Effect of spraying myrrha extract and organic fertilizer on the leaf content of nutrients and medicinally active compounds of pomegranate plant

Punica granatum L.

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Summary

The experiment was conducted at the Agricultural Research Station of the College of Agriculture - University of Basrah during the two growing seasons, 2021-2022, on pomegranate seedlings Punica granatum L. Shahrban cultivar, in order to study the effect of spraying myrrha extract with three concentrations (0, 3, 6) $g.L^{-1}$ and the effect of spraying the organic fertilizer Foliarex was also applied at three concentrations (0, 5, 10) $ml.L^{-1}$ and the effect of the interaction between them in the leaf content on some nutrients and medicinally active compounds in the leaves of pomegranate seedlings, with two periods of spraying the fall season starting from 2022/10/15 and the spring season starting 2022/3/15 with 12 sprayings, between one spraying and another one week, the experiment was factorial with two factors according to the randomized complete block design (R.C.B.D). and the Least Significant Difference (L.S.D) test was used to compare the averages of the treatments and at the level of probability (0.05), the results showed that the spraying of seedlings Pomegranate with myrrha extract at a concentration of 6 $g.L^{-1}$ led to a significant increase in some nutrients studied such as the percentage of nitrogen, phosphorus, protein and medically effective compounds such as phenols, tannins and alkaloids, while spraying with the organic fertilizer Foliarex at a concentration of 10 $ml.L^{-1}$ led to an improvement in most of the nutrients and effective compounds The effect of the interaction led to a treatment of (6 $g.L^{-1}$ myrrha extract and 10$ml.L^{-1}$ organic fertilizer) significantly superior in most of the studied traits compared with the control treatment.

Key words:myrrha, organic fertilizer, Medically effective compounds, pomegranate
* Retrieved from the first researcher's thesis

Introduction

The pomegranate plant *punica granatum* L. belongs to the pomegranate family punicaceae, Pomegranate trees are widely cultivated in the subtropical regions of the world between latitudes 41 north and south of the equator, and they can grow at high altitudes of up to 2400 m, as well as in coastal areas, it is also characterized by their tolerance of soil salinity, Pomegranate tree is one deciduous trees in cold regions and evergreen to partially deciduous in tropical and subtropical regions [11].

Due to the side effects that industrial chemicals pose, which may be negative, the tendency has been to find alternatives from natural compounds that can perform an effect similar to that of industrial chemical compounds. There are many plant extracts that have a role in encouraging growth characteristics, this is due to the fact that these plants containing a number of natural chemical compounds that differ according to the plant parts, stages of growth and environmental conditions to which plants are exposed [1].

Myrrha is a resinous secretion by exudation from the cracks that usually form in the bark of the Commiphora myrrha tree automatically or by human intervention and contains mineral elements, carbohydrates, proteins and other organic compounds [2].

Recently, it has been noticed that many farmers are paying attention to agriculture that does not pollute the environment, including the shift towards organic fertilization and avoidance of chemical fertilizers, which may cause health problems for humans in the long run [12]. From the plant's need for nutrients when conditions are not suitable in the soil to absorb those nutrients .

[3] found that when spraying green plant organic fertilizer with three concentrations (0, 5 , 10) $ml.L^{-1}$ on olive trees (Ashrasi cultivar), the concentration of 10 $ml.L^{-1}$ led to a significant increase in the percentage of nitrogen, phosphorus and potassium compared with the control treatment. [4] found in a study of the effect of the organic fertilizer Botryful at concentrations of (5,4,3,0) $ml.L^{-1}$ on the woolly peach plant Red June, a significant effect at a concentration of 5 $ml.L^{-1}$ on the content of the leaves of the nutrients nitrogen and phosphorus And potassium compared with the control treatment, [5] in the study of the effect of liquid humus fertilizer LiQ-HUMAS on the content of total alkaloids of Indian ginseng at a concentration of 0.75 ml.L-1 significantly affected the content of leaves of alkaloids compared with the control treatment.

Materials and methods-:

Experiment site, plant preparation and cultivation medium

The field experiment was conducted at the research station of the College of Agriculture, University of Basra during the growing season (2021-2022) for the purpose of knowing the effect of spraying myrrha extract and foliarex organic fertilizer on some anatomical and chemical indicators of the pomegranate plant *Punica granatum* L. Under the environmental conditions of southern Iraq, a selection of Twenty-seven seedlings of pomegranate, cultivar Shahrban, from one of the private nurseries in Basra city, two years old, homogeneous in terms of size and vegetative growth. And then planting it directly in the ground by planting 2.5 * 2.5 m in the hollow that has been prepared and mixing an agricultural medium for it consisting of fine sand with peat moss in a ratio of 3:1 and distributing the mixture over the hollow in the amount of (50-60) kg per hole.The date for planting seedlings in the ground was 10/5/2021.

Table (1) Some chemical properties of irrigation

Valuable	properties of water
7.18	Reaction degree (pH)
8.37	Electrical conductivity (E.C.) DC Siemens. M^{-1}
26.56	Cl^{-}(mg. L^{-1})
13.97	Ca^{+2}(mg.L^{-1})
28.87	No_3^{-}(mg. L^{-1})
98.97	Na^{+}(mg. L^{-1})
39.34	K^{+}(mg. L^{-1})
32.12	Mg^{+2}(Mg. L^{-1})

It was analyzed in the central laboratory of the College of Agriculture, University of Basrah

Prepare the transactions for the experiment

Fertilizer treatments used in spraying were prepared by dissolving 3 and 6 $g.L^{-1}$ of myrrha in distilled water to prepare the extract. Likewise, liquid organic fertilizer was prepared at concentrations of 5 and 10 $ml.L^{-1}$, and the volume allocated for each tree was 1 liter. As for the comparison treatment, which Use distilled material only.

Statistical design and analysis

The experiment data was analysed statistically as a factorial experiment according to the Randomized Complete Block Design (R.C.B.D.) and the experiment had two factors for each factor, three concentrations and three replications, so the total number of experimental units = 27 experimental units. The statistical analysis of the experimental data was carried out using the statistical program (Genstat v.12), and the least significant difference was (0.05).

Estimation of elemental (NPK) and protein content in leaves

Take 0.2 g of dried leaves, grind them and digest them according to the method of [14] using sulfuric and perchloric acid.

Percentage of Nitrogen in leaves(%)

Nitrogen in the digested samples was determined using the Micro Kjeldhal apparatus as described in [18].

Percentage of phosphorus in leaves:(%)

It was estimated using a spectrophotometer at a wavelength of 700 nm according to the method of [15].

Percentage of potassium in leaves(%)

It was estimated using a flame photometer according to the method of [18].

Percentage of protein in leaves (%)

The percentage of protein = the percentage of nitrogen x 6.25 [6].

Estimation of medicinally active compounds in leaves

Estimation of phenols in leaves(%)

Estimate the amount of phenolic compounds as shown by [13].

Estimation of tannins in leaves(%)

This method was followed by [7] :

$$\text{Tannins\%} = \frac{T2-T1}{\text{sample weight}} X100$$

Estimation of alkaloids in leaves

Estimation of alkaloids using the method of [16] :

$$\text{The percentage of alkaloid} = \% \frac{\text{extract weight(g)}}{\text{Plant sample weight (g)}} X\ 100$$

Results and discussion

First: The effect of spraying myrrha extract and the organic fertilizer Foliarex and the interaction between them on the chemical properties of the leaves of pomegranate seedlings, Shahrban cultivar.

The data of Table (2) indicate that there is a significant difference between the concentrations of myrrha extract in their effect on the percentage of nitrogen concentration in the leaves. It is noted that the concentration treatment of $6g.L^{-1}$ was significantly superior compared to the rest of the treatments, amounting to 3.40%, while the control treatment gave the lowest rate In the percentage of the nitrogen element amounted to 3.01%.

As for the effect of organic fertilizer concentrations Foliarex, the data of the table show that the concentration treatment 10 $ml.L^{-1}$ was significantly superior in the percentage of the nitrogen element concentration compared to the rest of the treatments, as it gave the highest percentage, followed by the treatment of concentration 5 $ml.L^{-1}$ in the same effect (3.81, 3.33)%, respectively, while the control treatment gave the lowest rate of nitrogen concentration, which was 2.42%.

As for the effect of the overlap treatments, the treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the highest percentage of nitrogen concentration amounting to 4.07% compared to the rest of the treatments, and the control treatment gave the lowest rate in the percentage of nitrogen concentration. It reached 2.39%.

Table (2) The effect of spraying myrrha extract and the organic fertilizer Foliarex and the interaction between them on the nitrogen concentration (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
2.42	2.45	2.43	2.39	0
3.33	3.39	3.35	2.97	5
3.81	4.07	3.68	3.69	10
	3.40	3.15	3.01	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.29	0.17	0.17	

The data of Table (3) indicate that there is a significant difference between the concentrations of myrrha extract in their effect on the percentage of phosphor concentration in the leaves. It is noted that the concentration treatment of 6 $g.L^{-1}$ was significantly superior compared to the rest of the treatments that gave 0.253%, while the control treatment gave the lowest percentage. In elemental phosphorus concentration amounted to 0.179%.

As for the effect of the organic fertilizer Foliarex concentrations, the data of the table show that the concentration treatment of 10 $ml.L^{-1}$ was significantly superior in the percentage of phosphorous element concentration in the leaves compared to the rest of the treatments, followed by the treatment of the concentration of 5 $ml.L^{-1}$ in the same effect of (0.256, 0.214)% respectively, while the control treatment gave the lowest percentage in the concentration of phosphorous, which amounted to 0.181%.

As for the effect of the overlap treatments, the treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the highest percentage in the concentration of the element phosphorus amounted to 0.340%, compared to the rest of the treatments, and the control treatment gave the lowest percentage in the concentration of the element phosphorus amounted to 0.181%.

Table (3) Effect of spraying myrrha extract and organic fertilizer Foliarex and the interaction between them on the concentration of phosphorus (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
0.181	0.182	0.182	0.181	0
0.214	0.238	0.206	0.199	5
0.256	0.340	0.216	0.212	10
	0.253	0.201	0.197	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.023	0.013	0.013	

The data of Table (4) indicate that there is no significant difference between the concentrations of myrrha extract in their effect on the percentage of potassium concentration in the leaves.

As for the effect of the organic fertilizer Foliarex concentrations, the data of the table show that the concentration treatment 10 $ml.L^{-1}$ was significantly superior in the percentage of potassium element concentration compared to the rest of the treatments, followed by the treatment of 5 $ml.L^{-1}$ in the same effect, amounting to (2.91, 2.60)% respectively, while the control treatment gave the lowest percentage of potassium concentration, which reached 2.13%.

As for the effect of the overlap treatments, the treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the highest percentage in potassium concentration amounting to 3.08% compared to the rest of the treatments, and the control treatment gave the lowest percentage in potassium concentration amounting to 2.12% .

Table (4) The effect of spraying myrrha extract and the organic fertilizer Foliarex and the interaction between them on the potassium concentration (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
2.13	2.13	2.13	2.12	0
2.60	2.77	2.51	2.51	5
2.91	3.08	2.82	2.83	10
	2.66	2.49	2.49	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.23	0.13	N.S	

The results of table (5) indicate that there is a significant difference between the concentrations of myrrha extract in their effect on the percentage of protein in the leaves, as it is noted that the concentration treatment of 6 $g.L^{-1}$ was significantly superior compared to the rest of the treatments, which gave the highest rate of 21.29%, while the treatment of The lowest percentage of protein was 18.86%.

As for the effect of the concentrations of the organic fertilizer Foliarex, the data of the same table show that the concentration treatment 10 $ml.L^{-1}$ was significantly superior in the percentage of protein in the leaves, compared with the rest of the treatments, as it gave the highest percentage, followed by the treatment of the concentration 5 $ml.L^{-1}$ in the same effect, which amounted to (23.84, 20.86)% respectively, while the control treatment gave the lowest percentage of protein, amounting to 15.17%.

As for the effect of overlapping treatments, treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the highest percentage of protein amounting to 25.45% compared to the rest of the treatments, and the control treatment gave the lowest percentage of protein amounting to 14.98%.

Table (5) The effect of spraying myrrha extract and the organic fertilizer Foliarex and the interaction between them on the protein concentration (%) in the leaves of pomegranate seedlings, Shahrban cultivar.

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
15.17	15.35	15.18	14.98	0
20.86	23.06	20.97	18.56	5
23.84	25.45	23.02	23.06	10
	21.29	19.73	18.86	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	1.84	1.06	1.06	

The content of myrrha extract of microelements such as iron, boron and cobalt has a role in fixing atmospheric nitrogen and this is reflected in increasing the percentage of nitrogen concentration in the leaves, as in Table (2), and thus increasing the amino acids, of which nitrogen is the main component of which enters into the formation of proteins and their increase, as in Table (5) which reflects positively on the growth of the plant [9], as well as the increase in the percentage of nitrogen, phosphorus and potassium as shown in Tables (2, 3, 4) respectively in the leaves due to the direct feeding of this element when sprayed on the leaves of what the organic fertilizer contains Foliarex of these elements, which led to an increase in the efficiency of the process of photosynthesis [8]. And the increase in the percentage of nitrogen, phosphorous and potassium is due to the activity of the vegetative growth of the plant caused by the necessary mineral nutrients that are included in the composition of the organic fertilizer Foliarex, which requires the withdrawal of the largest amount of these elements to meet the plant's need for it (5), phosphorus in the formation of cellular membranes such as the plasma membrane, mitochondria and chloroplasts as well as its entry into the formation of some energy-rich compounds as catalysts for enzymes, and potassium in the processes of opening and closing stomata and the process of food transfer and is responsible for the controlling osmosis in the cell [6].

Second:-The effect of spraying myrrh extract and the organic fertilizer Foliarex and the interaction between them on the medicinally effective compounds of the leaves of pomegranate seedlings, Shahrban cultivar

The data of Table (6) indicate that there is a significant difference between the concentrations of myrrha extract in its effect on reducing the percentage of total phenols in the leaves, It is noted that the concentration treatment of 6 $g.L^{-1}$ was significantly superior compared to the rest of the treatments that gave the lowest percentage of total phenols amounting to 4.72%, while the control

treatment gave the highest percentage of total phenols amounting to 5.41%.

As for the effect of organic fertilizer concentrations Foliarex, the data of the same table show that the concentration treatment 10 $ml.L^{-1}$ was significantly superior in reducing the percentage of total phenols compared with the rest of the treatments, as it gave the lowest percentage in this characteristic, followed by the treatment of concentration 5 $ml.L^{-1}$ in the same effect. It reached (4.50, 5.06)% respectively, while the control treatment gave the highest percentage of total phenols, amounting to 5.82%.

As for the effect of overlapping treatments, treatment (6 g.l-1 + 10 ml.l-1) was significantly superior as it gave the lowest percentage of total phenols amounting to 4.08%, compared to the rest of the treatments, and the control treatment gave the highest percentage of total phenols amounting to 5.88%.

Table (6) Effect of spraying myrrha extract and organic fertilizer Foliarex and the interaction between them on the percentage of total phenols (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
5.82	5.76	5.83	5.88	0
5.06	4.32	5.28	5.58	5
4.50	4.08	4.65	4.76	10
	4.72	5.25	5.41	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.38	0.22	0.22	

The data of Table (7) indicate that there is a significant difference between the concentrations of myrrha extract in its effect on reducing the percentage of total tannins in the leaves, as it is noted that the concentration treatment of 6 $g.L^{-1}$ was significantly higher compared to the rest of the treatments that gave the lowest percentage of 3.51%, while The concentration treatment of 3 $g.L^{-1}$ gave the highest percentage of total tannins, amounting to 4.27%.

As for the effect of organic fertilizer concentrations Foliarex, the data of the same table show that the concentration treatment 10 $ml.L^{-1}$ was significantly superior in reducing the percentage of total tannins compared to the rest of the treatments, followed by the treatment of concentration 5 $ml.L^{-1}$ in the same effect, amounting to (3.33, 3.96)% respectively, while the control treatment gave the highest percentage of total tannins, amounting to 4.54%.

As for the effect of the overlap treatments, the treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the lowest percentage of total tannins in the leaves amounting to 2.87%, compared to the rest of the treatments, and the control treatment gave the highest percentage of total tannins amounting to 4.60%.

Table (7) Effect of spraying myrrha extract and organic fertilizer Foliarex and the interaction between them on the percentage of total tannins (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L^{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
4.54	4.43	4.59	4.60	0
3.96	3.23	4.28	4.37	5
3.33	2.87	3.94	3.20	10
	3.51	4.27	4.05	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.22	0.13	0.13	

The data of Table (8) indicate that there is a significant difference between the concentrations of the myrrha extract in their effect on the percentage of total alkaloids in the leaves, as it is noted that the concentration treatment of 6 $g.L^{-1}$ was significantly superior compared to the rest of the treatments and gave the highest percentage, followed by the concentration treatment of 3 $g.L^{-1}$ reached (1.55, 1.44)% respectively, while the control treatment gave the lowest percentage of total alkaloids, amounting to 4.27%..

As for the effect of the concentrations of the organic fertilizer Foliarex, the data of the same table show that the concentration treatment of 10 $ml.L^{-1}$ was significantly superior in the percentage of total alkaloids compared with the rest of the treatments, as it gave the highest percentage, followed by the treatment of the concentration of 5 $ml.L^{-1}$ in the same effect, which amounted to (1.55, 1.42)% respectively, while the control treatment gave the lowest percentage of total alkaloids, amounting to 1.27%.

As for the effect of the interaction treatments, the treatment (6 $g.L^{-1}$ + 10 $ml.L^{-1}$) was significantly superior as it gave the highest percentage of total alkaloids amounting to 2.70% compared to the rest of the treatments and the control treatment gave the lowest percentage of total alkaloids amounting to 1.06%.

Table (8) Effect of spraying myrrha extract and organic fertilizer Foliarex and the interaction between them on the percentage of alkaloids (%) in the leaves of pomegranate seedlings, Shahrban cultivar

Average organic fertilizer	Concentration of myrrha extract ($g.L_{-1}$)			Foliarex Organic Fertilizer Concentrate ($ml.L^{-1}$)
	6	3	0	
1.27	1.43	1.32	1.06	0
1.42	1.54	1.45	1.27	5
1.55	2.70	2.54	1.41	10
	1.55	1.44	1.24	Myrrha extract average
	overlap	The organic fertilizer	Myrrha extract	L. S.D. 0.05
	0.05	0.05	0.05	

Reducing the percentage of phenols and tannins, as shown in Tables (6, 7), may be due to the reduction of stress on the plant and the abundance of nutrients, because the percentage of phenols is inversely related to the nutritional status of the plant. Magnesium elements have a direct role, which participates in the demolition and construction of phenolic compounds through the process of glycolysis and the production of the compound Phosphoenol pyruvate, which creates shikimic acid, which creates the two amino acids, tyrosine and phenylalanine [10], since the alkaloids are metabolites of the alpha-amino acids such as Ornithine, Lysine, and the aromatic acids phenylalanine, tyrosine, and tryptophan [1]. Therefore, the increase in the amount of alkaloids formed in the leaves, as shown in Table (8), is attributed to the joint role of these elements in improving the vital processes related to the synthesis of these compounds as initiating elements in the formation of alkaloids. [17] indicated that fertilizing plants with nutrients in appropriate quantities increases the amount of alkaloids formed through its role in increasing amino acids as materials that enter into the construction of alkaloids.

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