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NANO- AND BIO-FERTILIZATION EFFECTS ON THE GROWTH TRAITS OF ORANGE SEEDLINGS

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SUMMARY

The experiment commenced in the shade house at Al-Mahaweel Horticulture Station, located in the north of Babylon Province, Iraq, from April to November 2021. It studied the effect of bio fertilizers and nanofertilizers on traits of some root and vegetative growth of orange (*Citrus sinensis* L. *Osbeck*). The experiment had a factorial setup (4 × 4) with a completely randomized design and three replicates. The two factors included nanofertilizer with four levels at 0, 1, 2, and 3 g L^{-1} and corresponding symbols (N0, N1, N2, and N3, respectively). The second factor was the bio fertilizers with four levels (control and *azotobacter* at 10 ml L^{-1} pot, *mycorrhiza* at an average of 10 gm pot^{-1}, and a mixture of *mycorrhiza* and *azotobacter* as ground additive), symbolized as F0, F1, F2, and F3, respectively. The results showed the treatment of F3 excelled, giving the highest rate for the traits of the number of leaves, plant height, leaf area, root length, and the number of roots. Their corresponding values reached 125.93 leaves $plant^{-1}$, 72.30 cm, 83.17 cm^2, 121.52 cm, and 7.89 roots $plant^{-1}$, respectively. The treatment of N3 stood out, and it gave the highest rate for the traits of leaf number, plant height, leaf area, root length, and numbers, with values at 66.15 leaves $plant^{-1}$, 139.01 cm, 94.64 cm^2, 138.38 cm, and 9.195 roots $plant^{-1}$), respectively. Meanwhile, the bi-interaction treatment (F3 + N3) was superior, providing the maximum values for all studied traits.

Keywords: Orange (*Citrus sinensis* L.), bio fertilizers, nanofertilizers, growth traits

Key findings: The treatment of F3 was excellent, and it gave the highest rate for the traits of the number of leaves, plant height, leaf area, root length, and the number of roots.

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INTRODUCTION

Oranges belong to the Rutaceae family, containing the most important species. *Citrus* is the local orange, and its original habitat is the tropics and subtropics. It also has great nutritional value, as it was evident that 100 g of orange fruits contain 46 kilocalories, water, protein, amino acids, vitamins such as vitamins B1, B2, A, and C, and ascorbic acid—as each 100 ml of juice contains 40–50 mg (Hussein *et al.,* 2007).

The correct and balanced feeding process is what provides the plant with all its nutritional needs, which is important in increasing the plant's growth rate, completing and forming flowers, and producing high-quality fruits. Considering said specifications combined with spraying growth regulators, such as nanofertilizers, are the considered modern fertilization methods of the present time. They reduce loss of plant nutrients on one hand and reduce the economic costs to the farmer through optimal use of fertilizers in small quantities with high absorption efficiency (Kang *et al*., 2005).

It is also possible to extract small amounts of these nanofertilizers, and they are highly efficient in absorption. Adding these helps accelerate the development of the plant (Abdullah, 2014). With nanofertilizers considered as modern fertilizers of the present era, they reduce the loss of food juices due to their readiness and easy absorption. Moreover, used in small quantities, they increase the high absorption efficiency, as well as the absorption of other elements working to achieve nutritional balance in the soil. This research worked on increasing the growth and development of the plant by applying the mycorrhizal fungi and azotobacter bacteria obtained from isolating microorganisms (Ditta and Arshad, 2015).

MATERIALS AND METHODS

The experiment took place in the Horticulture Department in the District of Mahaweel for two seasons (2021 and 2022), within the Al-Mahaweel Horticulture Research Station of the Ministry of Agriculture, Iraq. The research studied the response of orange seedlings to bio fertilizers and nanofertilization based on the traits of root and vegetative growth of seedlings.

Plant height (cm) measurement started from a height of 2–5 cm above the soil surface. Counting total leaf number comprised the number of leaves per seedling. For leaf area, using the planimeter device measured the different leaves taken from each plant. The calculation of average per seedling occurred for each experimental unit.

Experimental design

A factorial experiment design with three factors followed a randomized complete block design (RCBD). Each factor included 48 seedlings, three seedlings per unit with two factors. The first factor represents spraying with nanofertilizer with four levels (0, 1, 2, and 3 g L^{-1}). The second factor is the biofertilizers with four levels and a mycorrhiza and azotobacter as a ground additive, in addition to the control treatment, with corresponding symbols of F0, F1, F2, and F3. Samples taken from the seedlings underwent analysis at the Al-Qasim Green University's Laboratory of the Department of Horticulture and Landscape Design to determine the physical and chemical properties (Table 1) (Black, 1965; Page *et al.,* 1982).

RESULTS AND DISCUSSION

The results showed bio fertilization had a significant effect on the increase in the number of leaves, as the F3 bio fertilization treatment excelled (Table 2). The highest average number of leaves reached 72.30 leaves $plant^{-1}$, while the control treatment F0 obtained the lowest number of leaves, reaching 55.08 leaves $plant^{-1}$. The results also revealed spraying of nanofertilizer significantly affected a rise in the number of leaves. It outperformed the N3 and gave the highest average of 66.15 leaves $plant^{-1}$. The N0 treatment provided the lowest number of leaves, reaching 60.80.

Table 1. Chelated content of nanofertilizers.

Element	B	Zn	Mn	Cu	Fe
Percentage	0.5%	1.5%	1.5%	0.5%	8%

Table 2. Effect of nano- and bio-fertilization and their interaction on the number of leaves of citrus seedlings.

Nanofertilization / Bio-fertilization	N0	N1	N2	N3	Means
F0	53.15	55.16	55.79	56.22	55.08
F1	58.27	60.27	62.91	66.13	61.90
F2	58.37	61.13	62.42	68.14	62.52
F3	73.42	70.48	71.18	74.11	72.30
Means	60.80	61.76	63.08	66.15	
$LSD_{0.05}$	F = 5.302	N = 5.302		F*N = 10.604	

Table 3. Effect of nano- and bio-fertilization and their interaction on the plant height of citrus seedlings.

Nanofertilization / Bio-fertilization	N0	N1	N2	N3	Means (cm)
F0	95.16	108.56	110.38	129.6	110.93
F1	99.71	114.3	114.64	135.63	116.07
F2	101.58	117.66	117.4	138.77	118.85
F3	102.97	124.12	124.59	152.03	125.93
Means (cm)	99.86	116.16	116.75	139.01	
$LSD_{0.05}$	F = 4.636	N = 4.636		F*N = 9.273	

The outcomes have a two-way interaction effect, with the interaction treatment F3 + N3 as distinct, giving the highest average number of leaves. It reached 74.11 leaves plant^{-1}, while the comparison treatment showed an average of 53.15 leaves. Boosting leaf number may be due to bio fertilization and spraying with nanofertilizers has an effect on regulating the vital processes in the plant, including the process of carbon metabolism. Likewise, they lead the regulation of physiological processes, which, in turn, cause an increase in the number of leaves in plants (Diallo *et al.*, 2014; Chen *et al.,* 2017).

The results revealed the plant's high biological activity (Table 3). The F3 fertilized donations naturally had the topmost average of plant height (125.93 cm), while the F0 control plant had a height of 110.93 cm. The results also showed spraying with nanofertilizer altered plant height. The N2 gave the highest rate of 139.01 cm. The rates of the overlay effect appeared, and the overlay coefficient was F3 + N3. The reason for this is because of the role of bio- and nanofertilization in increasing plant growth and development due to the result of interactions and interventions between other growth regulators (Al-Khafaji, 2014).

The outcomes also detailed the increase in leaf area as a characteristic of nanomaterials' ability to stimulate vegetative cells. This stimulation divides through the direct effect on the leaf formation areas and the speed of their division and enhancement of leaf area (Table 4) (Buzea *et al.*, 2007). It was noteworthy that the biomarker F3 had the highest leaf average of 83.17 cm, while F0 had the lowest leaf average of 74.69 cm^2, with the N0 leaf treatment recorded at 65.92 cm^2. Furthermore, the results of the interaction showed the size of the binary interaction (F3 + N3) reached and gave the most leaves, averaging at 40.

Table 4. Effect of nano- and bio-fertilization and their interaction on the leaf area of citrus seedlings.

Nanofertilization / Bio-fertilization	N0	N1	N2	N3	Means (cm^2)
F0	63.12	70.09	78.12	87.42	74.69
F1	65.78	75.79	90.33	92.32	81.06
F2	67.14	77.38	76.33	96.44	79.32
F3	67.62	84.01	78.67	102.39	83.17
Means (cm^2)	65.92	76.82	80.86	94.64	
$LSD_{0.05}$	F = 3.20	N = 3.20		F*N = 6.40	

Table 5. Effect of nano- and bio-fertilization and their interaction on the root length of citrus seedlings.

Nanofertilization / Bio-fertilization	N0	N1	N2	N3	Means (cm)
F0	94.67	126.41	123.17	134.5	119.69
F1	95.86	117.64	111.19	136.63	115.33
F2	96.33	121.01	116.45	137.94	117.93
F3	99.8	121.95	119.88	144.43	121.52
Means (cm)	96.67	121.75	117.67	138.38	
$LSD_{0.05}$	F = 5.07	N = 5.07		F*N = 10.14	

Table 6. Effect of nano- and bio-fertilization and their interaction on the number of roots of citrus seedlings.

Nanofertilization / Bio-fertilization	N0	N1	N2	N3	Means
F0	5.22	6.45	6.8	8.92	6.8475
F1	5.51	7.73	7.24	9.05	7.3825
F2	5.83	8.42	8.33	9.13	7.9275
F3	5.38	8.63	7.86	9.68	7.8875
Means	5.485	7.8075	7.5575	9.195	
$LSD_{0.05}$	F = 0.48	N = 0.48		F * N = 0.97	

The results indicated bio fertilization influenced root length (Table 5). The treatment with F3 fertilization gave the highest average root length of 121.521 cm, while the treatment with the lowest F0 obtained the shortest root length of 119.69 cm. Moreover, results showed spraying with nanofertilizer modified root length (96.67 cm). The findings also provided a bilateral interaction. The binary interaction treatment (F3 + N3) gave the tallest average root length (144.43 cm), while the control treatment reached an average root length of 94.67 cm. This is consistent with the findings of Al-Khafaji (2014).

Researchers saw the F3 treatment gave the premier average number of roots (7.88 roots), while the control treatment F0 provided the lowest average number of roots (6.84 roots) (Table 6). Similarly, the results signified spraying with nanofertilizer had an effect on the number of roots, as the highest treatment was N3 and the highest percentage was 9.19 roots, while the rest showed the lowest number. The same treatment also gave the most roots with the interaction. The changes occurring in the root system are due to an increase in the photosynthesis process (Singh *et al.,* 2017).

Nano- and bio fertilizers' effect on vegetative growth

A result of the previous experiment revealed the change in vegetative traits due to the fungi mycorrhiza and *azotobacter* individually or in combination. The mycorrhiza works a strong relationship between the plant and fungus. Likewise, fungi help in the absorption of present elements, such as phosphorus from the soil (Demir, 2004). Fungi prevail in the root topsoil. They also help provide an amount of released growth regulators (gibberellins, auxins, and cytokinins) (Hamdan, 2011), and this action has a role in the protraction of plant cells. As for *Azotobacter*, its role is similar to mycorrhiza. It aids cell division, photosynthesis, and the synthesis of RNA and DNA (Mrkovacki and Milić, 2001). Moreover, this element is an essential composition of amino acids, improving symbiotic relationships. Additionally, it leads to an increase in the absorption of nutrients as a result of their absorption in the growth medium (Devlin and Witham, 1983).

In addition to their role in the secretion of growth regulators, such as auxin, cytokinin, and ghrelin, they play an important role in increasing cell division and development. All of the above can contribute significantly to increasing the plant's ability to absorb water and nutrients from the soil solution surrounding the roots of infected plants (Taiz and Zeigar, 2002). This will reflect positively in enhancing the vegetative traits, which can be a result of the interaction of azotobacteria and mycorrhizal fungi. Consequently, it results from the mutual relationship between them, which greatly improved the size and shape of seedlings because of double pollination between these fertilizers.

As for the nanofertilizer and its effect on vegetative traits, the reason may be ascribable to the small size of the particles of this fertilizer and its content of microelements crucial in the growth and development of the plant (boron, iron, zinc, manganese, and copper). Boron plays an effective role in some physiological processes, such as photosynthesis and energy production (ATP), which are necessary in the plant's vital processes. Furthermore, it helps in building the nucleic acids DNA and RNA essential for cell division, which encourage the movement and transfer of nutrients within the plant. Aside from the significant role in cell division and elongation, it positively affects an increase in the percentage of auxins, especially indole acetic acid (IAA) (Al-Nuaimi, 1999; Focus, 2003).

The zinc element is important in activating many enzymes, including the enzymes found in chloroplasts, which are crucial in the process of photosynthesis and necessary for the elongation of the stem or other plant cells (Alloway, 2004). This indicates positively improving the vegetative characteristics of the seedlings (Zagzog and Gad, 2017).

Manganese also plays an influential role in building the chlorophyll molecule. Its key role is in the process of photosynthesis as a result of its participation with chlorine in the photolysis of the water molecule (photolysis) and obtaining electrons in the process called the Hill reaction. The Hill reaction is the conversion of light energy into chemical energy in the form of ATP and $NADPH_2$, causing an increase in the studied vegetative properties of the seedlings treated with this fertilizer (Abu-Dahi and Al-Younes, 1988).

Finally, the nanofertilizer contains copper elements, and the reason for the increase in the studied vegetative characteristics may be due to the role of this element in the chloroplast, photosynthesis, and the oxidation-reduction process. It contributes to the production of manufactured foodstuffs used in the growth and development of seedlings. The valuable roles of these elements and their presence in one fertilizer greatly contributed to the improvement of the growth and development of these seedlings (Abu-Dahi and Al-Younes, 1988).

CONCLUSIONS

The study concludes that the treatment with mycorrhiza was significantly superior and gave the highest values for the studied traits. It was also significantly superior to the nanofertilization treatment at a concentration of 3 g L^{-1}.

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