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STUDY OF THE EFFECT OF FOLIAR SPRAY WITH NANO FERTILIZER AND BIOLOGICAL FERTILIZATION IN SOME CHARACTERISTICS OF VEGETATIVE AND ROOT GROWTH OF ORANGE SEEDLINGS

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Abstract

The research was carried out in the lath house of the Technical College, Al-Musaib during the 2018-2019 growth season to study the effect of bio-composting and foliar spraying with nanoparticle on the vegetative and root growth of grafted Orange seedlings on *Citrus aurantium* origin the experiment included two factors, the first is the use of four types of earthly addition with biological fertilizer these are (control treatment, *Azotobacter* bacteria 10 ml. L^{-1}, mycorrhiza fungi 10 g, treatment of interference between *Azotobacter* bacteria 10 ml. L^{-1}, Mycorrhiza fungi 10 g) to the one pot As for the second factor, it is sprayed with chelated nano fertilizer (micronutrients) with several concentrations (control treatment, 1 g.L^{-1} l, 2 g.L^{-1}.3 g.L^{-1}). Therefore, the experiment included (16) treatments, three replicates, and three seedlings per refined, for a total of 144 seedlings per experiment. The seedlings were sprayed three times for the spring and autumn dates. Untreated seedlings (control) were sprayed with distilled water only. The experiment was performed according to Completely Randomized Design (CRD) with two factors (4 × 4), the results were analyzed using the ANOVA table of variance analysis according to the Excel program. Statistical differences between the treatments were tested using the least significant difference (L.S.D) at the 5% probability level. The results of this study can be summarized as follows: (1) The combined treatment with Mycorrhiza and *Azotobacter* resulted in a significant increase in the vegetative traits, which include plant height, stem diameter (vegetative), as well as an increase in the root and represented traits (number of roots, length of the main root, dry weight of the root system) and chemical characteristics (leaf content of chlorophyll). (2) The reason for spraying with nano fertilizer 3 g.L^{-1} significant increase in the vegetative and root traits and the leaf content of chlorophyll). (3) The treatment of the interaction between the treatment with Mycorrhiza and *Azotobacter* combined with spraying with nanostructures achieved 3 gm.L^{-1} significant increase in vegetative traits plant height, stem diameter reached (54.23 cm, 5.20 cm). The same treatment also gave a significant increase in the root traits (main root length, number of roots, and dry weight of root root) (51.80 cm, 3.99, 9.87 g). The same treatment gave a significant increase in the leaf content of chlorophyll (60.93SPAD).
***Keywords*:** Oranges, nanofertilizers, *Azotobacter*, Mycorrhiza and spray dates.

Introduction

Citrus comes from the Rutaceae family**,** It includes four genera, the most important of which is the Citrus genus, of which the local Orange is considered to be the tropical habitat and the sub-tropical regions, which lie between latitudes 40° north and south of the equator. It also has great nutritional importance, as the benefit found in (100) grams of orange fruits is about (46) kilocalories and contains 86% water and 0.9% protein 0.1% as it contains other amino acids as well as a group of vitamins, the most important of which is Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), Vitamin A (Carotene) Antioxidant and Vitamin C as each 100 ml of juice contains 50-40 mg of this vitamin (Unctad), 2007). Biofertilizers are among the types that have received wide attention in recent research, because they are cheap and environmentally friendly compared to mineral fertilizers, which play an important role in stabilizing some elements, including nitrogen, which in turn leads to increased absorption of phosphorous from the soil. It is believed that the absorption of potassium and other elements present in the soil is linked to the improvement of the budget Nutritional absorption of the two components leading to increased plant growth and development, Among these Boifertilizers are Mycorrhiza fungi and *Azotobacter* bacteria obtained from isolation and propagation by microorganisms in suitable farms, and then kept in appropriate conditions until they are used as polluting roots or soil (Mahmoud *et al*., 1997). Nano fertilizers are also among the modern technologies at the present time due to their importance and speed in increasing the readiness and absorption of nutrients, which work to reduce losses and stabilize them in the soil in the case of adding them in a timely manner and usually use them in small quantities and are highly efficient in absorption, and can be added in several forms as an element Or a group of elements, which in turn leads to increased growth, as well as an increase in the quantity and quality of the outcome, as well as reduces the rate of environmental pollution (Abdullah, 2014). This study came with the aim of: Studying the effect of biological fertilizers (Mycorrhiza fungi and *Azotobacter* bacteria) individually or in combination and their effect on the vegetative and root growth of orange seedlings, and revealing the role of overlapping experiment factors in the evolution and growth of grafted orange seedlings on the origin of bitter orange.

Materials and Methods

Experiment execution site

The experiment was conducted in the lath house of the Technical College, Al-Musaib, Al-Furat Al-Awsat technical university for the period from April 2018 to November 2018.

Initialization study requirements

Initialization seedlings

Attended a local seedlings of orange, grafted on the origin of *Citrus aurantium*, at the age of one year, which was obtained from the horticultural department in Al-Hindia, The holy city of Karbala was planted in plastic bags with sizes (25 * 30) cm, the seedlings were transferred to plastic bushings with a capacity of (7) kg, containing a mixture of river mixture and peatmoss (2: 1), as 144 seedlings of homogeneous growth and size were chosen as much as possible and service operations were carried out equally and for all the transactions, which included combating the leaves digger with a twin insecticide. Double Spiders active substance in the form of concentrated emulsion Ec% 4 and spray on plants at a rate of 2 ml.L $^{-1}$ water and used a 2 liter hand sprinkler. Samples were taken from the growing agricultural medium in which the seedlings were mixed and then mixed homogeneously. At the College of Agriculture, University of Kufa, soil and water department laboratories were analyzed to find out some of their physical and chemical characteristics. Table (1) according to the methods mentioned (Black, 1865) and (Page and others, 1882) and the results of the analysis Shown in Table (1).

Table 1 : Some of the chemical and physical properties of the growing soil have seedlings.

Separate	Unit	Quantity
sand	g.kg^{-1} soil	818
silt	g.kg^{-1} soil	111
clay	g.kg^{-1} soil	70
texture	--------	loamy sand
properties	Unit	Quantity
Electrical conductivity EC	Decimes. M^{-1}	1.5
Degree of reaction pH	---------	7.2
Ready nitrogen	g.kg^{-1}	3.7
Ready phosphorous	mg. Kg^{-1}	6.0
Ready potassium	mg. Kg^{-1}	3.47
Organic matter	g.kg^{-1}	7.0

Table 2 : Contents of Chelated Nano Fertilizers (KHAZRA)

Element	Boron	Zinc	Manganese	Copper	Iron
Percentage	% 0.5	% 1.5	%1.5	% 0.5	% 8

Experimental design and experiment treatments

Perform the research as a global experiment (4 * 4) Completely Randomized Design and with three replicates, as each repeater includes 48 seedlings with 3 seedlings for each experiment, and two factors represent the first spraying with nano fertilizer and in four concentrations are 3, 2, 1, 0 g. L^{-1} and the second factor with four additions, which included (Mycorrhiza at a rate of 10 g. pot and *Azotobacter* 10 ml. L^{-1} pot and a mixture between Mycorrhiza and *Azotobacter* as a terrestrial addition) in addition to the control treatment.

Spray dates

Spray the manure compost on three dates, it was the first spray on 15/4/2018 and the second spray 15 days after the first spray and the third spray on 15/5/2018 for the spring date, after that the spray was stopped from the sixth month until the ninth month because of the high temperatures as for the autumn date, spraying was carried out with three dates as well, starting on 1/8/2018 for the first date 1/8/2018 and 30/8/2018. Also, the control treatment was sprayed with distilled water only, I used a 2 liter manual sprinkler, and the spraying process was carried out in the morning until complete wetness. For seedlings, bubblegum was added at a rate of drops to increase the surface tension The watering process of the seedlings was conducted one day before the spraying process to increase the efficiency of the plants in absorbing the sprayed material as moisture has a role in the process of bulging guard cells and opening gaps, as well as the fact that watering before spraying works to reduce the concentration of solubles in the leaf cells so that the penetration of ions of the spray solution to Leaf cells (As-Sahaf, 1989). The measurements were taken on 20/11/2018.

Studied traits: vegetative traits

Plant height (cm)

The height of the seedlings was measured after the end of the experiment and measured from the original contact area with the graft and at a height of 15 cm above the soil surface.

Main stem diameter (cm)

Seedling diameters were measured using the Vernier and a height (cm) from the vaccination area and the rate was used for each experimental unit.

Root traits

Length of main root (cm)

The length of the main root was measured using the metric tape measure from the root nodes region to the root end and by average for each experimental unit.

Number of roots: The number of roots was calculated for each seedling.

Root dry weight (g)

Cut the root mass from the bottom of the stem of the original, then put it in perforated paper bags in an electric oven (Oven) for the purpose of removing moisture at a temperature of 70 degrees Celsius for 48 hours until the weight is proven and take the dry weight using a sensitive electric balance (As-Sahaf, 1989)

Chemical characteristics:

Chlorophyll leaf content (SPAD)

The leaf content of chlorophyll was estimated using a Chlorophyll Meter device of the type SPAD 502 (SPAD: Soil Plant Analysis Device) and supplied by the Japanese company Minolta Co.LTD. The reading of three leaves was taken from different places for each seedlings, calculating the average for each seedlings, then calculating the average for each seedlings. Pilot unit (Jemison and Williams, 2006).

Results

The vegetative traits

Height of plant (cm):

The results of Table (3) show the effect of Mycorrhiza or *Azotobacter* and nano fertilizer on the average height of the plant for orange seedlings. The combined ground addition treatment with Mycorrhiza and *Azotobacter* combined gave the highest average mean for the height of the plant reached (42.73 cm), while the control treatment was recorded (30.39 cm) which is the lowest value With regard to foliar spraying with manure, the concentration (3 g.L^{-1}) surpassed the other concentrations and gave the highest average of the same trait (43.60 cm) compared to the control treatment that gave the lowest average of that trait (32.24 cm). As for the treatment of interference between the biological fertilizers (The Mycorrhiza and *Azotobacter*) and nano fertilizer, the highest value recorded for the treatment of Mycorrhiza and *Azotobacter* combined with nano fertilization at a concentration of (3 g.L^{-1}) for the same characteristic (54.23 cm), while the lowest value for the control treatment (25.78 cm) was recorded.

Stem diameter (cm)

The results of Table (4) show that the ground addition of Mycorrhiza and *Azotobacter* and foliar spray with nanofertilizer significantly affected the average stem diameter of orange seedlings. Cm), and with regard to foliar spraying with manure, the concentration exceeded (3 g.L^{-1}) and gave the highest average mean for the same trait reached (673. cm) compared to the control treatment that gave the lowest average for that trait (2.53 cm) as for the treatment of interference, the highest value was recorded for the treatment of Mycorrhiza, *Azotobacter* and nano fertilization treatment at a concentration of (3 g.L^{-1}) in the average stem diameter was (5.20 cm), compared to the control treatment that recorded the lowest value for that characteristic (1.48 cm).

Root traits

Length of main root (cm)

It is evident from the results in Table (5) that the use of Mycorrhiza in combination with *Azotobacter* gave a significant difference in the average length of the main root amounted to (45.44 cm) compared to the control treatment that gave (29.32 cm). In the case of foliar spraying with nano fertilizer, the addition of nano fertilizer at a concentration of 3 g.L^{-1} resulted in an increase of (40.54 cm) compared to the control treatment that gave the lowest average of (32.03 cm)

As for the bilateral interaction between the two factors of the experiments with Mycorrhiza and *Azotobacter* combined with foliar spraying with nano fertilizer, the results in the same table indicate the superiority of the seedlings to which the Mycorrhiza and *Azotobacter* were added and sprayed with nanostructures at a concentration of 3 g.L^{-1} and gave (51.80 cm) compared to the control treatment that gave (26.42 cm)).

Number of roots

The results showed in Table (6) that there were significant differences in the number of roots when adding the Mycorrhiza and *Azotobacter* to orange seedlings, as it led to an increase of (3.05) compared to the control treatment that gave (1.67) also, the addition of nano fertilizer spray on the vegetable total at a concentration of 3 g.L^{-1} had an effect on the same trait, as it gave an increase of (2.86) compared to the control treatment that gave (2.09). We also notice from the same table that the interaction between the factors of the experiment had a significant effect in the same trait, as the treatment of seedlings with microscopy and *Azotobacter* combined with foliar spraying with nano fertilizer at a concentration of 3 gm.

Root dry weight (g)

It is clear from the results of Table (7) that the dry weight of the root group of seedlings increased with the addition of Mycorrhiza and *Azotobacter* combined, as this treatment led to a significant superiority of (8.61 g) compared to the control treatment that gave (3.65 g). Also, all the manure-spraying treatments were superior to non-spraying in this capacity, and the treatment with a concentration of 3 g.L^{-1} resulted in a significant increase of (7.29 g) compared to the control treatment that gave (5.43 g). also, the interaction between the factors of the experiment showed a positive effect in this characteristic, as the treatment to which biomagnification added to Mycorrhiza and *Azotobacter* was combined with nano fertilizer at a concentration of 3 g. L^{-1} amounted to (9.87 g) compared to the control treatment that gave (3.00 g).

Chemical characteristics

The leaves content of chlorophyll (SPAD)

The results are shown in Table (8) related to the addition of Mycorrhiza and *Azotobacter* to the seedlings combined, as it led to a significant increase in the leaf content of chlorophyll amounted to (SPAD 52.89) compared to the control treatment that gave (38.48SPAD) The same characteristic was also increased when spraying with nanostructures. The concentration was recorded at 3 gm. L^{-1} the highest average was (SPAD 50.13) compared to the comparison treatment that gave (SPAD 43.11). We also note from the same table that the interaction between the factors of the experiment had a significant effect in the same trait, as the treatment between the biofertilization with Mycorrhiza and *Azotobacter* and spraying with nano fertilizer at a concentration of 3 g gave a-1 liter increase (60.93SPAD) compared to the control treatment that gave (SP 32.81).

Table 3 : Effect of Biofertilization with Mycorrhiza and *Azotobacter* and Foliar Sprinkling with Nano Fertilizers and Interference between them in Average Height of Plant (cm) for Orange Seedlings

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	25.78	30.57	31.54	33.67	30.39
F1	32.87	37.11	38.24	46.04	38.56
F2	35.81	38.91	40.40	40.84	38.99
F3	34.51	39.93	42.28	54.23	42.73
Average	32.24	36.63	38.11	43.69	
L.S.D 0.05	F= 2.674	N= 2.674	F*N= 5.349		

Table 4 : Effect of Nanofertilization with Mycorrhiza and *Azotobacter* and nano fertilizer leaf foliage and their interference in the average stem diameter (cm) of orange seedlings

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	1.48	2.21	2.27	2.39	2.08
F1	2.76	2.89	3.02	3.44	3.02
F2	2.58	3.33	3.13	3.66	3.18
F3	3.31	3.52	3.76	5.20	3.94
Average	2.53	2.98	3.05	3.67	
L.S.D 0.05	F= 0.284	N= 0.284	F*N=0.568		

Table 5 : Effect of bio-fertilizing with Mycorrhiza and *Azotobacter* and foliar spraying with nanostructures and their interference in the average length of the main root (cm) of orange seedlings

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	26.42	28.01	30.51	32.34	29.32
F1	30.66	36.98	38.08	38.62	36.08
F2	35.98	35.27	35.52	39.43	36.55
F3	35.09	46.52	48.38	51.80	45.44
Average	32.03	36.69	38.12	40.54	
L.S.D 0.05	F=2.649	N=2.649	F*N=5.298		

Table 6 : Effect of Biofertilization with Mycorrhiza and *Azotobacter* and Foliar Fertilizer Spreading with Nano Fertilizer and their Interference in the Average Number of Roots of Orange Seedlings.

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	1.34	1.77	1.80	1.80	1.67
F1	2.19	2.30	2.56	2.67	2.43
F2	2.21	2.67	2.95	3.01	2.71
F3	2.65	2.74	2.83	3.99	3.05
Average	2.09	2.37	2.53	2.86	
L.S D .0.05	F=0.251	N=0.251	F*N=0.502		

Table 7 : Effect of Biofertilization with Mycorrhiza and *Azotobacter* and Nebulization of Foliar Fertilizer and their Interference with Dry Weight of Root Total (gm) for Orange Seedlings

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	3.00	3.77	3.86	3.99	3.65
F1	5.51	6.20	6.49	7.69	6.47
F2	5.52	7.08	7.24	7.64	6.87
F3	7.69	8.18	8.73	9.87	8.61
Average	5.43	6.30	6.58	7.29	
L.S.D 0.05	F=0.983	N=0.983	F*N=1.966		

Table 8 : The effect of biofertilizing with Mycorrhiza and *Azotobacter* and nanofertilization spraying and their interaction on the leaf content of chlorophyll (SPAD) for orange seedlings.

Nanofertilization / Biofertilization	N0	N1	N2	N3	Average
F0	32.81	35.97	40.00	45.17	38.48
F1	43.94	44.41	45.45	45.11	44.72
F2	46.53	47.74	48.67	49.30	48.06
F3	49.17	50.49	50.99	60.93	52.89
Average	43.11	44.65	46.28	50.13	
L.S.D 0.05	F= 3.147	N= 3.147	F*N= 6.293		

Discussion

The effect of Mycorrhiza, *Azotobacter* and nano fertilizer on vegetative traits:

A review of the previous results showed the increase in the vegetative traits that can be attributed to the role of Mycorrhiza and *Azotobacter* either individually or collectively in improving the physical and chemical properties of the pot soil as a result of the important roles played by these fertilizers, which leads to an increase in the readiness of the nutrients in the soil and their absorption by the root hairs of the plant as well as The role of mycorrhiza fungi in improving the symbiotic relationship between plants and fungi and the role of these fungi in absorbing phosphorous from soil (Demir, 2004), These fungi extend for several centimeters from the surface of the roots and work to withdraw nutrients from the outer area of the affected roots (Al-Wahaibi, 2008). These fungi also increase the absorption area due to their heights due to the increase in the surface area of the roots and work to produce a good amount of liberated growth regulators in the center Growth (gibberellin, auxin and cytokinin) (Badawi, 2008 and Hamdan, 2011) These secretions play an important role in the elongation of plant cells as a result of increased division of plant cells as well as stimulating root capillaries, which is reflected positively on the process of absorption of nutrients and then an increase in vegetative traits represented by plant height, stem diameter, number of leaves, leaf area and dry weight of the total Vegetative. As for the role of *Azotobacter* bacteria, its role does not differ from the role of mycorrhiza fungi. The reason for the increase can be attributed to its role in increasing the absorption of nutrients such as nitrogen as a result of biological component fixation of nitrogen, which plays an important role in cell division and the important physiological processes of the roots in the soil and that the nitrogen component is involved in building Its chlorophyll molecule as well as in building nucleic acids RNA and DNA (Mrkovacki and Milic, 2001).

The effect of Mycorrhiza, *Azotobacter* and nano fertilizer on root traits:

The results showed that the significant increase in all root traits (number of roots, length of roots, dry weight of the root system) may be due to the role of Mycorrhiza and *Azotobacter* individually or collectively in secreting growth hormone stimuli (gibberellin and indole acetic acid and cytokine) which improved soil fertility and plant growth And increase the readiness of the nutrients of the plant in the medium of growth according to many mechanisms, including an increase in the acidity of the medium, the production of chelating compounds, and the presence of root nodes on the roots as a result of the presence of these fungi and bacteria on them, which contributes significantly to improving plant growth and root traits, Or, perhaps, the reason is that the microorganism and *Azotobacter* significantly helped increase the absorption of nutrients from the medium, improve water relations, increase the surface area of plant-affected roots and increase the surface area of the root tissue involved in the absorption process (Rajanka *et al*., 2007). In addition, the fungal strands attached to the affected root spread to far distances from the root, bypassing the nutrient depletion areas around the root (Kumari *et al*., 2008) as they capture nutrients and transport them to the host root tissue and the fungus in return gets the carbohydrates necessary for its growth (Aseri *et al.*, 2009).

Effect of Mycorrhiza, *Azotobacter* and nano fertilizer on the leaf content of mineral elements and the leaf content of chlorophyll

The results showed a significant increase in the leaf content of chlorophyll and mineral elements (nitrogen, phosphorus, potassium, boron, iron and manganese). The reason may be attributed to the role of *Azotobacter* and Mycorrhiza bacteria in forming root nodes on the roots that affected them and thus an increase in nutrient absorption from the culture medium, including phosphorus And nitrogen Which contributes greatly to increasing these macronutrients and raising the efficiency of photosynthesis process as a result of the mutual effect between these microscopic neighborhoods and their mutual and joint role. So the mycorrhiza prepares the important phosphorus that bacteria need in their activity and important processes within the growth medium in addition to that the bacterium isobuterin contributed greatly to stabilizing the nitrogen component As a result of the role of these two elements in many important biological processes within the plant, this increases the amount of mineral elements processed in the plant parts (Ishac, 2000). Also, the zinc element is no less than the other elements in the compost, as it is involved in opening the stomata as a component of the carbonic anhydrase that is needed to maintain (HCO) in the guarded cells. It is also a factor affecting the absorption of potassium by the guard cells, which increases the efficiency of photosynthesis and increases The leaf content of the mineral elements (Chamani *et al.*, 2015).

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