect of different sprays on some fruit physical parameters of Wonderful pomegranate

	Fruit height(cm)		Fruit diameter(cm)		Fruit shape index		Fruit weight(gm)		Fruit number/tree		Yield/tree (kg)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1	7.17^{f}	7.27^{e}	7.60^{e}	7.67^{f}	0.94^{c}	0.95^{cd}	299.5^{i}	310.0^{d}	105.0^{i}	115.0^{i}	31.43^{g}	35.68^{j}
T2	8.20^{e}	8.40^{d}	8.63^{d}	8.80^{e}	0.95^{c}	0.95^{bcd}	330.0^{h}	340.0^{c}	135.0^{h}	136.0^{h}	44.57^{f}	46.25^{i}
T3	8.80^{cd}	9.00^{c}	9.23^{c}	9.33^{cd}	0.95^{bc}	0.96^{abc}	345.4^{fg}	350.0^{c}	153.7^{e}	156.7^{f}	53.07^{e}	54.82^{g}
T4	9.13^{c}	9.27^{c}	9.57^{c}	9.63^{c}	0.95^{abc}	0.96^{abcd}	360.0^{de}	370.0^{b}	162.3^{d}	167.7^{e}	58.45^{d}	62.05^{e}
T5	8.40^{de}	8.43^{d}	8.70^{d}	8.93^{de}	0.97^{ab}	0.94^{d}	335.0^{gh}	350.0^{c}	141.3^{g}	148.0^{g}	47.35^{f}	51.82^{h}
T6	8.80^{cd}	8.90^{c}	9.20^{c}	9.30^{cd}	0.96^{abc}	0.96^{abcd}	351.7^{ef}	370.0^{b}	148.3^{f}	153.7^{f}	52.17^{e}	56.87^{f}
T7	10.90^{a}	10.03^{b}	11.17^{a}	10.33^{b}	0.98^{a}	0.97^{ab}	380.0^{bc}	392.3^{a}	177.3^{ab}	182.7^{b}	67.39^{b}	71.67^{b}
T8	11.13^{a}	11.33^{a}	11.47^{a}	11.67^{a}	0.97^{ab}	0.97^{a}	395.0^{a}	397.7^{a}	181.0^{a}	193.7^{a}	71.50^{a}	77.02^{a}
T9	9.67^{b}	9.83^{b}	10.10^{b}	10.27^{b}	0.96^{abc}	0.96^{abcd}	370.0^{cd}	388.3^{b}	167.7^{c}	171.7^{d}	62.04^{c}	66.66^{d}
T10	10.00^{b}	10.03^{b}	10.47^{b}	10.43^{b}	0.96^{bc}	0.96^{abcd}	385.0^{ab}	393.7^{a}	173.7^{b}	177.0^{c}	66.86^{b}	69.68^{c}

Means with different letters are significantly different at $p < 0.05$

and number and yield of Wonderful pomegranate. All treatments significantly influenced the fruit height and diameter compared to the control. For sole spray treatments, the highest value of fruit height and diameter was recorded with amino acid at 2% followed by amino acid 1% then irradiated licorice extract with no significant differences. Non-irradiated licorice extract and nutrients had a similar positive effect but less than the other treatments. For combined spray, all combinations significantly increased the fruit weight and number compared to sole spray with superiority to nutrients + amino acid 2% then nutrients + amino acid 1% and irradiated licorice extract. Regarding the fruit shape index, only the combined spraying of nutrients and amino acids at both rates utilized led to an increase in height by a greater percentage than the diameter, which led to a significant increase in the fruit shape index compared to the control. Compared to other sole spray and control, spraying Wonderful pomegranates with amino acid at 2% or irradiated licorice extract produced the highest average fruit weight. Moreover, among combined sprays, spraying each one along with nutrients produced the largest fruit weight. Nutrients, non-irradiated licorice and amino acids at 1% all had similar effects on the average fruit weight.

Regarding the effect of sole treatment spray on fruit number and yield, the highest values were obtained by amino acid at 2%, amino acid 1%, irradiated licorice extract, non-irradiated licorice extract, nutrients and then the control with significant difference. Combined spray of nutrients + amino acid at 2% gave a significant higher fruit number and yield compared to other combined treatments. The beneficial effect of amino acid at 1% or irradiated licorice extract in combination with nutrients was comparable but significantly higher compared to nutrients + non-irradiated licorice extract. Moreover, when combined sprays are applied, the majority of the examined physical fruit quality parameters react more favorably than when each constituent is applied separately.

Fruit quality parameters

Data of Table 5 illustrated the effect of nutrients; amino acids and irradiated licorice extract foliar spraying on number of sunburned, cracked pomegranate fruits and percentage of marketable and unmarketable fruits. All sole foliar sprays greatly decreased the number of sunburned and cracked fruits compared to the control with superiority of amino acids spray (2% and 1%) followed by licorice extract spray (irradiated and non-irradiated) then nutrients spray. For combination spray, all treatments significantly reduced the number of sunburned and cracked fruits compared to the control with no significant difference in between. Moreover, fruit cracking and sunburn are reduced when combined spraying is used instead of just one of its constituent parts. However, the reduction in these two outcomes was not statistically significant when a combination of nutrients and amino acids was used instead of amino acids alone in the 1st season only. The effect of different foliar treatments on the percentage marketable and un-marketable fruits followed the same trend as number of sunburned, cracked fruits. In this respect, all sole treatments in general induced a significant increase in the percentage of marketable fruit and decrease in the percentage of un-marketable fruits compared to the control in both seasons. The greatest positive effects were in favor of amino acid at both levels followed by licorice extracts and then nutrients. Also, combination treatments exerted more significant positive effect compared to those of sole application except with

Table 5 Effect of different sprays on some fruit quality parameters of Wonderful pomegranate

	Fruit sunburn%		Fruit cracking%		Marketable fruits%		Un-marketable fruits%	
	2021	2022	2021	2022	2021	2022	2021	2022
T1	13.62^{a}	12.20^{a}	15.25^{a}	13.09^{a}	79.37^{f}	79.93^{e}	20.63^{a}	20.07^{a}
T2	8.15^{b}	8.09^{b}	9.14^{b}	8.83^{b}	84.41^{e}	87.49^{d}	15.59^{b}	12.51^{b}
T3	2.61^{e}	3.41^{d}	3.90^{e}	4.04^{d}	94.79^{b}	94.25^{b}	5.21^{e}	5.75^{d}
T4	2.67^{e}	2.59^{e}	3.08^{ef}	3.18^{de}	95.69^{ab}	95.82^{ab}	4.31^{ef}	4.18^{de}
T5	6.85^{c}	6.08^{c}	7.78^{c}	6.77^{c}	89.60^{d}	90.98^{c}	10.40^{c}	9.02^{c}
T6	4.94^{d}	5.42^{c}	5.40^{d}	5.86^{c}	93.25^{c}	91.53^{c}	6.75^{d}	8.47^{c}
T7	2.26^{e}	1.28^{f}	2.81^{ef}	2.18^{ef}	96.43^{ab}	97.45^{a}	3.57^{ef}	2.55^{e}
T8	1.84^{e}	1.21^{f}	2.21^{f}	1.72^{f}	97.24^{a}	97.94^{a}	2.76^{f}	2.06^{e}
T9	2.98^{e}	2.07^{ef}	3.78^{e}	2.33^{ef}	94.84^{b}	96.80^{a}	5.16^{e}	3.20^{e}
T10	2.30^{e}	1.55^{f}	3.45^{e}	2.26^{ef}	96.16^{ab}	97.48^{a}	3.84^{ef}	2.52^{e}

Means with different letters are significantly different at $p < 0.05$

Table 6 Effect of different sprays on some fruit chemical parameters of Wonderful pomegranate

	Anthocyanin		Total sugar		TSS (%)		Total acidity (%)		TSS/Acidity (%)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1	17.50^{g}	16.50^{i}	10.20^{f}	10.40^{g}	13.23^{f}	14.20^{f}	1.90^{a}	1.87^{a}	6.97^{e}	7.62^{f}
T2	20.00^{f}	20.50^{g}	11.00^{e}	11.20^{f}	14.50^{e}	15.00^{e}	1.75^{b}	1.70^{b}	8.30^{d}	8.86^{e}
T3	22.00^{e}	23.50^{f}	11.30^{cde}	11.30^{ef}	15.00^{de}	16.00^{cd}	1.55^{c}	1.57^{c}	9.69^{c}	10.19^{d}
T4	23.50^{cd}	24.17^{e}	11.47^{bcd}	11.50^{cde}	15.50^{cd}	16.67^{b}	1.40^{e}	1.38^{d}	11.08^{b}	12.06^{bc}
T5	19.83^{f}	20.00^{h}	11.23^{de}	11.30^{ef}	14.50^{e}	15.50^{de}	1.51^{cd}	1.60^{c}	9.62^{c}	9.71^{d}
T6	22.50^{de}	23.50^{f}	11.33^{cde}	11.40^{def}	15.23^{de}	16.00^{cd}	1.50^{cd}	1.52^{c}	10.17^{c}	10.51^{d}
T7	24.50^{c}	26.00^{c}	11.70^{ab}	11.80^{ab}	16.50^{b}	17.00^{b}	1.40^{e}	1.35^{de}	11.79^{b}	12.59^{b}
T8	27.67^{a}	28.83^{a}	11.90^{a}	12.00^{a}	18.00^{a}	18.00^{a}	1.30^{f}	1.26^{e}	13.85^{a}	14.29^{a}
T9	24.00^{c}	25.50^{d}	11.50^{bcd}	11.58^{cd}	16.17^{bc}	16.50^{bc}	1.43^{de}	1.42^{d}	11.33^{b}	11.66^{c}
T10	26.00^{b}	26.83^{b}	11.60^{abc}	11.65^{bc}	16.50^{b}	17.17^{b}	1.40^{e}	1.41^{d}	11.76^{b}	12.23^{bc}

Means with different letters are significantly different at $p < 0.05$

amino acid sprays where addition of nutrients exerted little or no significant effect.

Fruit chemical parameters

Table 6 demonstrated the juice chemical parameters including anthocyanin, total sugar, TSS, total acidity and TSS/ acid ratio of Wonderful pomegranate as affected by different treatments spray. In our study, all sole spray treatments increased juice content of anthocyanin when compared to the control for the two seasons under study. The highest values of anthocyanin content was obtained with amino acid at 2%, followed by irradiated licorice extract and amino acid at 1%, then nutrients with the least value with non-irradiated licorice at both seasons. For combination treatments, foliar spray of nutrients + amino acid at 2% gave the highest anthocyanin content followed by nutrients + irradiated licorice extract then nutrients + amino acid 1% and nutrients + non-irradiated licorice extract that showed comparable effect. Regarding the juice content of total sugar and TSS, all sole spray significantly increased both parameters compared to the control but with little significance between them. The highest values of total sugar and TSS with combined treatment spray were obtained with nutrients + amino acids at 2% followed by the other combined treatments with no significance differences. Data analysis revealed that different treatments spray affect juice total acidity (%) and TSS/acidity (%) in an inverse manner. All treatments gave a significantly reduced total acidity (%) as well as elevated TSS/acidity (%) compared to the control. Among sole treatments, the lowest value of total acidity (%) and the highest value TSS/acidity (%) obtained by amino acid 2% followed by irradiated licorice extract, amino acid 1% and non-irradiated licorice extract with no significance differences and then nutrients spray. While among the combined treatments, nutrients + amino acids

Table 7 Effect of different sprays on some leaf chemical properties of Wonderful pomegranate

	Total chlorophyll		N%		P%		K%		Fe (ppm)		Zn (ppm)		Mn (ppm)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1	44.0^{f}	46.0^{h}	1.84^{i}	1.94^{g}	0.198^{f}	0.210^{e}	1.17^{f}	1.14^{g}	240.9^{i}	243.4^{i}	31.1^{f}	31.7^{e}	21.2^{h}	23.3^{e}
T2	51.3^{e}	52.3^{g}	1.96^{h}	2.08^{f}	0.209^{ef}	0.217^{de}	1.24^{ef}	1.22^{fg}	263.7^{h}	260.7^{h}	32.7^{ef}	33.6^{de}	25.2^{g}	25.2^{e}
T3	56.3^{d}	57.0^{ef}	2.19^{e}	2.23^{e}	0.256^{d}	0.269^{c}	1.42^{de}	1.45^{d}	273.3^{f}	274.9^{f}	34.5^{de}	35.2^{d}	27.9^{f}	29.3^{d}
T4	60.0^{bc}	61.3^{c}	2.22^{d}	2.33^{d}	0.261^{d}	0.270^{c}	1.45^{cd}	1.57^{c}	277.3^{e}	276.2^{e}	37.6^{bc}	39.5^{c}	31.3^{d}	32.4^{c}
T5	55.0^{d}	56.0^{f}	2.12^{g}	2.21^{e}	0.216^{e}	0.230^{d}	1.38^{de}	1.27^{ef}	269.1^{g}	266.4^{g}	33.7^{e}	34.6^{d}	27.3^{f}	26.2^{e}
T6	57.7^{cd}	58.0^{de}	2.17^{f}	2.22^{e}	0.255^{d}	0.268^{c}	1.39^{de}	1.31^{e}	271.4^{f}	273.7^{f}	34.1^{de}	35.2^{d}	29.2^{e}	30.2^{cd}
T7	61.0^{b}	62.7^{b}	2.41^{b}	2.53^{b}	0.296^{ab}	0.313^{ab}	1.74^{b}	1.81^{b}	287.8^{b}	286.9^{b}	39.5^{ab}	42.2^{b}	37.3^{b}	37.6^{b}
T8	64.7^{a}	65.0^{a}	2.51^{a}	2.63^{a}	0.301^{a}	0.328^{a}	1.98^{a}	2.07^{a}	293.9^{a}	298.6^{a}	41.4^{a}	45.6^{a}	41.3^{a}	42.1^{a}
T9	59.3^{bc}	59.0^{d}	2.33^{c}	2.43^{c}	0.272^{cd}	0.280^{c}	1.47^{cd}	1.57^{c}	279.7^{d}	281.4^{d}	36.2^{cd}	39.3^{c}	34.9^{c}	36.4^{b}
T10	61.0^{b}	62.0^{bc}	2.42^{b}	2.53^{b}	0.281^{bc}	0.300^{b}	1.61^{bc}	1.64^{c}	282.0^{c}	283.4^{c}	37.2^{c}	41.1^{bc}	37.9^{b}	38.7^{b}

Means with different letters are significantly different at $p < 0.05$

2% significantly decreased total acidity (%) and increased TSS/acidity (%) compared to the other combined treatments which showed non-significant differences between them. For all juice chemical parameters, adding nutrients to amino acid at either levels or licorice extracts whether irradiated or not had a significant positive effect when compared to application of each one alone.

Leaf chemical properties

Regarding leaf total chlorophyll content in Table 7, all treatments led to a significant increase compared to the control. For sole treatments, the highest chlorophyll content was recorded with amino acid at 2% followed by irradiated licorice, amino acid at 1%, non-irradiated licorice then nutrients. The best combination spray was nutrients plus amino acid at 2% that gave the highest chlorophyll content compared to other combination sprays.

According to data of Table 7, most of the foliar treatments positively affect the content of N, P, K, Fe Zn and Mn in the leaves of Wonderful pomegranate. For N%, all sole treatments significantly increased N% compared to the control with the highest value obtained by amino acid at 2% while the least value was recorded with nutrients. The highest increment in N% with combination treatment was for nutrients + amino acid while, the least was for nutrients + non-irradiated licorice extract. Regarding P%, K% and Zn (ppm), sole application of amino acids (2% and 1%) and licorice extract (irradiated or non-irradiated) induced a significant increment of P%, K% and Zn content with no or little significance in between while nutrients spray had no significant effect on any one of them. Combined spray of nutrients + amino acids at both levels was superior to nutrients + licorice extract whether irradiated or not in significantly elevating N% and P% compared to the control and sole treatments.

Concerning Fe, all sole and combined treatments significantly enhanced Fe content. Amino acid at 2% alone gave the highest Fe content among sole treatments while the lowest value was for nutrients. For combined sprays, the highest and lowest Fe content were recorded with nutrients + amino acids at 2% and nutrients + non-irradiated licorice, respectively. Amino acid spray at 2% induced the highest increment in Mn content among sole treatments followed by irradiated licorice extract, amino acids at 1%, non-irradiated licorice and then nutrients that showed a little significant effect. Nutrients + amino acids at 2% showed a more positive effect on Mn content compared to other combined treatments which have a comparable effect. Also, addition of nutrients to amino acids (both levels) or licorice extract (irradiated and non-irradiated) lead to a significant increment of the studied leaf mineral content compared to their sole application.

Discussion

Pomegranate is considered one of the royal fruits, as all its parts play a vital role in the development and maintenance of human health. The fruit enhances the brain activities, helps get ridding of intestinal worms and detoxifies the body and blood. The peel is used as an aid in controlling sugar and blood pressure levels for diabetics and hypertensive patients, and controlling sore throat infections. The white membrane is effective in promoting the healing of wounds and ulcers of mucous membranes in addition to its antidiarrheal effect (Khan et al. 2020).

Elements in plants are categorized based on their biochemical behavior and physiological function. Primary organic plant components include C, H, O, N, and S, which are essential for amino acids, proteins, enzymes, and nucleic acids. These nutrients are absorbed through oxidation–reduction processes. Phosphorus, boron, and silicon are two elements with notable biochemical similarities, taken up as inorganic anions or acids and chemically linked to sugars to form phosphate, borate, and silicate esters. Essential minerals include K, Na, Ca, Mg, Mn, and Cl, which are taken up in soil solution and ionic form in plant cells. Cations and chelates form ions, while Fe, Cu, Zn, and Mo transport electrons. Micronutrients are essential in small amounts, while macronutrients are essential in large quantities. Variations in mineral nutrient levels in plant shoots indicate their function (Marschner and Zed 2023).

Several environmental factors have a negative impact on plant growth, development, and crop output, with temperature stress being a particularly significant constraint. Plant thermal acclimation mechanisms involve the buildup of compatible nitrogen-rich solutes, such as amino acids. These solutes enhance stress tolerance by serving as osmolytes, regulating ion transport, and stomatal opening. Additionally, they have a protective effect on enzymes and influence gene expression and redox homeostasis. The rise in free amino acids is a result of the breakdown of functioning proteins or the suppression of new protein production (Botta 2012).

In our investigation, we found that spraying Wonderful pomegranate with nutrients (B, Zn, Fe, Ca, Mn, and Cu) greatly enhanced the fruit's physical, quality and chemical characteristics, as well as its vegetative development, total chlorophyll, and certain leaf nutritional content. The increases in leaf total chlorophyll and nutrient content that were observed were comparable to those reported by Fadam and Hamad (2023) who sprayed Selimi pomegranate seedlings with chelated iron or nutrient solution. Numerous investigations have validated the advantageous impacts of nutrients on the vegetative development of pomegranates, along with the physical and chemical characteristics of their fruits, particularly fruit cracking and sunburn on various pomegranate cultivars. These findings align with the findings of this study on the Wonderful Pomegranate. El-Salhy et al. (2022) reported a significant improvement of the vegetative growth, chemical fruit quality and yield of Wonderful, Manfalouty and Higazy pomegranate cultivar with reduction of fruit cracking percentage in response to spraying with 0.1% nano-B or 1% nano-Ca or both together. Also, foliar spraying of Wonderful pomegranate trees with either boric acid, calcium chloride, zinc sulphate, copper sulphate or manganese sulphate significantly enhanced the vegetative growth, fruits physical and chemical parameters and yield with reduced percentage of fruit cracking, sunburn and unmarketable fruit (Abd El-wahed et al. 2021). Moreover, spraying pomegranate cv. Bhagwa with KNO_3, $CaCl_2$ or H_3BO_3 had a significant positive effect on vegetative growth, fruit size, and weight, yield, fruit chemical properties and fruit cracking percentage with a more positive effect when sprayed together (Kumar et al. 2020).

The improved growth may be ascribed to the presence of Ca, which is necessary for the optimum processes of cell division, elongation, and cell wall construction (Ashraf et al. 2018). Zinc is involved in the synthesis of the amino acid tryptophan, which serves as a precursor for the production of the hormone indole acetic acid. This hormone promotes cell elongation and accelerates the pace of growth. Furthermore, Zn has a significant role in the activation of several enzymes, including peptidases, proteinases, and enolase. It also modulates reactions related to photosynthesis, protein synthesis, DNA and RNA metabolism (Dickinson et al. 2003; Alloway 2008).

The favorable effect of nutrients spray on yield and physical fruit parameters of Wonderful pomegranate could be referred to Mn which involved in photosynthesis process and activation of plenty of plant enzymes. In addition, Fe is essential for cytochromes synthesis which modulate photosynthesis and respiration as well as synthesis of chlorophyll that modulate plant metabolism (Rout and Sahoo 2015). Besides, Zinc promotes tissue growth through the synthesis of tryptophan involved in the synthesis of indole acetic acid. It has also a great role in protein, carbohydrates and nucleic acid metabolism in addition to its participation as integral part of some enzymes or as activator to others (Alloway 2008). Nutrients play an important role in protecting fruits from sunburn and cracking, and most of the effect is attributed to zinc. The role of zinc in improving fruit cracking goes back to enhance cell division, strengthen the cell wall and diminish the formation of abscission layer. Moreover, it modulates the uptake of water and nutrients as well as enhances enzymes activation and Indole acetic acid synthesis (Mengel et al. 2001; Kumar et al. 2017). Besides, boron is positively involved in the process of cell wall synthesis and water uptake (Kumar et al. 2017). Enhanced nutrient content in leaves can be attributed to stimulated vegetative growth, which is accompanied by greater absorption of nutrients needed to meet the plant's requirements for the physiological processes (Kobayashi et al. 2019).

Spraying Wonderful pomegranate with amino acids spray showed a great improvement of vegetative growth, fruit quality parameters, yield and leaf mineral content as well as a significant reduction of fruit cracking and sunburn that leads to increase the percentage of marketable fruits. These results were similar to those obtained by Randive et al. (2023) where spraying pomegranate trees with amino acids (2 g/L.) improved the vegetative growth and some fruit quality parameters. Also, foliar spraying of "Mollar de Elche" pomegranate with γ-Aminobutyric (0, 10, 50 and 100 mM) increased tree fruit number, yield, total anthocyanin and total phenolic compounds in rate dependent manner (Lorente-Mento et al. 2023). Similarly, Kurhade et al. (2022) declared the positive effect of amino acids spraying of Pomegranate cv. Phule Bhagwa Super on fruit number, weight and yield. The increase in the values of the studied parameters increases as the level of sprayed amino acids increased from 150 to 250 ppm, with a significant difference in favor of the higher level. Manfalouty pomegranate trees sprayed with amino acids (0, 0.5, 1 and 1.5%) showed improved physical and chemical fruit characteristics with reduced fruit cracking percentage with no or little significance between different amino acids levels (Khodair and El-Rahman 2021). Moreover, amino acids spraying (1 and 2%) of Manfalouty pomegranate improved yield, fruit length, diameter and weight, fruit TSS, total sugars and reducing sugars percentages while the percentages of fruit cracking and acidity were significantly decreased with more favorable effect for the lower amino acids level (Mohamed et al. 2020).

Amino acids play several vital roles in plants, including activation of natural hormones synthesis such as IAA, cytokinins, ethylene and GA3, cell multiplication, organic food synthesis and enzymes which in turn improves vegetative growth and nutritional status of the plant, which enhances the physical and chemical characteristics of the resulting fruits (Ahmed and Abd El-Hameed 2003). The positive effect of amino acids on protein synthesis based on its stimulating effect on nitrate reductase and glutamine synthetase enzymes up regulating nitrogen metabolism (Liu et al. 2014; Mobini et al. 2014). Besides, amino acids improved pollen tube ovule penetration and delayed ovule senescence thus increasing fruit set which means increased fruit number per tree. Such increment of fruit number as well as increased fruit weight translates into an increase in yield (Kurhade et al. 2022).

One of the positive effects obtained in this study is the effect of licorice extract spray, especially irradiated, on growth, fruit quality, yield, nutritional status and marketable fruit percentage of Wonderful pomegranate. Similar findings were documented by Anbhu et al. (2022) on Bhagwa, Hasan and Kader (2022) on Sawa, Nooruldeen et al. (2022) on Salemy and Hussein et al. (2021) on Salimi pomegranate cultivar where spraying of pomegranate trees with different rates of licorice extract significantly improved fruit physical and chemical characteristics, yield, nutritional status and cracking percentage in particular the higher rates. Moreover, spraying young pomegranate seedlings of the 'Salemi' cultivar with licorice extract (10 g.L-1) caused a significant increase in the height of the seedlings, as well as in the number of leaves and branches, leaf area, and total chlorophyll of the leaves when compared to the control (Al-Sereh et al. 2020). Spraying irradiated licorice extract led to an increase in the benefit in improving vegetative growth, nutritional status, physical and chemical characteristics of fruits, yield and nutritional content of leaves, as well as reducing the percentage of sun-burned and cracked fruits compared to non-irradiated ones. This improvement was parallel to those obtained with Ahmed and Abd El-Hameed (2003) who sprayed red globe grapevine with irradiated licorice extract (4 and 8 g. L-1) and yeast extract, resulting in a significant improvement in vegetative growth and leaf total chlorophyll and NPK percentages. Moreover, it increased berry volume, weight, TSS%, anthocyanin content, bunch weight and yield while number of sun-burned bunches and berry acidity% decreased compared to yeast extract spray only. Both irradiated licorice extract levels had a similar enhancing effect, though in some cases the high level was superior.

The stimulating effect of licorice on vegetative growth is due to its rich content of phenolic compounds, polysaccharides, amino acids, minerals, vitamins and growth phytohormones stimulating the cell division and elongation (Zadeh et al. 2013) while the improvement of the physical properties is attributed to the presence of the magic ingredient, mevalonic acid, which works to increase the synthesis of gibberellin, leading to increased cell division and expansion increasing fruit size and weight thus yield, in addition to containing abundant amounts of salts and carbohydrates that increase vegetative and root growth, which is accompanied by increased the ability to absorb a large amount of soil nutrients and water that is transferred to the fruits, thus increases yield parameters and productivity (Pérez and Gómez 2000; Casanova et al. 2009). The enhanced fruit total soluble solids in response to foliar licorice spray could be related to the enhancement of the leaf area and the leaf chlorophyll content that increased the synthesis of carbohydrates and absorption of soil nutrients while reduced total acidity may be due to acids exhaustion in many physiological processes like respiration (Alsalhy and Aljabary 2020). Irradiation of medicinal plants is a sensitive process because each dose of radiation has a different effect on different plants, and different doses have an asymmetric effect on the same plant in terms of its content, especially volatile components, with the appearance of new components and the disappearance of others that were present. As for licorice, gamma-irradiation with 10 kGy does not lead to the disappearance of any of the volatile compounds, with the emergence of new compounds such as benzaldehyde and a noticeable increase in many compounds such as indole. Once again, irradiated licorice at a dose of 10 kGy showed the highest content of the largest number of compounds present with maximum yield of its volatile compounds by 12% compared to non-irradiated one (Gyawali et al. 2008).

Our results declared that combination spray of nutrients mixture with amino acids or irradiated licorice extract gave better effect compared to their sole application. These results were align with El-Badawy, (2019) where spraying Canino apricot trees with micronutrients mixture and amino acids significantly improved leaf area, yield, fruit weight, TSS%, total sugars % and leaf N, P and K percentages and leaf Fe and Zn content. Combined spray in particular (150 ppm micronutrients and 3 ml/L amino acids) gave more positive effect compared to either micronutrients mixture or amino acids with more pronounced effect in comparison to the micronutrient mixture. Similar findings were obtained by Kheir et al. (2021) on mango (cv. Fagri Kalan) sprayed with micronutrient mixture and amino acids. Data showed that, when compared to applying the micronutrient mixture and amino acids alone, spraying the combination of the two significantly improved vegetative development, nutritional status, leaf nutrient content, physical and chemical fruit quality, and yield. Moreover, the highest level of both the micronutrient mixture and amino acids produced the best results.

Conclusions

Foliar sprays of "Wonderful" pomegranate with irradiated licorice or amino acids along with some nutrients resulted in marked improvements in vegetative growth (the length of the shoot, the number of leaves, and the area of the leaf increased almost double), physicochemical quality of fruits (the yield increased more than doubled) and nutrient content of leaves. In addition, there was a significant reduction in fruit cracking and sunburn by more than seven-folds resulting in an increase in the percentage of fruit fit for marketability and consumption. Therefore, these sprays can be regarded as a secure and efficient option for fertilization, ensuring that they do not harm the environment and help the plant cope with climate change effectively and obtain a crop with good characteristics that does not conflict with the financial interests of the producers.

Abbreviations

T1	Control (Only water was sprayed on the trees)
T2	Nutrients (B, Zn, Fe, Ca, Mn, Cu)
T3	Amino acids at 1%
T4	Amino acids at 2%
T5	Non-irradiated licorice at 8 mg/L
T6	Irradiated licorice at 8 mg/L
T7	Nutrients + Amino acids at 1%
T8	Nutrients + Amino acids at 2%
T9	Nutrients + Non-irradiated licorice at 8 mg/L
T10	Nutrients + Irradiated licorice at 8 mg/L

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Author contributions
MFA: Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data Curation, Project administration, Writing—Original Draft,. RMA: Visualization, Methodology, Validation, Investigation, Resources, Writing—Review & Editing. SZH: Methodology, Resources, Data Curation, Writing—Original Draft, MSG: Methodology, Resources, Data Curation, Writing—Original Draft, WA: Methodology, Resources, Data Curation, Writing—Review & Editing. AGS: Methodology, Resources, Data Curation, Writing—Original Draft.

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Declarations

Ethics approval and consent to participate
The samples were taken on a private land, and the owners had granted us permission to pick the pomegranates fruits and conduct testing on them.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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