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Effect of organic fertilizers and amino acids on the vegetative growth of young pomegranate trees *Punica granatum* L. cv. "Wonderful"

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Abstract

The experiment was conducted on a pomegranate field located in a deciduous fruit orchard at the Agricultural Research and Experimentation Station, which is associated with the College of Agriculture at the University of Kirkuk in the Republic of Iraq. The experiment took place during the 2023 season. The study incorporated 2 variables. The experiment consisted of 2 factors: the first factor included 5 levels of organic fertilizers (0, 250 and 500 g per tree of poultry waste and 15 and 30 g per tree of humic acid) and the second factor involved spraying with 3 concentrations of amino acids (0, 5 and 10 mL L^{-1}). The experimental design used was a Randomized Complete Block Design (RCBD) within factorial experiments, with 2 factors and 3 replicates. The study involved a total of 90 young pomegranate trees, with 2 trees assigned to each experimental unit. The data were analyzed using Duncan's multinomial test at a significance level of 0.05. Applying 500 g of poultry waste per tree resulted in a notable enhancement in vegetative development, namely in stem length. The leaf area and dry weight of the leaves were measured at 6.45 cm^2 and 54.67 % respectively. The level of 30 g per tree of humic acid resulted in a significant increase in the stem diameter, branch length and branch diameter, which was recorded at 23.16 mm, 39.98 cm and 6.30 mm respectively, compared to the control treatment. The application of a 10 mL L^{-1} concentration of amino acids led to a notable enhancement in the observed traits (stem length and branches, their diameters, leaf area and dry weight of leaves) in comparison to the control treatment. The mutual influence of organic fertilizer and spraying with amino acids resulted in a noticeable and statistically significant effect. This effect was particularly evident when using a level of 500 g per tree of poultry waste as fertilizer and a concentration of 10 mL L^{-1} of amino acids for spraying, as it positively impacted most parameters.

Keywords

Pomegranate; poultry waste; humic acid; amino acids

Introduction

The pomegranate, *Punica granatum* L., is one of the types of trees that is classified as a tree in temperate and subtropical regions and belongs to the pomegranate family, Punicaceae. The original homeland of the pomegranate is Iran and its surrounding areas and from there its cultivation moved to North Africa, the Mediterranean basin, Europe and America and its cultivation is currently famous in Iraq, Egypt, the Kingdom of Saudi Arabia, Syria,

Lebanon, Iran, Spain, Italy, Cyprus, in addition to the state of Florida and some southern states in America etc. (1). The pomegranate was mentioned in the Holy Qur'an 3 times and it was also mentioned in the rest of the other heavenly books (2). The importance of pomegranates comes from the fact that its fruits contain many nutrients, vitamins (vitamin C), mineral salts, pigments, fats, carbohydrates, sugars, acids, fibers and proteins, which vary in the number of nutrients they contain depending on the variety (3, 4). Pomegranates also have some medical benefits. The medicinal effects of the tree are attributed to the presence of many components, including anthocyanins, vitamins and phenolic substances. These compounds have been demonstrated to work as antioxidants and inhibitors of infections, making them beneficial in promoting health (5).

Poultry waste is a fertilizer that contains more nutrients than the rest of the animal waste and therefore it is used more widely and in greater quantities compared to the rest of the animal waste (6). One of the organic fertilizers is humic acid that contain many elements that work to improve soil fertility and increase the availability of nutrients, thus positively affecting plant growth and productivity. Humic materials work to reduce the hydrogen ion concentration (pH) of the soil (7, 8). In addition, they enhance the soil's physical, chemical, biological and nutritional properties by loosening it, breaking down clay particles and increasing its ability to retain water. Additionally, it serves as a conduit for the transportation of nutrients from the soil to plants (9, 10). According to researchers (11), amino acids are essential stimulants that are swiftly absorbed and transported within plants. They also directly impact enzymatic activity by speeding up the process of nutrient absorption and transfer within the plant and supplying the energy it needs to function. The aim of the study was improving the vegetative growth of young pomegranate trees of the Wonderful variety by adding organic fertilizers (poultry waste and humic acid) and spraying amino acids at different concentrations and determining the best levels among the factors studied individually or in combination.

Materials and Methods

The Agricultural Research and Experimentation Station of the College of Agriculture at the University of Kirkuk conducted this experiment in a pomegranate field, one of the deciduous fruit orchards, located in the Al-Sayadah area. The experiment was conducted during the 2023 agricultural season on young pomegranate trees of the Wonderful variety, 2 years old when planted in the orchard, to know the effect of organic fertilizers, acids, amino acids in the vegetative growth properties of young pomegranate trees, planted with dimensions of 4 × 3.5 m. Operations related to agricultural services were conducted out on the pomegranate trees well, including weeding, removing bushes, ploughing the soil, removing cancers and pruning broken and infected branches and the irrigation used in the orchard was drip irrigation. The experiment was being carried out using a randomized complete block design (R.C.B.D.).

Transactions studied in the experiment

The first was adding organic fertilizers at 5 levels:

1. Without adding (control) T0.
2. Adding 250 g per tree of poultry waste, T250.
3. Adding 500 g per tree of poultry waste, T500.
4. Adding 15 g per tree of humic acid, T15.
5. Adding 30 g per tree of humic acid, T30.

The second was spraying amino acids at 3 concentrations:

1. Without spraying (control), A0.
2. Spraying 5 mL L^{-1}, A5.
3. Spraying 10 mL L^{-1}, A10.

Stem length augmentation (cm)

The stem length of each experimental unit was assessed using a metric tape at the start and conclusion of the experiment and the rate of augmentation in stem length was computed.

Stem diameter (mm)

The stem diameter was measured at the start and end of the experiment using a Vernier electronic device at a distance of 5 cm from the soil surface and the rate of growth in stem diameter was calculated.

Length of the branches (cm)

For every experimental unit, 4 branches were chosen and their lengths were measured at the beginning of March and December.

Diameter of the branches (mm)

The diameter of the above selected branches was measured for each experimental unit using the Vernier electronic device, at a distance of 1 cm from the point of contact with the branch growing on it during the beginning of the season in March and the end of the experiment in December.

Area of the leaf (cm^2)

20 fully expanded leaves were collected from the central part of the young shoots and from all sides of the tree and at different heights during the first week of July and the method applied was adopted from a previous study (12)

Percentage of dry weight of leaves (%)

The dry matter percentage was determined using the following mathematical equation:

$$\text{Dry matter percentage (\%)} = \frac{\text{Dry weight of leaves}}{\text{Wet weight of leaves}} \times 100 \quad \text{……..(Eqn. 1)}$$

Results

Increase in stem length (cm)

The findings given in Table 1 demonstrated that there was a significant difference in the rate of increase in stem

length between the experimental treatments. Specifically, the T500 poultry waste treatment outperformed all other treatments, reaching 131.16 cm or 30.04 % more than the control treatment, while the control treatment T0 had the lowest rate of this trait, measuring 100.86 cm.

The results provided in the Table 1 also demonstrated the impact of spraying amino acids on a significant increase in the rate of stem length; the control treatment A0 produced the lowest rate in this characteristic, measuring 109.03 cm, while the A10 concentration significantly outperformed the other treatments, reaching 124.83 cm, an increase of 14.49 % over the control treatment.

Furthermore, the results from the Table 1 indicate that the combination of organic fertilizer and amino acid spraying had a significant positive effect on stem length. Specifically, the treatment involving T500 A0 resulted in the highest stem length of 137.10 cm, which is a 73.17 % increase compared to the control treatment.

Increase in stem diameter (mm)

According to the statistical analysis in Table 2, organic fertilization significantly outperformed all experimental treatments in terms of the rate at which stem diameter increased. The humic acid T30 treatment, for example, outperformed all experimental treatments, reaching 23.16 mm at a rate that was 19.38 % higher than the control treatment did not show any significant differences compared to the therapy. T250 poultry waste treatment produced the smallest average stem diameter, measuring 18.07 mm, compared to T500 poultry waste.

Table 2 results shown below indicate the effect of spraying with amino acids and the presence of a significant superiority in increasing the stem diameter of pomegranate trees, as the A10 treatment gave the highest values, reaching 22.22 mm, with an increase of 10.87 % compared to trees not sprayed. The A5 treatment resulted in the smallest stem diameter rate, reaching 19.74 mm.

As for the effect of the bilateral interaction between the treatments of adding organic fertilizers and spraying amino acids, it was shown that there was a significant increase in stem diameter, as the T30 A10 intervention treatment was significantly superior in giving it the largest stem diameter, which reached 24.33 mm, with a 43.45 % growth rate over the observation treatment; the two T500 A0 interference treatments did not differ substantially from one another. In comparison to other treatments, T0 A0 gave the smallest average stem diameter of 16.96 mm during the study season.

Increase in the length of the branches (cm)

Table 3 presents the results, which demonstrate a significant difference in the rate of increase in branch length between the experimental treatments. The T30 humic acid treatment was the most successful treatment, reaching 39.98 cm and 9.38 % more than the comparison treatment; the control treatment, T0, showed the least increase, with the branches reaching 36.55 cm.

The results displayed in the table demonstrate a clear advantage in the rate of branch length growth when amino acids are applied. Specifically, concentration A10 exhibited the highest increase in branch length, reaching 38.80 cm, with a growth rate of 3.43 % compared to the control treatment. On the other hand, concentration A5 resulted in the lowest branch length of 37.04 cm. The results of the combined effect of organic fertilizers and amino acid spraying on the rate of branch length increase were significant. The T30 A10 intervention treatment yielded the highest values, reaching 42.16 cm, with a growth rate of 23.41 % compared to the control treatment. The control treatment T0 A0 exhibited the minimum average branch length of 34.16 cm.

Increase in the diameter of the branches (mm)

According to the data in Table 4, the amount of organic fertilizers had a moderate impact on branch diameter growth. The humic acid treatment T30 outperformed all other experimental treatments combined, reaching 6.30

Table 1. The effect of organic fertilizer (poultry waste and humic acid) and amino acid spraying and their interaction on the increase in stem length (cm) in young pomegranate trees of the Wonderful variety.

Organic fertilizer +Amino acids	Ao (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	79.16 ^{g}	98.76 ^{f}	124.66 ^{bc}	100.86 ^{d}
T250 (250 g per tree poultry waste)	104.13 ^{ef}	105.66 ^{ef}	117.00 ^{cd}	108.93 ^{c}
T500 (500 g per tree poultry waste)	137.10 ^{a}	120.73 ^{bc}	135.66 ^{a}	131.16 ^{a}
T15 (15 g per tree humic acid)	101.76 ^{ef}	109.50 ^{de}	118.00 ^{cd}	109.75 ^{c}
T30 (30 g per tree humic acid)	123.00 ^{bc}	123.16 ^{bc}	128.83 ^{ab}	125.00 ^{b}
Average	109.03 ^{b}	111.56 ^{b}	124.83 ^{a}	

Table 2. The effect of organic fertilizer (poultry waste and humic acid) and amino acid spraying and their interaction on the increase in stem diameter (mm) in young pomegranate trees of the Wonderful variety.

Organic fertilizers +Amino acids	A0 (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	16.96 ^{g}	17.30 ^{fg}	23.93 ^{abc}	19.40 ^{b}
T250 (250 g per tree poultry waste)	17.63 ^{fg}	17.96 ^{efg}	18.63 ^{ef}	18.07 ^{c}
T500 (500 g per tree poultry waste)	24.16 ^{ab}	21.93 ^{d}	22.66 ^{cd}	22.92 ^{a}
T15 (15 g per tree humic acid)	18.60 ^{ef}	19.16 ^{e}	21.53 ^{d}	19.76 ^{b}
T30 (30 g per tree humic acid)	22.83 ^{bcd}	22.33 ^{d}	24.33 ^{a}	23.16 ^{a}
Average	20.04 ^{b}	19.74 ^{b}	22.22 ^{a}	

Table 3. The effect of organic fertilizers (poultry waste and humic acid) and amino acid spraying and their interaction on increasing the length of branches (cm) in young pomegranate trees of the Wonderful variety.

Organic fertilizers +Amino acids	A0 (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	34.16 ^{g}	36.13 ^{f}	39.36 ^{bc}	36.55 ^{c}
T250 (250 g per tree poultry waste)	36.86 ^{ef}	36.50 ^{f}	36.80 ^{ef}	36.72 ^{c}
T500 (500 g per tree poultry waste)	40.36 ^{b}	37.10 ^{def}	38.13 ^{cde}	38.53 ^{b}
T15 (15 g per tree humic acid)	36.80 ^{ef}	37.06 ^{def}	37.56 ^{def}	37.14 ^{c}
T30 (30 g per tree humic acid)	39.36 ^{bc}	38.43 ^{cd}	42.16 ^{a}	39.98 ^{a}
Average	37.51 ^{b}	37.04 ^{b}	38.80 ^{a}	

mm, a 28.57 % increase in diameter compared to the trees that received no additional fertilizer, while the control treatment T0 produced a lower average of 4.90 mm in this capacity.

The effect of spraying with amino acids shows that there is a narrow increase in the diameter of the branches, as the A10 treatment was mildly superior in giving it the largest diameter of the branches, reaching 5.94 mm, with a rise in 12.71 % in contrast to the other treatments being compared, while the control treatment A0 gave the smallest diameter of the branches, with a percentage of 5.27.

While the results of the double interaction between organic fertilizer levels and amino acid concentrations showed a significant effect in increasing the diameter of the branches, as the T30 A10 experimental treatments by a rate of 6.66 mm, an increase of 47.01 %, which did not differ significantly from the T500 A10 interaction treatment. The control treatment T0 A0 results in the least increase in branch diameter by 4.53 mm during the season.

Percentage of dry weight of leaves (%)

As can be seen in Table 5, there was a significant difference between the experimental treatments in terms of the percentage of dry weight in leaves. During the study season, the control treatment T0 had the lowest percentage of 51.56 % for this trait, while the T500 poultry waste treatment outperformed all other treatments with a percentage of 54.67 %.

With respect to the impact of the amino acid spraying, the results presented in the same table show a statistically significant difference: concentration A10 yielded the highest percentage of dry weight of leaves (54.01 %) in contrast to the control treatment A0, which yielded the lowest percentage of dry weight of leaves (51.92 %).

The results given in the same table demonstrated a significant superiority brought about by the dual interaction of spraying amino acid and adding organic fertilizer. With the highest percentage of dry weight of leaves (56.57 %), the T500 A10 treatment surpassed all other treatments, while the T0 A0 treatment produced the lowest percentage of results for this trait (47.79 %). Throughout the study season, there was no discernible difference between the T500 A10 and the T0 A10 treatments.

Table 4. The effect of organic fertilizers (poultry waste and humic acid) and amino acid spraying and their interaction on increasing the diameter of branches (mm) in young pomegranate trees of the Wonderful variety.

Organic fertilizers +Amino acids	A0 (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	4.53 ^{h}	4.80 ^{gh}	5.36 ^{de}	4.90 ^{d}
T250 (250 g per tree poultry waste)	4.86 ^{fgh}	4.93 ^{fg}	5.53 ^{cd}	5.11 ^{cd}
T500 (500 g per tree poultry waste)	5.86 ^{bc}	5.33 ^{de}	6.60 ^{a}	5.93 ^{b}
T15 (15 g per tree humic acid)	5.00 ^{efg}	5.23 ^{def}	5.56 ^{cd}	5.26 ^{c}
T30 (30 g per tree humic acid)	6.10 ^{b}	6.13 ^{b}	6.66 ^{a}	6. 30 ^{a}
Average	5.27 ^{b}	5.28 ^{b}	5.94 ^{a}	

Leaf area (cm^2)

The findings shown in Table 6 indicate that the inclusion of humic acid and poultry waste led to a substantial augmentation in leaf area. The T500 treatment resulted in the largest leaf area, measuring 6.45 cm^2, which represents a 17.48 % increase compared to the control treatment. The leaf area of the control treatment did not show a significant difference from the T15 humic acid treatment. The T250 treatment exhibited the most minimal values for this par-

Table 5. Effect of organic fertilization (poultry waste and humic acid) and amino acid spraying and their interaction on the percentage of dry weight of leaves (%) in young pomegranate trees of the Wonderful variety.

Organic fertilizers +Amino acids	A0 (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	47.79 ^{e}	50.68 ^{d}	56.21 ^{a}	51.56 ^{c}
T250 (250 g per tree poultry waste)	51.87 ^{cd}	51.43 ^{cd}	51.82 ^{cd}	51.70 ^{bc}
T500 (500 g per tree poultry waste)	55.12 ^{b}	52.32 ^{cd}	56.57 ^{a}	54.67 ^{a}
T15 (15 g per tree humic acid)	51.41 ^{cd}	51.81 ^{cd}	52.12 ^{cd}	51.78 ^{bc}
T30 (30 g per tree humic acid)	53.42 ^{bc}	51.89 ^{cd}	53.36 ^{bc}	52.89 ^{b}
Average	51.92 ^{b}	51.63 ^{b}	54.01 ^{a}	

ticular feature, measuring 5.44 cm^2.

The findings demonstrated that spraying amino acids increased leaf area significantly. Treatment A5 produced the lowest concentration for this trait, at an average rate of 5.41 cm^2, while concentration A10 produced the largest leaf area, at a rate of 6.28 cm^2, with an increase of 7.16 % over the control treatment.

Regarding the combined interaction between concentrations of amino acids and levels of organic fertilizers, there was a significant increase in the trait of leaf area. The T500 A10 treatment yielded the highest values for the trait, reaching 7.18 cm^2 with an increase rate of 48.96 %, while the control treatment T0 A0 produced the lowest values. The T500 A10 treatment outperformed all other combined treatments among the experimental treatments. The area of the leaf in the control treatment was 4.82 cm^2.

Discussion

The findings presented in Table 1-6 indicate that the addition of poultry waste to the ground improves the vegetative growth characteristics (stem length and diameter, branch length and diameter, area of the leaf and dry weight of the leaves). According to some authors (13), the improvement of the chemical, biological and physical properties of the soil as well as physiological activities like boosting the efficiency of the photosynthetic process in the leaves can be related to the increase in vegetative characteristics. When organic fertilizer is added, the height and diameter of the stem as well as the length and diameter of the branch were increased. . This could be because organic waste improves the physical characteristics of the soil, creating an environment that is favourable for root growth and spread and increases nutrient absorption (14, 15). The results of the study agree with the findings of authors on apple trees, golden deciduous trees and on pomegranate trees (16-19).

The results shown in Table 3-6 showed that the ground addition of humic acid caused significant superiority in many characteristics including stem length, branch length and diameter, area of leaf and dry weight of the leaves, which may be attributed to improving the characteristics of vegetative growth after adding acid. When humic acid enters the soil, as a result of its indirect effect on the soil by increasing the microbial activity of the soil, the effectiveness of nutrients and their absorption by the root hairs as a chelating agent, which increases the nutritional status in the leaves and thus the leaf area and dry weight of the leaves increase (20). These results are consistent with other studies on pomegranate trees and on seed orange (21, 23) and (23, 24) on Slimi pomegranate trees (22) and on apple trees including local Ibrahimi variety (24, 25).

The data shown in Table 1-6 indicate that spraying amino acids improved the characteristics of vegetative growth (stem length and diameter, branch length and diameter, leaf area and dry weight of leaves). This can be attributed to the role of amino acids in enhancing characteristics of vegetative development by stimulating crucial activities, particularly the processes of cell division and elongation as well as boosting enzyme activity (26). Amino acids are also essential components in the process of protein construction (27), the results agree with both on pomegranate tree cultivars Manfalouty and Wonderful (27, 28) and on apricot tree cultivar Zaghinia (29).

Conclusion

From the results obtained, we can conclude that the ground addition of organic fertilizers (poultry waste and humic acid) along with foliar spraying of amino acids has a positive effect on the vegetative growth characteristics compared to the control trees. It is also revealed that adding poultry waste at a level of 500 g per tree and adding humic acid at a level 30 gm per tree and spraying amino acids at a concentration of 10 mL L^{-1}, which gave the best treatments for increasing vegetative growth studied in the experiment, individually or overlapping.

Table 6. Effect of organic fertilizers (poultry waste and humic acid) and amino acid spraying and their interaction on leaf area (cm^2) of young pomegranate trees of the Wonderful variety.

Organic fertilizers +Amino acids	A0 (0 mL L^{-1})	A5 (5 mL L^{-1})	A10 (10 mL L^{-1})	Average
T0 (0 g control treatment)	4.82 ^{e}	5.08 ^{de}	6.56 ^{bc}	5.49 ^{bc}
T250 (250 g per tree poultry waste)	5.30 ^{de}	5.46 ^{cde}	5.56 ^{cde}	5.44 ^{c}
T500 (500 g per tree poultry waste)	6.71 ^{b}	5.47 ^{cde}	7.18 ^{a}	6.45 ^{a}
T15 (15 g per tree humic acid)	6.23 ^{bcd}	5.65 ^{bcde}	5.98 ^{bcd}	5.95 ^{ab}
T30 (30 g per tree humic acid)	6.25 ^{bcd}	5.38 ^{de}	6.13 ^{bcd}	5.92 ^{b}
Average	5.86 ^{b}	5.41 ^{bc}	6.28 ^{a}	

Authors' contributions

Both authors contributed equally.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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