Response of two Endive cultivars to Bio fertilizer and spraying with total amino acids in some qualitative indicators

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Abstract:

A field experiment was conducted on two Endive varieties in one of The Development Project in the desert area of the Plant Production Department in the Holy Karbala Agriculture Directorate during the agricultural season 2019-2020. The experiment was a factorial experiment according to the split split plot design using a randomized complete block design (R.C.B.D.) and with three replications, the averages were compared using the LSD test with a probability level of 0.05.

Most of the overlap treatments showed significant superiority in the triple overlap treatment of the Endivien variety, as it gave the highest values in the quality indicators of the leaf content of Carbohydrates to 2.53 mg100gm-1, nitrates to 79.10 mg gm-1 dry weight, total amino acids to 218.00 µm. gm fresh weight-1, and chlorophyll to 83.82 mg100gm-1 fresh weight.

Keywords: Endive cultivar, bio fertilizers, amino acids, qualitative indicators.

Introduction:

Aromatic and leafy plants are among the important plants in our daily lives because of the use of their extracts in various medical treatments as well as fresh use in many countries of the world, including Iraq. Among those leafy plants are watercress, red basil, lemongrass and dandelion. The Endive plant. Cichorium endivia L. is one of the leafy crops that belongs to the Asteraceae family, comprises about 70 genera and 1500 species (3) and it is considered one of the plants with nutritional and medicinal content as well as economic return, as it contains many effective and specific compounds that act as antioxidants. It is one of the largest producers in the European Union for the plant in many countries Such as Spain, France and Italy, as it amounts to 97% of the cultivated areas in Italy (17.(

Biological fertilization is one of the modern agricultural activities affecting the growth and yield of plants, especially vegetable plants, as its addition leads to providing the plant with nutrients and increasing its readiness in the soil, for the purpose of creating the appropriate conditions for bringing about changes in the biological content of the soil and the surrounding area of the rhizosphere. Biofertilizer were used, through which plant roots or soil are inoculated with microorganisms (6) . Among those microorganisms that improve soil properties and contribute to the processing of nutrients for plants are Azotobacter and Azospirillum,Bacillus spp Bacteria(19 and 13.(

The use of foliar spraying is an important factor in plant nutrition and is not a substitute for terrestrial addition (18), so spraying was used with amino acids on the leaves, as they enter into the building of cell membranes and play an important role in enzymatic reactions within plant cells, as well as being representative A chelating substance for each of zinc, iron, manganese, magnesium and calcium, its importance is summarized in the

modification of the osmotic potential. Plant tissue through its various physiological activities, so it plays an important impact in all stages of the plant, as it is one of the important means in improving and increasing plant productivity (1). The spraying of amino acids on the vegetative system showed positive effects in improving vegetative growth and thus reflected positively on the nougat content of the plant (12),Therefore, the study aimed to study the effect of levels of biofertilization and spraying with total amino acids on two cultivars of Endive and its effect on the leaves content of some specific components of the plant and to show the best interactions between the experimental factors.

Materials and methods

A field experiment was conducted during the fall planting season 2019-2020 on endive. The experiment included two varieties of the local Endive plant and Endivien (V1 and V2), while the bio-fertilization included treatments without fertilization, the bio-fertilization using Azotobacter chroococccun by adding 10 gm for each plant, and the bio-fertilization using the Bacillus subtilis bacterium, which was added by injection in the root growth area at a level of 10 mm. Plant-1. A mixture of biofertilizer composed of two types of bacteria 10 mm. Plant-1 for each type and its symbol (A0, A1, A2, A3). While the third factor was spraying four levels of total amino acids on the shoots, which are (0, 2, 4 and 6 ml. L-1) has its symbol (B0, B1, B2, and B3) Straight. A factorial experiment was carried out according to the split- split plot design, using a randomized complete block design (R.C.B.D.), with three replications, and each repetition contains 32 experimental units. The number of experimental units was 96, with 15 plants per experimental unit, and the averages were compared using the LSD test at a probability level of 0.05.

Measured traits:

-1Measuring the total chlorophyll content of the leaves according to (8.(

-2Measuring the carbohydrate content of leaves according to (7.(

-3Measuring the protein content of leaves according to (9 .(

-4Measuring the amino acid content of leaves according to (4.(

-5Measuring the nitrate content of the leaves according to (5.(

Results and Discussion:

-1Total chlorophyll content of leaves (mg 100 gm fresh weight-1(

The results shown in Table (1) indicate that the Endivien cultivar had a significant superiority over the local cultivar in increasing the total chlorophyll content of the leaves to 56.51 mg.100 gm fresh weight-1 compared to the local plants in which the studied trait decreased to 50.01 mg.100 gm fresh weight-1. As for the effect of adding biofertilizer, the bio-combination was significantly superior in increasing the chlorophyll content of the leaves to 69.19 mg.100gm fresh weight-1, compared with the comparison treatment in which the chlorophyll content of the leaves decreased to 40.47 mg.100gm fresh weight-1.

As for the effect of spraying the total amino acids, the concentration of 6 ml L-1 was significantly superior in increasing the chlorophyll content of the leaves, reaching 58.71 mg.100gm fresh weight-1 compared with the comparison treatment, which decreased to 46.80 mg.100gm-1. While the interaction between the cultivar and the addition of bio fertilizer led to the superiority of the Endivien variety over the local one with the combination of the biofertilizer Azotobacter chroococcum at the level of 10 g + Bacillus subtilis 10 mm. Plant-1 significantly increased the leaves' chlorophyll content to 74.68 mg. 100 g fresh weight -1 , While the comparison treatment showed a decrease in the studied trait to 36.41 mg.100 gm fresh weight-1 for the local variety for the two consecutive seasons. As for the effect between the three factors, the Endivien variety

increased significantly on the local chlorophyll content in the leaves, reaching 83.82 mg.100g fresh weight-1 at the level of the biosynthesis Azotobacter chroococcum and Bacillus subtilis at the level of 10g + 10 ml. L-1 and spraying amino acids at a concentration of 6ml . L-1, compared with the control treatment, which decreased to 34.83 mg.100 gm fresh weight-1. Leaves content of total soluble carbohydrates (mg 100 gm -1(

Table (1): Effect of cultivar, biofertilization and spraying of total amino acids on the leaves content of total chlorophyll mg. 100 gm. fresh weight-1

Cultivar V	**Bio fertilizer A**	**AMINO ACID B**				**Cultivar ×Bio fertilizer**	**Mean of cultivar**
		0(B0)	**2 (B1)**	**4(B2)**	**6(B3)**		
Local V_1	**Control (A0)**	34.83	36.18	36.64	38.00	36.41	50.01
	Azotobacter (A1)	39.40	43.48	54.52	46.02	45.86	
	Bacillus (A2)	50.60	45.55	52.97	67.18	54.07	
	Azotobacter + Bacillus (A3)	53.38	52.11	66.32	82.99	63.70	
Endivien V_2	**Control (A0)**	45.18	46.53	44.48	41.94	44.54	56.51
	Azotobacter (A1)	41.74	47.37	56.19	49.68	48.75	
	Bacillus (A2)	48.81	58.35	65.07	60.07	58.07	
	Azotobacter + Bacillus (A3)	60.45	72.68	81.78	83.82	74.68	
Mean of amino acid		46.80	50.28	57.25	58.71	**Cultivar = 2.602**	
Cultivar ×amino acid	Local V 1	44.55	44.33	52.61	58.55	**L.S.D Cultivar** × amino acid **2.706.**	
	Endivien V 2	49.05	56.23	61.88	58.88		
	Mean of fertilize	**L.S.D 23.96 =** amino acid **7.71 =**					
Fertilizer × amino acid	**Control (A0)**	40.01	41.36	40.56	39.97	40.47	
	Azotobacter (A1)	40.57	45.42	55.36	47.85	47.30	
	Bacillus (A2)	49.71	51.95	59.02	63.62	56.07	
	Azotobacter + Bacillus (A3)	56.91	62.40	74.05	83.40	69.19	
	L.S.D Fertilizer × amino acid 3.971 = Fertilizer 2.352 =						

The results shown in Table (2) show that the Endivien cultivar was significantly superior to the local cultivar in increasing the carbohydrate content of the leaves to1.7629 mg.100gm-1 compared with the comparison plants in which the studied trait decreased to1.6715 mg.100 gm-1. As for the effect of The addition of bio-fertilizer, the bio-combination was significantly superior in increasing the carbohydrate content of the leaves to 2.0913 mg.100gm-1 compared with the control treatment in which the carbohydrate content of the leaves decreased to 1.4688 mg.100gm-1 for the season. . As for the effect of spraying total amino acids, the concentration of 6 ml L-1 was significantly superior in increasing the carbohydrate content of the leaves, reaching 1.8363 mg.100gm-1, compared with the comparison treatment, which decreased to 1.5900 mg.100gm-1 for the season. The results of the interaction between biofertilization and amino acids showed that the concentration of the biological combination of Azotobacter chroococcum and Bacillus subtilis at the level of 10 g + 10 ml.L-1 was significantly superior to the concentration of amino acids at the concentration of 6 ml. L-1 gave the carbohydrate content in the leaves to 2.3283 mg.100gm-1 compared with the comparison value, which decreased to 1.4100 mg.100gm-1.As for the three-way interaction between the cultivar, the biofertilizer and the application of amino acids, as the variety Endivien increased significantly over the sweetener in terms of carbohydrate content in the leaves, reaching 2.5300 mg.100gm-1 at concentration 6 ml. L-1 for the season, compared to the control treatment, which decreased to 1.3700 mg.100gm-1 for the local.

-Percentage of protein in leaves(%)

The results shown in Table (3) indicate that the local cultivar was significantly superior to the Endivien cultivar in increasing the percentage of protein in the leaves, reaching 11.436% compared to the Endivien plants in which the studied trait decreased to 10.548%.

The addition of the biofertilizer Azotobacter chroococcum at the level of 10 g with Bacillus 10 ml.L-1 increased the percentage of protein, reaching 14.241%, compared to the comparison treatment, in which the percentage of protein decreased to 8.770 % . It was also found that the spraying of total amino acids was significantly superior at the level of concentration of 6 ml.L-1 in increasing the percentage of protein in the leaves, reaching 12.507%, compared with the comparison treatment, which decreased to 9.866 for the season.

While the interaction between the cultivar and the addition of biofertilizer gave the superiority of the local variety with the bio-combination significantly in increasing the percentage of protein, reaching 15.564%, while the comparison treatment showed a decrease in the studied trait to 8.710% for the Endivien variety. .

While the response between the three factors led to a significant increase in the local cultivar on Endivien in the percentage of protein in the leaves, reaching 16.49 % at the level of the biosynthetic Azotobacter chroococcum and Bacillus subtilis at the level of 10 g + 10 ml.L-1 and spraying amino acids at a concentration of 6 ml L-1 for both seasons, sequentially, compared with the control treatment, which decreased to 7.93% for the Endivien variety.

Table (2): The effect of cultivar, biofertilization and spraying of total amino acids on the leaves content of total soluble carbohydrates mg 100gm -1 for Endive plants

Cultivar V	Bio fertilizer A	AMINO ACID B				Cultivar × Bio fertilizer	Mean of cultivar
		0(B0)	2 (B1)	4(B2)	6(B3)		
Local V_1	Control (A0)	1.3833	1.4133	1.4500	1.4500	1.4242	1.6879
	Azotobacter (A1)	1.5467	1.5133	1.5633	1.6067	1.5575	
	Bacillus (A2)	1.6833	1.7533	1.8333	1.8467	1.7792	
	Azotobacter + Bacillus (A3)	1.7867	1.8500	2.1533	2.1733	1.9908	
Endivien V_2	Control (A0)	1.4367	1.5567	1.5400	1.5200	1.5133	1.8160
	Azotobacter (A1)	1.5633	1.6400	1.6300	1.7467	1.6450	
	Bacillus (A2)	1.7600	1.8267	1.9367	1.8633	1.8467	
	Azotobacter + Bacillus (A3)	1.9533	2.1467	2.4067	2.5300	2.2592	
Mean of amino acid		1.6392	1.7125	1.8142	1.8421	Cultivar 0.080=	
Cultivar × amino acid	Local V 1	1.6000	1.6325	1.8363	1.7692	L.S.D Cultivar × amino acid	
	Endivien V 2	1.6783	1.7925	1.8783	1.9150		
	Mean of fertilize	L.S.D = 060		0 . amino acid 0.022 =			
Fertilizer × amino acid	Control (A0)	1.4100	1.4850	1.4950	1.4850	1.4688	
	Azotobacter (A1)	1.5550	1.5767	1.5967	1.6767	1.6013	
	Bacillus (A2)	1.7217	1.7900	1.8850	1.8550	1.8129	
	Azotobacter + Bacillus (A3)	1.8700	1.9983	2.2800	2.3517	2.1250	
	L.S.D Fertilizer × amino acid = 0.029			Fertilizer =0.016			

Table (3): Effect of cultivar, biofertilization and spraying of total amino acids on the percentage of protein in the leaves of Endive plants

Cultivar V	Bio fertilizer A	Amino Acid B				Cultivar ×Bio fertilizer	Mean of cultivar
		0(B0)	2 (B1)	4(B2)	6(B3)		
Local V_1	Control (A0)	7.933	8.497	9.163	9.727	8.830	11.436
	Azotobacter (A1)	10.120	8.747	10.203	12.040	10.277	
	Bacillus (A2)	10.457	8.530	11.140	14.163	11.072	
	Azotobacter + Bacillus (A3)	14.497	15.267	15.997	16.497	15.564	
Endivien V_2	Control (A0)	7.600	8.433	8.537	10.270	8.710	10.548
	Azotobacter (A1)	7.267	12.993	10.540	10.163	10.241	
	Bacillus (A2)	10.080	9.687	10.370	11.160	10.324	
	Azotobacter + Bacillus (A3)	10.977	9.767	14.890	16.037	12.917	
Mean of amino acid		**9.866**	**10.240**	**11.355**	**12.507**	**L.S.D. Cultivar = 0.223**	
Cultivar × amino acid	Local V 1	10.752	10.260	11.626	13.107	**L.S.D Cultivar** × amino acid = 0.202	
	Endivien V 2	8.981	10.220	11.084	11.907		
	Mean of fertilize	**L.S.D 0 .351 =** amino acid **0.123 =**					
Fertilizer × amino acid	Control (A0)	7.767	8.465	8.850	9.998	8.770	
	Azotobacter (A1)	8.693	10.870	10.372	11.102	10.259	
	Bacillus (A2)	10.268	9.108	10.755	12.662	10.698	
	Azotobacter + Bacillus (A3)	12.737	12.517	15.443	16.267	14.241	
	L.S.D Fertilizer × amino acid = 0.243 Fertilizer =0.129						

-Leaves content of total amino acids (µm.gm fresh weight-1(

The results shown in Table (4) No significant between the two cultivars of total amino acids in leaves .

Biofertilization was significantly superior when adding Azotobacter as it reached 210.7 µm.gm fresh weight-1 of the studied indicator, compared with the control treatment that decreased to 158.2 µm.gm fresh weight-1, the effect of spraying total amino acids showed a significant superiority at the level of 4 ml.L-1 as it reached 203.7 µm.gm fresh weight-1 compared to the unsprayed treatment in which the studied index decreased to 160.3 µm.gm fresh weight-1. The interaction between the cultivar and biofertilization had a significant effect on the amino acid content of the leaves, as the treatment V1A1 excelled significantly in the studied indicator, reaching 241.8 µm.gm fresh weight-1, while the treatment V1A0 showed a decrease, reaching 140.6 µm.gm

fresh weight-1 for the season. And the interaction between cultivar and total amino acid sprays had a significant effect in treatment V2B3 as it reached 221.2 µm.gm fresh weight-1, while treatment V1B0 decreased as it gave 157.6 µm.gm fresh weight-1. The triple interaction between the study factors showed a significant effect on treatment V1A1B2 as it gave 341.3 µm.gm fresh weight-1, compared with treatment V1A0B0, which decreased to 80.0 µm. gm fresh weight-1.

Table (4): Effect of cultivar, biofertilization and spraying of total amino acids on the measurement of total amino acids in leaves µm. gm fresh weight-1

Cultivar V	Bio fertilizer A	Amino Acid B				Cultivar ×Bio fertilizer	Mean of Cultivar
		0(B0)	2 (B1)	4(B2)	6(B3)		
Local V_1	Control (A0)	80.0	146.1	182.3	154.1	140.6	181.6
	Azotobacter (A1)	136.0	254.8	341.3	235.0	241.8	
	Bacillus (A2)	250.5	177.4	147.4	155.0	182.6	
	Azotobacter + Bacillus (A3)	164.2	127.3	204.8	148.9	161.3	
Endivien V_2	Control (A0)	88.0	161.2	200.8	252.9	175.7	180.9
	Azotobacter (A1)	218.4	125.3	126.6	248.5	179.7	
	Bacillus (A2)	179.8	169.2	208.7	189.3	186.8	
	Azotobacter + Bacillus (A3)	166.0	147.8	218.0	194.2	181.5	
Mean of amino acid		**160.3**	**163.6**	**203.7**	**197.2**	**Cultivar N.S.**	
Cultivar × amino acid	Local V 1	157.6	176.4	218.9	173.3	**L.S.D Cultivar** × amino acid **17.59**	
	Endivien V 2	163.0	150.9	188.5	221.2		
	Mean of fertilize	**L.S.D Cultivar** × amino acid **= 23.96** amino acid = **7.71**					
Fertilizer × amino acid	Control (A0)	84.0	153.7	191.5	203.5	158.2	
	Azotobacter (A1)	177.2	190.1	234.0	241.7	210.7	
	Bacillus (A2)	215.1	173.3	178.1	172.2	184.7	
	Azotobacter + Bacillus (A3)	165.1	137.5	211.4	171.5	171.4	
	L.S.D Fertilizer × amino acid 15.21 = Fertilizer 8.27 =						

-5Nitrate content of the leaves (mg gm dry weight-1(

The results shown in Table (5) indicate that the Endivien cultivar had a significant superiority in increasing the nitrate content of

the leaves, reaching 71.68 mg.gm dry weight-1, compared with the plants of the local variety, which decreased to 53.74 mg.gm dry weight-1. Biofertilization was significantly superior when adding Azotobacter + Bacillus as it reached 71.51 mg.gm dry weight-1 compared with the comparison treatment, which decreased to 56.70 mg.gm dry weight-1 for the studied indicator. The effect of spraying total amino acids showed a significant superiority at the level of 6 ml L-1, as it recorded 68.65 mg.gm dry weight-1, compared with the comparison treatment in which the leaf nitrate content decreased to 56.57 mg gm dry weight-1.

As for the interaction between the cultivar and biofertilization, it had a significant effect on the nitrate content of the leaves, as the treatment V2A2 excelled significantly in the studied indicator, reaching 75.50 mg.gm-1 dry weight, while the treatment V1A1 showed a decrease, reaching 32.04 mg.gm dry weight-1. The interaction between biofertilization and spraying of total amino acids showed a significant effect in treatment A3B3 as it reached 77.85 mg.gm dry weight-1, while it decreased in treatment A0B0 as it gave 48.50 mg.gm dry weight-1.

The triple interaction between the study factors significantly affected treatment V2A2B2, as it gave 84.07 mg.gm dry weight-1, compared with treatment V1A1B0, which decreased to 25.97 mg gm dry weight-1.

Although there are simple variations in the chemical and qualitative indicators between the two cultivars of the study, it is noted that there is a discrepancy between them in the superiority of some of the studied indicators. Its effect on the transformation of nutrients through metabolic processes and its reflection on the specific characteristics of the plant. This behavior may interact to a large extent with the surrounding environmental conditions, and therefore the content of chemical compounds in the plant is affected by such genetic factors or the environment (15). Therefore, their genetic behavior may differ or harmonize with this environmental condition, which affects the qualitative or chemical indicators

The addition of bio fertilizers also had an important role in improving the chemical indicators, and this was more evident and effective in the combination of Azotobacter and Bacillus. Rhizosphere and thus has increased the ability of the roots to absorb these elements and raise their level within the plant tissue and thus has affected the efficiency of the photosynthesis process and its entry into energy compounds, which lead to an increase in the production of nutrients within the plant, which positively affects the increase in the rate of vegetative growth and then improve the characteristics of the yield quantity and quality (11) , On the other hand, the combination of fertilizers and the synergistic effect between these organisms has contributed to increasing the percentage of carbohydrates, proteins and amino acids and then increasing the building of vital compounds and their accumulation within the plant tissue in terms of increasing the dry matter in the plant, and this is agreement with Al-Ibrahemi (2) who found that bio fertilization had an effective effect in increasing the chemical compounds in plants and their accumulation.

The role of spraying total amino acids in improving the quality indicators may be attributed to its effect on the construction of many chemical compounds through the vital processes that occur inside the plant, either as initiators of such compounds or as part of their composition (10), as well as its role in activating the enzyme system of the plant, especially the oxidation-reduction system, increasing the ability of cells to divide and elongate (14), and raising the efficiency of carbon metabolism and the manufacture of carbohydrates and proteins, which contribute to increasing the dry weight of plants (16.(

Table (5): Effect of cultivar, biofertilization and spraying of total amino acids on the nitrate content of leaves mg gm-1 dry weight

Cultivar V	Bio fertilizer A	AMINO ACID B				Cultivar ×Bio fertilizer	Mean of Cultivar
		0(B0)	2 (B1)	4(B2)	6(B3)		
Local V_1	Control (A0)	41.20	44.47	58.33	57.00	50.25	53.74
	Azotobacter (A1)	25.97	27.40	29.33	45.47	32.04	
	Bacillus (A2)	51.77	64.57	71.33	65.60	63.32	
	Azotobacter + Bacillus (A3)	64.03	62.63	74.20	76.60	69.37	
Endivien V_2	Control (A0)	55.80	62.30	64.27	70.20	63.14	71.68
	Azotobacter (A1)	73.63	76.07	72.63	75.33	74.42	
	Bacillus (A2)	63.03	74.97	84.07	79.93	75.50	
	Azotobacter + Bacillus (A3)	77.13	63.67	74.73	79.10	73.66	
Mean of amino acid		56.57	59.51	66.11	68.65	Cultivar = 1.938	
Cultivar ×amino acid	Local V 1	45.74	49.77	58.30	61.17	L.S.D Cultivar × amino acid 2.226	
	Endivien V 2	67.40	69.25	73.93	76.14		
	Mean of fertilize	L.S.D = Cultivar × amino acid =4.462				amino acid 1.642 =	
Fertilizer × amino acid	Control (A0)	48.50	53.38	61.30	63.60	56.70	
	Azotobacter (A1)	49.80	51.73	50.98	60.40	53.23	
	Bacillus (A2)	57.40	69.77	77.70	72.77	69.41	
	Azotobacter + Bacillus (A3)	70.58	63.15	74.47	77.85	71.51	
	L.S.D Fertilizer × amino acid 3.175 = Fertilizer 1.570=						

Conclusions :

Based on the results from the above study, we can conclude the following:

-1The Endivien variety had a significant and clear effect on the sweetener on the leaf content of some qualitative characteristics.

-2The biofertilizer Azotobacter chroococcum at 10 gm and Bacillus subtilis 10 ml. plant-1 had a significant superiority over some specific compounds and chlorophyll.

-3 The interaction between adding Azotobacter chroococcum at 10gm and Bacillus subtilis at 10 ml Plant-1 and spraying amino acids at 6 ml L-1 had an effect on improving plant growth, which increased the

content of amino acids, nitrates, protein and chlorophyll.

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