

Effect of foliar application chitosan, nitrogen, and humic acid on the mineral content of young pomegranate trees. c.v. Salimi

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Abstract

The experiment was conducted in one of the deciduous fruit orchards (pomegranate field) at the Agricultural Research and Experiment Station of the College of Agriculture - Kirkuk University, located in the Al-Sayada area during the 2025 growing season in Kirkuk Governorate/ Iraq, from 15/4/2025 to 1/12/2025, on young pomegranate trees of the Salimi variety, aged (two years). To determine the improvement of the chemical properties of young pomegranate trees of the Salimi variety in their early stages of growth in the orchard, spraying the first factor, chitosan, at concentrations of (0, 100, 200) mg L⁻¹, the second factor, nitrogen, at concentrations of (4, 8) g L⁻¹, and humic acid at concentrations of (6, 3) g L⁻¹, in addition to a control treatment, (60) pomegranate trees with good vegetative growth and free from diseases and insects were selected. The experiment was carried out according to R.C.B.D (Randomized Complete Block Design) within factorial experiments with two factors and four replicates, and the treatments were randomly distributed within each replicate of the experimental unit. The results showed that spraying chitosan at a concentration of 200 mg L⁻¹ led to a significant improvement in most of the chemical properties represented by (the total chlorophyll content of the leaves, the nitrogen percentage in the leaves, the phosphorus percentage in the leaves, the potassium percentage in the leaves, the total sugars in the branches, and the protein in the leaves), which amount to (18.35 mg/100 g⁻¹ fresh weight, 0.85%, 0.13%, 2.74%, 7.58%, 5.30%) respectively. Spraying with nitrogen at a concentration of 8 g L⁻¹ led to a significant improvement in the trait (nitrogen content in leaves, phosphorus content in leaves and protein content in leaves), reaching (1.25%, 0.12% and 7.79%) respectively, while spraying with humic acid at a concentration of 6 g L⁻¹ led to a significant improvement in the trait (total chlorophyll content of leaves). The percentage of potassium in the leaves and the percentage of total sugars in the branches were (17.75 mg/100 g⁻¹ fresh weight, 2.53% and 7.24%) respectively.

Keywords: Chitosan, Nitrogen, Humic Acid , Salimi , Mineral Content

The Article is extracted from the master's thesis of the first Author

Introduction

The pomegranate (*Punica granatum* L.) is a fruit native to South Asia, and can now be grown in tropical and subtropical regions, producing about seven thousand metric tons annually. It is distinguished by its rich flavor and properties. Its functionality, which has made it widely accepted in various countries, as well as its health benefits, has industrial applications in food technology, cosmetics and pharmaceuticals.[1] The number of fruit-bearing pomegranate trees in Iraq is estimated at (6,495,705) million trees for the summer season of 2020. Pomegranate trees come in second place in terms of production compared to the rest of the other types of fruit in Iraq. Diyala Governorate ranked first in terms of the number of fruit-bearing trees, as it reached (3,439,563) million trees, and in second place it is followed by Salah al-Din Governorate, which reached (2,513,673) million trees, while Kirkuk Governorate comes in eleventh place with the number of trees (5,780) thousand trees [2]. Chitosan (CHT) is a natural biopolymer. Due to its unique properties and multiple biological activities, it is used in various fields, most notably biomedicine, agriculture, and the food industry. It is a biosafe, biodegradable, and tissue-compatible compound. It has been proven to activate plant growth and stimulate its defense response, thus enhancing its resistance to various pathogens, including bacteria, fungi, worms, parasites, and viruses [3]. Nitrogen is a major nutrient and one of the essential macronutrients that plants need in huge quantities. It comes second only to carbon and oxygen in the dry weight of plants and is an essential mineral element for plants [4]. It is the main component of protein, nucleic acids, phospholipids, chlorophyll, hormones, vitamins, and alkaloids. It participates in all

stages of plant growth and development. Nitrogen is responsible for the production of chlorophyll, which increases the rate of photosynthesis, gives plants their green color, and improves crop quality and yield. Furthermore, it enhances plant resistance to environmental stresses by promoting protein synthesis, activating enzymes necessary for stress resistance, and regulating osmotic pressure [5]. Humic acid (HA) is one of the most important types of organic fertilizers used in agriculture. It is a large-molecule substance formed primarily from plant and animal remains through microbial decomposition or transformation and a series of biochemical processes [6]. As an organic acid soluble in alkaline solutions, it is characterized by a complex structure and lacks a fixed molecular weight or formula [7]. It is a naturally occurring organic acid and one of the compounds of humic matter resulting from the decomposition of organic matter. Its composition includes carbon, hydrogen, nitrogen, and oxygen in varying proportions, resulting in the formation of compounds with varying molecular weights. Commercially, it is usually produced from Leonardite rock and from some materials that can be obtained from Leonardite mines containing up to 60% humic and fulvic acids [8]. Due to the importance of the pomegranate plant and its many benefits, our study aimed to improve its mineral properties by adding different concentrations of chitosan, nitrogen, and humic acid individually and finding the best combination of them in an overlapping manner.

Materials and working methods

The experiment was conducted in one of the deciduous fruit orchards (pomegranate field) at the Agricultural Research and Experiment Station of the College of Agriculture - Kirkuk University, located in the Al-Sayada area during the 2025 growing season, about (7.5 km southwest of Kirkuk), located at latitude (35.39) and longitude (44.34) Kirkuk, for the period from 15/4/2025 to 1/12/2025 on young pomegranate trees of the Salimi variety, aged (two years). To improve the chemical composition of young pomegranate trees of the Salimi variety in their early growth stages in the orchard, chitosan and mineral and organic fertilizers (nitrogen and humic acid) were sprayed at varying concentrations. Regular maintenance of the pomegranate trees, including weeding and weed removal, was carried out as needed. Using machinery and manual labor, the affected branches were pruned. (60) healthy, disease-free, and vegetatively sound pomegranate trees were selected for the experiment. These trees were trained on a single trunk using the central leader system and planted at (4 x 3.5 m) dimensions in a rectangular shape. Drip irrigation was used, and the trees were then sprayed with a fungicide after pruning. Insect control, including spider mites, was carried out using the insecticide (MARIO) manufactured by [Company Name - missing from original text] he company (Agrobest) applied a concentration of (1.8%) on (July 1, 2025) and (August 1, 2025) to all trees in the orchard. The study included two agents: chitosan, which was sprayed in three applications at concentrations of (0, 100, and 200) mg/L, with a time interval of 21 days until complete saturation, starting from (April 20, 2025). The second factor, nitrogen and humic acid, was sprayed with (0, 4 nitrogen, 8 nitrogen, 3 humic acid, and 6 humic acid) g L^{-1} to the experimental units starting on April 21, 2025. The

experiment was conducted according to R.C.B.D (Randomized Complete Block Design) as a factorial experiment with two factors and four replicates. The treatments were randomly distributed, and one tree was used for each experimental unit. Thus, the number of trees included in the experiment was 60 ($5 \times 3 \times 2 \times 2$), and the data were analyzed according to system [9], and the means were compared according to Duncan's Multiple Range test at the (0.05) level, as mentioned in [10].

characteristics studied:

Digestion of plant samples: Each sample of dried leaves was taken in the first week of July of the 2025 season, then ground in an electric grinder, and (0.2 g) of each sample was taken and digested by adding (5 ml) of concentrated sulfuric acid (3 ml) and perchloric acid. The digestion flask was heated gently until white vapors rose to obtain a clear, transparent solution. After that, the solution was filtered, and the nutrients in the digestion solution were estimated [11]. The work was done in the laboratories of the College of Agriculture - Kirkuk University.

1. Leaf total chlorophyll content (mg g^{-1} soft weight): Estimate the total chlorophyll content in pomegranate leaves according to the method [12].
2. Percentage of nitrogen in leaves (%): Nitrogen was estimated using the Micro-Kjeldahl apparatus and according to the method mentioned in [13].
3. The percentage of phosphorus in the leaves (%): It was estimated by the colorimetric method and reading the light absorption at a length of 410 nanometers using a spectrophotometer of type (UV-1100) as mentioned [14].

4. Percentage of potassium in the leaves (%): Potassium was estimated using a flame photometer microprocessor as described in [14].
5. Total sugars in branches (%): Total sugars were estimated according to the method [15].
6. Percentage of protein in leaves (%): The percentage of protein in leaves was estimated based on dry weight according to the method [13].

Results and discussion

1- Total chlorophyll content of leaves (mg/100g⁻¹ soft weight):

The results obtained and shown in Table (1) indicate that there is a significant increase in the total chlorophyll content of the leaves when sprayed with chitosan, as the treatment with 200 mg L⁻¹ gave the highest results for this trait, reaching 18.35 mg 100 g⁻¹ fresh

weight, while the control treatment gave a percentage of 13.67 mg 100 g⁻¹ fresh weight. Nitrogen and humic acid spraying also had an effect. The treatment with 6 g L⁻¹ humic acid resulted in the highest increase in leaf chlorophyll, reaching 17.75 mg/100 g⁻¹ fresh weight, while the control treatment resulted in the lowest total leaf chlorophyll content, at 14.38 mg/100 g⁻¹ fresh weight.

The results of the dual interaction between chitosan and nitrogen/humic acid spraying showed a significant effect on total leaf chlorophyll content. The interaction treatment of 200 mg/L⁻¹ chitosan with 6 g L⁻¹ humic acid resulted in the highest chlorophyll content, at 20.28 mg/100 g⁻¹ fresh weight, while the control treatment resulted in the lowest, at 10.97 mg/100 g⁻¹ fresh weight.

Table (1) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the total chlorophyll content of leaves (mg/100g⁻¹ fresh weight) in young pomegranate trees of the Salimi variety.

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	10.97e	12.95de	19.21ab	14.38bc
4 (g L ⁻¹) Nitrogen	14.16cde	15.94bcd	17.80abc	15.96b
8 (g L ⁻¹) Nitrogen	12.71de	15.76bcd	16.47abcd	14.98b
3 (g L ⁻¹) Humic Acid	15.02bcde	17.85abc	18.00abc	16.96ab
6 (g L ⁻¹) Humic Acid	15.48bcd	17.51abc	20.28a	17.75a
Chitosan effect	13.67c	16.00b	18.35a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%.

2- Nitrogen content in the leaves (%) :

Table (2) shows that spraying chitosan on young pomegranate trees of the Salimi variety led to a significant increase in the percentage of nitrogen in the leaves, as the treatment of spraying 200 mg L⁻¹ was superior by a percentage of 0.85%, while the treatment of spraying zero mg L⁻¹ gave a percentage of 0.44%. Similarly, with regard to spraying with mineral and organic fertilizers, there was a significant superiority in the percentage of nitrogen in the leaves, as the treatment of

spraying 8 g L⁻¹ of nitrogen gave the highest increase of 1.25%, while the control treatment gave the lowest results of 0.18%.

As for the dual interaction between spraying chitosan, nitrogen, and humic acid, the interaction treatment between 100 mg/L⁻¹ of chitosan with 8 g L⁻¹ of nitrogen was superior, giving the greatest increase in the percentage of nitrogen in the leaves, reaching 1.39%, while the control treatment gave the lowest percentage of nitrogen in the leaves, reaching 0.16%.

Table (2) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the percentage of nitrogen in the leaves (%) in young pomegranate trees of the Salimi variety.

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect of nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	0.16e	0.18e	0.19e	0.18d
4 (g L ⁻¹) Nitrogen	0.18e	0.19e	0.99bcd	0.45c
8 (g L ⁻¹) Nitrogen	1.23ab	1.39a	1.12abc	1.25a
3 (g L ⁻¹) Humic Acid	0.33e	0.73d	0.82cd	0.63bc
6 (g L ⁻¹) Humic Acid	0.32e	0.79cd	1.12abc	0.74b
Chitosan effect	0.44c	0.66b	0.85a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%

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3- Phosphorus content in the leaves:(%)

Table (3) shows that spraying chitosan on young pomegranate trees of the Salimi variety

resulted in a significant difference in leaf phosphorus content between the experimental treatments. The treatment with 200 mg/L⁻¹ showed the highest increase at 0.13%, while

the treatment with 0 mg/L⁻¹ showed an increase of 0.04%. The dual interaction between chitosan spraying, nitrogen, and humic acid also had a superiority. The interaction treatment between 200 mg L⁻¹ of

chitosan with 6 g L⁻¹ of humic acid gave the greatest increase in phosphorus percentage, reaching 0.18%, while the interaction treatment between zero mg L⁻¹ of chitosan and the control treatment gave 0.03

Table (3) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the percentage of phosphorus in the leaves (%) in young pomegranate trees of the Salimi variety

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect of nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	0.03de	0.05cde	0.07bcde	0.05bc
4 (g L ⁻¹) Nitrogen	0.02e	0.06cde	0.12abcd	0.07b
8 (g L ⁻¹) Nitrogen	0.07bcde	0.15ab	0.13abc	0.12a
3 (g L ⁻¹) Humic Acid	0.02e	0.05cde	0.16ab	0.07b
6 (g L ⁻¹) Humic Acid	0.04cde	0.07bcde	0.18a	0.10ab
Chitosan effect	0.04c	0.08b	0.13a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%

4- Potassium content in the leaves (%):

Table (4) shows, through statistical analysis, a significant increase in potassium levels in the leaves of young pomegranate trees (Category Salimi) when sprayed with chitosan. The highest increase, 2.74%, was observed when spraying with 200 mg/L⁻¹, while the control treatment resulted in the lowest potassium level at 1.81%.

The results also showed a significant effect of mineral and organic fertilizers on potassium levels. Spraying with 6 g L⁻¹ of humic acid resulted in a significant increase of 2.53%, while the control treatment resulted in an increase of 1.97%. Regarding the dual interaction between the effect of chitosan and the effect of nitrogen and humic acid, a significant increase in the percentage of potassium in the leaves was found, as the

interaction treatment between 200 mg L⁻¹ of chitosan with 6 g L⁻¹ of humic acid gave the greatest increase in the percentage of potassium, reaching 2.93%, while the

interaction treatment between zero mg L⁻¹ of chitosan with zero g L⁻¹ of the control treatment and 4 g L⁻¹ of nitrogen gave the lowest results, reaching 1.69%.

Table (4) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the percentage of potassium in the leaves (%) in young pomegranate trees of the Salimi variety.

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect on nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	1.69c	1.73c	2.48ab	1.97c
4 (g L ⁻¹) Nitrogen	1.69c	1.73c	2.85a	2.09bc
8 (g L ⁻¹) Nitrogen	1.77c	1.82c	2.55ab	2.05bc
3 (g L ⁻¹) Humic Acid	1.77c	2.53ab	2.90a	2.40ab
6 (g L ⁻¹) Humic Acid	2.11bc	2.55ab	2.93a	2.53a
Chitosan effect	1.81c	2.08b	2.74a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%

5- Percentage of total sugars in the branches (%):

Table (5) shows that the percentage of total sugars in the branches increased significantly with increasing levels of chitosan spraying on young pomegranate trees of the Salimi variety. The treatment with 200 mg/L⁻¹ showed the highest increase, reaching 7.58% in the branches, while the control treatment yielded the lowest increase at 6.55%.

Regarding the application of mineral and organic fertilizers, a significant increase in the percentage of total sugars in the branches was observed. The treatment with 6 g L⁻¹ of humic acid resulted in the highest increase, reaching 7.24%, while the control treatment yielded 6.83%.

The results in the same table showed significant differences between the binary interactions of chitosan, nitrogen, and humic acid spray concentrations. The interaction treatment of 200 mg L⁻¹ of chitosan with 6 g L⁻¹ of humic acid resulted in an increase in the

percentage of total sugars in the branches, reaching 7.91%, while the interaction treatment of zero mg L⁻¹ of chitosan combined

with a treatment of zero mg L⁻¹ of mineral and organic fertilizers resulted in 6.15%.

Table (5) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the percentage of total sugars in the branches (%) in young pomegranate trees of the Salimi variety.

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	6.15c	6.86b	7.48ab	6.83b
4 (g L ⁻¹) Nitrogen	6.79bc	6.95b	7.84a	7.19ab
8 (g L ⁻¹) Nitrogen	6.69bc	7.31ab	6.96b	6.99b
3 (g L ⁻¹) Humic Acid	6.83b	7.08b	7.69ab	7.20ab
6 (g L ⁻¹) Humic Acid	6.29c	7.52ab	7.91a	7.24a
Chitosan effect	6.55b	7.14b	7.58a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%

6- Percentage of protein in leaves (%):

The results shown in Table (6) indicate that the protein percentage in leaves increased significantly when chitosan was sprayed on young pomegranate trees of the Salimi variety. The highest increase, 5.30%, was observed with a 200 mg L⁻¹ spray, while a 0 mg L⁻¹ spray resulted in a 2.77% increase. The same table also shows that spraying with mineral and organic fertilizers significantly increased

the protein percentage. Spraying 8 g L⁻¹ of humic acid resulted in the highest increase in leaf protein, reaching 7.79%, while the control treatment resulted in a 1.10% increase. As for the effect of the dual interaction between spraying chitosan, nitrogen and humic acid, the interaction treatment between 100 mg/L of chitosan with 8 g L⁻¹ of nitrogen was superior in giving the greatest increase in the percentage of protein in the leaves, reaching 8.69%, while the control interaction treatment of chitosan interacting with the control treatment of nitrogen and humic acid reached 1.00%.

Table (6) Effect of spraying chitosan and mineral and organic fertilizers (nitrogen and humic acid) and the interaction between them on the percentage of protein in the leaves (%) in young pomegranate trees of the Salimi variety.

chitosan Nitrogen and humic acid	0)mg L ⁻¹ (	100)mg L ⁻¹ (	200)mg L ⁻¹ (	Effect nitrogen and humic acid
0 (g L ⁻¹) Comparison treatment	1.00de	1.12d	1.19d	1.10d
4 (g L ⁻¹) Nitrogen	1.12d	1.19d	6.19b	2.83c
8 (g L ⁻¹) Nitrogen	7.69b	8.69a	7.00b	7.79a
3 (g L ⁻¹) Humic Acid	2.06d	4.56c	5.12c	3.91bc
6 (g L ⁻¹) Humic Acid	2.00d	4.94c	7.00b	4.65b
Chitosan effect	2.77c	4.10b	5.30a	

The averages that have the same letters for each factor do not differ significantly from each other according to Duncan's multiple range test at a probability level of 5%.

Discussion

The results of the study, as shown in Tables (1, 2, 3, 4, 5, and 6), indicate that chitosan spraying significantly improved the following characteristics: total chlorophyll, nitrogen content in leaves, phosphorus content in leaves, potassium content in leaves, total sugar content in branches, and protein content in leaves. This may be attributed to chitosan, a biomolecule with important properties for enhancing plant development and growth [16]. Its effect on plant growth may stem from increasing nutrient and water absorption by modulating osmotic pressure, reducing the accumulation of harmful free radicals by

increasing various enzymes and antioxidant activity [17], and consequently increasing the content activity of nitrogen-assimilation enzymes such as (nitrate reductase, glutamine synthase and protease), which improve nitrogen transport in functionally active leaves and improve photosynthesis, which is reflected in the overall growth and development of the plant [18]. Chitosan also acts as a plant biostimulant, helping to activate cell division and elongation and increase the absorption of nutrients such as nitrogen, phosphorus and potassium, thus increasing the mineral content as shown in Tables (2, 3 and 4), strengthening vegetative growth and reducing stress, which leads to an increase in chlorophyll and an improvement in the overall characteristics of the plant as shown in Table (1), and activating enzymes and physiological processes within

the plant, which is reflected positively in the mineral content of the plant. Chitosan also has a positive effect on the chlorophyll content and the total nitrogen and phosphorus content [19]. These results are consistent with the findings of [20] in their study on the growth and fruiting of pomegranate trees of the Salimi variety. This is consistent with what [19] concluded when they studied pomegranate trees. The results shown in Tables 2, 3, and 6 indicate a significant advantage of nitrogen treatments in the mineral growth characteristics (leaf nitrogen percentage, leaf phosphorus percentage, and leaf protein percentage). This can be attributed to the essential role of nitrogen in the formation of amino acids, proteins, and chlorophyll, leading to increased efficiency of photosynthesis and stimulation of vegetative growth. Increased nitrogen levels contribute to improved leaf nitrogen content (2) by increasing cell division and elongation, which in turn leads to increased vegetative growth and a higher mineral content. Furthermore, nitrogen activates enzymes and physiological processes within the plant, further contributing to increased leaf mineral content. The increased leaf nitrogen may also be due to the plant's improved ability to absorb nitrogen from the soil, thus improving the plant's nutritional status and positively impacting leaf mineral content [21 and 22]. These results are consistent with those obtained in [23]. Pear trees and it was consistent with what was stated [24] on pomegranate trees, the Wonderful variety. The same applies to humic acid, as it exhibits a significant advantage in chemical properties such as total chlorophyll, nitrogen and potassium content in leaves, total sugar content in branches, and protein content in leaves. This is because humic acid contains potassium, which plays a crucial role in regulating stomata opening and closing, facilitating the entry of carbon dioxide essential for photosynthesis, and consequently

increasing chlorophyll and metabolic processes, leading to their accumulation in plant tissues [25]. Furthermore, humic acid activates various processes. The physiological processes within the plant increase the nutrient content of the leaves. Since humic acid contains nutrients, it increases their absorption by the trees and their accumulation in the leaves [26 and 27]. These results are consistent with those of [28] in their study of pomegranate trees, and agree with the findings of [29] in their study of pomegranate trees.

Conclusions

We conclude from the experiment that spraying with chitosan, nitrogen, and humic acid had an effective role in most of the mineral content characteristics of young pomegranate trees of the Salimi variety. Spraying with chitosan at a concentration of 200 mg L^{-1} , spraying with nitrogen at a concentration of 8 g L^{-1} , and spraying with humic acid at a concentration of 6 g L^{-1} had a significant effect on most of the characteristics studied during the study.

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