International Journal of Plant & Soil Science

Volume 38, Issue 5, Page 43-53, 2026; Article no.IJPSS.157190
ISSN: 2320-7035

Effects of Atonik Growth Regulator and Chemical Fertilization on Vegetative Growth and Chemical Composition of Lemon (*Citrus limon* L.) Seedlings

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i56063

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/157190

Original Research Article

Received: 05/03/2026
Published: 27/04/2026

Abstract

Background: An important indication of agricultural development is foliar (non-root) nutrition. Research and experiments have shown that it is possible to provide plants with different nutrients by spraying them with solutions of these elements.

Aim: The purpose of this study is to ascertain the impact of chemical fertilizer and tonic acid spraying, as well as the interaction between the two, on the chemical content and vegetative growth characteristics of lemon seedlings.

Methods: the study was conducted during 2023-2024 season to study a tonic acid and chemical fertilizer effect on lemon seedling vegetative growth and chemical contents (nitrogen, potassium, chlorophyl and phosphorus). A factorial experiment was arranged using three levels of Atonic (0, 5, and 10 mg/ L^{-1}) and three levels of fertilizer (0, 0.7, and 1.4 g $plant^{-1}$).

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Cite as: *Saadoune, S. Y., & Mohammed, M. J. (2026). Effects of Atonik Growth Regulator and Chemical Fertilization on Vegetative Growth and Chemical Composition of Lemon (Citrus limon L.) Seedlings. International Journal of Plant & Soil Science, 38(5), 43–53. https://doi.org/10.9734/ijpss/2026/v38i56063*

Results: The results showed that chemical fertilization significantly improved vegetative growth, particularly plant height, while Atonic had a moderate effect on growth traits. Both treatments significantly enhanced chlorophyll and nutrient contents (N, P, and K). The interaction between Atonic and fertilization further improved plant performance, with the highest values recorded under combined treatments.
Conclusion: The study showed that combined effect of application of tonic acid and chemical fertilizer enhance significantly the vegetative growth and chemical contents of lemon seedling.

Keywords: Foliar; lemon seedling; chemical fertilizer; tonic acid.

1. Introduction

Citrus trees are evergreen fruit trees that are members of the Rutaceae family, which consists of various genera, including Fortunella, Poncirus, and Citrus. Due to their ability to adapt to a variety of environmental circumstances, species in the genus Citrus are found all over the world (Hazarika, 2023). Tropical locations between 40°N and 40°S latitude, as well as subtropical (arid) and subtropical climates, are common places to grow citrus. Orange, mandarin, grapefruit, and sour are the four groups that make up the genus Citrus. There are multiple species in each group, each of which has a wide range of cultivars and variations (Hamid et al., 2024). Citrus limon Burmann, also known as the sour lemon, is a species of citrus in the genus Citrus. Its polyembryonic seeds, which produce scarlet new growths, are native to India (Kalal et al., 2023).

Plant growth regulators are non-nutritive organic substances, occurring naturally or synthetically, that have a high capacity to influence plant growth even at very low concentrations. These substances stimulate various plant physiological processes (El-Saadony et al., 2025). The growth regulator Atonic is one such agent; it is an aromatic nitrogen compound. Its use increases plant vitality without causing any malformation or toxicity. In a study conducted by El-wahed et al., (2005) on chamomile plants, spraying them with Atonic at concentrations of 60, 120 ml, and 0 ml/L showed a significant increase in plant height and number of branches.

Foliar (non-root) nutrition is a crucial indicator in agricultural development. Experiments and research have demonstrated the possibility of supplying plants with various nutrients by spraying them with solutions of these elements, which are then absorbed by the leaves and other plant parts above the soil surface, such as stems and fruits (Fazeli-Nasab et al., 2025). This is particularly relevant for certain elements that become fixed when added to soils with a high pH (alkaline soils), such as those found in Iraqi soils, thus rendering them unavailable for absorption by the plant (Hussain et al., 2026).

Fertilization of various types—chemical, organic, and biological—is among the most important agricultural practices applied to citrus seedlings to improve their nutritional status, which positively impacts their vegetative growth (Wan et al., 2021). Nitrogen is one of the essential elements required by plants, as it promotes vegetative growth and strengthens the root system. It is a primary component of cell protoplasm after water, constituting 2-4% of the plant's dry matter. The importance of nitrogen is also significant (Bhatla & Kathpalia, 2023). The importance of phosphorus to plants stems from its role in the synthesis of most vital plant components, such as proteins, enzymes, nucleic acids (DNA and RNA), amino acids, and plant hormones. Phosphorus is essential for several vital plant processes, including photosynthesis, carbohydrate synthesis and breakdown, energy transport within the plant, and cell division. It is also a component of nucleic acids, energy-carrying compounds, and some enzymes. Furthermore, it accelerates early root formation, growth, and spread in the soil, as well as promoting flowering in trees. Phosphorus is second only to nitrogen in terms of the amount of nutrient required by plants (Khan et al., 2023). This study aims to determine the effect of spraying with tonic acid and adding chemical fertilizer and the interaction between them on the characteristics of vegetative growth and the chemical content of lemon seedlings

2. Methodology

2.1 Experimental Site and Soil Sample Preparation

The experiment was conducted in the wooden greenhouse of the College of Agriculture, Samarra University, during the growing season of October 2023 - May 2024. Fifty-four two-year-old lemon seedlings, planted in 5 kg plastic containers, were used. The seedlings were selected for their uniform size whenever possible. These seedlings were then transplanted into larger 15 kg plastic containers filled with a nutrient-rich soil mix. Typical agricultural practices were followed, including irrigation, weed control with herbicides at the recommended rates, and insect and disease control. Soil samples were collected for chemical and physical analysis and analyzed at the Department of Soil Science and Water Resources, College of Agriculture, Tikrit University (Table 1).

- Soil analysis was conducted in the Department of Soil Science and Water Resources, College of Agriculture, Tikrit University.

Table 1. Some chemical and physical properties of the study soil

Properties		Value
pH		7.55
EC		70MS
Organic matter		1.12g/Kg^{-1}
Soil component	Sand	37%
	Silver	32%
	Clay	31%
Texture	sandy clay mixture.	
Nitrogen		0.14%
Phosphorous		0.9%
potassium		1.3%

The study included the following treatments:

1- Atonic spray treatment: Atonic is a growth regulator, specifically an aromatic nitrogen compound. The treatments were as follows:

1- Spraying with water only (control treatment), denoted as T0.
2- Spraying with atonic acid at a concentration of 5 mg/L, denoted as T1.
3- Spraying with atonic acid at a concentration of 10 mg/L, denoted as T2. Three sprayings were carried out on April 15, 2023, with the second and third sprays spaced 21 days apart.

Chemical fertilizer application treatment: The treatments were as follows:

1- Spraying with water only (control treatment), denoted as D0.
1- 2. Chemical fertilizer application at a level of 0.7 g. Plant -1, designated D1
2- Add chemical fertilizer at a level of 1.4 g. Plant -1, designated D2

2.2 Vegetative Growth Characteristics

2.2.1 Average Increase in Plant Height (cm)

Prior to treatment in March of the 2023 season, the plant height above the grafting point was measured with a measuring tape. At the conclusion of the growing season in April 2024, the plant length was measured, and the following formula was used to determine the rate of increase:

Increase in Plant Height = (Plant Height at the End of the Experiment - Plant Height at the Beginning of the Experiment) (Kamireddy et al., 2023).

2.2.2 Average Increase in Stem Diameter (mm)

A Vernier caliper was used to measure the stem diameter five centimeters above the grafting point prior to treatment. The following formula was used to determine the rate of increase in stem diameter: Stem Diameter Increase = (Stem Diameter at the End of the Experiment - Stem Diameter at the Beginning of the Experiment).

The following formula was used to determine the rate of increase in the number of leaves per seedling (branch * seedling - 1): New leaves are equal to the difference between the number of leaves at the start and the number of leaves at the end of the experiment (Opsahl & Benner, 1999).

2.3 Chemical Content

2.3.1 Nitrogen Content of Leaves (%)

Every experimental unit had its own sample taken, dried, and ground. Concentrated sulfuric and perchloric acids were used to digest a 0.2 g sample of the powdered sample. Upadhyay and Sahu (2012) outline the procedure that was subsequently followed while using the Microkjeldahl apparatus.

2.3.2 Estimation of Potassium Content in Leaves (%)

Using a flame photometer, type 378 Elicocl, the amount of potassium in leaves was calculated using the procedure outlined in Bhargava and Raghupathi (1999). Leaf Phosphorus Content (%) was calculated using colorimetric analysis and a 100 1v lab EMC spectrophotometer to measure light absorption at a wavelength of 410 nm, following the procedure outlined in Bhargava and Raghupathi (1999).

2.4 Statistical Analysis

In order to determine the impact of different factors (T, Dand C * U) on the study parameters, the Statistical Packages for the Social Sciences -SPSS (SPSS, 2019) program was utilized (factorial experiment: 3 x 3). When comparing means in this study, the Least Significant Difference (LSD) test was utilized.

3. Results

3.1 Plant Height

The results in table in (2) showed significant effect of spraying with Atonic acid and chemical fertilizer on plant height with LSD value (0.05). According to chemical fertilizer, the results showed increase in mean of plant height with chemical fertilizer increasing in which the highest plant height was recorded in D3 treatment (1.806cm), followed by D2(1.239cm) then D1 with (1.224 cm). Also for atonic acid, increase of acid concentration investigate significant increasing in plant height in which T3 showed high mean of plant height (1.661cm) followed by T2(1.587cm) and the lowest mean recorded in T1(1.020 cm).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect. The treatment(T2*D3) scored highest level of plant height (1.914cm), followed by (T3*D3) with(1.877cm), while the lowest level was recorded in (T1*D1) treatment. These results means presence of synergistic effect between two treatments which lead to best results on plants than using each one of them alone.

Table 2. Effect of T, D and interaction in plant high (cm)

D	**T**			**Mean**
	T: 1	**T: 2**	**T: 3**	
D: 1	0.584	1.363	1.724	1.224
D: 2	0.850	1.484	1.382	1.239
D: 3	1.627	1.914	1.877	1.806
Mean	1.020	1.587	1.661	---
L.S.D. * ($P \leq 0.05$)	C: 0.0202 * , U: 0.0202 * , C*U: 0.035 * .			

3.2 Stem Diameter

The results in table in (3) showed significant effect of spraying with Atonic acid and chemical fertilizer on Lemon Stem diameters with LSD value (0.05). According to chemical fertilizer, the results showed increase in mean of plant Stem diameters with chemical fertilizer increasing in which the highest plant Stem diameters was recorded in D3 treatment (223.08mm), followed by D2(179.67mm) then D1 with (152.85mm). Also, for atonic acid, increase of acid concentration investigate significant increasing in plant Stem diameters in which T3 showed high mean of plant Stem diameters (195.79mm) followed by T2(186.02mm) and the lowest mean recorded in T1(173.79mm).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on plant Stem diameters. The treatment(T3*D3) scored highest level of plant height (246.77mm), followed by (T2*D3) with(232.33mm), while the lowest level was recorded in (T2*D1) treatment(140.43mm). These results means presence of synergistic effect between two treatments which lead to best results on plants than using each one of them alone.

Table 3. Effect of T, D and interaction in stem diameter

D	**T**			
	T: 1	**T: 2**	**T: 3**	**Mean**
D: 1	147.40	140.43	170.73	152.85
D: 2	183.83	185.30	169.87	179.67
D: 3	190.13	232.33	246.77	223.08
Mean	173.79	186.02	195.79	---
L.S.D. * (P≤0.05)	C: 12.48 *, U: 12.48 *, C*U: 21.62 * .			

3.3 Chlorophyl

The results in table in (4) showed significant effect of spraying with Atonic acid and chemical fertilizer on content of chlorophyl with LSD value (0.05). According to chemical fertilizer, the results showed increase in mean of plant chlorophyl content with chemical fertilizer increasing in which the highest plant chlorophyl content was recorded in D3 treatment (45.31%), followed by D1(44.97%) then D2 with (41.64%). Also, for atonic acid, increase of acid concentration investigate significant increasing in plant chlorophyl content in which T3 showed high mean of plant chlorophyl content (48.57%) followed by T2(45.91%) and the lowest mean recorded in T1(37.44%).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on plant chlorophyl content. The treatment(T3*D1) scored highest level of plant chlorophyl content (51.37%), followed by (T3*D3) with (49.56%), while the lowest level was recorded in (T1*D1) treatment(34.67%). These results mean presence of synergistic effects between two treatments which lead to best results on plants than using each one of them alone

Table 4. Effect of T, D and interaction in CHO

D	**T**			**Mean**
	T: 1	**T: 2**	**T: 3**	
D: 1	34.67	48.87	51.37	44.97
D: 2	37.67	42.50	44.76	41.64
D: 3	40.00	46.36	49.56	45.31
Mean	37.44	45.91	48.57	---
L.S.D. * (P≤0.05)	C: 0.385 *, U: 0.385 * , C*U: 0.667 * .			

3.4 Nitrogen

The results in table in (5) showed significant effect of spraying with Atonic acid and chemical fertilizer on Lemon nitrogen content with LSD value (0.05). According to chemical fertilizer, the highest Lemon nitrogen content was recorded in D2 treatment (0.762%), followed by D1(0.300%) then D3 with (0.267%). While for atonic acid, increase of acid concentration investigate significant increasing in Lemon nitrogen content in which

T3 showed high mean of Lemon nitrogen content (0.740%) followed by T2(0.304%) and the lowest mean recorded in T1(0.285%).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on Lemon nitrogen content. The treatment(T3*D2) scored highest level of Lemon nitrogen content (1.392%), followed by (T3*D1) with (0.602%), while the lowest level was recorded in (T1*D1) treatment(0.119%). These results mean presence of synergistic effects between two treatments which lead to best results on plants than using each one of them alone.

Table 5. Effect of T, D and interaction in N %

D	T			Mean
	T: 1	T: 2	T: 3	
D: 1	0.119	0.179	0.602	0.300
D: 2	0.453	0.440	1.392	0.762
D: 3	0.281	0.292	0.226	0.267
Mean	0.285	0.304	0.740	---
L.S.D. * ($P \leq 0.05$)	C: 0.572 NS, U: 0.572 NS , C*U: 0.990 * .			

3.5 Phosphorus

The results in table in (6) showed significant effect of spraying with Atonic acid and chemical fertilizer on Lemon phosphorus content with LSD value (0.05). According to chemical fertilizer, the highest Lemon phosphorus content was recorded in D3 treatment (1.183%), followed by D1(0.958%) then D2 with (0.893%). While for atonic acid, decrease of acid concentration investigate significant increase in Lemon phosphorus content in which T1 showed high mean of Lemon phosphorus content (1.245%) followed by T2(0.940%) and the lowest mean recorded in T3(0.850%).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on Lemon phosphorus content. The treatment(T1*D3) scored highest level of Lemon phosphorus content (1.690%), followed by (T2*D3) with (1.110%), while the lowest level was recorded in (T3*D3) treatment(0.750%). These results mean presence of synergistic effects between two treatments which lead to best results on plants than using each one of them alone.

Table 6. Effect of T, D and interaction in P %

D	T			Mean
	T: 1	T: 2	T: 3	
D: 1	1.016	0.950	0.910	0.958
D: 2	1.030	0.760	0.890	0.893
D: 3	1.690	1.110	0.750	1.183
Mean	1.245	0.940	0.850	---
L.S.D. * ($P \leq 0.05$)	C: 0.0106 *, U: 0.0106 * , C*U: 0.0184 * .			

3.6 Potassium

The results in table in (7) showed significant effect of spraying with Atonic acid and chemical fertilizer on Lemon Potassium content with LSD value (0.05). According to chemical fertilizer, the highest Lemon Potassium content was recorded in D2 treatment (5.75%), followed by D1(5.18%) then D2 with (3.89%). While for atonic acid, decrease of acid concentration investigate significant increase in Lemon Potassium content in which T1 showed high mean of Lemon Potassium content (5.41%) followed by T2(4.68%) and the lowest mean recorded in T3(4.41%).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on Lemon Potassium content. The treatment(T3*D1) scored highest level of Lemon Potassium content (6.71%), followed by (T1*D2) with (6.34%), while the lowest level was recorded in (T2*D1) treatment(3.38%). These results mean presence of synergistic effects between two treatments which lead to best results on plants than using each one of them alone.

Table 7. Effect of T, D and interaction in K %

D	T			Mean
	T: 1	T: 2	T: 3	
D: 1	5.43	3.38	6.71	5.18
D: 2	6.34	5.41	5.52	5.75
D: 3	5.41	5.26	0.995	3.89
Mean	5.83	4.68	4.41	---
L.S.D. * (P≤0.05)	C: 0.114 *, U: 0.114 *, C*U: 0.197 * .			

3.7 Number of Branches

The results in table in (8) showed significant effect of spraying with Atonic acid and chemical fertilizer on Number of branches with LSD value (0.05). According to chemical fertilizer, the highest Number of branches was recorded in D2 and D3 treatment (5.00), followed by D1(3.89) then D2 with (3.89%). While for atonic acid, significant increase in Number of branches in which T1 and T3 (4.67) and the lowest mean recorded in T2(4.56%).

The interaction between atonic acid(T) and chemical fertilizer (D) showed significant effect on Number of branches. The treatment(T2*D3) scored highest level of Number of branches (7.00), followed by (T3*D2) with (6.67), while the lowest level was recorded in (T2*D1) treatment (2.33). These results mean presence of synergistic effects between two treatments which lead to best results on plants than using each one of them alone

Table 8. Effect of T, D and interaction in No of branch

D	T			Mean
	T: 1	T: 2	T: 3	
D: 1	5.33	2.33	4.00	3.89
D: 2	4.00	4.33	6.67	5.00
D: 3	4.67	7.00	3.33	5.00
Mean	4.67	4.56	4.67	---
L.S.D. * (P≤0.05)	C: 1.361 NS, U: 1.361 NS , C*U: 2.357 * .			

4. Discussion

The current study investigate the positive effect of sparing with tonic acid and chemical fertilizer on lemon seedling vegetative growth and chemical content such as nitrogen, potassium, carbohydrates, phosphorus and chlorophyl. These effect were more pronounced under combined treatment.

The role of plant growth regulators in promoting cell division and elongation is responsible for the improvement in vegetative growth features, especially plant height and stem diameter (Prisa et al., 2026). Recent research conducted by Domingues Neto et al., (2024) revealed similar results, citrus seedlings' growth and physiological performance were greatly enhanced by plant growth regulators.

In an experiment conducted by Orbovic et al., (2000) on rhizome lemons, applying urea either as a foliar spray or as a soil infusion, a concentration of 300 kg N/ha resulted in the highest increase in total dry weight and stem dry weight. Foliar treatment with 1.5% urea was also effective. The highest increase in stem diameter, root dry weight, and chlorophyll content, as well as the highest increase in stem diameter, number of leaves, and leaf area, was observed.

Kaur et al. (2024) noted that fertilizing seedlings of some citrus rootstocks (Rangpur, Cleopatra, mandarin, and lime) with three levels of phosphorus (20, 40, and 80 mg/kg^{-1} soil) improved branch and root growth. Al-Karaki (2013), in his experiment studying the fertilization of seedlings of sour orange (Citrus aurantium) by adding phosphorus at three levels (15, 45, and 90 mg P/kg^{-1} soil), concluded that the 45 mg/kg^{-1} fertilizer treatment improved the studied vegetative growth characteristics (stem height and diameter, and leaf area) compared to the other treatments, especially the control treatment.

Physiological parameters can change when more nutrients (N, P, and K) are available (Bleda et al., 2011). Both orange and lemon seedlings treated with soil conditioner and TF showed an increase in chlorophyll a, b, and total chlorophyll levels, as well as a higher stomatal conductance. However, it's important to note that modifications can vary between genotypes. Findings in Nemec and Vu (1990) were consistent with this idea, showing that citrus seedlings exhibited enhanced photosynthetic activity in response to elevated P concentrations. According to Bloomfield et al. (2014), phosphorus availability is commonly limited in tropical and subtropical soils, and seedlings grown in these regions generally show slower rates of photosynthesis than plants grown in other climates. Changes in chlorophyll content and stomatal conductance have been seen, and this may be due to an increase in nutrients that are available to plants.

Previous research has shown that enhanced nutrition availability boosts photosynthetic efficiency, which is supported by the considerable increase in chlorophyll content found in this study. Chlorophyll and the proteins involved in photosynthesis rely on nitrogen, which is an essential element (Xu et al., 2025). Recent research has shown that citrus seedlings' chlorophyll content and photosynthetic performance are much improved when given nitrate. This lends credence to the present results showing that fertilized plants had greater amounts of chlorophyll (Khan et al., 2025).

Foliar applications of salicylic acid enhanced olive trees' vegetative development traits, according to Abdel-Razek et al. (2013). Foliar treatment of salicylic acid to lemon seedlings significantly increased all vegetative growth parameters, including plant height, leaf number, and leaf area (Al-Abbasi et al., 2016). Zhu et al., (2021) found that tonic acid, when applied topically to citrus seedlings, significantly altered root traits.

The increase of nitrogen content in lemon plant treated with different treatments can explained by increase uptake of nutrients and process of assimilationNitrogen, more than any other element, is important for plant growth and development (2020). Nitrogen makes up 1% to 5% of all plant dry matter and is present in many plant hormones, proteins, chlorophyll (Chl), nucleic acids, amino acids, coenzymes, and secondary metabolites. Consequently, N is essential for several plant metabolic processes, including chloroplast production and photosynthesis (Huang et al., 2021). Xiong et al. (2021) found that citrus trees' spring shoot leaves underwent changes to their nitrogen partitioning when fertilized with the right quantity of nitrogen.

Fertilization increased phosphorus and potassium in this study. Meng et al. (2021) found that P deprivation reduces food absorption and photosynthetic performance, causing ROS generation and inhibiting C. grandis growth. For plant growth, phosphorus (P) is essential. It plays an important role in the metabolism of nitrogen compounds, carbohydrates, fats, and the transport of nucleic acids and proteins across the plasma membrane as well as in the synthesis of ATP and some secondary chemicals (Khare et al., 2025).

The rise in potassium levels seen in this study is also in line with what we already know about how potassium controls enzyme activity, stomatal activity, and the movement of carbohydrates. Potassium makes plants use water more efficiently and helps photosynthesis, which in turn helps plants grow and produce more (Sardans & Peñuelas, 2021).

5. Conclusion

The study concluded that combined effect of application of tonic acid and chemical fertilizer enhance significantly the vegetative growth and chemical contents of lemon seedling. The Optimal interaction showed best performance which improved metabolic activity and nutrient uptake and photosynthesis.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The peer review history for this paper can be accessed here:
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