Effect of spraying with nano-fertilizers and adding biofertilizers on some vegetative and chemical growth traits of olive seedlings of the Ashrasi cultivar.

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Abstract

This study was conducted at Al-Zafaraniya Station - Department of Horticulture - Ministry of Agriculture for the spring season 2022 to study the effect of spraying with nano-fertilizer S at three concentrations (0, 1, 2) g.L-1, and adding the biofertilizer F for mycorrhizal fungi and azotobacter bacteria at four levels (0, 50 spores. g-1 dry soil, 5 ml.L-1, 50 spores. g-1 dry soil + 5 ml L-1) on one-year-old olive seedlings of Ashrasi cultivar. A two-factor experiment was carried out in a randomized complete block design (RCBD). The number of experimental treatments was 12. For three replications, the experimental unit included 5 seedlings, so the total number of seedlings in the sector becomes 60 seedlings, and the total number of seedlings is 180. The data was analyzed according to the statistical program Gen Stat, and the coefficients were compared according to the L.S.D. at the probability level of 0.05 according to the randomized block design, and the results can be summarized as follows- :

The spraying treatment with nanofertilizer had a significant effect on all the studied traits, which included (stem diameter, number of leaves, dry weight of vegetative growth, and the leaves' content of chlorophyll and carbohydrates), where the S2 spraying treatment excelled at a concentration of 2 g.L-1, recording the highest averages compared to the no-adding treatment for all the studied traits. It also had a significant increase in the treatment of biofertilizers with mycorrhizal fungi and azotobacter aggregate F3, which added an enhanced effect to all the studied traits. The interaction treatment between the nanofertilizer at a concentration of 2 g.L-1 and the combined biofertilizer (fungi and bacteria) had a significant effect, recording the highest averages for all the studied traits, which included (stem diameter, number of leaves, dry weight of vegetative growth, and the leaves' content of chlorophyll and carbohydrates), which amounted to (12.03 mm). 6.40, 27.33 g, 64.00 SPAD, 23.20%) compared to the no-additive treatment, which recorded the lowest averages of (6.34 mm, 10.67, 168.67, 168.67 g, 42.67 SPAD, 14.43%), respectively.

Keywords: Olive Seedlings, nanofertilizer, biofertilizer

Introduction:

The olive tree is an evergreen fruit tree and is one of the most important oil crops in the world. The scientific name is Olea europaea L. and it belongs to the Oleaceae family. Its native habitat is the Mediterranean (Agaudaoud, 1991), and there are more than (40) cultivars of it in Iraq (Genaidy et al., (Genaidy et al., 1991). 2015. Due to the large number of cultivars, olive fruits were divided into oleaginous cultivars (for oil extraction) and table cultivars, in addition to dual-purpose cultivars. The area of olives in the world reached about 10,578,246 hectares, with a production of 19,464,495 tons (FAO, 2021).

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Its fruits are used as food, as is its oil, which is considered one of the best vegetable oils because it protects against atherosclerosis and treats heart disease (Preedy and Watson, 2010). Nanofertilizers have unique properties due to their small size and large surface area, which leads to an increase in the absorption surface and thus an increase. The process of photosynthesis and thus increasing plant production (Singh and Prasad, 2016). As noted by (ALalam and ALalaf, 2020), nanofertilizers, when added in the form of an element or a group, stimulate and increase vegetative growth and the plant's content of mineral elements and increase the effectiveness of nutrient use. Reducing environmental pollution. Biofertilizers help directly or indirectly in achieving food security (Mahmud et al., 2012), as they represent biological sources that contain different living microorganisms such as bacteria, fungi, or both together, as they work to enhance the growth of the root system to increase the ability to absorb water and nutrients. They are characterized by their ability to release nutrients from plant residues and raw materials, prepare them freely for the plant, enhance growth, and provide primary nutrients to the host plant (Hari et al., 2010 and Mosa et al., 2014). Biofertilizers carry different types of microorganisms, including fungi with symbiotic living and mutual benefit. (such as mycorrhizal fungi), and another free-living species in the soil, whose activity increases as a result of plant roots secreting substances that encourage biological activity, as in Osobacter bacteria, which fix atmospheric nitrogen (Hayat et al., 2010). This study was carried out to determine the effect of nanofertilizers and fungal and bacterial biofertilizers, individually or in combination, on the growth of some vegetative and chemical traits of olive seedlings of Ashrasi cultivar.

Materials and methods

The experiment was conducted in la of the lathhouse on farm, Department of Horticulture - Ministry of Agriculture, for the spring season of 2022. To study the effect of spraying nanofertilizers (microelements) and ground treatment with biofertilizers (Mycorrhizal inoculum and Azotobacter inoculum) on some vegetative and chemical traits on olive seedlings of the Ashrasi cultivar. A factorial experiment was carried out using a randomized complete block design (RCBD). The number of experimental parameters was 12 = 4 x 3, with three replications. The experimental unit included 5 seedlings, so the total number of seedlings in replicate became 60 seedlings, and the total number of seedlings in the experiment was 180. The data was analyzed according to the Gen Stat statistical program, and the parameters were compared. According to L.S.D. at a probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000.(

Study factors:

The first factor: nano-fertilizer (IQCOMBI), which contains some minor elements as shown in Table (1). The nano-fertilizer was added sprayed at three concentrations (2.1.0 g.L-1) and four sprays in a season and on dates of 1/3, 20/3, and 10/4. , 4/30 and symbolized by S2,S1,S0.

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Table (1) Contents of nanofertilizers (IQCOMBI)

Element	B	Cu	Fe	Mn	Zn
Percentage	0.2%	0.5%	6%	6%	6%

The second factor: Biofertilizers, which were obtained from the Agricultural/Saffron Research Department of the Ministry of Science and Technology, which included four levels: control treatment (without vaccine) and its symbol F0. The mycorrhizal vaccine, Glomus spp., was prepared at a concentration of 50 spores/g-1 of dry soil, and was coded as F1. And the chroccomus Azotobacte vaccine was prepared at a concentration of 1×910, and 5 ml.L-1 of liquid was added to each plant, and its symbol was F2. The vaccine interaction between the mycorrhizae was 50 spores/ 1 gm - dry soil + Azotobacter bacteria inoculum 5 ml.L1-) and is symbolized by the symbol F3.

The experiment began on 2/18/2022 for the spring season and began by transferring all olive seedlings (a local cultivar, Ashrasi), one year old, selected as homogeneous in size as possible, and contained in plastic bags with their soil, into a pot with a capacity of 7 kg. During the transfer, biofertilizer treatment was applied at its four levels, where The mycorrhizal fungus was added to the pot soil in the area in contact with the roots, and the pot soil was injected with azotobacter bacteria in the area surrounding the roots, as well as the interaction treatment according to the method proposed by (Gerdmann and Nicolson, 1963 and Matysiak and Falkowski, 2010). The addition was according to the experimental parameters for each pot and the filling was completed. The pot is filled with soil made of peat moss and sand at a ratio of 3:1. The first spraying of the nanofertilizer treatment was carried out in the early morning on 3/1/2022 with a 2-litre hand sprinkler until the seedlings were completely wet, while the control treatments were sprayed with water only. The seedlings were watered the day before the spraying process to increase the opening of the stomata, and the concentration of solutes in the leaf cells decreases, thus increasing the penetration of ions from the spraying solution into the wall of the leaf cells (Al-Sahhaf, 1989). All agricultural service operations for the seedlings continued in a homogeneous manner for all seedlings, and the soil was also analyzed. Used by taking a sample to determine the texture of the soil and some chemical and physical properties, Table (2). The readings for the studied characteristics were taken on 1/7 and during the first week of July 2022.

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Table (2) Some physical and chemical properties of thresher soil

Property	Value		Unit
PH: degree of soil reaction	7.1		
EC electrical conductivity	1.35		(ms/cm)
Organic matter	8.49		%
	Available nitrogen		
	NH4	47.6	
	No3	63.2	
Available ions	Available potassium	128.9	ppm
	Available Phosphor	13.2	
	Sand	793	
Soil separators	clay	184	$g.kg^{-1}$
	silt	59	
Soil texture	Sandy loam		

Studies traits in the experiment:

-1Main stem diameter (mm(

The main stem diameter of the seedlings was measured using a Calipers Vernier foot. The seedlings were selected randomly and an average was taken for each experimental unit in one replicate, then an average was extracted for each treatment.

-2Number of leaves/seedling

The number of leaves was calculated for each randomly selected seedling, then an average was extracted for each replicate, and the average was calculated for each treatment.

-3Dry weight of vegetative growth (g. Seedling - 1(

After uprooting the seedlings and separating the shoots from the roots, the shoots were taken and placed in perforated paper bags and placed in an electric oven at a temperature of (70 degrees Celsius) for (48) hours to get rid of moisture until the weight was constant (Al-Sahhaf, 1989), then readings were taken. Dry shoots were measured with a sensitive balance and a rate was extracted for each treatment.

-4Relative content of chlorophyll in leaves (SPAD unit(

The chlorophyll content in the leaves of olive seedlings was estimated using a device (SPAD CCM-200 Plus) from Apogee Instrument Inc., where readings were taken from (6) leaves from each seedling for each experimental unit of each replicate, and an average was extracted for each treatment as measured in SPAD units. (Jemisona and Williams 2006(

-5Percentage of total carbohydrates in leaves

The method of Joslyn (1970) was used to estimate the total carbohydrate contents in the leaves

Results and discussion:

Stem diameter (mm.(

The results in Table (3) showed that the nano-fertilizer spraying treatment significantly affected the main stem diameter. The S2

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spraying treatment outperformed all treatments and gave its highest value of (10.67) mm compared to control treatment S0, which gave its lowest value of (8.17) mm

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Table (3) Effect of nano and bio fertilizer on stem diameter (mm) of olive seedlings (Ashrasi cultivar).

Nano fertilizer	**Biofertilizer**				**average**
	F0	**F1**	**F2**	**F3**	
S0	6.34	8.07	8.77	9.51	8.17
S1	7.63	9.17	10.04	10.37	9.30
S2	8.90	10.42	11.32	12.03	10.67
average	7.62	9.22	10.04	10.63	9.38
LSD*0.05*	S=0.163	F=0.188			F×S= 0.326

Adding the biofertilizer treatment significantly increased stem diameter, and the addition treatment F3 was significantly excelled on all treatments and gave the highest value amounting to (10.63) mm compared to control treatment F0 which gave its lowest value amounting to (7.62) mm. The S2F3 interaction treatment of the factors achieved a significant effect on the stem diameter characteristic, as it gave the highest average for the characteristic, amounting to (12.03) mm, compared to the S0F0 interaction treatment, which recorded the lowest stem diameter, amounting to (6.34) mm.

Number of leaves/seedling

The results of Table (4) showed that the number of leaves trait was significantly affected by spraying with nanofertilizer, as the nanofertilizer spraying treatment S2 excelled on all spraying treatments and recorded the highest average for the trait, reaching (218.08) compared to control treatment S0, which recorded the lowest average number of leaves, reaching (185.17). The addition of the biofertilizer achieved a significant effect on the number of leaves, as the F3 biofertilizer treatment outperformed all addition treatments and gave the highest average number of leaves, reaching (221.11

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Table (4) The effect of nano and bio fertilizer on leaf number of olive seedlings (Ashrasi cultivar).

Nano fertilizer	**Biofertilizer**				**average**
	F0	**F1**	**F2**	**F3**	
S0	168.67	184.33	175.33	212.33	185.17
S1	186.33	204.83	194.67	218.33	201.04
S2	194.67	226.67	218.33	232.67	218.08
average	183.22	205.27	196.11	221.11	201.43
LSD*0.05*	S= 1.520	F =1.755			F×S=3.041

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Compared to control treatment F0, which gave the lowest average for the trait (183.22). The interaction treatment of the factors showed a significant effect on the number of leaves, as the interaction treatment S2F3 had the highest average number of leaves, reaching (232.67), compared to the triple treatment S0F0, which gave the lowest average number of leaves, reaching (168.67.(

Dry weight of vegetative growth (g(

The results in Tables (5) show that the nanofertilizer has a significant effect on the dry weight of the vegetative growth, where the nanofertilizer treatment with a concentration of 2 g.L-1 was excelled on the rest of the treatments and gave its highest value of (22.75 g) compared to control treatment that gave its lowest value of (12.50 g). As for adding biological fertilizers, it had a significant effect on the dry weight of vegetative growth, and the F3 treatment gave its highest value, amounting to (20.11 g), compared to control treatment, which recorded its lowest value, amounting to (15.44 g.(

Table (5) Effect of cultivar, nanofertilizer, and biofertilizer on the dry weight of vegetative growth of olive seedlings (Ashrasi cultivar).

Nano fertilizer	**Biofertilizer**				**average**
	F0	**F1**	**F2**	**F3**	
S0	10.67	12.00	13.33	10.67	12.00
S1	15.67	17.33	18.00	15.67	17.33
S2	20.00	22.67	21.00	20.00	22.67
average	15.44	17.33	17.44	20.11	17.58
LSD*0.05*	S= 0.740		F= 0.855		F×S=1.480

Table (5) showed that the interaction between the experimental treatments had a significant effect on the dry weight of vegetative growth. The S2F3 treatment gave its highest value, amounting to (27.33 g), compared to the no-add treatment, which recorded its lowest value, amounting to (10.67 g.(

Relative content of chlorophyll in leaves (SPAD unit(

The results in Tables (6) showed that there is a significant effect of spraying with nano-fertilizer on the relative content of chlorophyll, as it was observed that the nano-fertilizer spraying treatment S2 was significantly superior and gave the highest average of (SPAD 55.58) compared to control treatment S0 which gave the lowest average of (48.25 SPAD). As for the biofertilizer addition parameters, it was found that there was a significant effect of the addition on the relative content of chlorophyll

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Table (6) Effect of nano and bio fertilizer on the relative content of chlorophyll of olive seedlings (Ashrasi cultivar).

Nano fertilizer	Biofertilizer				average
	F0	**F1**	**F2**	**F3**	
S0	42.67	47.00	49.67	53.67	48.25
S1	45.33	50.33	53.00	56.67	51.33
S2	47.67	53.67	57.00	64.00	55.58
average	42.22	50.33	53.22	58.11	51.72
LSD*0.05*	S= 0.685	F= 0.791			F×S=1.369

the adding treatment F3 created a significant increase in the length of the main root, it was excelled and recorded the highest average for the trait (58.11 SPAD) compared to control treatment F0, which gave the lowest average (45.22 SPAD). Table (5) showed the significant effect of the interaction treatment between treatments on the relative content of chlorophyll, where the S2F3 treatment excelled significantly and gave the highest average for the trait, which was (64.00 SPAD), compared to the S0F0 treatment, which recorded the lowest average, which was (42.67 SPAD.(

Percentage of total carbohydrates in leaves.

The results in Table (7) showed that spraying with nanofertilizer had a significant effect on the percentage of carbohydrates, as the nanofertilizer spraying treatment S2 achieved the highest percentage of carbohydrates, superior to the rest of the spraying treatments and amounting to (20.39%) compared to control treatment S0, which gave the lowest amount of (15.93). %). The results in Table (7) indicated that the biofertilizer treatment had a significant effect on the percentage of carbohydrates, so the F3 treatment excelled and gave the highest average of (19.89%) compared to control treatment, which recorded a lower average of (15.59%). It was found that the interaction treatment had a significant effect on the percentage of carbohydrates if it recorded the highest average amounting to (23.20%) compared to the S0F0 treatment which recorded the lowest percentage of carbohydrates amounting to (14.43%.(

Table (7) Effect of nano and bio fertilizer on the percentage of total carbohydrates in the leaves of olive seedlings (Ashrasi cultivar).

Nano fertilizer	Biofertilizer				average
	F0	**F1**	**F2**	**F3**	
S0	14.43	15.41	16.43	17.46	15.93
S1	15.20	16.79	17.84	19.02	17.21
S2	17.16	19.40	21.80	23.20	20.39
average	15.59	17.20	18.69	19.89	17.84
LSD*0.05*	S=0.396	F=0.458			F×S= 0.792

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Discussion:

The results reached in Tables (3-7) during the spring season of 2022 indicated that the spraying treatment with nano-fertilizer and biofertilizer and the interaction between them had a significant effect on all the studied traits of vegetative growth and the leaves' content of some chemical traits, and the reason may be due to Nanofertilizers are carriers of nutrients with sizes of 1-100 nanometers. When the size is reduced, the surface area increases very significantly and thus it is able to enter the plant cells through the stomata in the leaves easily (Prasad et al., 2012 and Hong et al., 2013). This was confirmed by (Sekhon, 2013). 2014) that nano-fertilizer leads to an increase in photosynthesis because it has unique characteristics that lie in its small surface area and high absorption. The reason is also due to the added fertilizer mixture that was sprayed and contains some nutrients from microelements, as it stimulated the growth of seedlings by enhancing physiological and biological processes, such as increasing the efficiency of photosynthesis and structural activities, thus improving plant growth (Abdel-Aziz et al., 2018), and it may return The reason is the role of iron contained in the fertilizer mixture, which leads to improving plant growth, both vegetative and radically. This is through regulating vital activities and increasing the efficiency of photosynthesis. It activates enzymes involved in many physiological processes, such as nucleic acids, amino acids, and energy compounds, and thus stimulates cell divisions and elongation. It stimulates cambium activity, so vegetative growth rates increase (Mahil, 2019; Gyana and Sunita, 2015). It also participates in building and activating Although it is not part of the chlorophyll pigment, it acts as an enzyme companion. It is also involved in the formation of porphyrin compounds that make up the chlorophyll pigment, and it works on the synthesis of chloroplasts, which are highly important in the process of photosynthesis, which play a role in producing carbohydrates for plants (Nadia et al., 2013, Gyana and Sunita). , 2015). Or it may be due to the role of zinc in that it forms the amino acid trypophan, which is responsible for the formation of IAA and prevents its oxidation, as it encourages cell division, elongation and expansion, affects the plasticity of the cell wall, reduces the resistance of the wall to tension, and increases the water permeability of the cells, increasing the size of the cells and thus increasing the vegetative growth of the plant. From the number of leaves, stem diameter, and wet and dry weight of seedlings, it is involved in the formation of chlorophyll pigment through its direct effect on the process of forming amino acids and energy compounds. It also builds carbohydrates (Aloni et al., 2006, Marschner, 2012, Hafeez et al., 2013). Copper works by increasing activity. The physiological function of the plant is also involved in the formation of plastids and enzymes and plays an important role, directly and indirectly, in the formation of the chlorophyll pigment, protecting it from premature destruction, and maintaining its stability, as there is about 70% of copper in the plastids of plant leaves, and it has a role in the manufacture of carbohydrates and sugars (Odeh and Shamsham, 2011). As for manganese, it regulates the processes of photosynthesis necessary for plant growth and development and increases the efficiency of its work, through its participation with chlorine in the analysis of the water molecule and the formation of amino and nuclear acids and

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respiratory enzymes. Thus, it increases cell activity and raises the efficiency of the plant's vegetative growth. It is included in the formation of carbohydrates and is characterized by the fact that it It participates in the process of absorbing nitrogen, which is involved in the formation of chlorophyll and the construction and production of carbohydrates (Al-Tahafy, 2004, Mousawi et al., 2011, and Iyad, 2018). This agreed with (Jaber, 2022) when spraying fig seedlings with nanofertiliser, microelements. He agreed with (Al-Taie, 2020). Adding a biofertilizer, whether fungal or bacterial, singly or in combination, may be a reason for improving the vegetative growth and chemical content of olive seedlings. This may be due to the role of the added fungi, represented by the mycorrhizal fungus, which actually plays a role in stimulating the growth of seedlings by increasing the efficiency of the plant's vital activities. As a result of the symbiotic relationship between the host and the fungus and improving the root system of the plant and increasing the absorption of micro and macro nutrients and passing them to the plant cells, the fungus colonizes the roots of the plant and grows and coexists, taking the carbohydrates, amino acids and vitamins it needs from the plant, and in return it increases and improves the absorption of the macro and micro nutrients it needs. The plant by increasing the surface area of the roots of the host plant through fungal hyphae, which act as root hairs and act as an auxiliary agent to the root system, extending outward around the root if they reach more than (8 cm) than the host's root hairs reach and thus increase the efficiency of nutrients, and highlight The importance of mycorrhizal fungi is their ability to absorb phosphorus in their walls through their secretion of the phosphatase enzyme, which leads to an increase in the solubility of phosphorus and the ease of its absorption by the roots and its transfer to plant cells (Siddiqui et al., 2008; Hemalatha et al., 2010; Kolta and Yoram, 2010; Hussain et al., 2015; Cabanás, et al. ,2018.(

Phosphorus is involved in the processes of photosynthesis and energy production through the formation of the energy compounds ATP and NADPH2, and is involved in the formation of nucleic acids (RNA), which are important in the process of protein formation. It increases the physiological processes, giving the plant additional growth power, increasing the number of branches, and helping to increase the efficiency of photosynthesis. And the construction of plastids increases the production of chlorophyll pigment in the leaves (Abu Dahi and Al-Younis, 1988). Likewise, the reason for the high rates of vegetative growth and the content of leaves of chlorophyll and carbohydrates may be due to the influence of the oozobacteria, as their importance in fixing atmospheric nitrogen is highlighted, thus providing olive seedlings with this element necessary for the vital processes of the plant, as it participates with magnesium in the formation of the chlorophyll molecule and is involved in the processes of building carbohydrates and forming Amino acids that are involved in the formation of proteins (Hayat et al., 2010) and work on the formation of enzymes necessary for the vital processes of the plant, in addition to being involved in the formation of energy compounds, which leads to an increase in the entire process of photosynthesis, which leads to the number of leaves and an increase in dry matter in the leaves (Wesang et al., 2013) and this is what El-Khashab Abou (2003) agreed with when inoculating olive seedlings with

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bacteria. He agreed with (Al-Abbasi and Al-Zuhairi, 2018(

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