

Foliar Application of Proline, Glutathione, and Nano-Ascorbic Acid on Strawberry Growth under Well Water Irrigation.

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Abstract

The experiment was conducted in the Horticulture and Forestry Division of the Najaf Agriculture Directorate from (17/11/2024) to (1/4/2025) to study the effect of spraying proline acid at a concentration of (0, 200, 400 mg L⁻¹), the antioxidant glutathione (0, 100, 200 mg L⁻¹), and ascorbic acid (0, 50, 100 mg L⁻¹) on some chemical indicators of Ruby Gem strawberry seedlings irrigated with saline well water. This study aimed to evaluate the effect of foliar application of nano-proline acid, nano-glutathione, and nano-ascorbic acid—individually and in combination—on the chemical and vegetative characteristics of Ruby Gem strawberry seedlings irrigated with saline well water, in order to identify the most effective treatments for improving plant growth and tolerance under salinity stress conditions.

The experiment was designed as a factorial experiment using a completely randomized block design for the first factor, nano-proline acid; the second factor, nano-glutathione; and the third, nano-ascorbic acid. This brought the number of treatments to 27, randomly distributed across three blocks, resulting in a total of 81 experimental units. The chemical characteristics were analyzed using the Genstat statistical analysis program, and treatment means were compared using Duncan's multiple range test at a 5% probability level.

The most important results can be summarized as follows:

1. Proline showed a significant effect on all vegetative and chemical characteristics of the strawberry. The traits (leaf number, leaf area, average dry weight of vegetative growth, percentage of dry matter, and leaf nitrogen, phosphorus, and potassium contents) were highest at 400 mg L⁻¹, reaching (35.31 leaves plant⁻¹, 83.97 cm² plant⁻¹, 4.68 g, 39.5%, 1.933%, 0.471%, and 1.839%), respectively.

2. Spraying with nano-glutathione at 200 mg L⁻¹ resulted in a significant increase in these traits, reaching (37.43 leaves plant⁻¹, 75.92 cm² plant⁻¹, 3.89 g, 42.5%, 1.778%, 0.463%, and 1.637%).

3. Spraying with nano-ascorbic acid at 100 mg L⁻¹ also improved these parameters, achieving (35.59 leaves plant⁻¹, 80.97 cm² plant⁻¹, 4.02 g, 40.0%, 1.928%, 0.486%, and 1.863%).

4. The bi-factor interactions among the three experimental factors resulted in significant differences in most vegetative and chemical growth indicators.

5. The triple interaction (400 mg L⁻¹ proline + 200 mg L⁻¹ glutathione + 100 mg L⁻¹ ascorbic acid) produced the highest values for most studied traits, recording (54.77 leaves plant⁻¹, 135.3 cm² plant⁻¹, 6.57 g, 52.2%, 3.486%, 0.224%, and 2.266%).

Keywords:

Strawberry, Saline water, Nano-proline acid, Nano-glutathione, Nano-ascorbic acid, Vegetative growth, Chemical traits, Salinity tolerance.

1- Introduction

Strawberry (*Fragaria* spp.) is a perennial herbaceous plant distinguished by its attractive appearance and delicious fruits. It is one of the

most widely cultivated small fruit crops in the world because of its high nutritional and medicinal value. It belongs to the order Rosales, family Rosaceae, subfamily

Rosoideae, and genus *Fragaria*. Its English name is “Strawberry,” and in Turkish, it is known as “Chiliak,” a name also used in Iraq. The genus includes more than 150 species that are often difficult to distinguish from one another. North America is considered the native home of strawberries (18, 29).

Salinity stress is one of the most serious abiotic stresses affecting strawberry growth and productivity, particularly in arid and semi-arid regions where the use of saline water for irrigation is common. Excessive salt concentrations in the soil solution negatively influence water uptake, photosynthesis, and nutrient balance, leading to a decline in plant growth and fruit yield. Therefore, it is essential to employ effective strategies to mitigate the adverse effects of salinity on this sensitive crop.

Among these strategies, foliar application of amino acids such as proline has proven beneficial under various stress conditions (14). Proline is a simple, nonpolar amino acid that plays a key role in maintaining osmotic balance and protecting cells against oxidative damage. Its accumulation in plant tissues enhances stress tolerance by stabilizing proteins and membranes (20, 27). Likewise, antioxidants such as glutathione (GSH; glutamyl-cysteinyl-glycine) and ascorbic acid (vitamin C) are essential components of the plant’s defense system. Glutathione is a tripeptide with strong reducing power that participates in detoxification processes (11), while ascorbic acid regulates redox homeostasis and supports plant growth and stress responses (28).

Recent advances in nanotechnology have introduced new opportunities to enhance the efficiency of these bioactive compounds. Nanoparticles, typically ranging from 1 to 100 nanometers in size, exhibit unique physicochemical properties that increase their reactivity, stability, and absorption efficiency in plant tissues (25, 26). Applying amino acids and antioxidants in nano form may therefore improve their effectiveness in alleviating salinity stress.

Although many studies have investigated the role of proline, glutathione, and ascorbic acid in mitigating salinity stress in plants, limited research has focused on their nano-form applications and their combined interactive effects on the vegetative and chemical traits of strawberry (*Fragaria × ananassa*) under saline irrigation conditions.

This study was therefore conducted to evaluate the individual and combined effects of nano-proline acid, nano-glutathione, and nano-ascorbic acid on some chemical and vegetative characteristics of Ruby Gem strawberry seedlings irrigated with saline well water

2- Materials and Methods

The experiment was conducted on Ruby Gem seedlings propagated with runners as a factorial experiment within a Randomized Complete Block Design (RCBD). The experiment includes three factors: the first factor is treating the seedlings with proline at a concentration of 0, 200, or 400 mg L⁻¹. The second factor is spraying the seedlings with glutathione acid at a concentration of 0, 50, or 100 mg L⁻¹. The third factor is spraying the seedlings with ascorbic acid at a concentration of 0, 100, or 200 mg L⁻¹. Each treatment within a block contained

six experimental units, resulting in a total of 486 seedlings, in addition to seedlings as guard lines on both sides of the sectors. The planting lines are planned and prepared in the greenhouse in three lines, leaving a distance of 1 m between each line. The soil and irrigation water used were analyzed before starting the experiment by taking random samples. Planting was carried out in a greenhouse in agricultural rows covered with black plastic mulch. Soil mulch was sprayed after the appearance of fully developed true leaves, and the application was repeated every month. As for the type of water used (well water), spraying was carried out in the early morning until the vegetative growth was wet, with the addition of a spreading agent (Tween 20) at a rate of 0.1%. The results were analyzed using the ANOVA test using the GeneStat statistical program, and comparisons between treatments

were made using the least significant difference (LSD) test at a probability level of 5% (7).The soil was analyzed in the laboratory of the Directorate of Agriculture in Najaf

Governorate to measure the physical and chemical properties, and the results were as shown in Table (1).

Table (1) Analysis of some chemical and physical properties of the soil

Soil		
Traits	values	Units
Soil separators	Sand	380
	silt	308
	Clay	312
Texture	Clay loam	----
pH	6.9	
Electrical Conductivity	2.1	DS.m ⁻²
EC		
CO3	Nil	
Nitrogen	0.261	PPm
Phosphorus	0.245	PPm
Potassium	92.2	PPm
Calcium	4	mmol.L ⁻¹
Magnesium	9.6	mmol.L ⁻¹
Nitrogen	5	mmol.L ⁻¹
HCO3	0.7	mmol.L ⁻¹
SO4	0.42	mmol.L ⁻¹

2-1 Traits studied

The studied traits included the following:

1. Average number of leaves per plant (leaf plant⁻¹) (22)
2. Average leaf area (cm² plant⁻¹) (22)
3. Average dry weight of vegetative growth (g) (9)
4. Percentage of dry matter in vegetative growth (%) (10)
5. Leaf nitrogen content (N%) (12)
6. Leaf phosphorus content (P%) (9)
7. Leaf potassium content (K%) (9)

3-Results and discussion

3-1Average number of leaves (leaf/plant-1)

Table (1-3) shows that spraying plants with nano-proline for the first season significantly increased the number of leaves.plant, as the 400 mg L-1 treatment recorded the highest average number of leaves, reaching 35.31 leaves.plant, compared to the lowest number in the control treatment, which reached 32.41 leaves.plant⁻¹.

While spraying with nano-glutathione had a significant effect at a concentration of 200 mg L⁻¹, as it recorded the highest average number of leaves, reaching 37.43 leaves.plant⁻¹, compared to the control treatment, which gave The lowest rate was 30.60 leaves.plant-1 for the first season. From the same table, we note that spraying nano-ascorbic acid had a significant effect on the number of leaves for the first season, as the concentration of 100 mg L⁻¹ recorded the highest average number of leaves, reaching 35.59 leaves.plant⁻¹, compared to the lowest number recorded in the control treatment, which reached 32.63 leaves.plant-1. The dual interaction between nano-proline and nano-glutathione showed a significant effect, as the highest rate was recorded at the concentration (400 mg L⁻¹ + 200 mg L⁻¹), reaching 38.22 leaves.plant⁻¹, compared to the lowest rate of the control treatment, which reached 30.34 leaves.plant-1

for the first season. The interaction treatment of spraying nano-proline and nano-ascorbic acid also showed a significant effect for the first season, as the interaction recorded the highest average number of leaves at the concentration (400 mg L⁻¹ + 100 mg L⁻¹), which reached 41.70) leaves.plant⁻¹ (compared to the lowest rate recorded for the control treatment, which was 27.62 leaves.plant⁻¹) for the first season. The interaction between nano-glutathione and nano-ascorbic acid recorded the highest average leaf number at the concentration (200 mg L⁻¹ + 100 mg L⁻¹),

reaching 39.27 leaves.plant⁻¹, compared to the lowest rate of 29.23 leaves.plant⁻¹ in the control plants for the first season, respectively. According to the data available in the table, the triple interaction (proline + glutathione + nano-ascorbic acid) recorded the highest average leaf number at the concentration (400 mg L⁻¹ + 200 mg L⁻¹ + 100 mg L⁻¹) for the first season, reaching 54.77 leaves.plant⁻¹, compared to the lowest rate in the control plants, which was 24.01 leaves.plant⁻¹, respectively.

Table (3-1) Effect of spraying with proline and glutathione Nano ascorbic acid and their interaction on the average number of leaves (leaf/plant-1) of the Ruby Gem variety irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹			Prolin x Ascorbic
		0	100	200	
0	0	24.01	29.97	28.88	27.62
	50	29.70	40.31	37.02	35.68
	100	29.97	38.54	35.86	34.79
200	0	36.72	28.79	34.96	33.49
	50	29.19	35.35	40.67	35.07
	100	29.85	33.58	27.17	30.20
400	0	30.90	30.65	45.74	35.76
	50	32.86	32.23	31.84	32.31
	100	32.17	38.15	54.77	41.70
Glutathion average		30.60	34.17	37.43	
Glutathion x Prolin		Glutathion mg.L⁻¹			Prolin
Prolin mg.L ⁻¹	0	30.34	36.27	30.62	32.41
	200	31.92	33.68	37.85	34.48
	400	30.84	36.87	38.22	35.31
Glutathion x Ascorbic		Ascorbic mg.L⁻¹			Ascorbic
Glutathion mg.L ⁻¹	0	29.23	35.45	33.21	32.63
	100	33.49	35.96	32.58	34.01
	200	30.67	36.76	39.27	35.59
L.S.D. 0.05					
L.S.D Glutathion=4.453					
L.S.D Prolin=4.453					
L.S.D Ascorbic =4.453					

L.S.D Glutathion x Prolin = 7.713
L.S.D Glutathion x Ascorbic = 7.713
L.S.D Prolin x Ascorbic =7.713
L.S.D Glutathion x Prolin x Ascorbic =13.359

3-2 Average leaf area (cm².plant-1)

The results in Table 3-2 indicate a significant difference between the nanoproline spray treatments. Plants sprayed at a concentration of 400 mg L⁻¹ had the highest average leaf area (83.97 cm².plant-1), compared to the lowest average recorded in the control treatment (62.10 cm².plant-1) for the first season. While spraying with nano-glutathione at a concentration of 200 mg L⁻¹ significantly increased the average leaf area, reaching 75.92 cm² plant-1, compared to the lowest leaf area in the control treatment, which reached 66.37 cm² plant-1 for the first season. The nano-ascorbic acid spray treatment at a concentration of 100 mg L⁻¹ outperformed the treatment, recording the highest average leaf area of 80.97 cm² plant-1, compared to the lowest average in the control treatment, which reached 69.10 cm² plant-1 for the first season, respectively.

The interaction between nano-proline and nano-glutathione was superior, recording the highest average at the interaction (400 mg L⁻¹ + 200 mg L⁻¹), reaching 86.2 cm² plant-1 for the first season, compared to the control treatment, which gave the lowest average at

the interaction, which reached 47.6 cm² plant-1 for the first season. The dual interaction between nano-proline and Nano-ascorbic acid had a significant effect on the interaction plants (400 mg L⁻¹ + 100 mg L⁻¹), recording the highest average leaf area of 120.67 cm².plant-1, compared to the lowest average of 56.53 cm².plant-1 for the control plants for the first consecutive season.

The interaction between nano-glutathione and nano-ascorbic acid also outperformed, recording the highest average leaf area for the interaction (200 mg L⁻¹ + 100 mg L⁻¹), recording 87.4 cm².plant-1, compared to the lowest average of 61.7 cm².plant-1 for the control plants for the first consecutive season.

As for the triple interaction, the results in the same table showed that the interactions (nano-proline 400 mg L⁻¹ + nano-glutathione 200 mg L⁻¹ + nano-ascorbic acid 100 mg L⁻¹) differed. Significantly, the average leaf area was significantly higher, with the interaction treatment recording the highest average leaf area for the first season (135.3 cm² plant-1), compared to the control treatment, which recorded the lowest average (40.3 cm² plant-1) for the first season, respectively.

Table (3-2) Effect of spraying with proline, glutathione, and nano-ascorbic acid, and their interaction, on the average leaf area (cm² plant-1) of Ruby Gem strawberries irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹				Prolin x Ascorbic
		0	100	200		
		0	0	40.3	44.9	
0	50	79.9	83.9	60.2	74.67	
	100	68.6	71.1	59.0	66.23	
	200	74.4	57.6	65.8	65.93	
200	50	64.9	71.5	65.3	67.23	
	100	58.3	65.6	64.5	62.80	
	400	49.8	61.9	91.0	67.57	
400	50	69.5	61.4	111.7	80.87	

	100	91.6	135.1	135.3	120.67
	Glutathion	66.37	72.56	75.92	
Glutathion mg.L⁻¹					
	Glutathion x Prolin	0	100	200	Prolin
Prolin mg.L⁻¹	0	47.6	73.5	65.2	62.10
	200	65.9	73.3	85.0	74.73
	400	84.8	80.9	86.2	83.97
Ascorbic mg.L⁻¹					
	Glutathion x Ascorbic	0	50	100	Ascorbic
Glutathion mg.L⁻¹	0		65.2	80.4	69.10
	100	71.4	61.9	79.1	70.80
	200	71.7	83.8		80.97
L.S.D. 0.05					
L.S.D Glutathion=15.93					
L.S.D Prolin=15.93					
L.S.D Ascorbic =15.93					
L.S.D Glutathion x Prolin =27.59					
L.S.D Glutathion x Ascorbic =27.59					
L.S.D Prolin x Ascorbic =27.59					
L.S.D Glutathion x Prolin x Ascorbic =47.79					

3-3 Average Dry Weight of the Vegetative growth (g)

It is noted from Table (3-3) that the nanoproline spray treatment had a significant effect on the average dry weight of the vegetative growth, as the concentration (400 mg.L-1) significantly excelled it, gave the highest rate of 4.68 g, while the treatment without nanoproline spray (0 mg.L-1) gave the lowest rate of 3.20 g for the first season in Ruby Gem seedlings irrigated with saline water. while, the antioxidant nanoglutathione had an insignificant effect on the average dry weight of the vegetative growth. The same table shows that spraying nano-ascorbic acid significantly affected the average dry weight of the vegetative growth of chicory plants at a concentration of 100 mg.L-1, with the highest rate reaching 4.02 g, compared to the control treatment, which gave the lowest significant difference of 3.38 g for the first season. As for the bi- interactions, the table below shows that the effect was significant when combining nano-proline amino acid with nano-glutathione, which gave the highest rate at a

concentration of 400 mg.L-1 + 200 mg.L-1, reaching 4.90 g, compared to the control treatment, which gave the lowest rate of 2.82 g for chicory seedlings irrigated with brackish well water. The interaction between nano-proline amino acid spraying and nano-ascorbic acid was superior, as the interaction plants (400 mg L-1 + 100 mg L-1) gave the highest average dry weight of the vegetative growth, reaching 6.08 g, compared to the control plants, which gave the lowest average dry weight of the vegetative growth, reaching 2.76 g, for the first season. The interaction between nano-glutathione acid and nano-ascorbic acid had a significant effect on the average dry weight of the vegetative growth at a concentration of 200 mg L-1 + 100 mg L-1, reaching 4.19 g, compared to the control treatment, which gave the lowest significant difference, reaching 3.17 g. The results in the same table showed that the triple interactions differed significantly in the average dry weight of the vegetative growth. The triple interaction of spraying nano-proline amino acid, nano-glutathione amino acid, and nano-ascorbic

acid recorded a significant increase in the average dry weight of the vegetative growth at the interaction (400 mg L⁻¹ + 200 mg L⁻¹ + 100 mg L⁻¹), recording the highest rate of

(6.57 g) compared to the lowest rate of (2.30 g) for the control treatment plants for the first season.

Table (4-3) Effect of spraying with proline, glutathione, nano-ascorbic acid, and their interaction on the average dry weight of the vegetative growth of Ruby Gem strawberries irrigated with well water for both seasons 2024-2025

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹			
		0	100	200	Prolin x Ascorbic
0	0	2.30	3.36	2.63	2.76
	50	3.95	2.99	3.42	3.45
	100	3.59	3.19	3.21	3.33
200	0	3.25	2.94	3.17	3.12
	50	2.57	3.58	3.95	3.37
	100	2.59	2.98	3.26	2.94
400	0	3.68	4.27	3.90	3.95
	50	4.86	4.22	4.86	4.64
	100	5.73	5.93	6.57	6.08
Glutathion		3.61	3.72	3.89	
		Glutathion mg.L⁻¹			
Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L ⁻¹	0	2.82	3.50	3.29	3.20
	200	3.58	3.17	3.24	3.33
	400	4.31	4.84	4.90	4.68
		Ascorbic mg.L⁻¹			
Glutathion x Ascorbic		0	50	100	averageAscorbic
Glutathion mg.L ⁻¹	0	3.17	3.52	3.44	3.38
	100	3.35	4.04	4.07	3.82
	200	3.91	3.95	4.19	4.02
L.S.D. 0.05					
L.S.D Glutathion= 0.928					
L.S.D Prolin= 0.928					
L.S.D Ascorbic = 0.928					
L.S.D Glutathion x Prolin =1.607					
L.S.D Glutathion x Ascorbic =1.607					
L.S.D Prolin x Ascorbic =1.607					
L.S.D Glutathion x Prolin x Ascorbic =2.783					

3-4 Percentage of Dry Matter in the Vegetative growth (%)

The results presented in Table (3-4) showed that spraying plants with nanoproline at a

concentration of (400 mg L⁻¹) had a significant effect, providing the highest percentage of dry matter in the vegetative growth, reaching (39.5%), compared to the

lowest percentage, reaching (38.1%), for the control plants irrigated with saline well water. The results in Table (3-4) also show that spraying with nano-glutathione at a concentration of 200 mg L⁻¹ significantly outperformed the control treatment, recording the highest percentage of 42.5% for the first season, compared to the lowest percentage of 36.8% for the control plant, the Ruby Gem variety, irrigated with brackish well water. Spraying plants with nano-ascorbic acid at a concentration of 100 mg L⁻¹ significantly increased dry matter percentage, recording the highest percentage of 40.0% for the first season, compared to the lowest percentage of 38.5% for the control plant, respectively. The interaction between nano-proline and nano-glutathione produced a significant increase in the interaction (400 mg L⁻¹ + 200 mg L⁻¹) for the first season, recording 43.9%, compared to the lowest percentage of 34.3% for the control plant. The results of the interaction between spraying nano-proline and nano-ascorbic acid on the Ruby Gem variety irrigated with a saline well indicated that the interaction (400 mg L⁻¹ + 100 mg L⁻¹) gave the highest

percentage of (43.2%) compared to the lowest percentage of (34.0%) in the control plants for the first season, respectively. On the other hand, the interaction between nano-glutathione and nano-ascorbic acid recorded the highest percentage when the interaction (200 mg L⁻¹ + 100 mg L⁻¹) for the first season reached (43.8%), compared to the lowest percentage recorded in the control plants, which reached (35.4%), respectively. The results in the same table also indicated that the triple interactions (nanoproteoline amino acid, nanoproteoglutathione amino acid, and nanoproteo ascorbic acid) differed significantly in the percentage of dry matter of the vegetative growth. The interaction (400 mg L⁻¹ + 200 mg L⁻¹ + 100 mg L⁻¹) recorded the highest percentage (52.2%), compared to the lowest percentage (27.9%) for the control plants in the first season of the control treatment.

Table (4-4) Effect of spraying with proline, glutathione, and nanoproteo ascorbic acid, and their interaction, on the percentage of dry matter of the vegetative growth (%) of Ruby Gem strawberries irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹				Prolin x Ascorbic
		0	100	200		
0	0	27.9	34.7	39.4	34.0	
	50	40.4	39.1	46.6	42.1	
	100	34.7	32.9	47.0	38.1	
200	0	45.6	41.3	40.1	42.3	
	50	30.2	40.9	39.1	36.7	
	100	44.5	35.7	32.0	37.4	
400	0	36.2	39.0	39.5	38.2	
	50	37.9	33.2	46.4	39.1	
	100	33.6	43.5	52.2	43.2	
Glutathion		36.8	37.8	42.5		
		Glutathion mg.L⁻¹				

Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L ⁻¹	0	34.3	35.6	44.3	38.1
	200	40.1	39.3	39.2	39.5
	400	35.9	38.6	43.9	39.5
		Ascorbic mg.L ⁻¹			
Glutathion x Ascorbic		0	50	100	Ascorbic
Glutathion	0	35.4	38.3	41.7	38.5
	100	36.1	37.7	41.9	38.6
	200	38.8	37.4	43.8	40.0
L.S.D. 0.05					
L.S.D Glutathion=5.35					
L.S.D Prolin=5.35					
L.S.D Ascorbic =5.35					
L.S.D Glutathion x Prolin = 9.27					
L.S.D Glutathion x Ascorbic =9.27					
L.S.D Prolin x Ascorbic =9.27					
L.S.D Glutathion x Prolin x Ascorbic =16.05					

3-5 Percentage of Nitrogen in Leaves (%)

The results in Table (3-5) show a significant difference in the percentage of nitrogen in leaves between the proline spray treatments on the vegetative growth of the Ruby Gem plant irrigated with saline well water. Plants sprayed at a concentration of 400 mg L⁻¹ outperformed, gave the highest percentage of nitrogen in leaves (1.933%), especially at a concentration of 200 mg L⁻¹, where the highest percentage was recorded at 1.865%, compared to the lowest percentage recorded for the control treatment, which was 1.040% for the first season, respectively. The nano-glutathione acid spray treatment was significantly superior at a concentration of 200 mg L⁻¹, recording the highest percentage at 1.778%, compared to the control treatment, which gave the lowest percentage at 1.330% for the first season, respectively. The nano-ascorbic acid spray treatment at a concentration of 100 mg L⁻¹ achieved the highest percentage (1.928%), compared to the lowest percentage (1.353%) for the control treatment. The interaction between nano-proline and nano-glutathione was superior, recording the highest percentage at the interaction (400 mg L⁻¹ + 200 mg L⁻¹) of

2.314%, compared to the lowest percentage (0.795%) for the control plants for the first season, respectively. The dual interaction of spraying different concentrations of nano-proline and nano-ascorbic acid had a significant effect on the interaction plants (400 mg L⁻¹ + 100 mg L⁻¹), recording the highest percentage (2.455%), compared to the lowest percentage (0.618%) for the control plants. The interaction between nano-glutathione and nano-ascorbic acid also excelled, recording the highest percentage at the interaction (200 mg L⁻¹ + 100 mg L⁻¹) of 2.270%, compared to the lowest percentage for control plants (1.243%) for the first season. The results of the same table showed that the triple interactions significantly differed in the percentage of nitrogen in the leaves when plants were sprayed with different concentrations of nano-proline, nano-glutathione, and nano-ascorbic acid. This resulted in a significant increase at the interaction (400 mg L⁻¹ + 200 mg L⁻¹ + 100 mg L⁻¹), recording the highest percentage of nitrogen in the leaves (3.486%), compared to the lowest percentage (0.15%) for the control plants and for the first season, respectively.

Table (3-5) The effect of spraying with proline, glutathione, nano ascorbic acid and their interaction on the percentage of nitrogen in the leaves (%) of the Ruby Gem variety of Strawberry irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹			
		0	100	200	Prolin x Ascorbic
0	0	0.158	0.658	1.038	0.618
	50	1.373	0.742	1.314	1.143
	100	1.299	1.485	1.291	1.359
200	0	1.802	1.778	1.687	1.755
	50	2.172	1.744	1.695	1.870
	100	2.264	1.610	2.034	1.969
400	0	1.360	1.792	1.908	1.687
	50	1.630	1.799	1.549	1.659
	100	1.545	2.333	3.486	2.455
Glutathion		1.330	1.549	1.778	
		Glutathion mg.L⁻¹			
Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L ⁻¹	0	0.795	1.110	1.214	1.040
	200	2.079	1.711	1.805	1.865
	400	1.512	1.975	2.314	1.933
		Ascorbic mg.L⁻¹			
Glutathion x Ascorbic		0	50	100	Ascorbic
Glutathion mg.L ⁻¹	0	1.243	1.273	1.544	1.353
	100	1.725	1.428	1.519	1.558
	200	1.703	1.809	2.270	1.928
L.S.D. 0.05					
L.S.D Glutathion=0.3529					
L.S.D Prolin=0.3529					
L.S.D Ascorbic =0.3529					
L.S.D Glutathion x Prolin =0.6113					
L.S.D Glutathion x Ascorbic =0.6113					
L.S.D Prolin x Ascorbic =0.6113					
L.S.D Glutathion x Prolin x Ascorbic =1.0588					

3-6 Phosphorus percentage in leaves (%)

The results in Table (3-6) indicate that spraying nano-proline at different concentrations affected the vegetative growth of the Ruby Gem cultivar, especially at the concentration (400 mg L-1), which significantly outperformed, recording the highest percentage of phosphorus in the leaves

(0.471%), compared to the lowest percentage recorded for the control plants (0.363%) for the first consecutive season. From the results presented in the table, it is noted that spraying nano-glutathione acid had a significant effect, as the concentration (200 mg L-1) recorded the highest percentage (0.463%), compared to the lowest percentage (0.363%) recorded for

the control plants for the first season. Spraying ascorbic acid on the vegetative growth had a significant effect, as the concentration (100 mg L⁻¹) recorded the highest percentage (0.486%), compared to the lowest percentage (0.331%) for the control plants. For the first consecutive season. The same table shows that the effect was significant when plants were sprayed with nano-proline and nano-glutathione, with a significant increase. The highest percentage was recorded in the interaction (400 mg L⁻¹ + 200 mg L⁻¹) at 0.582%, compared to the lowest percentage recorded in the control plants at 0.311% for the first consecutive season. The interaction between nano-proline and nano-ascorbic acid was also recorded, with the interaction plants (400 mg L⁻¹ + 100 mg L⁻¹) gave the highest percentage of phosphorus in leaves at 0.643%,

compared to the control plants, which gave the lowest percentage at 0.322% for the first season, respectively. The interaction between nano-glutathione and nano-ascorbic acid sprays also recorded significant differences in the percentage of phosphorus in the leaves of the treated plants. The interaction (200 mg L⁻¹ + 100 mg L⁻¹) recorded the highest percentage (0.662%), compared to the control treatment, which recorded the lowest percentage (0.321%). It is noted from the results in the same table that the triple interactions differed significantly in the percentage of phosphorus in the leaves. The interaction(400 mg L⁻¹ + 200 mg L⁻¹ + 100 mg L⁻¹) recorded the highest percentage (0.224%), compared to the lowest percentage (0.939%) for the control plants in the first season.

Table (3-6) The effect of spraying with proline, glutathione, nano ascorbic acid and their interaction on the percentage of phosphorus in the leaves (%) of the Ruby Gem variety of Strawberry irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹			
		0	100	200	Prolin x Ascorbic
0	0	0.224	0.362	0.381	0.322
	50	0.322	0.404	0.433	0.386
	100	0.389	0.271	0.478	0.379
200	0	0.321	0.350	0.239	0.303
	50	0.303	0.450	0.319	0.357
	100	0.435	0.304	0.570	0.436
400	0	0.419	0.353	0.331	0.368
	50	0.404	0.326	0.476	0.402
	100	0.454	0.537	0.939	0.643
Glutathion		0.363	0.373	0.463	
		Glutathion mg.L⁻¹			
Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L ⁻¹	0	0.311	0.346	0.431	0.363
	200	0.353	0.368	0.376	0.366
	400	0.425	0.406	0.582	0.471
		Ascorbic mg.L⁻¹			
Glutathion x Ascorbic		0	50	100	Ascorbic

Glutathion mg.L ⁻¹	0	0.321	0.355	0.317	0.331
	100	0.343	0.393	0.410	0.382
	200	0.426	0.371	0.662	0.486
L.S.D. 0.05					
L.S.D Glutathion=0.0929					
L.S.D Prolin=0.0929					
L.S.D Ascorbic =0.0929					
L.S.D Glutathion x Prolin =0.1608					
L.S.D Glutathion x Ascorbic =0.1608					
Ascorbic =0.1608					
n x Prolin x Ascorbic =0.2786					

3-7 Potassium percentage in leaves (%)

The results in Table (3-7) show that spraying nanoproline on the vegetative part of the studied strawberry significantly affected the percentage of potassium in the leaves for the first season, as the concentration (400 mg L⁻¹) gave the highest percentage, reaching (1.839%). Also, the concentration (200 mg L⁻¹) gave an increase in the percentage of potassium in the leaves, reaching (1.805%), compared to the control treatment, which gave the lowest percentage (1.061%). Spraying nanoglutathione at the concentration (200 mg L⁻¹) had a significant superiority, recording the highest percentage, reaching (1.637%), compared to the lowest percentage in the control plants, reaching (1.207%), for the first season, respectively. The treatment of spraying with nano ascorbic acid at a concentration of (100 mg L⁻¹) was superior, recording the highest percentage of (1.863%) compared to the lowest percentage of (1.186%) in the control plants during the first season, respectively. The dual interaction between nanoproline and nanoglutathione sprays recorded the highest percentage at the interaction (400 mg L⁻¹ + 200 mg L⁻¹), reaching 1.864%, while the concentration (400 mg L⁻¹ + 100 mg L⁻¹) recorded the highest percentage, reaching 1.860%, compared to the

lowest percentage for control plants, reaching 1.014% for the first season. The interaction between nanoproline and nanoascorbic acid sprays at different concentrations had a significant effect on the interaction plants (400 mg L⁻¹ + 100 mg L⁻¹), recording a percentage at different concentrations of 1.908%, compared to the lowest percentage for control plants, reaching 0.664% for the first season, respectively. The interaction between nano-glutathione and nano-ascorbic acid was also superior, with the highest percentage recorded at the interaction (100 mg L⁻¹ + 200 mg L⁻¹) reaching (1.922%), compared to the lowest percentage in the control plants, reaching (1.079%) for the first season. The same table indicates that the triple interactions resulting from spraying and adding the above materials caused significant differences in the percentage of potassium in the leaves, as the interaction (proline 400 mg L⁻¹ + glutathione 200 mg L⁻¹ + ascorbic acid 100 mg L⁻¹) nano recorded the highest percentage of (2.266%), while the interaction (proline nano 400 mg L⁻¹ + antioxidant glutathione nano 100 mg L⁻¹ + ascorbic acid nano 100 mg L⁻¹) recorded (2.262%) compared to the control treatment, which gave the lowest percentage of (0.035%) for the first season.

Table (3-7) The effect of spraying with proline, glutathione, nano ascorbic acid and their interaction on the percentage of potassium in the leaves (%) of the Ruby Gem variety of Strawberry irrigated with well water for both seasons 2024-2025.

Prolin mg.L ⁻¹	Ascorbic mg.L ⁻¹	Glutathion mg.L ⁻¹			
		0	100	200	Prolin x Ascorbic
0	0	0.035	0.807	1.151	0.664
	50	0.978	1.140	1.239	1.119
	100	1.310	1.145	2.031	1.496
200	0	1.717	1.231	1.238	1.396
	50	1.796	2.014	1.310	1.707
	100	1.889	0.272	2.029	1.397
400	0	1.179	1.972	1.398	1.516
	50	0.752	1.024	2.068	1.281
	100	1.197	2.262	2.266	1.908
Glutathion		1.207	1.319	1.637	
		Glutathion mg.L⁻¹			
Glutathion x Prolin		0	100	200	Prolin
Prolin mg.L ⁻¹	0	1.014	1.031	1.138	1.061
	200	1.801	1.771	1.843	1.805
	400	1.792	1.860	1.864	1.839
		Ascorbic mg.L⁻¹			
Glutathion x Ascorbic		0	50	100	Ascorbic
Glutathion mg.L ⁻¹	0	1.079	1.216	1.262	1.186
	100	1.582	1.728	1.657	1.656
	200	1.809	1.859	1.922	1.863
L.S.D. 0.05					
L.S.D Glutathion=0.1953					
L.S.D Prolin=0.1953					
L.S.D Ascorbic =0.1953					
L.S.D Glutathion x Prolin =0.3382					
L.S.D Glutathion x Ascorbic =0.3382					
Ascorbic x Prolin x Ascorbic =0.5858					

Disussion

Amino acids play an important role in improving vegetative growth characteristics in many vital activities occurring within plant tissues due to their influence on the formation and stimulation of enzyme systems, enzyme

cofactors, various purine and pyrimidine bases, and increasing the formation of nucleic acids DNA and RNA, whose activities are affected by their external environment (19)(16). The deterioration of plant growth characteristics at high concentrations of NaCl

may be due to the decrease in water potential in the growth medium and root system, which reduced the plant's ability to absorb water. This was also accompanied by a decrease in the absorption of nutrients from plant cells, leading to a decrease in the plant's fresh and dry weights (15). This result is consistent with the findings of (8) (2), who indicated improved growth and increased height of mung and sunflower plants after treatment with proline. The reason for the increased plant height may be due to the fact that proline is a source of nitrogen, as it contributes to protein synthesis, which plays an important role in supplying plants with the energy needed for growth and development, thus increasing plant height. The results are also consistent with those of (1), who found that spraying proline on the leaves of citrus rootstocks at a concentration of 100 mg.L⁻¹ increased the seedling height of five citrus rootstocks, and the results of (3), who reported that adding proline at concentrations of 50 and 75 mg.L⁻¹ increased the height of orange seedlings. In addition to the role of proline as a source of energy, the oxidation of a single molecule of it produces the reduced hydrogen receptor NADH, which provides energy for vital activities. Therefore, it has a role in the process of aerobic respiration, contributing to the provision of important energy in the absorption process in the roots (21). Plants possess defense mechanisms, such as increasing the cellular content of enzymatic and non-enzymatic antioxidants in response to salt stress (23). Externally added osmoprotectants are an important means of protecting plants from the harmful effects of oxidative stress induced by salinity (6). Several researchers have reported that proline, as an osmoprotectant, increases in concentration in plant tissues with increasing intensity and duration of exposure to salt stress

Conclusions

1-Nano-Proline Effect:

Spraying with nano-proline at 400 mg L⁻¹ significantly enhanced vegetative growth and

(13). The reason for the increase in vegetative characteristics may be due to the fact that glutathione consists of three amino acids: glutamate, glycine, and cysteine. The amino acid cysteine contains a sulfur bond, which is a sulfur donor that protects cells from free radicals. Glutathione also plays an important role in photosynthesis (17). Ascorbic acid has many roles. Physiologically, it acts as an enzyme coenzyme in the enzymatic reactions of carbohydrate and protein metabolism, in addition to its important role in respiration (24). (4) found that spraying Anna apple trees grafted onto MM106 stock with ascorbic acid at a concentration of 250 mg. 120 mg.L⁻¹ alone or with some nutrients led to an increase in the leaf area and length of new growths formed on trees during the two years of study, due to the role of ascorbic acid in encouraging the photosynthesis process through the presence of a strong relationship between leaf area and the increase in vegetative growth and the seedlings' content of ascorbic acid. (5) also found in his experiment on apple trees *Malus domestica* Borkh and two cultivars, the first of which were Anna cultivar trees and the second on Vistabella cultivar trees mounted on 106 MM stock, that spraying the leaves of both cultivars with ascorbic acid at a concentration of (120 mg.L⁻¹) alone or with some nutrients led to a significant increase in the nitrogen concentration in the leaves, the area of one leaf, the total leaf area of the trees, the diameter of the main stem, the length of the branches formed on the main stem of the trees of both cultivars and formed on them during the two years of study, and the concentration of sulfur, phosphorus, potassium, total chlorophyll, and total sugars in the leaves of Anna cultivar trees, the height of the trees, and the number of branches. Formed on the main stem of Vistabella trees.

chemical traits. For example, the number of leaves increased to 35.31 leaves per plant, leaf area reached 83.97 cm² per plant, and dry

weight of vegetative growth was 4.68 g, significantly higher than the control.

2. Nano-Ascorbic Acid Effect:

Foliar application of nano-ascorbic acid at 100 mg L⁻¹ improved all studied traits, including a leaf area of 80.97 cm² per plant and nitrogen content of 1.928%, indicating a strong physiological response compared to untreated plants.

3. Nano-Glutathione Effect:

Nano-glutathione at 200 mg L⁻¹ significantly increased vegetative and chemical indicators, such as 37.43 leaves per plant and 42.5% dry

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matter, demonstrating its antioxidant role in improving plant stress tolerance.

4. Combined Treatment Effect:

The combined application of nano-proline (400 mg L⁻¹), nano-glutathione (200 mg L⁻¹), and nano-ascorbic acid (100 mg L⁻¹) had a synergistic effect, maximizing growth and chemical traits. This treatment produced 54.77 leaves per plant, leaf area of 135.3 cm², and dry weight of 6.57 g, clearly surpassing all single treatments and the control, indicating the benefit of multi-factorial nano-supplementation.

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