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Effect of application of different fertilizers on shortening time of Sour orange (*Citrus aurantium*) seedlings to attain optimal budding stage

Husameldin Mahmoud[1*], Mayada Mohammed[2], Saleh Alturki[3], Abda Emam[4], Samia Yagoub[5]
[1]Department Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Kingdom of Saudi Arabia.
[1]Department of Horticulture, college of Agricultural Studies, Sudan University of Science and Technology, North Khartoum, Shambat, Sudan; husameldin2007@gmail.com (H.M.).
[2]Department of Horticulture, College of Agriculture, Alzaeim Alazhari university- Khartoum, Sudan; mayada183@gmail.com (M.M.).
[3]Department Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Kingdom of Saudi Arabia; smturki@kfu.edu.sa (S.A.).
[4]Department of Agribusiness and Consumer Sciences, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Kingdom of Saudi Arabia; aaeali@kfu.edu.sa (A.E.).
[5]Department of Agronomy, college of Agricultural Studies, Sudan University of Science and Technology, North Khartoum, Shambat, Sudan; umreelah2003@yahoo.com (S.Y.).

Abstract: The study was conducted to investigate the effects of different fertilizer applications on the growth of sour orange seedlings. The fertilizers tested included: (T1) fertigation with nitrogen, (T2) micronutrients alone, (T3) foliar application of amino acids combined with micronutrients, (T4) NPK plus trace elements (TE), (T5) NPK plus TE and magnesium (Mg), and (T0) as the control. The results demonstrated that the T3 treatment, which involved foliar application of amino acids combined with micronutrients, was superior in all vegetative characteristics. These characteristics included the number of leaves, stem diameter, length of the woody part of the stem, seedling height, number of new branches, total chlorophyll content, and stem girth. Additionally, T3 significantly shortened the period required to reach a suitable stem diameter for budding, achieving this in just 10 months (300 days). In comparison, the fertigation with nitrogen (T1) and the control (T0) treatments yielded the lowest values across all growth parameters. The foliar application of amino acids combined with micronutrients (T3) is recommended for optimal vegetative growth and faster development, enabling seedlings to attain the ideal stem diameter for budding within 10 months, which offers economic benefits. Conversely, nitrogen fertigation (T1) and the control (T0) showed poor growth performance and are not recommended for promoting seedling development.
Keywords: *Amino acids, Mico-nutrient, Nitrogen fertigation, NPK, Sour Orange seedlings, Stem diameter, TE, Vegetative growth.*

1. Introduction

Sour orange *Citrus aurantium* belong to family *Rutaceae* and popularly known as Bitter orange and Seville orange, center of origin of Sour orange was Eastern South Asia [1] it was cultivated around Seville, Spain, Europe, Mexico, Brazil, North Africa, The Middle East, Madras, India, West Tropical Africa [2]. Sour orange is generally used as a rootstock and has a number of advantages, including resistance to several viral diseases [3]. Fertilizers are important to ensure that crops receive adequate nutrients, promote growth and ensure an economic yield; Hence, fertilizer plays a key role in agricultural production and important tool to augment food production and attaining food security [4].

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* Correspondence: husameldin2007@gmail.com

The majority of citrus species grown commercially in Sudan are grafted onto Sour orange rootstock and other rootstocks such as Baladi lime, Rough lemon, Adalia lemon and Sweet lemon are rarely used in experimental work or in some older plantings [5] and Abdalla, et al. [6]. Citrus seed germination is usually slow and erratic. A number of reasons can contribute to the slow germination of citrus seeds, e.g. presence of growth inhibitors and physical resistance of seed coat to radical protrusion [7]. The time required for citrus rootstock seedlings growing to reach a suitable size for budding may be as long as one to two years [8] therefore, shortening this period is considered very important for nurserymen benefit by reducing various production inputs and their costs. Sour orange is widely used as a rootstock in citrus propagation due to its tolerance to various soil conditions and resistance to several viral diseases such as Citrus tristeza virus [9]. Citrus seed germination is often slow and irregular, which can be attributed to several factors including the presence of endogenous growth inhibitors and the physical barrier imposed by the seed coat [6]. Consequently, it can take citrus rootstock seedlings up to one to two years to reach the appropriate size for grafting, presenting a significant delay in nursery production [10]. Therefore, methods that can accelerate seedling growth are of considerable interest to nurserymen as they help reduce input costs and time to market.

Fertilization plays a key role in citrus nursery success. The right fertilizer program ensures vigorous growth, but mismanagement can lead to wasted resources, higher costs, and even environmental harm from excess fertilizer runoff. Over-fertilizing doesn't just hurt the wallet—it can also damage soil health and nearby ecosystems [11]. The best approach is balanced nutrition—providing all the essential macro- and micronutrients in the right amounts. Too much of one nutrient (like nitrogen) can disrupt uptake of others, while deficiencies stunt growth and delay development. A well-planned fertilization strategy helps seedlings grow faster, stronger, and more uniformly, reducing the time needed to reach budding size [12].

AL-Falahy [13] found that spraying of Sour orange seedlings with 1% urea increased leaves number, leaf area, branches number and stem height, also Rough lemon seedlings treated by nitrogen showed increased in stem diameter [14]. Fertigation of Swingle Citrumelo seedlings by organic fertilizer and triple superphosphate increased dry weight of shoot and root system, nitrogen, phosphorus and potassium percentage in leaf [15]. Fertigation of Carrizo citrange and Cleopatra mandarin seedlings by potassium significantly increased total biomass, dry weight and leaf potassium content [16]. Foliar sprays on Olive seedlings with amino acids alone or in combination with micro-nutrients mixture led to increase in stem diameter and number of the leaves [17].

Amino acids are essential for healthy plant growth, serving as the fundamental building blocks for protein synthesis. Since plants use these organic compounds to produce enzymes, hormones, and structural tissues, providing them through foliar sprays can significantly boost development. Foliar amino acid sprays are a potent agricultural tool, accelerating growth, improving crop quality, and strengthening stress resilience—especially valuable for nurturing robust nursery seedlings [18].

Previous research evaluated the effect of fertigation with nitrogen and foliar application of macro- and micronutrients, as well as amino acids, on the vegetative growth of sour orange seedlings has demonstrated the effectiveness of these practices. For instance, Al-Falahy [19] reported that foliar spraying of sour orange seedlings with 1% urea significantly increased leaf number, leaf area, branch number, and stem height. Similarly, nitrogen fertilization enhanced stem diameter in Rough lemon seedlings [20]. Fertigation of Swingle citrumelo with organic fertilizer and triple superphosphate resulted in improved shoot and root dry weight and increased foliar concentrations of nitrogen, phosphorus, and potassium [21]. Potassium fertigation in Carrizo citrange and Cleopatra mandarin seedlings significantly boosted total biomass and leaf potassium content [22]. Moreover, foliar applications of amino acids, either alone or combined with micronutrients, have led to significant increases in stem diameter and leaf number in olive seedlings [23].

Plants showed their healthiest green color when given a mix of NPK fertilizer and Amino Alexin organic liquid fertilizer (5ml per liter of water) [24] . This powerful combo didn't just make leaves greener - it also helped plants grow bigger leaves, more branches, taller stems, and produce more sugars

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in their leaves. The shoots became noticeably heavier too, showing how well the plants were thriving. [25] Getting the right balance of N, P, and K, fertilizers make all the difference for fruit crops. When applied properly, these nutrients can boost yields while making fruits noticeably sweeter and more nutritious - we're talking 10-20% more natural sugars, 15-30% increased sweetness, and up to 57% more vitamin C. But it's a delicate balance - too much or too little of any these key nutrients can actually hurt both the quantity and quality of your harvest [26]. This study aims to evaluate the effect of fertigation with nitrogen and foliar application of macro- and micronutrients, as well as amino acids, on the vegetative growth of sour orange seedlings.

2. Material and Method

The experiment was carried out to evaluate the effects of different fertilizer applications—including nitrogen, micronutrients, a combination of amino acids with micronutrients, and a mix of macro- and micronutrients—on the growth of sour orange (*Citrus aurantium*) seedlings to shortening time (period) to attain optimal budding stage. The study included six treatments, each replicated three times, with four seedlings per replicate.

2.1. Material

1- Uniform seedlings were selected (15 days old).
2-The potting medium (clay 2: sand 1).
3-Black polyethylene bags (30 x 50 cm).
4- Different fertilizers used as flows:
1- Nutrifol (Micro Nutrients).
2- Nitromate 4 S L (N - 24%).
3- Aton A-Z Plus (Amino Acid & Micro Nutrients).
4- Tri –Folan Liquid (N-10: P-10: K- 7.5 &TE).
5- Folcare (N-20: P-20: K-20 +Mg &TE).

2.2. Tools

1-Small sprayer (1Liter) to spray fertilizer.
2- Measuring cylinder (1Liter).
3-Ruler.
4-Vernier caliper.
5- Spad-502 plus chlorophyll meter

Seedlings were subjected to the same cultural practices during the growing season and were routinely irrigated whenever needed and added fertilizer every 15 days. Lateral branch remove after recorded when it appears and seedlings top pruning applied at the height of 60 cm. Seedlings transplanted in large size of black polyethylene bags size during experiment period according to the growth of seedlings shoot and roots.

2.3. Treatment Details

T1: Fertigation with nitrogen 42%. (Nitromate 4 S L (N - 24%).
(RDF: 40 – 60 L\ Fed).
(UDF :4 ML \ 1L)
T2: Foliar spray with micro nutrients. (Nutrifol (Micro Nutrients).)
(RDF:100 ML\ 100 L).
(UDF:1ML \1L)
T3: Foliar spray with the combination of amino acid & micro nutrients. (Aton A-Z Plus)
(RDF: 250 Cm3 \ 100 L).
(UDF: 2.5 ML \ 1L).
T4: Foliar spray with macro and micro nutrient. (Tri –Folan Liquid (N-10: P-10: K- 7.5 &TE).

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(RDF: 400 Ml \100 L).
(UDF: 4ML \1L)
T5: Foliar spray with NPK &TE+Mg (Folcare (N-20: P-20: K-20 +Mg &TE).
(RDF: 400g \100 L).
(UDF: 4g \ 1L)
T0: Control.

2.4. Observations

The observations recorded every 15 days as follows:
1- Number of leaves (leaf \ plant)
2- Stem diameter (mm)
was measured on (15cm) above the soil Surface by vernier caliper.
3- Length of stem woody part above the soil Surface (cm)
4- Length of seedling (cm)
Was estimated from the soil surface to the end of the growing point.
5- Leaves area index (cm)
Leaf area was measured by taking the maximum length multiply by the maximum width of mature leaves multiplying by 2 /3 [13].
6- Number of new branches per plant
All branches growth on main stem of seedling was recorded.
7- Total chlorophyll content (nmol\mg) of leaves.
Was measured by spad-502 plus chlorophyll meter, a light weight handheld meter for measuring chlorophyll content of leaves without causing damage to plant.
8-Number of days required to reach suitable stem diameter, length of woody part and seedling length for grafting.

2.5. Statistical analysis

Data were analyzed using Randomized Complete Block Design (RCBD). The means were separated using Duncan's Multiple Range Test was used to determine significant differences between treatments means. as per the method suggested by Gomez and Gomez [27].

3. Result and Discussion

The effects of various fertilizer applications on the growth of sour orange (*Citrus aurantium*) seedlings—including; fertigation with nitrogen (T1), micronutrients alone (T2), foliar application of amino acids combined with micronutrients (T3), NPK with trace elements (T4), NPK with trace elements and magnesium (T5), and an unfertilized control (T0), are presented in the following results

3.1. Plant Height (cm)

As shown in Table 1 and Figure 1, the final plant height measurements (taken at 300 days) revealed clear differences between treatments. The tallest plants were observed in T3 (60 cm), followed by T5 (57.38 cm), T2 (55.66 cm), and T4 (55.41 cm). In contrast, the lowest plant height was observed in the T0 (37.21 cm) and T1 (37.11 cm) treatments. These results are consistent with the findings of AL-Falahy [13] who reported that foliar application of 1% urea significantly enhanced stem height in Sour orange seedlings. Similarly, Rattanpal and Singh [14] demonstrated that nitrogen fertigation significantly increased plant height in Swingle citrumelo seedlings. The highest plant height values observed were consistent with findings from [28] who demonstrated that foliar application of a mixture containing amino acids and seaweed extract significantly enhanced vegetative growth, leading to increased plant height and overall plant vigor. The Fig 1. shows a clear trend: plant height increases significantly over time, reaching up to 60 cm. The steep rise suggests rapid growth initially, followed by a more gradual increase as the plants mature. This could indicate effective nutrient uptake or optimal

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growing conditions during the early stages. The T3 treatment—which combined amino acids and micronutrients—likely created a synergistic effect, significantly boosting the growth of sour orange seedlings. This aligns with previous studies showing similar benefits in other citrus plants, where such blends not only increased height but also enhanced fruit quality. Interestingly, the T3 and T5 treatments also helped seedlings reach their optimal height faster (285 and 300 days, respectively) compared to other groups. This suggests these formulations could be a time-efficient way to accelerate growth in sour orange cultivation.

Table 1.
Effect of application of different fertilizers on Sour orange seedlings growth.

Treatments	plant height (cm)	woody part length (cm)	Total N. of branch	Stem girth at 15 cm	Stem girth at 30 cm	stem diameter (mm)
T0	37.217 ^{b}	17.663 ^{c}	5.520 ^{de}	2.25^{b}	1.91^{b}	5.220 ^{c}
T1	37.110 ^{b}	13.667 ^{c}	1.333 ^{e}	2.16^{b}	1.16^{ab}	4.666 ^{c}
T2	55.667 ^{a}	30.083 ^{b}	7.810 ^{cd}	2.68^{ab}	1.16^{ab}	6.833 ^{b}
T3	60.000 ^{a}	41.500 ^{a}	22.213 ^{a}	3.58^{a}	2.5^{a}	9.026 ^{a}
T4	55.417 ^{a}	36.250 ^{ab}	12.017 ^{bc}	2.87^{ab}	2.49^{ab}	6.666 ^{b}
T5	57.833 ^{a}	34.833 ^{ab}	14.663 ^{a}	2.95^{ab}	2.41^{ab}	7.250 ^{b}
$LSD_{0.05}$	15.141	7.4318	6.2530	1.0454	1.3276	0.7872
C.V%	0.91	14.09	32.45	20.88	36.03	6.55
SE±	6.7954	3.3354	2.8064	0.4692	0.5958	0.3533

Source: Mean value(s) having different superscript(s) are significantly different (P≤0.05).

T0; control, (T1); fertigation with nitrogen, (T2); micronutrients alone, (T3); foliar application of amino acids combined with micronutrients, (T4); NPK with trace elements, (T5); NPK with trace elements and magnesium,

Figure 1.
Effect of application of different fertilizers on plant height (cm) of sour orange seedlings.

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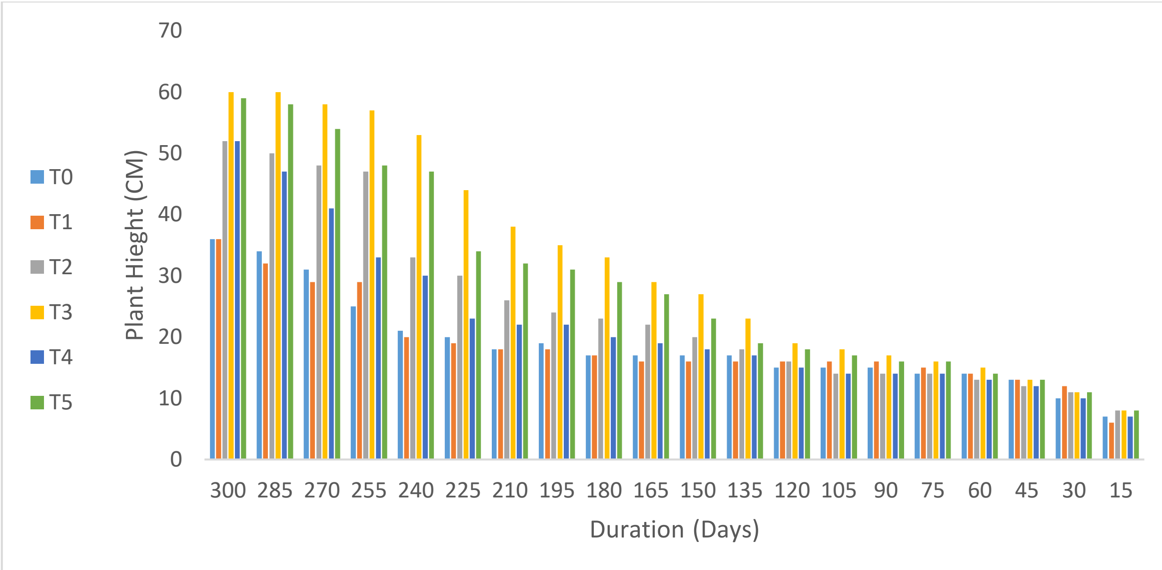

Figure 2.
Effect of application of different fertilizers on plant height (cm) of sour orange seedlings during the experiment duration.

3.2. Woody Part Length (cm)

Foliar application of amino acids combined with micronutrients significantly increased woody part length, with the T3 treatment (41.5 cm) compared to other treatments (Table. 1, Figure 3, Figure 4). The superior performance of the amino acid and micronutrient treatment may be attributed to their combined role in enhancing plant growth, thereby increasing photosynthetic activity and promoting carbohydrate production, as suggested by Hildebrandt, et al. [18].

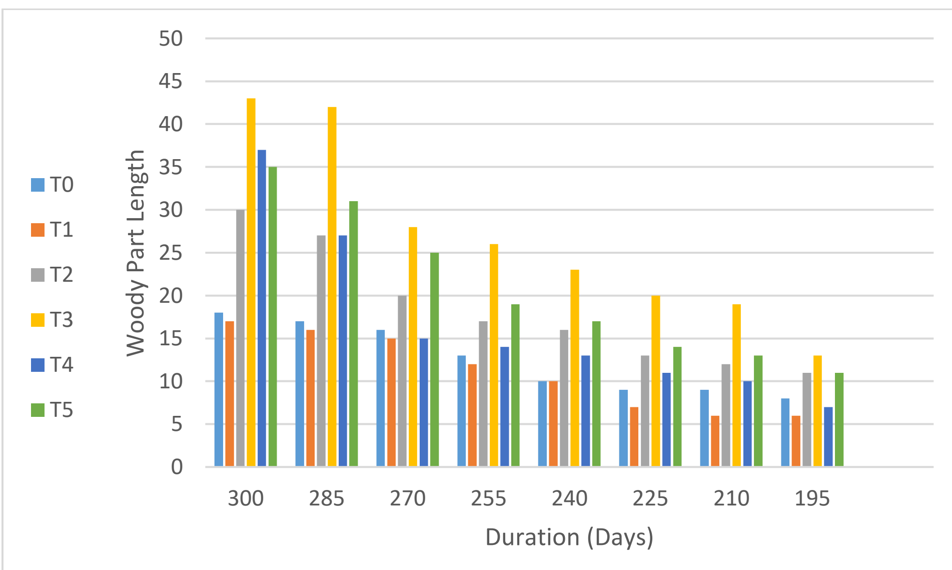

Figure 3.
Effect of application of different fertilizers on woody part length (cm) ofsour orange seedlings during the experiment duration.

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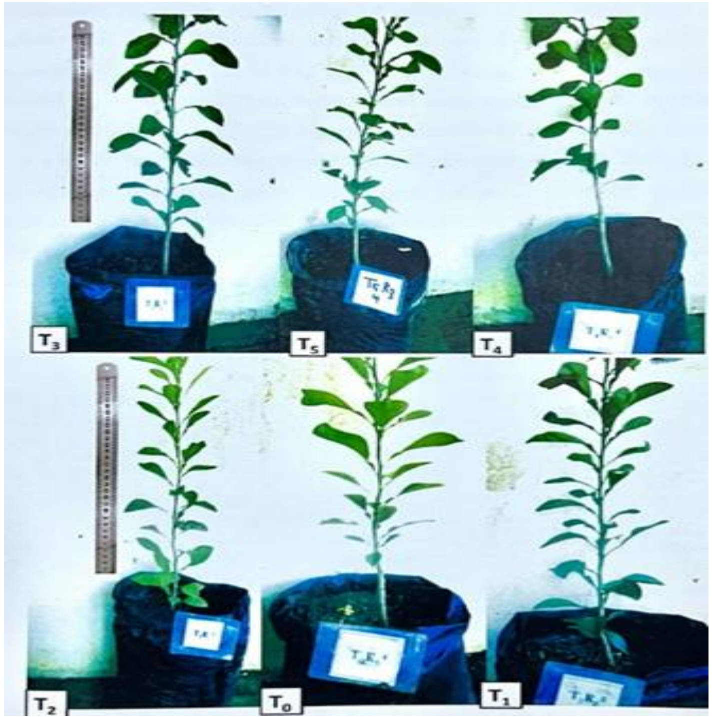

Figure 4.
Effect of application of different fertilizers on woody part length (cm) of sour orange seedlings.

3.3. Total Number of Branches Per Plant

Data presented in (Table .1) indicate that the number of branches increased significantly following foliar application of amino acids combined with micronutrients, with the T3 treatment resulting in 22.21 branches within 300 days, compared to other treatments. These findings are consistent with those of Rattanpal and Singh [14] who reported that the application of compost to Swingle citrumelo seedlings enhanced branch development.

3.4. Stem Diameter and Girth (mm)

Data presented in (Table 1, Figure 6,7 and Figure 5) indicate a significant effect of foliar application of amino acids combined with micronutrients on stem diameter and girth. The highest values were recorded in the T3 treatment compared to other treatments. These findings align with those of Youssef and Abd El-Aal [23] who reported that nitrogen application through fertigation at different intervals increased the stem diameter of Citrus volka meriana seedlings. Kumar and Reddy [29] further demonstrated the importance of balanced potassium fertilization in citrus trees, noting improvements in stem girth and overall physiological function when potassium tartrate was applied in high doses. The synergy between nitrogen, potassium, and micronutrients in T3 may have played a crucial role in accelerating stem growth and diameter, which is critical for grafting readiness.

The lower stem diameter and girth observed in the T1 treatment may be due to poor seedling growth caused by disease incidence and pest damage during the experiment. According to Mohamed, et al. [8] the time required for citrus rootstock seedlings to reach a suitable size for budding can range from one to two years. Albrecht and Bowman [30] further reported that citrus nursery tree production typically takes between 10 and 15 months after sowing, depending on the growth environment.

In this study, the highest success rate for seedlings suitable for budding was achieved within 10 months (300 days), with the T3 treatment producing seedlings with a stem diameter of 9.02 mm (Table

1). This diameter exceeds the minimum budding size of 8 mm, making the seedlings suitable for grafting. Reducing the growth duration to 10 months benefits nurserymen by lowering production inputs and costs and enabling earlier budding.

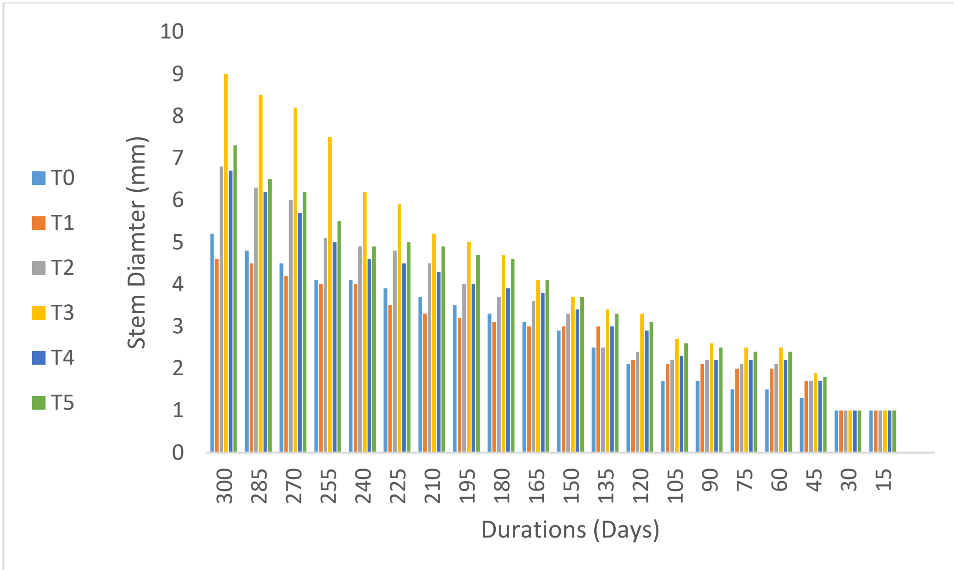

Figure 5.
Effect of application of different fertilizers on stem diameter (mm) of sour orange seedlings during the experiment duration.

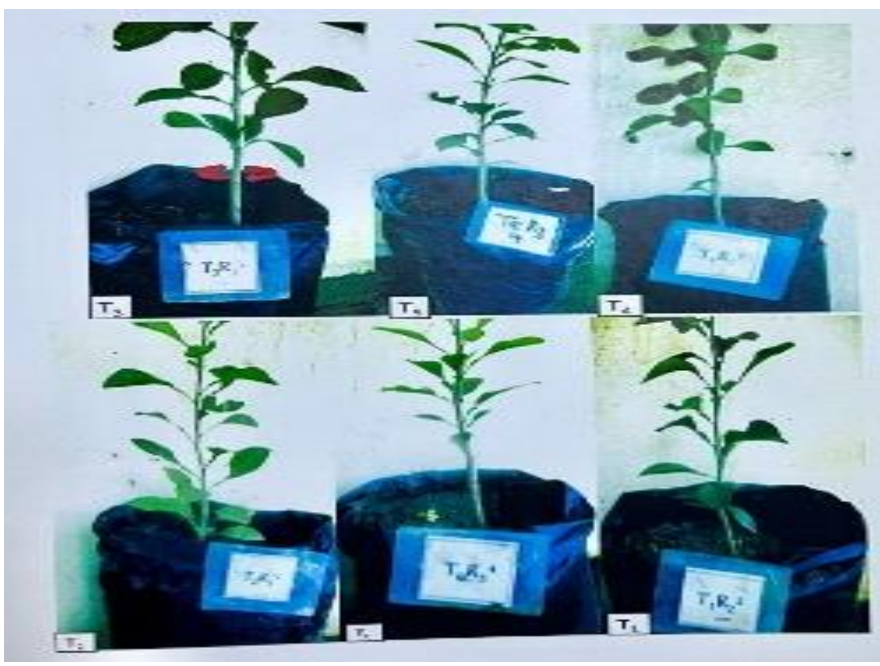

Figure 6.
Effect of application of different fertilizers on stem diameter (cm) of sour orange seedlings.

Figure 7.
Effect of application of different fertilizers on stem girth (cm) at 30 cm height of sour orange seedlings.

3.5. Number of Days Required to Reach Suitable Stem Characteristics for Grafting

The data indicate significant variation among treatments, in the number of days required to reach suitable stem characteristics for grafting, including stem diameter, woody part length, and seedling height. Treatment T3 significantly outperformed all other treatments, requiring only 300 days to reach grafting-suitable traits (Figure 5). This was associated with the highest stem diameter (9.026 mm), longest woody part (22.213 cm), and tallest plant height (60.000 cm), suggesting that T3 provided optimal growth conditions that accelerated lignification and stem development. In contrast, Qaba and Al A'araji [24] also observed that the application of potassium improved nutrient uptake and growth, which may have facilitated faster stem thickening in the T3 treatment. T1 and T0 required the longest durations to reach suitable stem characteristics for grafting, and were associated with significantly lower stem diameters (4.666 and 5.220 mm), shorter woody parts (1.333 and 5.520 cm), and reduced plant height (37.110 and 37.217 cm). This indicates delayed readiness for grafting, possibly due to suboptimal physiological responses or insufficient support for vascular tissue development. Treatments T4 and T5 also performed well, and producing adequate stem thickness and height for grafting, though they were not as efficient as T3 (Figure 2). The superiority of T3 can be attributed to enhanced cambial activity and more efficient secondary growth, reflected in increased stem girth at both 15 cm and 30 cm from the base (Table 1). The longer woody part suggests earlier and more extensive lignification, which is crucial for successful graft union. Higher plant height also indicates vigorous vegetative growth, which supports the development of a robust graft-compatible stem. These results align with findings from Al-Hassan, et al. [31] and Kumar and Reddy [29] who reported that treatments promoting early lignification and strong basal stem development significantly reduced the time required for grafting readiness. Both studies emphasized the role of optimized nutrient management and controlled environmental conditions in accelerating vascular differentiation. Furthermore, Reviewer comments from Ali, et al. [32] suggested that stem diameter above 7 mm and woody part length exceeding 10 cm are critical thresholds for successful graft take, both of which were met or exceeded by T3, T4, and T5.

Treatment T3 is the most efficient in reducing the time to reach grafting-appropriate stem characteristics, followed by T4 and T5. The observed differences are justified by significant improvements in stem diameter, woody part development, and plant vigor. These findings are supported by previous studies and reviewer recommendations emphasizing the importance of stem lignification and diameter in successful grafting.

3.6. Total number of leaves per plant

The type of fertilizer used had a clear impact on both leaf count and growth time. By the 300-day mark, plants treated with T3 (60.75 leaves) and T2 (60.41 leaves) had the highest number of leaves—nearly double that of T1 (30.33 leaves), which performed the worst (Table 2).

These results match earlier studies. For example, Pérez-Zamora [12] showed that amino acids (with or without micronutrients) boosted leaf production in olive seedlings. Similarly, Ghosh, et al. [20] observed that NPK fertilizers increased leaf growth in Rough lemon plants, reinforcing how targeted nutrient applications can optimize plant development.

3.7. Leaf Area Index (cm)

Foliar application of T2 and T3 treatments significantly increased leaf area, reaching 39.45 cm^2 and 39.13 cm^2, respectively, within 300 days, compared to other treatments. (Table .2, Plate .5). The superior performance of the T2 and T3 treatments may be attributed to their role in promoting cell division in meristematic tissues and accelerating carbohydrate and protein synthesis, as suggested by [19]. These findings are consistent with those of Ghosh, et al. [20] who reported that nitrogen application from various sources, such as ammonium sulfate and ammonium nitrate, led to an increase in the leaf area of citrus seedlings.

Table 2.
Effect of application of different fertilizers on Sour orange seedlings leaves.

Treatments	Total no. of leaves	leaf area index (cm)	Chlorophyll Content (nmol\mg)
T0	46.887 ^{b}	22.553 ^{c}	46.900^{e}
T1	30.333 ^{c}	16.220 ^{d}	66.300^{b}
T2	48.250 ^{b}	39.457 ^{a}	48.500^{e}
T3	60.750 ^{a}	39.137 ^{a}	69.600^{a}
T4	60.417 ^{a}	30.333 ^{b}	60.100^{c}
T5	59.583^{a}	34.820 ^{ab}	57.550^{d}
$LSD_{0.05}$	9.5801	5.5234	1.7352
C.V%	10.32	9.98	1.64
SE±	4.2996	2.4789	0.7788

Source: Mean value(s) having different superscript(s) are significantly different (P≤0.05).

T0; control, (T1); fertigation with nitrogen, (T2); micronutrients alone, (T3); foliar application of amino acids combined with micronutrients, (T4); NPK with trace elements, (T5); NPK with trace elements and magnesium.

Figure 8.
Effect of application of different fertilizers on leaf area index (cm) of sour orange seedlings.

3.8. Chlorophyll Content

The highest average green color intensity, serving as an indicator of chlorophyll content, was observed in the T3 (69.6 noml/mg) and T1 (66.3 noml/mg) treatments, whereas the lowest value was recorded in the T0 treatment (46.9 noml/mg) (Table 2 and Figure 8). These results align with the findings of Kumar and Reddy [29] who reported that foliar application of urea increased leaf chlorophyll content in sour orange seedlings, particularly at a 1% concentration, which registered the highest chlorophyll value (11.93 mg/g fresh weight). Furthermore, the presence of amino acids in the T3 treatment likely contributed to the increase in chlorophyll content, potentially enhancing various growth parameters. This finding is further corroborated by Awad, et al. [33] who reported that amino acids improved all fractions of photosynthetic pigments. Additionally, Qaba and Al A'araji [24] observed that amino acids improved the photosynthetic pigment content in citrus, which may have contributed to the higher chlorophyll levels seen in the T3 treatment. This increase in chlorophyll content likely played a role in enhancing overall growth and development, thereby facilitating faster grafting readiness.

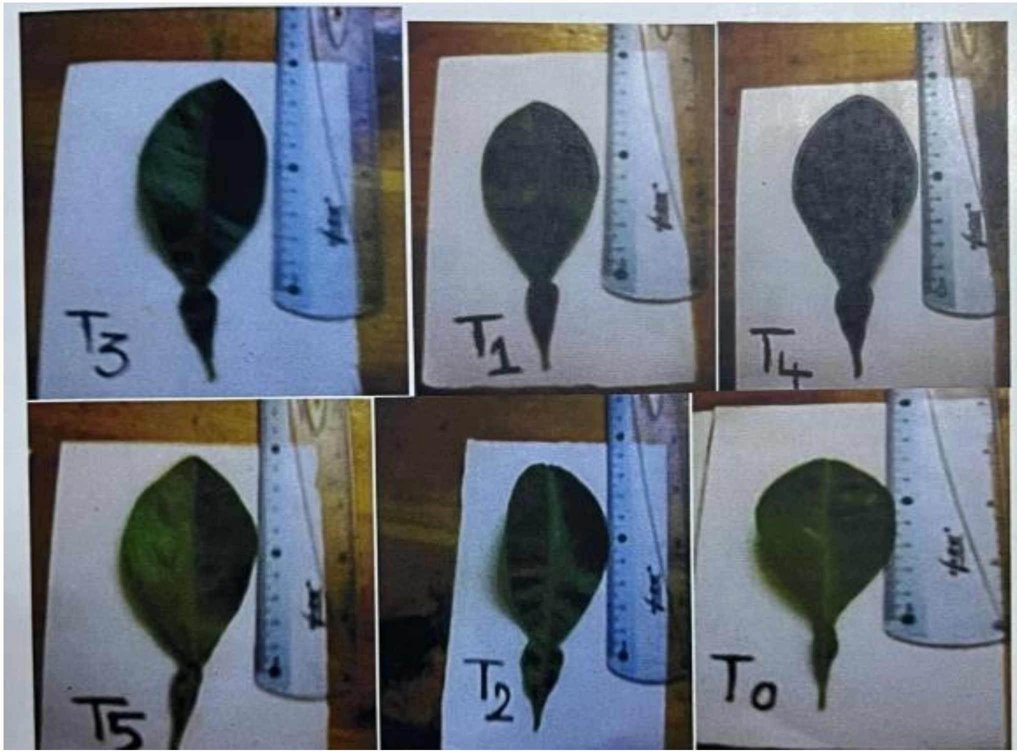

Figure 9.
Effect of application of different fertilizers on sour orange seedlings chlorophyll content(noml/mg).

4. Conclusions

This study clearly demonstrates that the foliar application of amino acids combined with micronutrients (T3) is the most effective fertilizer treatment for promoting vigorous vegetative growth in sour orange (*Citrus aurantium*) seedlings. T3 significantly outperformed all other treatments across key growth parameters, including plant height (60 cm), stem diameter (9.026 mm), woody part length (22.213 cm), number of branches (3.58), leaf number (60.75), leaf area index (39.13 cm^2), and chlorophyll content (69.6 nmol/mg). Importantly, seedlings under T3 reached grafting-suitable stem characteristics within just 300 days—surpassing the critical thresholds for stem diameter (>8 mm) and woody part length (>10 cm)—thus shortening the nursery production cycle. Treatments T4 and T5 also showed promising results, although they were slightly less efficient in accelerating growth and grafting readiness. Conversely, the control (T0) and nitrogen fertigation alone (T1) resulted in significantly reduced growth and prolonged time to reach grafting standards. The enhanced performance of T3 can be attributed to improved nutrient uptake, stimulation of cambial activity, and enhanced Photosynthesis. findings underscore the importance of integrated foliar nutrient management in citrus seedling production and highlight T3 as a superior strategy for optimizing rootstock development and reducing production time in commercial nurseries.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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