Effect of Biofertilizer and Nitrogen nano Fertilizer Spraying on Anatomical Qualities of Shaddock and Grapeferuit Seedlings

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Abstract:
The experiment was conducted in the lath house of the Department of Horticulture and Garden Engineering of the Faculty of Agriculture–University of Tikrit in the agricultural season 2023 to study the response of shaddock and grapefruit seedlings to bio-addition and spraying with nitrogen nan fertilizer, where 108 seedlings of shaddock and grapefruit were brought on a homogeneous sized with 1.5 years old . The results obtained can be summarized as follows: The spraying of nitrogen nanofertilizer with a concentration of (3 ml L-1) resulted in a significant increase in both the length * width of the bottom surface of the normal cells, reaching 178.6 µm, the average thickness of theupper cuticle reached 4.389 µm, the average thickness of the lower cuticle reached 4.500 µm, the average thickness of the upper skin reached 22.17 µm, the average thickness of the lower skin reached 22.00 µm, and the average thickness of the mesophilic tissue reached 238.33 µm . The addition of biofertilizer at a concentration of (12 g seedling -1) led to a significant increase in both the average length * width of the bottom surface of ordinary cells, which amounted to 151.3 µm , and a modification in the thickness of the mesophyll tissue, which amounted to 235.67 µm. When adding biofertilizer concentrate (6 g seedling-1), the increase was in the rate of increase in the thickness of theupper cuticle as it reached 4.175 µm, and the average thickness of the lower cuticle as it reached 4.022 µm, and the average thickness of the upper cuticle as it reached 21.28 µm adjusted in the thickness of the lower cuticle as it reached 19.61 µm. The Interaction between biofertilizer and nitrogen nanofertilizer, especially at concentration (12 g seedling -1with 3 ml -1), led to a clear moral effect in both the length * width of the bottom surface of the normal cells, which was 188.8 µm ,and the average thickness of the mesophyll tissue, which was 245.50 µm .

Keywords: Shaddock , Grapefruit, seedlings, bio-fertilizers, nanofertilizer.

Introduction:

Citrus is one of the evergreen fruit trees belonging to the family Rutaceae, which includes several genera, the most important of which are the genus Citrus , the genus Fortunella, and the genus Poncirus. The species belonging to the genus Citrus are widely distributed throughout the world due to their adaptation to a wide range of environmental conditions. The genus Citrus includes four groups, namely the orange group, the Indian lemon group, the tangerine group , and the acid group, and each group includes a number of species that include many varieties and breeds (2004 , Zhang, Ismail. (

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The grapefruit trees citrus is one of the citrus fruits belonging to the family Rutaceae ,which is characterized by the presence of oil glands in most of its parts, which gain the special aromatic smell that distinguishes them from the rest of the other types of fruit, their fruits are of a special type of grapefruit Berry called Hesperidium (Alkhafaji et al.,1990), and citrus fruits are of great importance among fruit trees for their nutritional, medical, environmental and economic importance, and are rich in vitamins, especially vitamin C , as well as rich in mineral elements and are considered one of the rich andnecessary sources of building a human body (Ahmed and David , 2020and Dongre et al.,2023. (

An effective means of addressing the problems of some soils and increasing their productivity is the use of biofertilizers, which are a food source for the plant and are cheap and environmentally safe compared to chemical fertilizers. In addition , they increase the efficiency of the use of chemical fertilizers in nutrient-deficient soils. Chauhanet et. al., (2010) found that bio-composting is the most promising alternative in reducing the use of chemical fertilizers and reducing the sources of environmental pollution. It is done by adding bio-vaccines to the soil, seedlings or seeds, and it is complementary to chemical and organic fertilizers. It is used in many countries of the world such as America, India and Russia (Lopez et al., 2008). Alwan et al. (2011) indicated that biofertilizers cannot be used as a substitute for chemical fertilizers, but rather they are fertilizers complementary to mineral fertilizers , as they contribute to increasing the effectiveness and efficiency of chemical fertilizers in low-fertility soils , as well as being an important means of preserving the environment and its role in improving the quality of the crop compared to the use of chemical fertilizers.Biofertilizers also positively affect the resistance of cannabinoids to pathogens, in addition to their direct impact as fertilizers for plantsand provide the nutrients necessary for plant growth, the most important of which are nitrogen , carbon, hydrogen, oxygen, phosphorus and sulfur to be a food source for plants (Wambacq et al., 2022 and Amen and AL-Hamdani,2022.(

It must be noted that nanofertilizers are important in the agricultural field , and through this they reduce inputs and increase outputs(plant pesticides, insecticides and fertilizers), especially the low economic cost as a result of the low incidence of epidemic diseases in various crops , in addition to increasing the efficiency of manufactured fertilizers, their low cost and the resistance of the agricultural product to environmental conditions (Mehrotra et al. , 2010, Al-Dujaili and Al-Hamdani,(2024) , and nanotechnology provides materials and nanoscale devices that have a key role in agriculture, for example, nanoscale biosensors to detect moisture content, as well as the nutrient status in soil and water management (Silva et al., 2011).Therefore, the uptake and accumulation of nanomaterials depends mainly on the type of plant and the chemical composition and size of nanoparticles (Khiew et al.2011). Secondary molecules help eliminate bacterial , fungal , and viral diseases that improve vegetative and germinal growth traits (Abedi and Naderi, 2012) . Among Mishra et al. (2017), mineral fertilizers are the key to food production despite low nutrient absorption efficiency and high losses. However , nanotechnology can enhance crop productivity and reduce nutrient loss . This has led to increased interest in nanofertilizers and NATO-backed fertilizers. Hence the concept

of fertilizers (Dimkpa and Bindraban, 2017). Nanofertilizers may be the best thing to happen in modern agriculture , because they have the ability to improve soil fertility and provide nutrients (Prasad et al. , 2017; Al-Hamdani and Al-Jubouri ,2020).
The aim of the research is to know the response of shaddock and grapefruit seedlings to adding biofertilizer and spraying with different concentrations of nano-nitrogen fertilizer and to know their effect on anatomical characteristics.

Materials and methods

The experiment was carried out in the lath house of the Department of Horticulture and Garden Engineering of the Faculty of Agriculture – University of Tikrit. In the 2023 growth season, 108 seedlings of Shaddock and Grapefruit were used in the size of one and a half years old and grafted on the Rootstock sour orange (Citrus aurantium L(.

from one of the private nurseries in Balad and planted inside plastic pots with a capacity of (5) kg , and then transferred to larger plastic pots with dimensions of (20cm in diameter and 30cm in height) with an anchor capacity of (15) kg and a medium of cultivation containing mixed soil + sodomy with a volume ratio of 2:1 sodomy: sodomy.

The study included the following factors:

a- The first factor included and symbolized Grapefruit and Shaddock seedlings (V1, V2) in succession

b- The second factor is ground fertilization with the BioHealth biostimulant (it is water-soluble granules containing 75% Humic acid, 10% Trichoderma harzinum fungus, Bacillus subtillus bacteria, 5% marine algae extract, and 10% moisture. It is produced by the German company HUMINTECH)is biofertilizer at levels of (12, 6, 0) g. Sapling-1 (B0, B1, B2) sequentially.

c- Factor III nitrogen nanofertilizer with levels of (3, 1.5, 0) ml-1 and its symbol (N0, N1, N2) respectively

The seedlings were sprayed in the morning until completely wet using a 2-liter hand spray on the same day that the biofertilizer was added . The first spray was on 2/2. The second spray was 21 days after the first and third spray at the same time.

Soil samples were taken from the soil from the center of agriculture randomly and the mixture was mixed completely and 1 kg was taken from it and sent to the laboratory of the Department of Soil and Water Resources at the Faculty of Agriculture – University of Tikrit to analyze the sample and know its chemical characteristics, and the seedlings were divided into three main repeats, where one repeater included (18) treatments and each experimental unit containing 2 seedlings. The total number of seedlings in the experiment was 108 seedlings. The soil service process was carried out each of hoeing, weeding and controlling insects and diseases when needed. The experiment was conducted according to the Split-Plot Design system by three iterations and the transactions were distributed randomly using the RCBD design.

The traits studied :

Anatomical Characteristics :

Preparation of Transverse Sections

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The cross-sections of the species under study (leaves) were studied based on soft samples that are easy to obtain from the field by immersing them in NaOH solution at a concentration of 1% for (24-48) hours, then rinsing with distilled water to remove the base solution from them and then fixing them in FAA solution for (18-24) hours. When used, they are rinsed with 70% alcohol and passed with the rest of the steps or the dry samples are boiled with water for (5) minutes and then transferred to 70% alcohol until they are used , and the sections were attended according to the following steps

Fixation: Soft parts of the paper were taken and cut into small pieces ranging in length from (2-5) mm , then transferred to small bottles Vials and placed in them about 20 milliliters of the stabilizer solution Formaldehyde Acetic Alcohol, which was prepared according to the Johnson method (Johnson, 1940) for a period of between (18-24) hours

(1 Washing and Dehydration: The samples were washed twice with 70% ethyl alcohol to ensure that the traces of the stabilizer were removed and preserved in 70% alcohol, and the water was disposed of by passing the samples in an ascending series of different concentrations of ethyl alcohol (80% , 90%, 95%) two hours at each concentration , then placed in absolute ethyl alcohol for two hours to dispose of the remaining water in the preserved samples .

(2 Clearing and Infiltration: After the process of washing and infiltration, the process of clarification and infiltration was carried out by passing the samples with a series of absolute ethyl alcohol and xylene in volumetric proportions (1:3 , 1:1, 3:1) and then with pure xylene for two hours each according to the SAS method (Sass, 1958), then half of the xylene containing the samples was poured and instead a quantity of liquid paraffin was added in the oven at a temperature of (55-60)OML for one hour in order to replace the evaporated xylene, then paraffin was poured and instead pure liquid paraffin was added inside the oven , after that the samples were left in the oven for a period of (4-5) days to remove traces of xylene , then paraffin was poured and instead of it pure liquid paraffin and the samples were left in the oven for two hours (this process was repeated 5-6 times). The last time I left a full night,(Al-Mashhadani, 1992 (

(3 Embedding and Mounting: Custom molds of suitable sizes were prepared and a quantity of hot molten wax was poured into them at a degree of (60-55) Om, and the samples were transferred to square-shaped molds and placed in the desired direction and the samples were marked and left in a cold place for a full day to ensure sufficient hardness, then the wax molds were removed while retaining their identities and thus became ready for cutting , and then the wax molds were installed on wooden pieces designated as carriers after being trimmed using a blade for that purpose until the molds became parallel to the rectangles to be ready for cutting with the rotary microtome type Bright and thickness (10-15) micrometers.

After cutting, the tapes containing the samples were placed in a water bath with a temperature ranging between (40-45) Om in order to flatten the tapes , and then the tapes were carried on clean glass slides pre-painted with a thin swab of Glycerin-Albumin adhesive and the slides were placed on a hot plate with a degree of (40-45) Om for a period of (4-12) hours for the purpose of fixing the tapes on the slides and removing wrinkles.

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i- Dewaxing and Staining: Based on what was mentioned by Sass, (1958. (

The slides then carried a permanent mounting by placing a drop of adhesive (Canada balsm) on the clips, gently placing the Cover Slide and transferring the slides to a hot plate with a temperature of (40-45) O for a full day to be dried . Then I examined the clips under the Olympus composite light microscope and photographed with the camera that was installed on the composite microscope.

Epidermis Preparation

I prepared the skin from soft samples, it took part of the middle of the full-growth paper so that it included the middle sweat, part of the blade and the edge, and I used the stripping off method to get the upper and lower skin using two-tip tongs and brushed the skin on a glass slide and put a drop of glycerin on it, then I covered it with the slide cover and then it was ready for examination and study . The models were examined under the Olympus type composite microscope using the inserted ophthalmic lens scale, and (5-10) skin cells were measured, and the dimensions of the radial and transverse walls and the shape of the Ordinary Epidermal Cells were studied, and the dimensions of the guard cells and the shape of the stoma complexes were studied, and the stoma area was calculated and then the stoma was repeated according to the equation :

Vent frequency = number of vents in mm2. According to Stace (1965 ,(

Capillary frequency = the number of capillaries in mm 2 and the terms in Dilcher (1974) were used.

The transparent wax coating method of the surface layer of the skin of the leaves was also used to study the phenotypic qualities of stoma and Khazraj normal skin cells (Al,2019 (

Results and Discussion

-1 Length * width of the bottom surface of ordinary cells (µm(

The results shown in Table (1) show that theitem did not achieve significant differences in the length * width of the bottom surface of the normal cells . As for nitrogen nanofertilizer spraying, it achieved significant differences in the average length * width of the bottom surface of ordinary cells ,

as the transaction N2 gave the highest rate of 178.6 µm, followed by a significant difference over the transaction N1, which gave a rate of 138.0 µm, while the transaction N0 gave the lowest rate of 98. 3 µm. It is noteworthy from the results of the same table that there are significant differences as a result of the addition of biofertilizer, as the transaction B2 recorded the highest rate of 151.3 µm, followed by a significant difference in the transaction B1 by giving it a rate of 135.6 µm, while the transaction B0 gave the lowest average length * width of the bottom surface of ordinary cells, as it reached 128.0 µm. The results of the same table indicate the moral impact of the Interaction between the variety and the spraying with nitrogen nanofertilizer, as the transaction V2N2 gave the highest rate in the length * width of the bottom surface of the normal cells, which was 185.0 µm, while the transaction V2N0 gave the lowest rate of 96.2 µm. The bilateral Interaction between the variety and the biofertilizer had a significant impact, as the transaction V2B2 gave the highest rate of 153.7 µm, while the transaction V1B0 gave the lowest rate of 124.3 µm .

As for the bilateral Interaction between nanofertilizer and biofertilizer, the results of Table (1) showed that there were significant differences in the average length * width of the bottom surface of normal cells, as the transaction N2B2 achieved the highest rate of

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increase of 188.8 µm, while the comparison transaction N1B1 gave the lowest rate of 86.8 µm. As for the triple Interaction of research transactions, the results showed that there were significant differences as a result of this Interaction , as the transaction V2N2B2 gave the highest rate of increase in the length *width of the bottom surface of normal cells amounted to 195.0 µm, while the transaction V1N0B0 gave the lowest rate of 85.0 µm .

Table 1. Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the length *width of the bottom surface of the normal cells of seedlings of Shaddock and crepefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			N×V
		B_0	B_1	B_2	
v_1	N_0	85.0	96.0	120.0	100.3
	N_1	125.0	133.0	144.0	134.0
	N_2	163.0	171.0	182.7	172.2
V_2	N_0	90.0	77.7	121.0	96.2
	N_1	134.0	147.0	145.0	142.0
	N_2	171	189	195	185.0
Effect of Class (V)					
B×V	V_1	124.3	133.3	148.9	.135
	V_2	131.7	137.9	153.7	141.1
Nano-Nitrogen Effect (N)					
B×N	N_0	87.5	86.8	120.5	98.3
	N_1	129.5	140.0	144 5	138.0
	N_2	167.0	180.0	188.8	178.6
Effect of biofertilizer(B)		128	.135	151.3	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**
23.13	16.35	13.35	13.35	9.44	9.44	N.S

-2 Average thickness of upper and lower cuticle (µm(

The results shown in Table (3and 2) show that the variety did not achieve a significant increase in the thickness of the upper and lower cuticle.

As for manure spraying with nitrogen nanoparticles, it achieved a significant increase in the thickness of the upper and lower cuticle , as the transaction N2 gave the highest rate of 4.389 and4.500 µm, followed by a significant difference from the transaction N1 by giving it a rate of 3.631 and3.639 µm, while the transaction N0 gave the lowest rate of 3.056 and 2.950 µm. It is noteworthy from the results of the same table the significant differences as a result of the addition of biofertilizer, as the transaction B1 recorded the highest rate of increase in the thickness of the upper and lower cuticle as it reached 4.175 and 4.022 µm, followed by a significant difference in the transaction B2 by giving it a rate of 3.706 , 3.894 and 3.172 µm, while the transaction B 0 gave the lowest rate of theupper cuticle as it reached 3.194 µm .

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The results of the same table also indicate the moral impact of the Interaction between the variety with the spraying with nitrogen nanofertilizer, as the treatment V2N2 and V1N2 gave the highest rate of increase in the thickness of the upper and lower cuticle of 4.767 and4.567 µm, while the treatment V1N0 gave the lowest rate of 3.033 and 2.733 µm. The bilateral Interaction between the variety and the biofertilizer had a significant impact, as the transaction V2B1and V1 B1 gave the highest rate of upper and lower cuticle thickness of 4.411 and 4.078 µm, while the transaction V1B0 gave the lowest rate of 3.011 and 3.144 µm . As for the bilateral Interaction between nanofertilizer and biofertilizer, the results of Table (3and 2) showed that there were significant differences in the increase in the thickness of the upper cuticle, as the treatment N2B1 achieved the highest rate of 5.333 and 5.533 µm, while the comparison treatment N0B0 gave the lowest rate of 1.900and 2.100 µm. As for the triple Interaction of the research coefficients, the results shown in Table (2) showed that there were significant differences as a result of this Interaction , as the transaction V2N1B1 and V1N2B1 gave the highest ratein the thickness of the upper cuticle of 6.000 and 5.967 µm, while the transaction V1N0B0 gave the lowest rate of 1.800 and2,000 µm.

Table 2. Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the average upper cuticle thickness of seedlings of Shaddock and crepefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			N×V
		B_0	B_0	B_0	
v_1	N_0	1.800	3.200	4.100	3.033
	N_1	3.133	4.033	3.267	3.478
	N_2	4.100	3.150	4.100	4.767
V_2	N_0	2.000	4.000	3.233	3.078
	N_1	4.100	6.000	4.200	3.783
	N_2	4.033	4.667	3.333	4.011
Effect of Class (V)					
B×V	V_1	3.011	4.411	3.856	3.759
	V_2	3.378	3.939	3.556	3.624
Nano-Nitrogen Effect (N)					
B×N	N_0	1.900	3.600	3.667	3.056
	N_1	3.617	3.592	3.683	3.631
	N_2	4.067	5.333	3.767	4.389
Effect of biofertilizer(B)		3.194	4.175	3.706	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**
0.737	0.521	0.425	0.425	0.301	0.301	N.S

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Table 3. Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the average thickness of the bottom quota of the seedlings of Shaddock and grapefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			
		B_0	B_0	B_0	
v_1	N_0	2.000	3.000	3.200	2.733
	N_1	4.100	3.267	4.100	3.822
	N_2	3.333	5.967	4.000	4.433
V_2	N_0	2.200	3.300	4.000	3.167
	N_1	3.300	3.500	3.567	3.456
	N_2	4.100	5.100	4.500	4.567
Effect of Class (V)					
B×V	V_1	3.144	4.078	3.767	3.663
	V_2	3.200	3.967	4.022	3.730
Nano-Nitrogen Effect (N)					
B×N	N_0	2.100	3.150	3.600	2.950
	N_1	3.700	3.383	3.833	3.639
	N_2	3.717	5.533	4.250	4.500
Effect of biofertilizer(B)		3.172	4.022	3.894	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**
0.753	0.532	0.435	0.435	0.307	0.307	0.251

-4 Average thickness of upper and lower skin (µm(

The results shown in Table (4) show that the category achieved a significant increase in the thickness of the upper and lower skin, as the categoryV2 achieved the highest rate in the thickness of the upper and lower skin, reaching 20.56 and19.48 µm, while the category V1 gave the lowest rate in the thickness of the upper and lower skin, reaching 19.52 and 18.22 µm.

As for the percentage of spraying with nitrogen nanoparticle fertilizer, it achieved significant differences in the thickness of the upper and lower skin, as the transaction N2 gave the highest rate of 22.17 and 22.00 µm, followed by a significant difference of the transaction N1 by giving it a rate of 20.83 and19.00 µm, while the transaction N0 gave the lowest rate of the thickness of the skin on the lower and 17.11 and 15.56 µm.

It is noteworthy from the results of the same table that there is a significant difference as a result of the addition of biofertilizer, as treatment B1 recorded the highest rate of skin thickness at 21.28 and 19.61 µm , followed by a significant difference in treatment B2 by giving it a rate of 20.33 and19.11 µm, while treatment B0 gave the lowest rate at 18.50 and 17.83 µm .

The results of the same table indicate the moral impact of the Interaction between the variety with manure spraying with nitrogen nanoparticles, as the treatment V2N2 gave the highest rate in the thickness of the upper and lower skin of 23.00and23.00 µm , while the

treatment V1N0 gave the lowest rate of 16.56 and 15.00 µm.

The bilateral Interaction between the variety and the biofertilizer had a significant impact, as the transaction V2B1 gave the highest rate of thickness of the skin on the top and bottom 21.67 and20.22 µm, while the transaction V1B0 gave the lowest rate of 17.33 and17.00 µm .

As for the bilateral Interaction between nanofertilizer and biofertilizer, the results of Table (5 and 4) showed that there are significant differences in the thickness of the skin on the top and bottom, as the treatment N1B1 and N2B1 achieved the highest rate of 24.00 µm, while the comparison treatment N0B0 gave the lowest rate of 15.50and 13.50 µm.

As for the triple Interaction of the research coefficients, the results showed that there were no significant differences for both workers in the thickness of the upper skin, as the coefficients gave V1N2B1 and V1 N2B1 a rate of 24.00 µm in the thickness of the skin. As for the thickness of the lower skin, the treatment V2N2B1 gave the highest rate of 25.00, while the treatment V1N0B0 gave the lowest rate of 15.00 and12.00 µm.

Table 4 Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the upper skin thickness of Shaddock seedlings and grapefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			N×V
		B_0	B_0	B_0	
v_1	N_0	15.00	16.67	18.00	16.56
	N_1	19.00	22.00	21.00	20.67
	N_2	18.00	24.00	22.00	21.33
V_2	N_0	16.00	19.00	18.00	17.67
	N_1	21.00	22.00	20.00	21.00
	N_2	22.00	24.00	23.00	23.00
Effect of Class (V)					
B×V	V_1	17.33	20.89	20.33	19.52
	V_2	19.67	21.67	20.33	20.56
Nano-Nitrogen Effect (N)					
B×N	N_0	15.50	17.83	18.00	17.11
	N_1	20.00	22.00	20.50	20.83
	N_2	20.00	24.00	22.50	22.17
Effect of biofertilizer(B)		18.50	21.28	20.33	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**
2.65	1.87	1.53	1.53	1.08	1.08	0.88

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Table 5. Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the lower skin thickness of the seedlings of Shaddock and crepefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			N×V
		B_0	B_0	B_0	
v_1	N_0	12.00	15.00	18.00	15.00
	N_1	20.00	19.00	17.00	18.67
	N_2	19.00	23.00	21.00	21.00
V_2	N_0	15.00	17.00	16.33	16.11
	N_1	19.00	18.67	20.33	19.33
	N_2	22.00	25.00	22.00	23.00
Effect of Class (V)					
B×V	V_1	17.00	19.00	18.67	18.22
	V_2	18.67	20.22	19.56	19.48
Nano-Nitrogen Effect (N)					
B×N	N_0	13.50	16.00	17.17	15.56
	N_1	19.50	18.83	18.67	19.00
	N_2	20.50	24.00	21.50	22.00
Effect of biofertilizer(B)		17.83	19.61	19.11	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**
2.56	1.81	1.48	1.48	1.04	1.04	0.85

-5 Average thickness of mesophyll tissue (µm(

The results of Table (6) show that thecategory achieved significant differences in the average thickness of the mesophyll fabric, as the category V2 achieved the highest average thickness of the mesophyll fabric, which reached 234.07 µm, while the category V1 gave the lowest average, which reached 217.22 µm.

As for the percentage of spraying with nitrogen nanoparticle fertilizer, it achieved morale in the thickness of the mesophyll fabric, as the transaction N2 gave the highest rate of 238.33 µm, followed by a significant difference in the transaction N1 by giving it a rate of 223.78 µm, while the transaction N0 gave the lowest rate of the thickness of the mesophyll fabric reached 214.83 µm. It is noteworthy from the results of the same table the significant differences as a result of the addition of biofertilizer, as the transaction B2 recorded the highest rate of thickness of mesophyll tissue as it reached 235.67 µm, followed by a significant difference from the transaction B 1 by giving it an average of 228.83 µm, while the transaction B0 gave the lowest rate of thickness of mesophyll tissue as it reached 212.44 µm .

The results of the same table also indicate the moral impact of the Interaction between the variety and the spraying with nitrogen nanofertilizer. The coefficient of figs V1N2 and V2N 2 gave the highest average thickness of the mesophyll tissue of 238.33 µm, while the transaction V1N0 gave the

lowest rate of 203.00 µm. The bilateral Interaction between the variety and the biofertilizer had a moral impact. The transaction V2B2 gave the highest average thickness of the mesophyll tissue of 244.00 µm, while the transaction V1B0 gave the lowest rate of 206.67 µm.

As for the bilateral Interaction between nanofertilizer and biofertilizer, the results of Table (6) showed that there are significant differences in the thickness of the mesophyll tissue, as the treatment N2B2 achieved the highest rate of 245.50 µm, while the comparison treatment N0B0 gave the lowest rate of 189.50 µm.

As for the triple Interaction of the research transactions, the results shown in Table (6) showed that there were significant differences as a result of this Interaction , as the transaction V2N 2 B 2 gave the highest rate of 257.00 µm, while the transaction V1N0B0 gave the lowest rate of 185.00 µm.

Table 6. Effect of Biofertilizer and spraying with Nitrogen nanofertilizer and the Interaction between them in the mesophyll of Shaddock seedlings and grapefruit.

Class (V)	Nano-Nitrogen (N)	Biofertilizer			N×V
		B_0	B_0	B_0	
v_1	N_0	185.00	197.00	227.00	203.00
	N_1	209.00	201.00	221.00	210.33
	N_2	226.00	255.00	234.00	238.33
V_2	N_0	194.00	246.00	240.00	226.67
	N_1	230.67	246.00	235.00	237.22
	N_2	230.00	228.00	257.00	238.33
Effect of Class (V)					
B×V	V_1	206.67	217.67	227.33	217.22
	V_2	218.22	240.00	244.00	234.07
Nano-Nitrogen Effect (N)					
B×N	N_0	189.50	221.50	233.50	214.83
	N_1	219.83	223.50	228.00	223.78
	N_2	228.00	241.50	245.50	238.33
Effect of biofertilizer(B)		212.44	228.83	235.67	

LSD

B×N×V	**B×N**	**B×V**	**N×V**	**B**	**N**	**V**

DISCUSSION

The improvement of the qualities studied in general as a result of treatment with nitrogen nanofertilizer may be due to the role of nitrogen fertilizer in increasing the majority of the physiological qualities of the plant, which reflects positively on the increase in the number of stoma and thus increase the frequency of stoma on the upper and lower surfaces.

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Or it may be attributed to the physiological role of nitrogen in the formation of amino acids, including the amino acid Tryptophane, which is the starting compound in the construction of oxuin that have a role in encouraging cell divisions and elongation, as well as its involvement in the formation of accompaniments and enzymatic aids such as NADP and nad necessary in the transfer of energy and availability . This may be attributed to the high response of mineral fertilization to the readiness of nitrogen in the soil solution, which improves the vegetative growth qualities to reflect on the anatomical qualities, as well as to the role of mineral fertilizer of the plant to carry out its vital activities, the most important of which is photosynthesis to reflect positively on vegetative and anatomical qualities (Taiz and Zeiger,(2012.(

It is also noted that nanofertilizers have a clear effect on the vegetative qualities studied. The reason may be due to the unique characteristics of nanofertilizers due to their small size, which makes it possible for them to be absorbed more efficiently by the plant, as well as increasing their surface area, which increased the absorption surface and their direct entry into the plant cells (Sabir et al., 2014), thus increasing the anatomical characteristic. These results are consistent with Al-Hamdani et al.,(2018), Al-Dujaili and Al-Hamdani,(2024 (

As for the impact of biofertilizer, it may be attributed to the fact that biofertilizer increases the concentration of some nutrients in the soil, especially nitrogen and phosphorus, through multiple mechanisms that increase the concentration of nitrogen and phosphorus in the plant to increase carbohydrates and protein in the plant, and thus reflect on vegetative growth traits and thus on anatomical traits (Amen and AL-Hamdani , 2022. (

This demonstrates the importance of biofertilizers (bacteria and fungi) in stimulating root growth through their secretion of growth regulators that increase cell division and form a good root pool that helps the plant absorb nutrients to carry out its vital processes (Pathak et al.,2017.(

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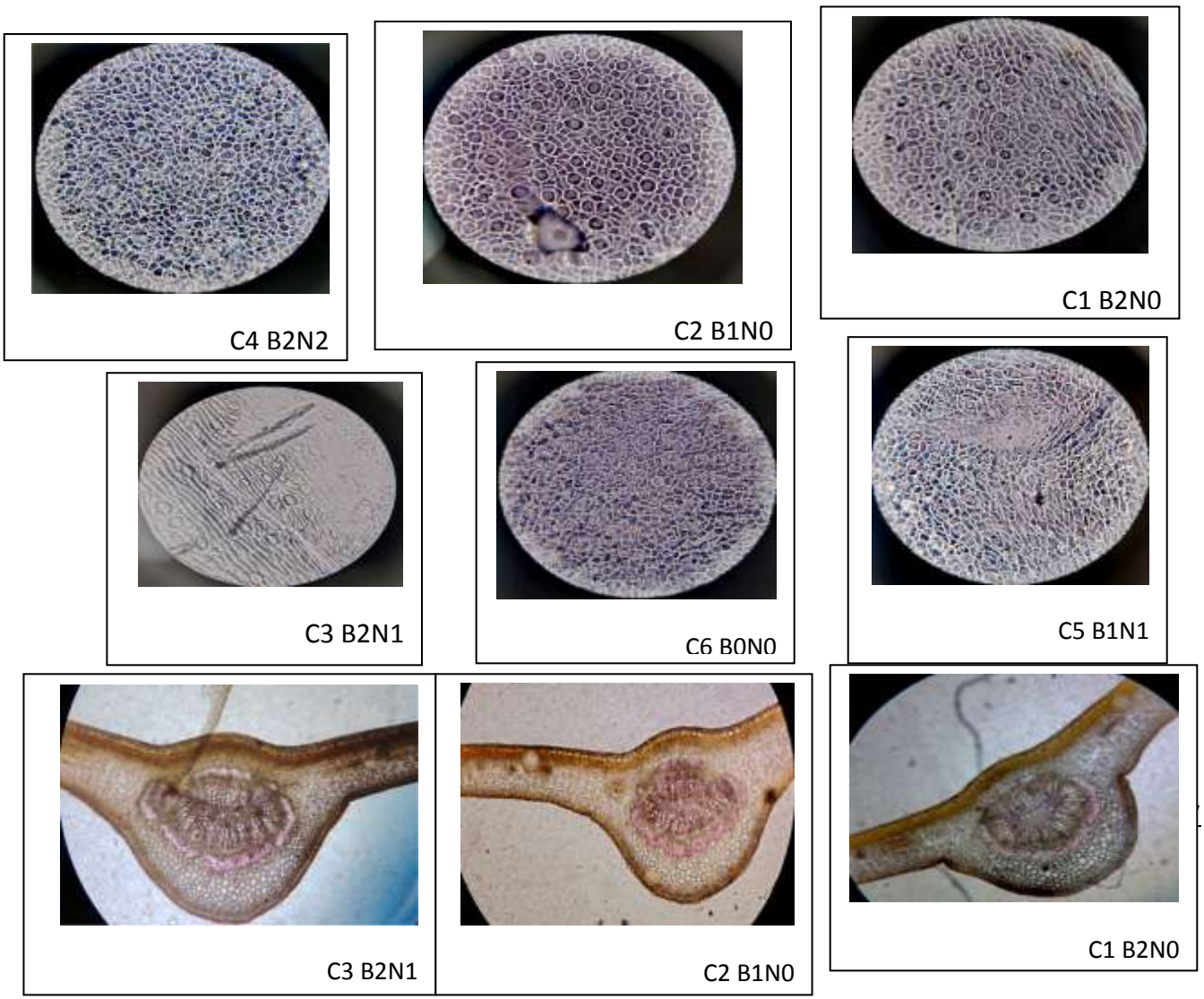

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