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Effect of root inoculation with Mycorrhizal fungus and foliar spraying with Moringa leaf extract on some vegetative characteristics and concentration of the active substance in Citrullus Colocynthis fruits

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***Abstract*---**A field experiment was conducted in the 2021 agricultural season in one of the agricultural fields in Anbar Governorate - Fallujah district to study the effect of treating roots with different concentrations of Mycorrhizal fungi and foliar spraying with different concentrations of Moringa leaf extract and the interaction between them on some vegetative characteristics and concentration of the active substance in Citrullus Colocynthis fruits. The experiment was carried out in a randomized complete block design R.C.B.D with three replications for each treatment. The experiment included 16 treatments resulting from the control treatment, inoculation with three concentrations of mycorrhizal fungi, foliar spraying with three concentrations of moringa leaf extract and the interaction between them, the averages were compared using the least significant difference L.S.D at a probability level of 0.05. The results showed that the inoculation of roots with mycorrhizal fungi gave significant differences in the studied traits, as the inoculation treatment with mycorrhizal fungi at concentration (20 g) for each plant achieved the highest values for some of the traits studied, which significantly outperformed the control treatment without addition in each of the number of flowers, leaf content of Chlorophyll and concentration of active substances (Citrullol, Cucurbitacine, Colocynthine, Colocynthitine). While foliar spraying with Moringa leaf extract at treatment (200 g/L) gave the best results for the characteristics of number of flowers, chlorophyll content of leaves and concentration of active substances (Cucurbitacine, Colocynthine), while the treatment

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(300 g / l) achieved the highest concentration of active substances (Citrullol, Colocynthitine), while the interaction treatment (20 g, 200 g/L) had a significant effect on plant height, number of flowers and leaf content of chlorophyll, and the same treatment recorded the highest concentration of active substances (Citrullol, Cucurbitacine, Colocynthine, Colocynthitine).

***Keywords*---**Citrullus colocynthis, Mycorrhizal, Moringa leaf extract.

Introduction

Bitter melon (*Citrullus Colocynthis*) is an important medicinal plant that belongs to the family of cucurbits, and it is one of the well-known plants in traditional medicine. Bitter melon grows in the hot and warm desert regions of Asia and Africa. It is also spread in the Arab region such as Morocco, Egypt and Sudan (AL- Gamdi et al., 2009). Sultan et al. (2010) indicated that the bitter melon plant is considered a medicinal plant because it contains the active medicinal compound Colocynthine and Colocynthitine. It was found that these two substances are a mixture of alkaloids, glycosides and an alcoholic substance called Citrullol. It contains Colocynth, which is a very bitter substance, and Colocynth has been found to be a mixture of alkaline substances and a glycoside (Ahmed, 2018). Hussain et al., (2014) noted that bitter melon has a wide range of medicinal uses and has been well studied for its anti-diabetic, anti-cancer, antioxidant, antimicrobial and anti-inflammatory activities.

Moringa oleifera belongs to the family Moringaceae and is a perennial plant (Karim et al., 2016) which is tolerant of both severe drought or moderate frost and thus can be widely cultivated all over the world (Gopalokrishnane et al., 2016). El-Hack (2018) notes that the Moringa plant contains many phytochemicals distributed in its leaves, pods, and roots. These key chemicals are glycosides, phenols, flavonoids, key nutrients, minerals and total protein. moringa leaf extract has been used for many studies as it is rich in many growth hormones, in particular the two oils, which have been indicated to increase the productivity of crops by 10-45%. of crops (Koul and Chase, 2015). The addition of Moringa leaf extract is a cheap and environmentally friendly organic technology that increases the growth of most vegetable crops such as turnips, cabbage, tomatoes and field crops including corn and beans. moringa leaves contains the growth hormone zeatin, which is one of the growth hormones (cytokinins) that are effective in the plant, which can improve plant growth by 25-30%. Natural of cytokinins, proteins, vitamins, phenols, essential amino acids and many minerals, making it a natural plant growth stimulant (Howladar, 2014).

Mycorrhiza is a group of soil fungi called Arbuscular mycorrihza fungi that form a symbiotic relationship with plants, as this relationship helps the plant to obtain phosphorous and protect it from biotic and abiotic stresses. It is known to promote plant growth by dissolving phosphate and producing plant hormones (Nanjundappa et al , 2019), as explained by Genre et al , (2020) that the term mycorrhizal fungi that has a symbiotic relationship with the roots of many vascular plants under natural conditions, and it is an unsatisfactory symbiotic

relationship to which the host plant responds, improving its growth and physiological characteristics and increasing its resistance to diseases and many Environmental factors such as drought, freezing and salinity. This research aims to study the effect of root treatment with Mycorrhiza and foliar spraying with moringa leaf extract on some vegetative characteristics and the concentration of active substances in the fruits of *Citrullus colocynthis* due to its high medical importance.

Materials and working methods

A field experiment was conducted for the cultivation of bitter melon in one of the agricultural fields in the city of Fallujah - Anbar Governorate. The soil was analyzed before the experiment was carried out by taking random samples from different sites of field soil with a depth ranging between (0-30) cm. The samples were mixed well to be a representative model of soil The field and then the samples were analyzed in the central laboratory of the College of Agriculture, University of Anbar to identify the physical and chemical properties of the soil as shown in Table No. (1)

Adjective	Value	measruing unit
EC	1.84	ds m^{-1}
PH	7.31	
N	33	mg kg^{-1} soil
P	5.13	mg kg^{-1} soil
K	75	mg kg^{-1} soil
Mud	170	gm kg^{-1}soil
Silt	360	
Sand	470	
Soil texture	Mix	

Table (1) shows the physical and chemical properties of soil

The treatments were distributed according to the Randomized Complete Block Design (R.C.B.D) with two factors and three concentrations for each factor in addition to the interactions between the two factors and three replications for each treatment. Bitter melon seeds were provided from the Desert Studies Center / University of Anbar, and the Mycorrhizal inoculum (roots of plants infected with Mycorrhizal fungi and Mycorrhizal spores and soil in the form of a homogeneous mixture) were provided from the College of Agriculture / University of Anbar. The inoculum was examined by wet sieving to ascertain the density of Mycorrhizal spores belonging to the genus Glomus sp. according to the method of (Gerdemann and Nicolson., 1963). Moringa leaves were also obtained from adult trees. The mature leaves were picked, then washed and cleaned well of dust and impurities, and after drying them, they were left to air dry in the normal air without exposing them to sunlight. After the leaves were completely dry, they were ground using an electric grinder and keeping the powder of the leaves in sterile bottles for use in the preparation of Moringa leaf extract for the purpose of foliar spray.

The farm land was well plowed by two orthogonal plows before planting and left for 5 days for the purpose of ventilation. Then the land was softened to get rid of clumps and remove impurities from it. Then the farm was divided into 8 stalks

and shoulders for the purpose of cultivation and irrigation with the length of 4 meters and the distance between them is 2 meters. A hole was dug at a depth of 5 cm and the distance between one hole and another is 40-50 cm. The mycorrhizal inoculum was added in three concentrations: 15 , 20 , 25 g (each one gram of the pollen contains 50 spores) to each hole before planting the seeds . Then the seeds were planted by three seeds for each hole, after which irrigation was carried out until saturation and the seeds were left in order to sprout . Germination was done and the first sprouts appeared after 5-8 days . After the germination of all the seeds and the appearance of vegetative buds, that is, after 14 days of planting, the plants were thinned to one plant per hollow.

The treatment was carried out with the second factor , which is spraying with the extract of Moringa leaves after 21 days of planting when the number of leaves became from 3 to 5 leaves, and the spraying process was carried out in three concentrations also 100, 200, 300 g/L spraying on the leaves until complete wetness after 48 hours of watering the plants, as the guard cells will be swollen and stomata open and have the ability to absorb . Then the plants were left to grow until the second treatment, two weeks after the first treatment, using foliar spray until completely wet. The spraying process was carried out using a manual sprayer with a capacity of 2 liters, and the spraying process was carried out in the early morning, since the plant was at the beginning of its activity and to avoid high temperature. Raise the ability of the plant to take advantage of the solution. The data for the studied traits were taken after 120 days of planting the plant and included the following :

1- Plant length : The measurement of the length of the main stem was taken starting from the area of contact with the soil and reaching the growing top of the plant using a graduated tape measure .
2-Number of flowers: The process of calculating the number of flowers was carried out 15 days after the appearance of the first flower bud.
3-The percentage of chlorophyll in the leaves: A measurement of the chlorophyll content in the leaves was taken using the (Chlorophyll Meter) model SPAD-502 supplied by the Japanese company Minolta .
4-Concentration of the active substance: 20 ul of the sample was injected into the HPLC system according to ideal conditions. The separation was carried out on a liquid chromatograph (HPLC) equipped with a Shimadzu A0-LC dual delivery pump, at a temperature of 25°C, a flow rate of 1.4 ml/min and a wavelength of 330 nm. The prepared peaks were monitored by UV-Vis 10 A- spectrophotometer SPD. The following active substances were measured:

- Citrullol
- Cucurbitacine
- Colocynthine
- Colocynthitine

Results & Discussion

1-Plant height (cm)

Table No. (2) shows that the average of plant height increased with the addition of mycorrhizal pollen and moringa leaf extract and the interaction between them, where the treatment (25 g) of mycorrhizal pollen gave the highest average plant height of 122.25 cm, which differed from the control treatment without adding, which gave the least average of plant height was 108.08 cm. While the plant height increased when treated with Moringa leaf extract (200 g/L) and the highest average plant height was obtained which reached 127.33 cm, while the control treatment without adding gave the average plant height reached 105.75 cm. Whereas, the interaction treatment (20 g and 200 g/L) between Mycorrhiza and Moringa, respectively, gave the highest significant difference in plant height of 145 cm, which differed from the control treatment without addition, which gave the least plant height of 100 cm.

Table No. (2) Effect of Mycorrhizal Vaccine and Moringa Leaf Extract
And the overlap between them in the length of the plant

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	100.000	106.00	111.33	115.00	108.08
15	105.00	120.00	116.00	113.00	113.50
20	104.00	115.00	145.00	119.33	120.83
25	114.00	115.00	137.00	123.00	122.25
MEAN	105.75	114.00	127.33	117.58	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	0.845	0.845	1.689		

2-Number of flowers (flower/plant)

It is noted from Table No. (3) that there is a significant effect of the treatments of inoculation with Mycorrhiza and spraying with Moringa leaf extract and the interactions between them on the number of flowers / plant , while treatment (15 g) with mycorrhizal pollen gave the least significant difference in the number of flowers 10,083 flowers/plant. The results of the same table also indicate the superiority of the treatment (200 g/L) of Moringa leaf extract, which gave the highest average number of flowers 13,083 flowers/plant compared to the control treatment without addition, which gave the lowest average number of flowers 10,167 flowers/plant, and the interaction treatment between (20 gm) , 200 g/L) between Mycorrhiza pollen and Moringa leaf extract had the highest significant difference as the number of flowers reached 17 flowers/plant, while the least average was in the control treatment without addition, as the number of flowers was 9 flower/plant.

Table No. (3) Effect of Mycorrhiza pollen and Moringa leaf extract and the interaction between them on the number of flowers (flower / plant)

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	10.000	9.000	10.000	13.000	10.500
15	9.333	9.667	10.333	11.000	10.083
20	9.333	12.000	17.000	12.000	12.583
25	12.000	10.667	15.000	12.000	12.417
MEAN	10.167	10.333	13.083	12.000	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	0.5115	0.5115	1.0230		

3-The chlorophyll content in the leaves

The results of Table No. (4) indicate a significant effect of the mycorrhizal inoculation and foliar spraying with Moringa leaf extract and the interaction between them on the percentage of chlorophyll in the leaves. In leaves 56,850, while the control treatment without addition gave the lowest average of chlorophyll that amounted to 48.408, while the foliar spraying treatment with Moringa leaf extract (200 g/L) achieved the highest mean of chlorophyll that amounted to 59.750 compared with the control treatment without addition which gave the lowest mean of chlorophyll 43.058 It is also noted from the results in the table below that the interaction treatment (20 g, 200 g/L) between the Mycorrhizal inoculation and Moringa leaf extract, respectively, achieved the highest significant difference in the percentage of chlorophyll 67,400, while the control treatment without adding the least significant difference in the percentage of chlorophyll 40,200.

Table No. (4) Effect of Mycorrhizal Vaccine and Moringa Leaf Extract
And the interaction between them in the percentage of chlorophyll in the leaves

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	40.200	46.200	57.200	50.033	48.408
15	41.333	47.400	52.700	57.400	49.708
20	44.400	54.400	67.400	61.200	56.850
25	46.300	50.300	61.700	57.100	53.850
MEAN	43.058	49.575	59.750	56.433	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	0.4211	0.4211	0.8422		

4-Concentration of the Citrullol in the fruits

It is clear from the results of Table No. (5) that there is a significant effect of the mycorrhizal inoculation treatments and the foliar spray treatment with Moringa leaf extract and the interactions between them in the concentration of Citrullol . The Mycorrhizal inoculation treatment (20 g) achieved the highest average concentration of the Citrullol amounting to 240.418 mg/ml, while the Mycorrhizal inoculation treatment achieved (15 g) the lowest average concentration of Citrullol was 151.032 mg/ml, and the results of the same table indicated the superiority of the foliar spray treatment (300 g/l) with the extract of Moringa leaves in giving the highest average concentration of Citrullol, which reached 265.213 mg/ml compared to With the control treatment without addition, which gave the lowest average concentration amounted to 106.213 mg/ml, while the interaction treatment (20g, 200g/l) gave the highest significant difference for the concentration of the Citrullol amounted to 352.570 mg/ml compared to the control treatment without addition that It gave the least significant difference in the concentration of the Citrullol, reaching 79.773 mg/ml.

Table No. (5) Effect of Mycorrhizal Vaccine and Moringa leaf extract and the interaction between them In the concentration of the Citrullol in the fruits

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	79.773	139.618	150.438	276.457	161.571
15	110.357	183.368	148.577	161.827	151.032
20	122.667	207.642	352.570	278.792	240.418
25	112.057	212.118	197.843	343.776	216.448
MEAN	106.213	185.686	212.357	265.213	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	0.1601	0.1601	0.3202		

5-The concentration of Cucurbitacine in the fruits

The results of Table No. (6) indicate that there are significant differences between the Mycorrhizal inoculation treatment and the foliar spray treatment with Moringa leaf extract and the interactions between them at the concentration of Cucurbitacine in the fruits. The results indicated the superiority of the Mycorrhizal inoculation (20 g) in giving the highest average concentration of Cucurbitacine, which amounted to 341.656 mg/ml. Compared with the control treatment without addition, which gave the least significant difference in the concentration of Cucurbitacine amounted to 193.101 mg/ml, while the foliar spray treatment (200 g/l) with Moringa leaf extract was superior in giving the highest average concentration of Cucurbitacine amounting to 310.339 mg/ml, while The control treatment without adding the lowest average concentration of Cucurbitacine was 179.470 mg/ml, as the results of the table below show that the interaction treatment (20g, 200g/l) between Mycorrhiza inoculum and Moringa leaf extract, respectively, achieved the highest significant difference in

the concentration of Cucurbitacine. It reached 499,048 mg/ml compared to the control treatment without addition, which gave the least average for the concentration of Cucurbitacine, which amounted to 158.563 mg/ml.

Table No. (6) Effect of Mycorrhizal Vaccine and Moringa leaf extract and the interaction between them In the concentration of Cucurbitacine in the fruits

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	158.563	166.267	233.374	215.200	193.101
15	177.940	262.441	202.113	220.595	215.772
20	203.659	246.218	499.048	417.697	341.656
25	177.717	293.180	307.819	226.546	251.316
MEAN	179.470	242.027	310.339	270.010	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	0.2975	0.2975	0.5950		

6- The concentration of Colocynthine in the fruits

It is evident from the results of Table No. (7) that there is a positive effect on the concentration of Colocynthine in the fruits of the bitter melon plant as a result of the treatment with Mycorrhizal pollen and the treatment of foliar spraying with Moringa leaf extract and the interactions between them . The treatment of inoculation with Mycorrhiza (20 g) out performed and achieved the highest average concentration of Colocynthine reached 523.9 mg/ml, while the comparison treatment without adding the lowest average concentration of Colocynthine reached 264.7 mg/ml, and the foliar spray treatment (200 g/l) was superior to Moringa leaf extract and achieved the highest average concentration of Colocynthine amounting to mg/ml. ml 512.9 compared with the comparison treatment without addition, which gave the lowest average concentration of the active substance Colocynthine reached 210.4 mg/ml, while the interaction treatment (20g, 200g/l) between the mycorrhizal vaccine and Moringa leaf extract, respectively, achieved the highest significant difference in the concentration of the active substance reached 692.3 mg/ml compared to the comparison treatment without addition, which gave the least significant difference in the concentration of the active substance Colocynthine, which was 188.7 mg/ml.

Table No. (7) Effect of Mycorrhizal vaccine and Moringa leaf extract and the interaction between them in the concentration of Colocynthine in the fruits

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	188.7	273.0	303.2	294.0	264.7
15	204.3	363.5	495.1	488.0	387.7
20	214.4	547.0	692.3	641.8	523.9
25	234.2	633.6	561.2	452.1	470.3

MEAN	210.4	454.3	512.9	469.0	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	10.98	10.98	21.97		

7- Concentration of Colocynthitine in the fruits

The results of Table No. (8) indicate that there are significant differences between the Mycorrhizal inoculation treatment and the foliar spray treatment with Moringa leaf extract and the interaction between them in the concentration of Colocynthitine in the fruits, where the Mycorrhizal inoculation treatments significantly affected the concentration of the active substance Colocynthitine. The Mycorrhizal inoculation treatment (20g) gave the highest average concentration of the active substance Colocynthitine reached 267.86 mg/ml, while the control treatment without adding the lowest average concentration of the active substance Colocynthitine, which amounted to 130.72 mg/ml, while the foliar spray treatment with Moringa leaf extract (300 g/L) achieved the highest average concentration of the active substance Colocynthitine, which reached mg/ml. 250.21 compared with the comparison treatment without addition, which gave the lowest average concentration of Colocynthitine amounting to 86.96 mg/ml, as it is noted from the results in the table below that the interaction treatment (20g, 200g/l) between the Mycorrhiza vaccine and Moringa leaf extract, respectively, achieved the highest difference. Significant in the concentration of Colocynthitine amounted to 397.23 mg/ml, while the control treatment without adding the least significant difference in the concentration of Colocynthitine , but g mg/ml 69.58 .

Table No. (8) Effect of Mycorrhizal vaccine and Moringa leaf extract and the interaction between them on Colocynthitine concentration in fruits.

Moringa leaf extract (g/l) / Mycorrhiza (g)	0	100	200	300	MEAN
0	69.58	129.10	138.20	186.00	130.72
15	80.64	241.64	190.55	223.34	184.04
20	95.22	242.08	397.23	336.89	267.86
25	102.40	363.45	237.06	254.60	239.38
MEAN	86.96	244.07	240.76	250.21	
LSD 5%	Mycorrhiza	Moringa	Overlap		
	3.269	3.269	6.539		

Discussion

The increase in vegetative growth characteristics, including plant length, may be a result of the beneficial effect of infecting the roots with mycorrhizal fungi and increasing the surface area of the roots through the extension of the fungal hyphae to more distances than the root hairs, thus providing the plant with the largest amount of nutrients, including nitrogen and phosphorus. Potassium is necessary for plant growth, which is reflected in the metabolic activities inside the plant and the improvement of plant growth characteristics, including an increase

in plant length and an increase in the number of leaves because of a direct relationship between them (Abdulsada et al., 2014). The increase in vegetative growth characteristics may also be attributed to the effect of the Moringa leaves extract, where Moringa leaves have multiple roles in the biological and physiological activities of the plant, as it accelerates the growth of young plants and growth indicators when adding Moringa leaf extract at several levels (Kumar and Tripathi, 2011). The use of Moringa leaf extract to fertilize the tomato plant led to an increase in the growth and productivity of this plant. It also agreed with the results obtained by Biswas et al., (2020) in the use of Moringa leaf extract on corn plant. The difference in the number of flowers is also due to the role of mycorrhizal fungi in the production of secondary compounds that lead to the synthesis of growth hormones, including GA3 in the rhizosphere and then transmitted to the plant tissues through a symbiotic relationship (Siddiqui et al., 2008), which leads to stimulating the production of flowering hormone (Floregin) in inoculated plants. Or this effect may be due to the Moringa leaves extract , as it contains Zeatin and many other growth hormones, such as Indole Acetic Acid and gibberellin (GAs), so it is strongly recommended to use the Moringa leaves extract as natural plant bio stimulants to stimulate plant growth (Rady et al., 2015 and Manzor et al., 2105). The Zeatin growth hormone promotes the growth of apical and lateral buds, causes stem growth, helps flowering, delays yellowing of leaves, (Kadam et al., 2018).

The increase in the chlorophyll content of leaves may be attributed to the symbiotic relationship between the mycorrhizal fungi and the host plant, which causes an increase in the length of the roots as a result of inoculation with the mycorrhizal fungi through the formation and extension of the hyphae in the soil, thus increasing the surface area of the roots, which encourages the plant to absorb nutrients such as Nitrogen, Phosphorous, Sulfur and some minor elements such as Zinc and Copper (Kapulnik and Koltai, 2010), which leads to an increase in plant growth by encouraging the formation of plant pigments, including chlorophyll and increasing the activity of the photosynthesis process (Ayoob et al., 2011). Or, the increase in the percentage of chlorophyll in the leaves may be attributed to the Moringa leaves extract, as the leaves of the moringa plant contain a high percentage of plant hormones, especially the oleaginous plant hormone (Iqbal et al., 2013). Increasing the vegetative total and thus stimulating the plants to the flowering process, and these results are consistent with the results obtained by Hijazi et al., (2015).

The increase in the concentration of active substances in the fruits of the bitter melon plant is attributed to the role played by the mycorrhizal fungi. Mandal et al. (2013) found that inoculation with mycorrhizal fungi leads to an increase in the size of the root system, which is reflected positively in the increase in the biomass of the vegetative group. The mycorrhizal fungi stimulate the root system to increase the absorption of water and nutrients such as Nitrogen, Phosphorous, Potassium, Magnesium, Iron, Manganese, Copper and Zinc, in addition to increasing the percentage of chlorophyll, total carbohydrates and jasmonic acid in the vegetative system . The mycorrhizal fungi also secrete the enzyme alkaline phosphatase, which converts phosphorous from the unready form of the plant to the ready (dissolved) form to facilitate the process of absorbing this element and benefiting from it in the growth process, this increase in nutrients, carbohydrates

and jasmonic acid leads to an increase in the synthesis of active compounds and the results we obtained agree with the findings of Lingua et al., (2013) in their study of the effect of Inoculation with Mycorrhiza on strawberry fruits as it agrees with what was reached by Hart et al., (2015) in their study of the effect of mycorrhizal on the tomato plant.

The increase in the concentration of active substances in the fruits of the bitter melon plant may also be attributed to the effect of the Moringa leaves extract. Where the results agreed with what was obtained by Bashir et al., (2016) when studying the effect of Moringa leaf extract on the growth and yield of tomato plant, and the results agreed with what was reached by Hala et al., (2017) in their study on the pepper plant., all these data indicate the role of Moringa leaf extract in increasing the concentration of the active substance in the fruits.

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