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Improving the mineral content of young pomegranate trees (*Punica granatum* L. var. Wonderful) by adding organic fertilizers and spraying with amino acids

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Abstract

The study was conducted in a pomegranate field orchard at the Agricultural Research and Experiments Station of the College of Agriculture, University of Kirkuk, Iraq, located in the Al-Sayadah area, during the 2023 growing season, on young pomegranate trees of var. Wonderful. The experiment included two factors: the first factor included five levels of organic fertilizers (0, 250, and 500 g/tree of poultry waste and two levels of 15 and 30 g/tree of humic acid) and three amino acid concentrations (0, 5, and 10 ml/L). Results showed that applying 500 g/tree of poultry waste significantly increased the mineral content of pomegranate trees (carbohydrate percentage in the branches, nitrogen in the branches, and the carbohydrate/nitrogen ratio in the branches) at rates of 4.667%, 1.721%, and 2.771%, respectively. There was also a significant increase in potassium content in the leaves at a rate of 0.655% with the application of 30 g/tree of humic acid. A concentration of 5 ml/L of amino acids enhanced the leaf protein percentage by 6.762%, while 10 ml/L was most effective for the majority of the studied characteristics (potassium in the leaves, carbohydrates in the branches, nitrogen percentage, and the carbohydrate/nitrogen ratio in the branches) at rates of 0.692%, 3.814%, 1.387%, and 2.760%, respectively, compared to unsprayed trees. As for the binary interaction between the two factors, it showed a significant effect on all the traits studied.

Keywords: pomegranate, Wonderful, organic fertilizers, chemical characteristics.

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1. Introduction

The pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and is widely valued for its edible fruits. This subtropical (or tropical) shrub can grow up to 5 meters in height and produces dense branches with numerous buds that emerge near the ground surface. The pomegranate's origins are believed to lie in the East, with some sources suggesting Iran as its native region. It has since spread across the Mediterranean basin, throughout the Arab world, India, and Afghanistan (Hassan, 1998). Pomegranate fruits hold significant nutritional value due to their high sugar content, reaching up to 16%—primarily in monosaccharide form—along with a protein content of approximately 1%. Organic acids, such as citric acid, constitute 0.5–2.0% of the fruit, and water content exceeds 75% of the fruit's total weight (Al-Issa *et al*., 2012). Organic fertilizers, including poultry waste, are known to enhance soil fertility and increase plants' nutrient absorption capabilities by providing macro- and micronutrients upon decomposition, ultimately boosting yields and improving crop quality (Granatstein, 2004). Recently, there has been a shift toward using organic fertilizers like humic acid, which results from the breakdown of organic matter. Humic acid improves plant growth by enhancing soil structure, facilitating root water and nutrient absorption, and increasing soil retention of these elements, while also promoting soil microorganism activity (Hakeem *et al*., 2023a; Rupiasih and Vidyasagar, 2005). Additionally, amino acids, which plants naturally produce, promote balanced growth, enhance nutrient uptake, increase cellular protein levels, and supply nitrogen crucial for plant development. Amino acids also mitigate ammonia toxicity in plant cells and act as fundamental building blocks for proteins and coenzymes, with a chelating effect on micronutrients (Medan and Al-Douri, 2021). This study aims to improve the chemical characteristics and mineral content of young Wonderful pomegranate trees by applying organic fertilizers (poultry waste and humic acid) and spraying with amino acids at varying concentrations. Additionally, the study seeks to identify optimal levels of organic fertilizers and amino acid sprays, as well as their interactions, in enhancing the chemical properties of pomegranate trees.

2. Materials and methods

This study was conducted in a pomegranate orchard at the College of Agriculture, University of Kirkuk, at the Agricultural Research and Experiment Station in the Al-Sayadah area (approximately 7.5 km southwest of Kirkuk) at an altitude of 350 m above sea level, with coordinates 35.39° latitude and 44.34° longitude. The research was carried out during the 2023 growing season on two-year-old pomegranate trees, var. Wonderful, planted in the orchard and trained to a single stem using the main leader method in sandy loam soil, spaced 4 × 3.5 m apart. The experiment examined the effects of organic fertilization and amino acid application on the mineral content of young pomegranate trees. The experimental design was a bifactorial randomized complete block design

(RCBD) comprising two main factors. The first factor was organic fertilizer application, with five levels: T_0 (control, no fertilization), T_{250} (250 g/tree of poultry waste), T_{500} (500 g/tree of poultry waste), T_{15} (15 g/tree of humic acid), and T_{30} (30 g/tree of humic acid). The second factor involved amino acid foliar spraying at three concentrations: A_0 (control, no amino acids), A_5 (5 ml/L), and A_{10} (10 ml/L).

2.1 *Studied characteristics*

Samples were collected on February 7th, 2023, and the following parameters were assessed:

1. Nitrogen in leaves: Nitrogen content was determined using the Micro-Kjeldahl method, as described by A.O.A.C. (1980).
2. Phosphorus in leaves: Phosphorus was measured colorimetrically with a spectrophotometer at a wavelength of 410 nm, following the method outlined by Estefan *et al*. (2013).
3. Potassium in leaves: Potassium content was assessed using a Microprocessor Flame Photometer, based on the method provided by Estefan *et al*. (2013).
4. Protein in leaves: Leaf protein content was calculated on a dry weight basis using the formula: protein percentage = nitrogen percentage × 6.25 (A.O.A.C., 2005).
5. Carbohydrates in branches: Total carbohydrates in the branches were measured on February 12th, using an EMC lab V-1100 digital spectrophotometer at 490 nm, following the method by Dubois *et al*. (1956).
6. Nitrogen in branches: Nitrogen content in the branches was determined using the Micro-Kjeldahl method (Al-Sahhaf, 1989) on February 12th, 2023.
7. Carbohydrate/Nitrogen (C/N) ratio in branches: This ratio was calculated by dividing the carbohydrate content by the nitrogen content in each sample's branches.

All data were statistically analyzed, with means separated using Duncan's multiple range test at a 5% probability level.

3. Results

3.1 Nitrogen in leaves

The results in Table (1) indicate that both organic fertilization and amino acid treatments significantly increased the nitrogen content in the leaves compared to the control. Notably, the T_{250} A_5 interaction (250 g/tree poultry waste with 5 ml/L amino acids) yielded the highest nitrogen content at 3.22%, while the T_0 A_5 interaction provided the lowest at 0.61%.

3.2 Phosphorus in leaves

As shown in Table 2, while individual treatments with organic fertilizers and amino acids did not significantly alter phosphorus levels compared to the control, a significant effect was observed in the interactions. Specifically, the T_0 A_5 interaction resulted in the highest phosphorus level (0.80%), while T_0 A_0 produced the lowest (0.25%).

Table (1): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on nitrogen percentage in leaves of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	0.63 b	0.61 b	1.27 b	0.83 a
T_{250} (250 g/tree of poultry waste)	0.81 b	3.22 a	0.86 b	1.63 a
T_{500} (500 g/tree of poultry waste)	1.06 b	0.88 b	0.85 b	0.93 a
T_{15} (15 g/tree of humic acid)	0.87 b	0.84 b	0.74 b	0.82 a
T_{30} (30 g/tree of humic acid)	1.02 b	0.87 b	1.27 b	1.05 a
Amino acid average	0.87 a	1.28 a	1.00 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

Table (2): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on phosphorus percentage in leaves of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	0.25 b	0.80 a	0.46 ab	0.50 a
T_{250} (250 g/tree of poultry waste)	0.49 ab	0.36 ab	0.53 ab	0.46 a
T_{500} (500 g/tree of poultry waste)	0.50 ab	0.65 ab	0.41 ab	0.52 a
T_{15} (15 g/tree of humic acid)	0.48 ab	0.61 ab	0.56 ab	0.55 a
T_{30} (30 g/tree of humic acid)	0.47 ab	0.45 ab	0.33 ab	0.41 a
Amino acid average	0.44 a	0.57 a	0.46 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

3.2 Potassium in leaves

Table (3) demonstrates that both organic fertilization and amino acid treatments significantly impacted potassium levels. The T_{30} treatment (30 g/tree humic acid) had the highest potassium content at 0.655%, while T_{250} had the lowest at 0.517%. Among amino acid treatments, A_{10} (10 ml/L) produced the highest potassium content (0.692%), and the T_0 A_{10} interaction gave the overall highest leaf potassium content at 0.900%.

3.3 Protein in leaves

According to Table (4), amino acid spraying significantly affected leaf protein content, with the A_5 treatment achieving the highest average (6.762%) and A_0 the lowest (5.344%). The T_{250} A_5 interaction produced the highest protein content (7.316%), indicating the effectiveness of this combination in enhancing protein levels in leaves.

Table (3): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on potassium percentage in leaves of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	0.399 d	0.508 cd	0.900 a	0.602 b
T_{250} (250 g/tree of poultry waste)	0.491 cd	0.524 cd	0.537 cd	0.517 c
T_{500} (500 g/tree of poultry waste)	0.658 bcd	0.513 cd	0.608 bcd	0.593 bc
T_{15} (15 g/tree of humic acid)	0.533 cd	0.670 bc	0.620 bcd	0.608 b
T_{30} (30 g/tree of humic acid)	0.591 bcd	0.579 bcd	0.795 ab	0.655 a
Amino acid average	0.534 b	0.559 b	0.692 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

Table (4): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on protein percentage in leaves of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	5.466 ab	6.636 ab	5.856 ab	5.986 a
T_{250} (250 g/tree of poultry waste)	5.386 ab	7.316 a	5.933 ab	6.212 a
T_{500} (500 g/tree of poultry waste)	5.020 b	7.080 ab	4.813 b	5.637 a
T_{15} (15 g/tree of humic acid)	5.476 ab	6.350 ab	6.920 ab	6.248 a
T_{30} (30 g/tree of humic acid)	5.373 ab	6.430 ab	5.933 ab	5.912 a
Amino acid average	5.344 b	6.762 a	5.891 b	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

3.5 Carbohydrates in branches

The analysis in Table (5) shows that T_{500} (500 g/tree poultry waste) significantly increased carbohydrate content in branches, reaching 4.667% compared to 2.657% in the control. Among amino acid concentrations, A_{10} resulted in the highest carbohydrate content (3.814%). The T_{500} A_{10} combination recorded the highest carbohydrate level at 5.233%, showing that this interaction effectively boosts carbohydrate accumulation in branches.

Table (5): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on carbohydrate percentage in branches of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	2.200 f	2.210 f	3.561 bc	2.657 c
T_{250} (250 g/tree of poultry waste)	2.295 ef	2.395 def	3.416 bc	2.702 c
T_{500} (500 g/tree of poultry waste)	4.790 a	3.978 b	5.233 a	4.667 a
T_{15} (15 g/tree of humic acid)	2.946 cdef	3.098 cd	3.276 bc	3.107 b
T_{30} (30 g/tree of humic acid)	3.000 cde	3.346 bc	3.583 bc	3.310 b
Amino acid average	3.046 b	3.005 b	3.814 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

3.6 Nitrogen in branches

As displayed in Table (6), T_{500} significantly increased branch nitrogen levels to 1.721% compared to 1.136% in the control, with the A_{10} amino acid concentration producing the highest nitrogen content at 1.387%. The T_{500} A_{10} interaction further amplified nitrogen levels, achieving 1.976%, highlighting the synergistic effect of high poultry waste and amino acid concentrations.

3.7 Carbohydrate/nitrogen ratio (C/N) in branches

The data in Table 7 reveal that the C/N ratio was significantly affected by organic fertilization, with T_{500} producing the highest ratio at 2.771%, while T_{250} had the lowest at 2.252%. The A_{10} concentration significantly enhanced this ratio to 2.760%, and the T_{500} A_5 interaction achieved the highest C/N ratio of 3.201%, suggesting an ideal combination for promoting balanced carbohydrate and nitrogen distribution.

Table (6): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on nitrogen percentage in branches of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	0.966 d	1.210 bc	1.233 bc	1.136 d
T_{250} (250 g/tree of poultry waste)	1.173 c	1.180 bc	1.233 bc	1.195 c
T_{500} (500 g/tree of poultry waste)	1.943 a	1.243 bc	1.976 a	1.721 a
T_{15} (15 g/tree of humic acid)	1.216 bc	1.223 bc	1.246 bc	1.228 bc
T_{30} (30 g/tree of humic acid)	1.256 bc	1.263 b	1.246 bc	1.255 b
Amino acid average	1.311 b	1.224 c	1.387 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

Table (7): Effect of organic fertilization (poultry waste and humic acid), amino acid spraying, and their interactions on carbohydrate/nitrogen ratio (C/N ratio) in branches (%) of young pomegranate trees (var. Wonderful).

Organic fertilization (poultry waste and humic acid)	Amino acid foliar spraying			Average
	A_0 (0 ml/L)	A_5 (5 ml/L)	A_{10} (10 ml/L)	
T_0 (0 g/tree as control)	2.316 b-e	1.826 e	2.885 ab	2.343 bc
T_{250} (250 g/tree of poultry waste)	1.956 de	2.029 c-e	2.770 ab	2.252 c
T_{500} (500 g/tree of poultry waste)	2.464 b-d	3.201 a	2.646 a-c	2.771 a
T_{15} (15 g/tree of humic acid)	2.420 b-e	2.534 b-d	2.628 a-c	2.528 a-c
T_{30} (30 g/tree of humic acid)	2.387 b-e	2.649 a-c	2.871 ab	2.635 ab
Amino acid average	2.309 b	2.448 b	2.760 a	

Means followed by different letters indicate significant differences at the 5% level (Duncan's multiple range test).

4. Discussion

The findings demonstrate that poultry waste and humic acid positively influence the chemical composition of young pomegranate trees, particularly when combined with amino acid foliar spraying. Poultry waste increases soil microbial activity, promoting hormone production (e.g., cytokinins, gibberellins, and auxins), enhancing nutrient absorption, and stimulating photosynthetic enzymes (Al-Hadethi, 2019; Medan *et al*., 2021). This results in increased carbohydrate accumulation in branches (Garcia *et al*., 2004). Humic acid, as shown in previous studies, facilitates nutrient retention and uptake, increasing leaf potassium levels and promoting biochemical activities within the plant (Osman, 2015; Medan, 2023). Amino acids improve plant enzymatic activity and nutrient transport, resulting in elevated nitrogen and protein levels in both leaves and branches. The significant improvements observed align with findings in pomegranate and apricot trees, demonstrating that combined organic fertilization and amino acid application can enhance nutritional content and growth performance (Abo-Ogiala, 2018; Tahir *et al*., 2019). These results confirm that specific combinations of poultry waste, humic acid, and amino acid concentrations offer an effective strategy for improving the chemical characteristics and nutritional status of pomegranate trees.

5. Conclusion

This study demonstrates that the application of organic fertilizers, specifically poultry waste and humic acid, in combination with amino acid foliar sprays, significantly enhances the mineral and biochemical properties of young pomegranate trees (var. Wonderful). Among the treatments, the highest levels of poultry waste (500 g/tree) and the highest amino acid concentration (10 ml/L) produced superior results across most parameters, including increased nitrogen, potassium, protein, and carbohydrate levels in both leaves and branches. These findings suggest that using 500 g/tree of poultry waste along with 10 ml/L of amino acid spray represents an optimal combination, promoting balanced nutrient content and overall growth in pomegranate trees. Furthermore, the study highlights the potential of organic fertilization and amino acid treatments as effective, environmentally friendly strategies to improve pomegranate tree health and productivity. Implementing these practices could be especially beneficial in sustainable agriculture and for enhancing crop quality and yield in similar subtropical fruit crops. Future research could explore the long-term impacts of these treatments and evaluate their effects under varying climatic and soil conditions.

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