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EFFECT OF BACTERIAL FERTILIZERS AND ORGANIC MEDIUM ON THE GROWTH AND FLOWERING OF RANUNCULUS PLANT (*RANUNCULUS ASIATICUS* L)

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Abstract

The research was conducted in the greenhouse of the Plant Production Techniques Department, Al-Mussaib Technical College for the autumn season 2019-2020. To study the effect of bacterial fertilizers and organic medium on the growth and flowering of *Ranunculus asiaticus*, Red cultivar in pots 22 cm in diameter, The research included the effect of two factors, the first factor the bio fertilizers bacterial, with four levels (without the bacterial vaccine, *Bacillus subtillus*, *Azospirillium brasilences*, *Azospirillium brasilences +Bacillus subtillus*) and which it were symbolized by (B0, B1, B2, B3) and the second factor, the organic medium that included (River soil + peat moss at a ratio of (2: 1), River soil + 3%, decomposed horse waste, River soil+ 3% residues from decomposing fungi (mushrooms), Agaricus bisporus, River soil + 5% decomposed horse waste, River+ 5% residues of the decomposing fungus (Agaricus bisporus) and which it were symbolized by (A0, A1, A2, A3, A4). factor experiment was conducted according to Completely Randomized Design (C.R.D) and by three replicates and each replicate contains (20 treatments), The averages were compared according to the L.S.D test under the 5% probability level .The results of this study can be summarized as follows: The treatment with bio fertilizers bacterial resulted in a significant increase in all the studied traits. where the treatment of B3 (mixture) recorded the highest average in plant height, leaf area, percentage of carbohydrates in leaves, number of flowers, flower diameter, vase life, anthocyanin pigment, the results were as follows: 44.80 cm, 1293 cm ^{2},12.87 flower.plant $^{-1}$,13.62%, 63.19 mm, 14.20 days, 2.47 mg.100 g^{-1}.The results showed the excelled of the organic medium treatments, where the A3 treatment (River soil + 5% decomposed horse waste) recorded the highest average for all the studied traits, plant height, Leaf area, percentage of carbohydrates in leaves, number of flowers, flower diameter, vase life, anthocyanin pigment and the results were as follows: 44.88 cm, 14.59 cm 2,14.23%, 13.38 flowers.plant^{-1}, 66.08 mm, 15.66 days, 2.75 mg.100 g^{-1}.The results of the bi-interaction between the biofertilizer bacterial and the organic medium showed significantly excelled in all the studied traits. where the A3B3 treatment excelled in the plant height, Leaf area, percentage of carbohydrates in the leaves, number of flowers, flower diameter, flowering age, anthocyanin stain and the results were as follows: 53.17 cm, 82.0 cm, 1691 cm^{2}, 15.88%, 17.17 flowers.plant^{-1}, 72.14 mm, 3.51 mg. 100 g dry weight.

Keyword: bacterial fertilizers, *Bacillus subtillus,* decomposed horse waste, *Azospirillium brasilences*

***Research paper from MSc thesis for the First author**

Introduction

Ranunculus asiaticus L, which belongs to Ranunculaceae family , is one of the annual winter flowering bulbs that prefers areas with moderate temperatures .The Mediterranean region is considered the origin country and it grows well in some areas of Iraq (Badr et al., 2010).Its economic importance is due to the fact that its flowers are suitable for commercial picking, As flowers are used in flower bouquets, either singly or after coordination with other flowers (Bernstein et al., 2011), The use of biofertilizers (bacterial) is necessary to limit the use of chemical fertilizers ,Among the bacterial fertilizers is the *Bacillus subtilis* bacteria, which is one of the genera that dissolves phosphates through their secretion of organic acids such as acetic, lactic, formic (Al-Serafy, 2010).Likewise, *Azospirillum* bacteria, which affect the plant's nutrition with nitrogen, fix atmospheric nitrogen, convert it into ammonium ion and nitrate, and increase its absorption by the plant (Vessey, 2003), Organic fertilizers of all kinds are an important and essential source of the macro and micro elements needed by the plant, in addition to improving the physical, chemical and biological properties of the soil through the breakdown of heavy soil particles, And improving its aeration also contributes to increasing biological activity within the area of root proliferation because it contains some beneficial microbes and stimulates bioprocesses (AlcaAntara et al 2016 ; Adiaha2017), The sources of organic matter vary from plant sources that come from plant roots and fallen leaves on the surface of the soil, which go through stages of biological decomposition by the action of microorganisms to plants grown to fluctuate in the soil, such as green compost plants and plant wastes that are added to the soil to increase its productivity and improve its qualities and animal sources come as a result of the effectiveness of reviving the soil and its cells and tissues after their death, as well as human and animal wastes added to the soil (Granatsteir, 2004; Al-Hasnawi et al,2019) and due to the importance of expanding the production of ornamental flowers and bulbs, including the Ranunculus plant, in Iraq. Therefore, this research aims to study the type of organic medium and bio fertilizers in the growth and flowering of the Ranunculus plant.

Materials and Methods

The research was conducted in the greenhouse of the Plant Production Techniques Department, Al-Mussaib Technical College for the autumn season 2019-2020, to study the effect of the Organic medium species and bio fertilizer on the growth and flowering of Ranunculus Red Cultivar, produced by Flower Holland . where the bulbs were soaked in water for 8 hours, and they were planted in plastic pots of 22 cm in diameter, where the experiment included two factors, The first factor includes adding two types of bio fertilizers bacterial loaded on peat moss and produced in the laboratories of the Agricultural Research Department of the Ministry of Science and Technology, namely *Bacillus subtillus* and *Azospirillium brasilences.* It was added, according to the experiment treatments of (10 g. pot^{-1}). It was taken into account when cultivating that the bacterial fertilizers were in contact with the bulbs by placing them below and above the bulb (Matysiak and Falkowski, 2010 and Allawi, 2013).Included on four levels are (without the bacterial vaccine, *Bacillus subtillus*, *Azospirillium brasilences*, *Azospirillium brasilences* +*Bacillus subtillus*) and which it were symbolized by (B0, B1, B2, B3). As for the second factor,

the organic medium that included (River soil + peat moss at a ratio of (2: 1), River soil + 3%, decomposed horse waste, River soil+ 3% residues from decomposing fungi (mushrooms), Agaricus bisporus, River soil + 5% decomposed horse waste, River+ 5% residues of the decomposing fungus (Agaricus bisporus) and which it were symbolized by (A0, A1, A2, A3, A4). A factor experiment was conducted according to(Completely Randomized Design (C.R.D) and by three replicates. The averages were compared according to the L.S.D test under the 5% probability level (Al-Sahuki and Waheeb, 1990). The data were analyzed using the ready-made statistical program Genstat. Samples were taken from organic medium (mushroom residues, decomposed horse waste) and river soil for analysis in the Central Laboratory for Graduate Studies, University of Baghdad, College of Agricultural Engineering Sciences (Table 1 and 2) before distributing it to the treatments.

Table (1) Chemical and physical properties for horse waste and mushroom residues

mushroom residues	horse waste	Units	Traits
6.21	4.16	$d.S.m^{-1}$	Electrical conductivity (EC)
7.4	7.28	---	PH
25.40	28.45	$g.Kg^{-1}$	Organic matter
20.14	15.40	---	The ratio of carbon to nitrogen
8.75	6.56	$g.Kg^{-1}$	Total nitrogen
0.17	0.56	$g.Kg^{-1}$	phosphorous availability
0.04	0.11	$g.Kg^{-1}$	Potassium availability

Table (2) the chemical and physical characteristics of the soil under study

Unites	Values	Measured chemical and physical properties
	7.43	PH
$g.Kg^{-1}$	8.31	Organic matter
dsm^{-1}	4.21	Electrical conductivity (EC)
$mg.Kg^{-1}$ soil	28	Nitrogen
	4.14	Phosphorous
	287.12	Potassium
		soil separates
$g.Kg^{-1}$ soil	816	sand
	400	Silt
	144	Clay
	sandy loam	Texture

Studied traits

Plant height (cm)

The plant height was measured from the soil surface to the top of the flower in the plant using the metric tape measure for all plants of the experimental unit, then according to the averages for each treatment.

Leaf area (cm2)

The leaf area was calculated by measuring it according to the method used by (Al-Zaidi, 2016) and described by (Sadik et al, 2011) depending on the leaf area and the number of leaves in the plant, where the average leaf area was calculated first by taking 4 full-width leaves from different parts From each experimental unit using the (Image J) computer program, the process was repeated for three times and the rate was taken and the leaf area was extracted.

Number of flowers per plant (flower .plant $^{-1}$)

According to the number of flowers for each plant and for all plants of the experimental unit, then the mean was calculated.

Flower diameter (mm)

It was measured at the two most distant points of the flower using the electronic footage of all plants of the experimental unit and then extracted the medium.

Vase life (day)

According to the number of days from the appearance of the color in the flower bud of the plant until the petals start to wilt, where the flower stems were cutting and their length standardized and placed in 300 ml plastic containers containing 250 ml distilled water, then the flowers were transferred to a cooled room by means of a cooling device , The temperature was set to 2 + 30 m by controlling the temperature of the device, the relative humidity of + 5% 40, the number of hours of lighting 14 hours under the fluorescent candles, and for all treatments uniformly until 50% of florets withered (Kazemi and others, 2012).

Determination of anthocyanin pigment in petals (mg.100g^{-1}dry weight)

The anthocyanin content of Ranunculus flowers was determined by taking 1 g of the dry tissue of the petals of and crushed in acidified ethanol (HCL is 1.5 nm and 95% ethyl alcohol) and (mM) mixture, 1 mL of it was taken and the volume completed to 10 mL, then measured by a Spectrophotometer at a wavelength of 520 nanometers (1977, Ranganna) and according to the equation anthocyanin dye = (mg.100 g dry weight).

Percentage of Carbohydrates in the Leaves (%)

The total carbohydrate content in the leaves was determined according to the method mentioned (Joslyn, 1970). A dried and milled sample weighing 0.2 g was taken from each treatment and 8 ml of 80% ethyl alcohol was added to it. Then it was placed in a water bath at 60 ° C for a period of 30 minutes, then a centrifugal process was conducted for 15 minutes at a speed of 3000 revolutions/second, the filtrate was withdrawn and these steps were repeated three times. Then collect the clear solution (the filtrate) in a volumetric flask and complete the volume to 25 ml by adding ethyl alcohol and withdraw 1 ml of the dilute solution and add 1 ml of a 5% phenol solution and 5 ml of concentrated sulfuric acid. After that, the optical absorption was read by a Spectrophotometer. At a wavelength of 560nm,The dissolved carbohydrates were estimated on the basis of the standard curve for pure glucose sugar prepared by dissolving different concentrations

of glucose (2,4,6,8,10) mg in 100 ml of distilled water, then the optical absorption readings were recorded at a wavelength of 560 nm for each solution and the data were recorded to make the curve. Glucose standard, then the percentage of dissolved carbohydrates.

Results and Discussion

Plant height (cm)

The results in Table (3) indicate that bio fertilizers had a significant effect on increasing plant height, where the mixture treatment B3 (*Azospirillium brasilences* + *Bacillus subtillus*) was significantly excelled and gave the highest plant height of 44.80 cm. It was followed by the treatment of the bio fertilizer B2 (*Azospirillium brasilences*), which gave a height amounted to (41.43) cm, while the control treatment B0 gave the lowest height of 32.93 cm. The use of organic medium had a significant effect on plant height, where the treatment of A3 (River soil + 5% decomposed horse waste) excelled and gave the highest averages of (44.88) cm. This was followed by the A2 treatment that gave a height that amounted to (41.88) cm, excelled on the treatment A4, which gave the lowest height of (33.42) cm. The bi-interaction between bio fertilizers and the organic medium achieved a significant increase in the height of the Ranunculus plant. The A3B3 treatment recorded the highest average of (53.17) cm and thus excelled on all the treatments, while the A4B0 treatment recorded the lowest average of plant height of (28.67) cm.

Table (3) The effect of biofertilizer bacterial and the organic medium and their interaction on plant height (cm) of *Ranunculus asiatcus*.

The effect of organic medium (A)	biofertilizer bacteria				organic medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
34.83	39.00	36.33	34.00	30.00	River soil + peat moss (A0)
39.42	45.00	42.33	37.67	32.67	River soil + 3%, decomposed horse waste(A1)
41.88	48.33	44.83	39.17	35.17	River soil+ 3% mushrooms residues(A2)
44.88	53.17	47.17	41.00	38.17	River soil + 5% decomposed horse waste(A3)
33.42	38.50	36.50	30.00	28.67	River soil+ 5% mushrooms residues(A4)

	44.80	**41.43**	**36.37**	**32.93**	**The effect of biofertilizer bacterial (B)**
			1.408	**A**	**L.S.D 0.05**
			1.259	**B**	
			2.816	**A*B**	

Leaf area (cm^2)

The results in Table (4) showed the significantly excelled of plants inoculated with biofertilizer in increasing the leaf area of Ranunculus , where treatment B3 significantly excelled and gave the highest average of leaf area reaching (1293) cm^2, while the control treatment gave the lowest leaf area of (939) cm^2.It noticed that the organic medium have a significant effect in increasing the leaf area of the plant, where the A3 treatment (river soil + 5% decomposed horse waste) was significantly excelled and gave the largest leaf area of (1459) cm^2, while the treatment gave A4 (river soil+ 5%residues from decomposing fungi (mushrooms)) The minimum leaf area was (881) cm^2.From the interaction of biofertilizers with the organic medium , it appears that the treatment A3B3 gave the highest values for this trait, reaching (1691) cm^2, and the treatment of A0B0, the lowest average for the leaf area was (684) cm^2.

Table (4) The effect of biofertilizer bacterial and the organic medium and their interaction on the leaf area (cm2)of the *Ranunculus asiatcus*.

The effect of organic medium (A)	**biofertilizer bacterial**				**organic medium)A(**
	Mixture (B3)	***Azospirillium brasilences*(B2)**	***Bacillus subtillus* (B1)**	**without the bacterial vaccine B0)(**	
1006	**1179**	**1072**	**1089**	**684**	**River soil + peat moss (A0)**
1071	**1398**	**880**	**1034**	**973**	**River soil + 3%, decomposed horse waste(A1)**
1223	**1200**	**1392**	**1192**	**1109**	**River soil+ 3% mushrooms residues(A2)**
1459	**1691**	**1534**	**1407**	**1202**	**River soil + 5% decomposed horse waste(A3)**

881	**998**	**1034**	**764**	**727**	**River soil+ 5% mushrooms residues(A4)**
	1293	**1183**	**1097**	**939**	**The effect of biofertilizer bacterial (B)**
383.5				**A**	**L.S.D 0.05**
343.1				**B**	
767.1				**A*B**	

Number of flowers (flower. Plant $^{-1}$)

The results in Table (5) show that bio-fertilizers achieved a significant increase in the number of flowers , where the results showed that the treatment B3 (Azospirillium brasilences +Bacillus subtillus) was significantly excelled to it, and it gave the highest number of flowering plants, which reached 12.87.Compared to the control treatment (B0), which gave the lowest number of flowers for a plant, reaching 7.97 flowers. Plant $^{-1}$. It is noticed that the addition of the organic medium had a significant effect in increasing the average of this trait of the plant. The treatment of A3 (River+ 5% residues of the decomposing fungus) significantly excelled on the rest of the other treatments in increasing the number of flowers on the Ranunculus plant and gave 13.38 flowers. Plant $^{-1}$ .where the control treatment A0, which gave the lowest number of flowers on the Ranunculus plant amounted to 7.50 flowers. Plant $^{-1}$.While the bi-interaction between biofertilizers and organic medium showed significantly excelled for this trait, the A3B3 treatment was significantly excelled. The highest number of flowers on the Ranunculus plant was 17.17 flowers. Plant $^{-1}$, compared to the control treatment A0B0 in which the number of flowers decreased on the Ranunculus plant, reaching 7.00 flowers. Plant $^{-1}$.

Table (5) The effect of biofertilizer bacterial and the organic medium and their interaction on the Number of flowers (flowers .plant^{-1})of the *Ranunculus asiatcus*.

The effect of organic medium (A)	**biofertilizer bacteria**				**organic medium (A)**
	Mixture (B3)	***Azospirillium brasilences*(B2)**	***Bacillus subtillus* (B1)**	**without the bacterial vaccine (B0)**	
7.50	**8.50**	**7.67**	**6.83**	**7.00**	**River soil + peat moss (A0)**
10.38	**13.00**	**9.17**	**12.00**	**7.33**	**River soil + 3%, decomposed horse waste(A1)**

10.88	**14.83**	**11.50**	**9.50**	**7.67**	**River soil+ 3% mushrooms residues(A2)**
13.38	**17.17**	**15.33**	**13.50**	**7.50**	**River soil + 5% decomposed horse waste(A3)**
10.25	**10.83**	**10.83**	**9.00**	**10.33**	**River soil+ 5% mushrooms residues(A4)**
	12.87	**10.90**	**10.17**	**7.97**	**The effect of biofertilizer bacterial (B)**
			1.512	**A**	**L.S.D 0.05**
			1.352	**B**	
			3.024	**A*B**	

Flower diameter (mm)

Table (6) indicate that biofertilizers have a significant effect in increasing the diameter of the Ranunculus flower. It reached 77.53 mm. The results showed the significant role of the organic medium in increasing the diameter of plant flowers, where the A3 treatment significantly excelled on the rest of the other treatments, and it recorded a significant increase in the highest diameter of the Ranunculus flowers by 66.08 mm, excelled the average treatment of A4 (River+ 5% residues of the decomposing fungus), which gave less Ranunculus flowers diameter: 52.43 mm.Whereas, the bi-interaction treatment A3B3 recorded the highest average diameter of Ranunculus flowers, at 72.14 mm, compared to the control treatment A4B0, which gave the lowest diameter of Ranunculus flowers of 48.24 mm.

Table (6) The effect of biofertilizer bacterial and the organic medium and their interaction on the Flower diameter (mm) of the *Ranunculus asiatcus.*

The effect of organic medium (A)	**biofertilizer bacterial**				**organic medium (A)**
	Mixture (B3)	***Azospirillium brasilences*(B2)**	***Bacillus subtillus* (B1)**	**without the bacterial vaccine (B0)**	
54.69	**58.72**	**55.59**	**53.80**	**50.64**	**River soil + peat moss (A0)**

58.33	**63.17**	**60.26**	**56.80**	**53.09**	**River soil + 3%, decomposed horse waste(A1)**
61.17	**65.80**	**63.72**	**58.78**	**56.40**	**River soil+ 3% mushrooms residues(A2)**
66.08	**72.14**	**67.95**	**63.75**	**60.47**	**River soil + 5% decomposed horse waste(A3)**
52.43	**56.11**	**54.58**	**50.78**	**48.24**	**River soil+ 5% mushrooms residues(A4)**
	63.19	**60.42**	**56.78**	**53.77**	**The effect of biofertilizer bacterial (B)**
4.53				**A**	**L.S.D 0.05**
4.05				**B**	
9.06				**A*B**	

Vase life (day)

The vase life trait is one of the important traits that determine the duration of the flower stay in the vase after cutting, which depends on the amount of nutrients absorbed by the plant and the environmental conditions and fertilization before cutting.The results in Table (7) showed that the treatment B3 was significantly excelled on the rest of the other treatments by giving it the longest vase life for Ranunculus (14.20) days, compared to the treatment of B0, which gave the shortest vase life for plant flowers of (10.62) days. The data show the significant effect of the organic medium on the prolongation of the vase life of the Ranunculus flowers, where the treatment(River soil + 5% decomposed horse waste) was significantly excelled and gave the longest vase life of flowers (15.66) days, compared to the treatment of A4 (River+ 5% residues of the decomposing fungus) It gave the lowest vase life (9.86) days. As for the bi-interaction between the biofertilizers and the organic medium , their effect was significant in increasing the vase life of the Ranunculus .The bi-interaction treatment, A3B3, was significantly excelled and it recorded the longest vase life, which reached (18.67) days for Ranunculus flowers, while the vase life of flowers decreased when the interaction treatment A4B0 gave (7.33) for Ranunculus flowers.

Table (7) The effect of biofertilizer bacterial and the organic medium and their interaction .on the vase life (day)of the *Ranunculus asiatcus*

The effect of organic medium (A)	**biofertilizer bacterial**				**organic medium (A)**
	Mixture (B3)	***Azospirillium brasilences*(B2)**	***Bacillus subtillus* (B1)**	**without the bacterial vaccine**	

				(B0)	
10.53	**11.78**	**10.33**	**10.66**	**9.33**	**River soil + peat moss (A0)**
11.39	**12.66**	**11.33**	**11.44**	**10.11**	**River soil + 3%, decomposed horse waste(A1)**
14.14	**15.55**	**14.33**	**13.67**	**13.00**	**River soil+ 3% mushrooms residues(A2)**
15.66	**18.67**	**15.44**	**15.22**	**13.33**	**River soil + 5% decomposed horse waste(A3)**
9.86	**12.33**	**10.44**	**9.33**	**7.33**	**River soil+ 5% mushrooms residues(A4)**
	14.20	**12.37**	**12.06**	**10.62**	**The effect of biofertilizer bacterial (B)**
0.84				**A**	**L.S.D 0.05**
0.75				**B**	
1.68				**A*B**	

Determination of anthocyanin pigment in petals (mg.100 g dry weight)

The results in Table (8) indicate that biofertilizers had a significant effect in increasing the anthocyanin pigment for the Ranunculus flower, where the results showed that the treatment B3 (*Azospirillium brasilences +Bacillus subtillus*) was significantly excelled, and it gave the highest percentages of the above pigment, which amounted to 2.47 mg. 100 g dry weight. Compared to the control treatment (B0), which gave the lowest percentage of anthocyanin, which was 1.37 mg. 100 g dry weight.The results of the table show that the treatment of the organic medium A3 significantly excelled on the rest of the treatments in other media, and it recorded the highest percentage of anthocyanin pigment, which reached 2.75 mg. 100 g dry weight, while the treatment of A4 gave the lowest percentage of anthocyanin stain (1.23) mg 100 g dry weight.The bi-interaction between biofertilizers and organic medium also showed significant differences between the treatments, where the percentage reached its highest average of 3.51 mg to 100 g dry weight when treatment A3B3, and the comparison treatment, A4B0, gave the lowest percentage of anthocyanin (0.77) mg. 100 g dry weight.

Table (8) The effect of biofertilizer bacterial and the organic medium and their interaction on the anthocyanin pigment in petals (mg. 100 g dry weight) of the *Ranunculus asiatcus*.

The effect of organic medium (A)	biofertilizer bacterial				organic medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
1.42	1.88	1.71	1.09	0.99	River soil + peat moss (A0)
1.92	2.33	2.17	1.81	1.37	River soil + 3%, decomposed horse waste(A1)
2.14	2.62	2.22	2.12	1.62	River soil+ 3% mushrooms residues(A2)
2.75	3.51	2.99	2.42	2.10	River soil + 5% decomposed horse waste(A3)
1.23	2.02	1.20	0.93	0.77	River soil+ 5% mushrooms residues(A4)
	2.47	2.06	1.67	1.37	The effect of biofertilizer bacterial (B)
0.53				A	L.S.D 0.05
0.47				B	
1.06				A*B	

Percentage of Carbohydrates in the Leaves (%)

The results in Table (9) showed that the role of the mixed bacterial vaccine (*Bacillus subtillus* + *Azospirillium brasilences*) in increasing the percentage of carbohydrates in the leaves. where the mixture treatment B3 recorded the highest average, reaching 62.13%, compared to the B0 treatment (without vaccine), which recorded the lowest values of 00.12%. Whereas, the results indicated that the treatment A3 (River soil + 5% decomposed horse waste) gave the highest values for this trait, which amounted to (14.23)%.The treatment of A4 recorded the lowest percentage of carbohydrates in the plant leaves, reaching (11.14)%, and thus it significantly differed from the other treatments. The same role also shows that the interaction between bio fertilizers and organic medium has an important role in increasing the percentage of carbohydrates in plant leaves. The interaction treatment differed between them, and all of them excelled on the control treatment

A4B0, which recorded the lowest percentage of carbohydrates in the leaves amounted to (10.03%), While the interaction treatment A3B3 significantly excelled on the rest of the interaction treatment in giving the highest percentage of carbohydrates which reached (15.88)%.

Table (9) The effect of biofertilizer bacterial and the organic medium and their interaction on the percentage of Carbohydrates in the Leaves (%)of *Ranunculus asiatcus*.

The effect of organic medium (A)	biofertilizer bacterial				organic medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
12.20	13.14	12.72	11.86	11.09	River soil + peat moss (A0)
13.06	13.32	13.20	12.96	12.76	River soil + 3%, decomposed horse waste(A1)
13.10	13.46	13.32	12.85	12.75	River soil+ 3% mushrooms residues(A2)
14.23	15.88	14.42	13.21	13.40	River soil + 5% decomposed horse waste(A3)
11.14	12.30	11.62	10.60	10.03	River soil+ 5% mushrooms residues(A4)
	13.62	13.06	12.30	12.00	The effect of biofertilizer bacterial (B)
0.45				A	L.S.D 0.05
0.40				B	
0.90				A*B	

Biofertilizers play an important and effective role in increasing the Studied traits. The results showed that the treatment of B3 mixture (Bacillus subtillus + Azospirillium brasilences) was significantly excelled on the control treatment in plant height trait, chlorophyll content, number of flowers, duration of flowering and number of tuberous roots . This excelled is due to the role of biofertilizers in improving the root growth of the plant by encouraging the formation of root hairs and increasing the surface of the root system, which leads to an increase in the absorption of water

and nutrients and thus improves the vegetative growth of the plant, Thus it improves plant height and leaf area (Bhat et al, 2019 and Yadav and Sarkar, 2019) as well as the role of Bacillus bacteria on Production of plant growth-regulating hormones (Gnamamanickm, 2006), The results of testing the hormonal content in the organic medium in which the Bacillus was grown by Al-Ashour (2009) demonstrated its ability to produce hormone-like substances such as Auxin, gibberellin, and Zeatin that help increase the average of cell division, expansion and elongation, and an increase in the level of metabolism within cells, and this is reflected in plant growth (Swain et al,2007), Also contribute increases plant height. Bio-fertilizers contribute to increasing the absorption of nutrients, which play an important role in increasing the In vegetative growth. In addition to the role of these organisms in the secretion of growth regulators, including gibberellins, which perform their role in building carbohydrates and proteins and increase their accumulation in the plant (Hobbie, 2006), and that the increase in flowering traits is due to perhaps the reason for the excelled that these types of bacteria belong to the group of growth-stimulating bacteria (PGPR) that increases the concentrations of nutrients due to their role in dissolving phosphate compounds and fixing atmospheric nitrogen and reducing the degree of soil reaction and thus increasing the release of nutrients, including potassium, or by secreting them many growth regulators and organic acids that work to increase the concentration of elements in the soil and thus reflect positively on plant growth (Alkhalil, 2011), This result agrees with (Al-Sharea, 2020) in its experiment on the Ranunculus plant when using *Bacillus subtillus* bacteria and with (Qasim,2014) on gladiolus when using *Azospirillum brasilense*. and (Sumaisem and Al-Rabie ,2013) on the *Catharanthus roseus* plant .From the results in Tables (3-8), it is noted that the organic medium A3 (River soil + 5% decomposed horse waste) A2 (River soil + 3% mushroom residues) on A0 in most of the characteristics of Ranunculus . This may be due to the content of the medium with a high percentage of organic matter and nutrients N, P, K compared to the medium A0 (River soil + Peat moss)., this works to improve soil fertility, provide nutrients, and increase its availability and absorption by the plant, which affects various biological activities such as cell division and elongation, building proteins and nucleic acids, and thus forming good vegetative growth (Al-Sawaf and Alwan, 2010). This result agrees with Abdallateef (2015) on Antirrhinum majus and with Khattak et al, (2011) on *Catharanthus roseus* L plant when using mushroom residues, and it also agrees with Hamad and Kfeesh (2015) when using horse waste on pomegranate trees and with E.laczi et al (2016) on the plant Chinese Cabbage.

References

Abdallateef, S. (2015) Influence of chelated Calcium and growing media on thegrowth and flowering of *Antirrhinum majus. The Iraqi Journal of Agriculture Sciences,* 46(5):813-818

Adiaha, M.S. (2017).The Role of Organic Matter in Tropical Soil Productivity . World Scientific News 86(1):1-66 .

Al-Ashour, Ali Jaber Jasim (2009). The possibility of using some local isolates of the genus Bacillus in controlling some plant pathogenic fungi. PhD thesis. Faculty of Science - University of Kufa.

AlcaAntara ,B; R. Mary;B. Almudena and L.Francisco(2016).Liquid Organic Fertilizers For Sustainable Agriculture :Nutrient Up.

Al-Hasnawi, H. A., Hussein, J. K., & Khaleel, T. H. (2019). Effect of growth regulators and preservative solution on vase life and water relation of gladiolus hybrida l. after cut flowers. The Iraqi Journal of Agricultural Science, 50, 182-191.

Alkhalil, Sherine Muzaffar Ali, 2011. Tyr Integration between mineral, organic and biological fertilization in tomato yield (Lycopersicon esculentum Mill) in greenhouses. Master thesis, Department of Soil Science and Water Resources - University of Baghdad - College of Agriculture

Allawi. Mohammed Mustafa . (2013). The effect of biological, organic and chemical fertilization on the architectural structure of roots and the yield growth of the pepper plant (Capsicum annuum L.), PhD thesis. faculty of Agriculture . University of Baghdad, Iraq.

Al-Sahuki, Medhat Wahib, Karima Muhammad. (1990). Applications in design and analysis of experiments. House of wisdom for printing and publishing. Ministry of Higher Education and Scientific Research, College of Agriculture. Baghdad University . Iraq.

Al-Sawaf, Muhammad Dawood Salim and Alwan, Nibras Ihsan Abdul-Jabbar (2010). The effect of some agricultural treatments on the growth, flowering and yield of the germs and creams of two varieties of the freesia plant, Freesia hybrida. Al-Rafidain Agriculture Journal, Volume 38, Annex 1, pp. 73-78.

Al-Serafy, Zakaria Massad. (2010). Fertilizer Chemistry, Faculty of Agriculture, Mansoura University, First Edition, Dar Al Kotob for Publishing and Distribution.

Al-Sharaa, Marwa Ali Abdul-Jabbar (2020). The effect of biological fertilizers and humic acid on the growth of Ranunculus asiaticus. Master thesis. faculty of Agriculture . Al-Qasim Green University. Iraq.

Badr, Mustafa and Mahmoud, Khattab, Tariq, Al-Qai'i, Muhammad Yaqut, Mustafa Raslan, Mustafa Badr, Muhammad Heikal and Alamuddin Noah. (2010) Flowers, ornamental plants, design and landscaping, ninth edition, Ma'arif facility in Alexandria

Bernstein, N., Ioffe, M., Luria, G., Bruner, M., Nishri, Y., Philosoph-Hadas, S., .and Matan, E. (2011). Effects of K and N Nutrition on Function and Production of Ranunculus asiaticus. Pedosphere, 21(3), 288-301.

Bhat, M.A.; R..Rasool and S.Ramzan.(2019).Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable and Eco-Friendly Agriculture. Acta Scientific Agriculture 3(1):23-25.

Contreras-Angulo,J.R.;T.M.Mata;P.Sara and P.Cuellar-Bermudez (2019). Symbiotic Co-Culture of Scenedesmus sp. And Azospirillum brasilense on N-Deficient Media with Biomass Production for Biofuels.Sustainability ,11(7):1-16. 27(2). 201-205.

Diwany, Sadiq Jaafar Hasan (2003) The role of organic matter and water quality in the mobility and distribution of salts in salt-affected soils. Master Thesis . Department of Soil Sciences - College of Agriculture / University of Baghdad.

E.LACZI. A , Apahidean, E. Luca, A. Dumitraş, P. Boancă.(2016) .Headed Chinese cabbage growth and yield influenced by different manure types in organic farming system..

GnamamanickS.S.(2006).PlantAssociatedBacteria.Springer,NetherlansPP.155-194.

Granatsteir, D. (2004). Center for sustaining Agriculture and Natural Resources. Washington State University, Wentchee, Wa.USA.

Hamad, Rasmi Muhammad, Kfeesh, Sari Ahmed. (2015) The effect of adding organic residues on some growth and production characteristics of pomegranate. University of Anbar. College of Agriculture. Anbar Journal of Agricultural Sciences.

Hobbie , E.A .2006.Carbon allocation to ectomycorrhiza fungi correlates with total belowground allocation in culture studies. Ecology 87,P:563-569.

Joslyn , M.A.(1970) .Methods In Food Analysis , Physical ,Chemical and Instrumental Methods of Analysis, 2nd ed.Acdemic Press. New York and London.

Khattak, A.; Ahmad, I.; Ul Amin, N; Wahid, F. and Ul Rahman, H. (2011) Effects of different amended organic media on the growth anddevelopment of *Vinca rosea* 'Victory'. Sarhad Journal Agriculture.

Matysiak,B. and G.Falkowski.2010.Response of three ornamental plants species to inoculation with Arbuscular Mycorrhiza fungi depending on compost addition to peat substrate and the rate of controlled release fwrtilizer.Journal of Fruit and Ornamental Plant Researach 18(2):321-333.

Qasim,M.; A,Younis .;Z.A, Zahir .; A,Riaz; H,Raza; and U,Tariq.(2014). Microbial Inoculation Increases The Nutrient Uptake Efficiency For Quality Production Of Gladiolus grandiflorus.

Sumaisem, Enas Abdel-Raouf Amarou Rabie, Muslim Abd Ali (2013): The effect of bacilloptrazole and the biomodulation Bacillus subtilis on the growth of the eye of the bison plant (Catharanthus roseus L). Faculty of Agriculture, University of Kufa.

Swain, M .R.; Naskar,K.S. and Ramesh,C.R.(2007).Indole-3-acetic acid production and effect on sprouting of yam (Dioscorea rotundata L.)minisetts by Bacillus subtilis isolated form culturable cow dung microflora .Polish Journal of Microbiology,56(2):103-110.

Vessey,JK.2003.Plant growth promoting rhizobacteria asbiofertilizer .plant and Soil 255,p:571-586.

Yadav, K; K.and S. Sarkar. (2019).Biofertilizers ,Impact on soil Fertility and Crop Productivity under Sustainable Agricultur.Environment and Ecology 37(1):89-93.