Impact of Natural stimulates and Antioxidant compounds on fruit quality and quantity of pomegranate trees cv. H116

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Abstract. This experiment was carried out during two seasons (2022 and 2023) to examine the effect of spraying pomegranate trees cv. H116 with extract algae at (2cm/l), salicylic acid at (0.5 g/l) and ascorbic acid at (0.5 g/l) either alone or in combination together on productivity, fruit properties and leaf mineral content. All treatments were applied as a foliar three times: at full blooming, after fruit sit and after 30 days from fruit set. The results showed that all treatments caused a significant increase in the yield and the leaf mineral content of trees compared to the control. Treated pomegranate trees with 0.5 g/l ascorbic acid + 0.5 g/l salicylic acid was the best treatment for enhancing the yield (kg), fruit weight (g) and fruit peel (%). Foliar application on pomegranate trees with (2 cm/l algae extract + 0.5 g/l ascorbic acid + 0.5 g/l salicylic acid) was the best treatment in this study to improve the fruit juice content and its quality and obtain maximum leaf content of nitrogen, phosphorus and potassium.

1 Introduction

The pomegranate (Punica granatum) L. belongs to the Punicaceae plant family and is mainly found in semi-arid, mild-temperate, and subtropical regions. Today pomegranate orchards are planted all over the world, but they are most popular in the Mediterranean region, where the fruit is of the highest quality [1, 2]. The pomegranate (Punica granatum L.) is highly adaptive to a wide range of environments, particularly [3].

Salicylic and ascorbic acids are examples of antioxidants that are safe for the environment and people (4). They play a role in protecting the cells from senescence and preventing free radicals from the oxidizing lipids, which make up the plasma membrane. Additionally, they contribute to reducing stress by improving the antioxidant system. According to [5], salicylic acid is essential for plant development, ion absorption, and transport. According to [6] salicylic acids have an impact in stomatal movement, photosynthesis, ethylene production, water relations in plants, and the reversal of ABA's influence on leaf abscission. Because of its beneficial effects on cell division, expansion,

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and specialization, it is also one of the substances that play a role in the regulation of plant growth and development [7]. According to [5] it improved the antioxidant enzymes' responses to salinity and drought stress in plants. It is responsible for enhancing natural hormones, which are essential for regulating the growth and development of plants [8]. In this respect, [9] mentioned that foliar sprays with salicylic acid induced positive effects on yield and fruit quality. [10] Stated that foliar sprays of salicylic acid at 100 ppm improving growth, yield, fruit quality of Manfalouty pomegranate trees.

Ascorbic acid is regarded as an organic and natural antioxidant compound [11]. Ascorbate is a major metabolite in the plant. This antioxidant and its association with other components of the antioxidant system, protect the plant against oxidative damage resulting from aerobic metabolism, photosynthesis and range of pollutants [12]. Ascorbic acid has auxinic action. [13] Reported that foliar spray ascorbic acid enhanced yield and fruit quality of Manfaloty pomegranate trees. [14, 9] pointed out that foliar spray with ascorbic acid improved growth, yield fruit quality of Wonderful pomegranate trees.

Marine algae extracts are among the organic sources used in agricultural production and are complementary to fertilizers and not a substitute for them [15]. Worldwide, more than 15 million tons of them are utilized in agriculture each year. These extracts are non-fertilizer substances that, at low concentrations, stimulate plant growth. They include micro- and macronutrients as well as multiple sets of substances that promote growth, including vitamins, amino and organic acids, cytokines, auxins, and compounds that resemble auxins. [16] It also contains betaines which are a source of nitrogen in the low concentrations and a regulator of osmosis in high concentrations. It is possible that this compound plays a role in the extracts' ability to increase plants' resistance to salinity, freezing, and drought [17].

Therefore, this study was conducted to investigate the effect of spraying algae extract and Antioxidant compounds (salicylic acid and Ascorbic acid) on fruit quality and quantity of pomegranate trees cv. H116

2 Materials and Methods

This study was achieved during two seasons (2022 and 2023) in a private orchard located at wady elnatroon city, El-Beheira governorate, Egypt. The study was achieved on 10 years old pomegranate trees of "H116" cultivar. The trees planted at 3X5m under drip irrigation system, apart grown in sandy soil. The chosen trees were uniform in form and received the common horticultural applications. Soil and water analysis were presented in Table (1) and Table (2) as analyzed according to [18].

Table 1. Some chemical properties of the orchard soil.

	Soluble salts		Cations				anions			
parameters	PH	EC	Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}	HCO_3^{-}	CO_3^{-2}	Cl^{-}	SO_4^{-}
Unit		Ds/m	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L
Sample	8.00	4.89	8.90	4.50	0.92	30.4	3.00	N.D	18.50	23.2
	Available levels of macro and micro nutrients									
parameters	N	P	K	Fe	Zn	Mn	Cu	$CaCO_3$	SAR	
Unit	Ppm	Ppm	ppm	ppm	Ppm	Ppm	ppm	%	%	
Sample	21.00	18.00	140.00	2.85	2.77	2.61	0.95	1.7	11.8	

Table 2. Chemical analysis of the used water well for the study:

parameters	Ph	EC	Total soluble salts	Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}	HCO_3^{-}	CO_3^{-2}	Cl^{-}	SO_4^{-}
Unit		(micro	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)

		mos)									
Sample	7.68	1032	660	60	24.5	120	4.4	---	160	200	80

2.1 Experimental design

The experiment was designed in the form of randomized complete block design (RCBD), the experiment contains eight treatments, each treatment includes three replicates, and each replicate is one tree. All pomegranate trees were treated with the horticultural program of the farm.

2.2 Treatments

This experiment was designed to study the effect of foliar application on H116 pomegranate trees with algae extract, salicylic acid and ascorbic acid at different applications (24 trees for all treatments) during two studied seasons.

The experiment included eight treatments, which were:

(T1) without foliar application (control).

(T2) foliar application with 2 cm/l algae extract

(T3) foliar application with 0.5 g/l ascorbic acid.

(T4) foliar application with 0.5 g/l salicylic acid.

(T5) foliar application with 2 cm/l algae extract + 0.5 g/l ascorbic acid.

(T6) foliar application with 2 cm/l algae extract + 0.5 g/l salicylic acid.

(T7) foliar application with 0.5 g/l ascorbic acid + 0.5 g/l salicylic acid.

(T8) foliar application with 2 cm/l algae extract + 0.5 g/l ascorbic acid + 0.5 g/l salicylic acid.

All treatments were applied as foliar application at three times:

First: at full blooming. Second: after fruit sit. Third: after 30 days from the second application

Measurements:

Trees were randomly selected to conduct the experiment during October before the new growing season, where three replicates were selected for each treatment, the tree represented a replicate, and the measurements were taken on these trees as follows:

2.3 Leaf mineral content

Leaves samples were taken during September from trees and from the four directions around the tree. Leaves were also taken from non-fruit-bearing branches and the leaves were mature (fourth leaf and fifth leaf from the top of the branch). Then, the leaves of each tree were mixed together. The leaves were dried in a drying oven on the temperature of 70^{o}C, where the concentration of nutrients in the dry leaves was estimated as follows:

Nitrogen was determined in leaf samples digested in sulfuric acid by the kjeldahl method according to [19]. The phosphorous element was estimated by a spectrophotometric, according to methods of [20]. As well as the determination of potassium by the Flame photometric device, according to methods of [21].

2.4 Fruit Yield

The yield of tree (kg) was calculated by calculating the average weight of fruit and the total number of fruits on the tree; thus, we get the total weight of the fruits on the tree (kg), during mid-August in each season.

2.5 Fruit physical properties

At the time of harvest (mid-August), four mature fruits were taken from each tree in order to make physical and chemical measurements on them. The physical measurements that were calculated: average weight of the fruit (gm), fruit grain and fruit peel percentage.

2.6 Fruit chemical properties

Several chemical measurements were taken in the fruits as follows:
The percentage of T.S.S (total soluble solids) in juice of fruit was measured using a manual refractometer. While, the acidity percentage in juice of fruit was estimated by titration method by sodium hydroxide, according to [22]. Meanwhile, the vitamin in pomegranate juice was estimated by titration method using dichlorophenol indophenol and the amount of vitamin was calculated for each 100 ml juice. Also, the concentration of anthocyanin pigment in juice of fruit was estimated using a spectrophotometer, according to [23].

2.7 Experimental Design and Statistical Analysis

The data obtained during the two seasons of the experiment were analyzed using the statistical program (CoStat), and the method of analysis was ANOVA (analysis of variances) according to [24]. In addition, the comparison between the averages within the data was done using the calculation method LSR (least significant ranges) with a probability of 5%, according to [25].

3 Result and discussion

Data from Table (3) indicated the effect of spraying pomegranate with algae extract and some antioxidants on productivity and fruit physical characteristic.

Concerning to the yield, It could be noticed that all treatments caused a significant increase in the yield value (kg/ tree) compared to the control in both seasons. For the average of both seasons the maximum increase in yield (about 90 % over the control) was obtained from trees treated with salicylic acid (0.5g/L) + ascorbic acids (0.5g/L).

For the fruit weight, the results for the average of two seasons indicated that all experimental treatments except ascorbic acids (0.5 g/L) treatment seemed to have an increase in fruit weight as compared with control. The maximum increase in mean fruit weight reached (about 20 % over the control) in fruits from trees sprayed with salicylic acid (0.5 g/L) + ascorbic acids (0.5 g/L).

Fruit grain and fruit peel (%), it could be noticed that there were inverse relationships between them while the percentage of fruit grain increase the percentage of fruit peel decrease. Treated pomegranate trees with salicylic and ascorbic acids gave the maximum percentage of fruit grain and the minimum percentage of fruit peel. On the other hand, the minimum percentage of fruit grain and the maximum percentage of fruit peel observed when treated with ascorbic acid 0.5 g/L.

Table 3. Effect of spraying algae extract, salicylic acid and ascorbic acid on yield (kg), fruit weight (g), fruit grain (%) and Fruit peel (%) of H116 pomegranate trees in 2022 & 2023 seasons.

Treatment	Yield / tree (kg)			Average fruit weight (g)			Fruit grain %			Fruit peel%		
	2022	2023	Ave.	2022	2023	Ave.	2022	2023	Ave.	2022	2023	Ave.
T1	22.33 d	20.00 d	21.17 e	282.62 e	328.36 b	305.49 b	56.12 bc	53.59 bc	54.86 ab	43.88 bc	46.41 ab	45.15 ab
T2	42.33	32.33	37.33	377.4	271.2	324.3	52.8	56.84	54.83	47.19	43.16	45.18

	a	bc	ab	0a	9c	5 ab	1 c	abc	ab	b	abc	ab
T3	32.33 c	22.33 d	27.33 d	355.3 6b	283.3 4c	319.3 5ab	46.9 8 d	51.48 c	49.23 b	53.01 a	48.51 a	50.76 a
T4	37.66 b	32.33 bc	35.00 bc	256.4 8 f	250.7 8d	253.6 3c	55.9 1 bc	59.26 ab	57.59 ab	44.09 bc	40.73 bc	42.41 ab
T5	37.66 b	34.00 b	35.83 bc	312.5 3d	330.5 7 b	321.5 5ab	68.4 7 a	51.11 c	59.79 a	31.53 d	48.89 a	40.21 b
T6	42.00 a	37.66 a	39.83 a	369.5 6ab	320.7 4b	345.1 5 ab	53.6 8 bc	52.54 bc	53.11 ab	46.32 bc	47.45 ab	46.89 ab
T7	42.00 a	39.66 a	40.83 a	357.0 9b	373.6 0 a	365.3 5 a	57.9 2 b	62.13 a	60.03 a	42.07 c	37.86 c	39.97 b
T8	35.00 bc	30.66 c	32.83 c	335.6 1c	279.1 6cd	307.3 9 b	55.9 4 bc	54.44 bc	55.19 ab	44.05 bc	45.55 ab	44.80 ab

Means having the same letter (s) within a column is not significantly different at 5% level.

The data in the table (4) indicate the effect of spraying pomegranate trees with algae extract and some antioxidants on the quantity of juice extracted from 100 gm of fruit grain and its quality.

For the juice content (ml/100gm), it could be seen that in the average of both seasons, spraying salicylic acid either alone or combination with algae and (algae 2cm^3/l + salicylic acid 0.5 g/L + ascorbic acid 0.5 g/L) seemed to have no much effect on the juice content compared with control, while the other treatments caused a significantly decreased in the juice content which extract from 100 gm fruit grain compared with control.

Total soluble solids percentage (T.S.S. %), it refers to the concentration of dissolved solids, such as sugars, in a liquid or fruit. From the data in the table, it could be seen that in the average of both seasons, there were no significant differences between all treatments and control except treated with algae +salicylic acid which gave the lowest value for the T.S.S.

Concerning to acidity, the data indicated that there was no significant difference between all treatments and untreated trees. Spraying pomegranate trees with ascorbic acid 0.5 g/L alone gave the minimum percentage for acidity (1.42%).

As for the TSS/Acid ratio, The TSS/Acid ratio is a key characteristic determining the taste, texture and feel of fruit segments. It is the sugar/acid ratio which contributes towards giving many fruits their characteristic flavor. It is also an indicator of commercial and sensory ripeness.

From the data in table it could be noticed that the TSS/Acid ratio for the average of both seasons was not much affected by spraying algae extract, salicylic acid, and ascorbic acid either alone or combined together and control, the highest TSS/Acid ratio was obtained in fruits from trees sprayed with ascorbic acid 0.5 g/L (10.96%)

Table 4. Effect of spraying algae extract, salicylic acid and ascorbic acid on fruit juice content T.S.S, acidity and T.S.S/acidity ratio of H116 pomegranate trees in 2022 & 2023 seasons.

Treatment	**Juice content ml/100gm**			**TSS (Brix)**			**Acidity %**			**TSS/acidity ratio**		
	2022	**2023**	**Ave.**	**2022**	**2023**	**Ave.**	**2022**	**2023**	**Ave.**	**2022**	**2023**	**Ave.**
T1	62.33 ab	57.66 b	60.00 a	15.36 a	15.40 ab	15.38 ab	1.92 c	1.83 cd	1.88 abc	8.02 cd	8.39 a-c	8.28 ab
T2	54.33 d	47.33 d	50.83 c	15.20 a	14.60 cd	14.90 bc	1.43 ef	1.87 c	1.65 bc	10.63 b	7.78 c	9.26 ab
T3	56.00 cd	58.33 ab	57.17 b	15.73 a	13.93 d	14.83 bc	1.28 f	1.58 e	1.42 c	12.30 a	8.85 a	10.96 a
T4	62.83 a	56.00 b	59.42 a	15.06 a	14.73 bc	14.90 bc	2.34 a	2.15 b	2.25 a	6.41 e	6.84 d	6.88 b
T5	58.16 abcd	52.00 c	55.08 b	14.66 a	14.20 cd	14.43 c	1.81 cd	1.66 de	1.74 bc	8.09 cd	8.54 abc	8.60 ab
T6	57.66 bcd	61.66 a	59.67 a	16.03 a	15.56 a	15.80 a	2.13 b	1.94 c	2.04 ab	7.56 d	8.03 bc	7.87 b
T7	60.33 abc	50.66 cd	55.5 b	14.93 a	15.40 ab	15.17 ab	1.71 d	2.38 a	2.05 ab	8.74 c	6.45 d	7.72 b

T8	61.83 ab	58.66 ab	60.25 a	14.93 a	15.86 a	15.40 ab	1.49 e	1.80 cd	1.63 bc	10.00 b	8.80 ab	9.87 ab

Means having the same letter (s) within a column is not significantly different at 5% level.

Data in table (5) showed the effect of spraying algae extract, salicylic acid and ascorbic acid on juice ascorbic acid content and juice anthocyanin content of H116 pomegranate trees in 2022 & 2023 seasons.

For ascorbic acid, data for the average of both seasons observed that spraying pomegranate trees with algae either alone or combination with salicylic acid or/and ascorbic acid caused a significantly decrease in the value of ascorbic acid compared with control, while spraying tress with salicylic acid (0.5 g/l) either alone or combined with ascorbic acid at (0.5 g/l) caused a significantly increase in the value of scorbic.

Concerning to Anthocyanin, Anthocyanins (ACNs) are the largest and most important group of flavonoids present in pomegranate juice, from the data of the average of both seasons it could be noticed that treated pomegranate trees with (algae 2cm^3/l + salicylic acid 0.5 g/L + ascorbic acid 0.5 g/L) gave the highest value for the anthocyanin, while treated trees with (algae 2cm3/l + ascorbic acid 0.5 g/L) gave the lowest value.

Table 5. Effect of spraying algae extract, salicylic acid and ascorbic acid on juice ascorbic acid content and juice anthocyanin content of H116 pomegranate trees in 2022 & 2023 seasons.

Treatment	Ascorbic acid (g/100g)			Anthocyanin (mg/g)		
	2022	**2023**	**Ave.**	**2022**	**2023**	**Ave.**
T1	4.55 a-c	3.03 b	3.79 b	19.80 b	19.12 bc	19.46 ab
T2	3.68 bcd	1.95 d	2.82 c	18.46 b	20.67 ab	19.57 ab
T3	2.92 d	3.25 b	3.09 c	23.51 a	16.17 d	19.84 ab
T4	4.75 ab	4.33 a	4.88 a	23.41 a	17.47 cd	20.44 ab
T5	3.91 abcd	2.60 c	2.93 c	15.98 c	13.63 e	14.81 c
T6	3.38 cd	3.03 b	2.71 c	18.55 b	17.02 d	17.79 bc
T7	4.97 a	3.25 b	4.44 a	20.38 b	21.75 a	21.07 ab
T8	3.57 bcd	2.60 c	3.09 c	24.32 a	21.68 a	23.00 a

Means having the same letter (s) within a column is not significantly different at 5% level.

Data in Table (6) indicate the effect spraying algae extract, salicylic acid and ascorbic acid on leaf mineral contents of H116 pomegranate trees in 2022 & 2023 seasons.

The results indicated that the leaf mineral content was significantly affected by all treatments in both seasons compared to the control. Treated pomegranate trees with (Algae 2cm^3/l + salicylic acid 0.5 g/L + ascorbic acid 0.5 g/L) gave the highest percentage for leaf nitrogen (1.43%) and leaf potassium (0.4%) and leaf phosphor (1.23%) compared with the control treatment which gave the lowest percentage of leaf content of N,P,K in the average for the both seasons.

Table 6. Effect of spraying algae extract, salicylic acid and ascorbic acid on leaf mineral contents of H116 pomegranate trees in 2022 & 2023 seasons.

Treatment	N %			P %			K %		
	2022	**2023**	**Ave.**	**2022**	**2023**	**Ave.**	**2022**	**2023**	**Ave.**
T1	0.756 g	0.760 g	0.758 g	0.103 h	0.104 g	0.103 h	0.65 g	0.59 f	0.62 f
T2	0.926 e	0.913 e	0.920 de	0.214 e	0.227 d	0.221 e	0.81 e	0.83 d	0.82 d
T3	0.893 ef	0.916 e	0.905 e	0.186 f	0.183 e	0.184 f	0.75 f	0.74 e	0.74 e
T4	0.863 f	0.853 f	0.858 f	0.137 g	0.134 f	0.136 g	0.71 f	0.70 e	0.71 e

T5	1.263 b	1.256 b	1.260 b	0.310 b	0.321 b	0.315 b	1.11 b	1.12 b	1.11 b
T6	1.073 c	1.096 c	1.085 c	0.286 c	0.288 c	0.287 c	0.97 c	0.96 c	0.96 c
T7	0.983 d	0.986 d	0.985 d	0.243 d	0.232 d	0.238 d	0.85 d	0.85 d	0.85 d
T8	1.406 a	1.453 a	1.430 a	0.384 a	0.411 a	0.398 a	1.22 a	1.25 a	1.24 a

Means having the same letter (s) within a column is not significantly different at 5% level.

The obtained results regarding the effect salicylic acid on yield and fruit quality go in line with the findings of [10] they reported that foliar sprays of salicylic acid improving the yield, fruit weight, reduces the total acidity% and increases TSS, TSS/ acid ratio, ascorbic acid and leaf mineral content of pomegranate trees. In addition to [26, 27, 9] found that salicylic treatments enhanced yield and fruit quality traits of pomegranate.

The obtained results regarding the effect of ascorbic on yield and fruit quality go in line with the findings of [14] on pomegranate. He mentioned that foliar sprays of ascorbic improved fruit quality of pomegranate fruit. [9] Observed that Spray Wonderful pomegranate trees with ascorbic at 1000 ppm twice a year i.e. at full bloom and four weeks later caused to enhanced tree growth, yield and fruit quality. [28] Found that spraying pomegranate trees with ascorbic acid at (750 or 1500 mg/l) caused to improve and increase the yield and weight percentage.

The results of the effect of algae extract are in agreement with those found by [29] on olive and [30] they reported that spraying pomegranate trees with algae led to a clear significant increase in all the vegetative growth and yield characteristics.

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