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Effect of Bio-Fertilizers and Agricultural Medium on the Growth and Flowering of the *Ranunculus Asiaticus*

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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 agricultural 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 agricultural medium that included (River soil + peat moss , River soil + 3%, decomposed horse waste ,River soil+ 3% mushrooms residues(Agaricus bisporus) , River soil + 5% decomposed horse waste ,River soil+ 5% mushrooms residues(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 0.05 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, relative chlorophyll content in leaves, the number of flowers, The duration of the flowers on the plant, the number of tuberous roots and the results were as follows: 44.80 cm, 35.92 spad unit , 12.87 flowers.plant^{-1},17.03 days, 7.92 roots.plant^{-1} respectively.The results showed the excelled of the agricultural medium treatments, where the A3 treatment (River soil + 5% decomposed horse waste) recorded the highest average for all the studied traits, plant height, relative chlorophyll content in the leaves, and,The number of flowers, The duration of the flowers on the plant, the number of tuberous roots and the results were as follows, 44.88 cm, 40.72 Spad unit , 13.38 flowers.Plant^{-1}, 18.83 days, 7.82 roots.plant^{-1}.The results of the bi-interaction between the biofertilizer bacterial and the agricultural medium showed significantly excelled in most the studied traitswhere the A3B3 treatment excelled in the plant height, the relative chlorophyll content in the leaves, the number of flowers,the duration of the flower stay on the plant,and the results were as follows:53.17 cm, 45.23 spad unit, 17.17 flower.plant^{-1},23.11 day. The treatment of the bi-interaction treatment A2B3, also excelled by giving the highest average number of tuberous roots 9.11 root.plant $^{-1}$.

Keyword:*bio-fertilizers; Bacillus subtillus; decomposedhorse waste; Azospirilliumbrasilences.*

1. 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 [1]. 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

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coordination with other flowers Some types of Ranunculus are used in alternative medicine as an anti-rheumatism, fever, and redness of the skin, and this comes through the presence of the substances Anemonin and Protoanemonin, which are used as a treatment for many diseases [2],[3]. 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 [4]. 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 [5]. Organic fertilizers of (decomposed horse waste, mushrooms residues) 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[6] , The sources of organic matter vary from plant sources that come from plant roots and fallen leaves on the surface of the soil. This goes 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. These 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 [7], [8]. When using biological fertilizer, the Ranunculus plant obtained an increase in the number of flowers and the diameter of tuberous roots 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 agricultural medium and addded difrented bacterial types of bio fertilizers in the growth and flowering of the Ranunculus plant.

2. 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 Agriculture 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) [9] As for the second factor, the agricultural medium that included (River soil + peat moss , River soil + 3%, decomposed horse waste ,River soil+ 3% mushrooms residues*(* *Agaricus bisporus*) , River soil + 5% decomposed horse waste,River soil+ 5% mushrooms residues (*Agaricus bisporus*) and which it were symbolized by (A0, A1, A2, A3, A4) Horse waste was collected from the stables of the Lebanese Equestrian Club in the Najaf provain. Experiment was applied 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 0.05 probability level (Al-Sahuki and Waheeb, 1990). The data were analyzed using the ready-made statistical program Genstat. Samples were taken from agricultural 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.

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Table 1. Chemical and physical properties for horse waste and mushroom residues

Traits	Units	mushroom residues	horse waste
Electrical conductivity (EC)	$d.S.m^{-1}$	4.4	4.16
PH	---	7.4	7.28
Organic matter	$g.Kg^{-1}$	25.40	28.45
The ratio of carbon to nitrogen	---	16.14	15.40
Total nitrogen	$g.Kg^{-1}$	8.75	6.56
phosphorous availability	$g.Kg^{-1}$	0.17	0.56
Potassium availability	$g.Kg^{-1}$	0.04	0.11

* It was analyzed in the Central Laboratories for Graduate Studies, University of Baghdad, College of Agriculture.

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	718	**Sand**
	195	**Silt**
	87	**Clay**
loamy sand		**Texture**

2.1.Studied traits

2.1.1.Plant height (cm)

2.1.2.Relative chlorophyll content in leaves (SPAD unit)

The intensity of the chlorophyll pigment in the leaves was estimated by a (Chlorophyll meter Model SPAD-502)) device from the Japanese company Minolta. Record the reading of four leaves per plant and extract the averages.

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

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

2.1.4.The duration of the flowers on the plant (day)

It was calculated from when each flowering on the plant until the petals begin to wither.

2.1.5.The number of tuberous roots.plant^{-1}

The average of tuberous roots growing for all experimental unit plants was extracted and then averages were calculated for each treatment.

3. RESULTS AND DISCUSSION

3.1. Plant height (cm)

The results in Table (3) indicate that bio fertilizers had a significant effecting 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 agricultural medium had a significant effecting 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 agricultural 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 agriculture medium and their interaction on plant height (cm) of *Ranunculus asiatcus*

The effect of agriculture medium (A)	biofertilizer bacterial				agriculture 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)**
1.40				A	**L.S.D 0.05**
1.25				B	
2.81				A*B	

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3.2. Relative chlorophyll content in leaves (SPAD unit)

The results in Table (4)showed that the addition of the bio fertilizer led to a significant increase in the relative chlorophyll content. where the treatment B3 significantly excelled and gave 35.92 Spad , while the control treatment B0 gave the lowest values of (27.89) Spad. It is also noticed from the results of the same table that the agricultural medium had a significant increase in the relative chlorophyll content in the leaves. The treatment A3 significantly excelled on the rest of the treatments and gave the highest average of (40.72) Spad. While the A4 treatment gave the lowest average of (25.44) Spad. The bi-interaction treatment differed significantly between the bio fertilizers and the agricultural medium and all the treatments excelled on control treatment A4B0, which gave the lowest chlorophyll content of (22.44) Spad, while the interaction treatment A3B3 recorded the highest average, reaching 45.23 Spad.

Table 4. The effect of biofertilizer bacterial and the agriculture medium and their interaction on Relative chlorophyll .content in leaves (SPAD unit) of *Ranunculus asiatcus*

The effect of agriculture medium (A)	biofertilizer bacterial				agriculture medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
25.76	29.49	25.62	24.89	23.04	**River soil + peat moss (A0)**
29.86	34.53	30.69	28.78	25.45	**River soil + 3%, decomposed horse waste(A1)**
36.94	40.96	38.87	35.64	32.27	**River soil+ 3% mushrooms residues(A2)**
40.72	45.23	42.37	39.03	36.24	**River soil + 5% decomposed horse waste(A3)**
25.44	29.40	26.33	23.60	22.44	**River soil+ 5% mushrooms residues(A4)**
	35.92	32.77	30.39	27.89	**The effect of biofertilizer bacterial (B)**
4.57				A	**L.S.D 0.05**
4.08				B	
9.14				A*B	

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

The results in Table (5) show that the bio fertilizers achieved a significant increase in the number of flowers formed on each plant. The mixture treatment B3 (*Bacillus subtillus* + *Azospirillium brasilences*) was significantly excelled and gave it the highest number of flowers, which reached 12.87 flower.Plant $^{-1}$compared to the control treatment B0 , which gave the lowest number of flowers, which reached 7.97 flower.Plant $^{-1}$. From the results in the same table, we notice the significant effect of the agricultural medium on the number of flowers formed on the plants. where the A3 treatment (River soil + 5% decomposed horse waste) significantly excelled on the rest of the medium(A0, A1, A2) by recorded the highest number of flowers formed, which reached 13.38 flower.Plant $^{-1}$and thus excelled on the control treatment A0 (River soil + peat moss), which recorded the lowest numbers, which are 7.50 flower.Plant $^{-1}$.Where, it showed the bi-interaction between the bacterial fertilizer B3 (mixture) and the culture medium A3 (River soil + 5% decomposed horse waste). It achieved the highest average of 17.17 flower.Plant $^{-1}$,

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compared to the control treatment A0B1 in which the number of flowers formed on plants decreased and reached 6.83flower.Plant $^{-1}$.

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

The effect of agriculture medium (A)	biofertilizer bacterial				agriculture 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)**
1.51				A	**L.S.D 0.05**
1.35				B	
3.02				A*B	

3.4. The duration of the flowers on the plant(day)

The data in Table (6) refer to the role of biological fertilizers in prolonging the duration of the Ranunculus flowers on the plant .The treatment of mixture B3 significantly excelled and gave the highest average of (17.03) days, excelled on the control treatment B0 that gave (11.88) days. The results of the table also show that the treatment of agricultural medium had an effect on prolonging the duration of flowers on the plant. where the A3 treatment (River soil + 5%decomposed horse waste) significantly excelled and gave (18.83) days, While the duration of flowers on the plant decreased to its lowest average in the treatment of A4 (River soil + mushroom residues(*Agaricus bisporus*)5%), amounting to (10.77) days. As it can be noted from the data of the bi-interaction between biofertilizers and agricultural medium, the A3B3 interaction treatment was significantly excelled on the rest of the treatments and gave (23.11) days. Where the flowering duration decreased when the interaction treatment A4B0 gave (9.19) days.

3.5. Number of tuberous roots.Plant $^{-1}$

Table (7)showed that the two treatments (B3)(*Bacillus subtillus* + *Azospirillium brasilences*) and B2 (*Azospirillium brasilences*) were excelled on the treatment of B1 (*Bacillus subtillus*) in the number of tuberous roots formed. where the treatment of B3 recorded the highest average of (7.92)) tuberous. plant $^{-1}$, compared to the control treatment that gave the lowest average (5.64) tuberous. plant $^{-1}$. The results also indicated the significant effect of the agricultural medium on the number of tuberous roots. The medium treatment A3 significantly excelled and was the highest average of tuberous roots (7.82) tuberous. plant $^{-1}$), while the control treatment A0 gave the lowest average of (5.86)) tuberous. plant $^{-1}$. The bi-interaction between bio fertilizers and the agricultural medium showed a significantly excelled for this trait. The treatment, A2B3, significantly excelled on the rest of the interaction treatments, and it recorded the highest

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Table 6. The effect of biofertilizer bacterial and the agriculture medium and their interaction on The duration of the flowers on the plant (day)of the *Ranunculus asiatcus*

The effect of agriculture medium (A)	biofertilizer bacterial				agriculture medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
12.45	15.45	13.11	10.92	10.33	River soil + peat moss (A0)
14.12	16.66	14.96	13.53	11.33	River soil + 3%, decomposed horse waste(A1)
14.72	17.13	14.44	14.33	12.99	River soil+ 3% mushrooms residues(A2)
18.83	23.11	19.89	16.78	15.55	River soil + 5% decomposed horse waste(A3)
10.77	12.78	11.36	9.77	9.19	River soil+ 5% mushrooms residues(A4)
	17.03	14.75	13.06	11.88	The effect of biofertilizer bacterial (B)
1.78				A	L.S.D 0.05
1.59				B	
3.57				A*B	

Table 7. The effect of biofertilizer bacterial and the agriculture medium and their interaction on Number of tuberous roots . plant $^{-1}$ of *Ranunculus asiatcus*

The effect of agriculture medium (A)	biofertilizer bacterial				agriculture medium (A)
	Mixture (B3)	*Azospirillium brasilences*(B2)	*Bacillus subtillus* (B1)	without the bacterial vaccine (B0)	
5.86	6.78	6.55	5.44	4.66	River soil + peat moss (A0)
7.05	7.44	7.44	7.67	5.67	River soil + 3%, decomposed horse waste(A1)
7.57	9.11	8.39	7.33	5.44	River soil+ 3% mushrooms residues(A2)
7.82	8.55	8.39	7.77	6.55	River soil + 5% decomposed horse waste(A3)
6.68	7.72	6.45	6.68	5.88	River soil+ 5% mushrooms residues(A4)
	7.92	7.44	6.98	5.64	The effect of biofertilizer bacterial (B)
1.14				A	L.S.D 0.05
1.02				B	
2.28				A*B	

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rate of the number of tuberous roots, reaching 9.11) tuberous. plant $^{-1}$, compared to treatment A0B0, which gave the lowest average of 4.66 tuber. Biofertilizers play an important and effective role in increasing the Studied traits. showed that the treatment of B3 (Bacillus subtillus + Azospirillium brasilences) was significantly excelled in traits of plant height , 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[10,11] as well as the role of Bacillus bacteria on Production of plant growth-regulating hormones [12] ,The results of testing the hormonal content in the agriculture medium[13] 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[14], which increases plant height. Bio-fertilizers contribute to increasing the absorption of nutrients, which play an important role in increasing the size and number of leaf cells. 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[15] 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[16] ,The results also showed that bio fertilizers had a significant effect in increasing the number of tuberous roots (Tables 8). This is due to its role in improving the biological and chemical properties of the soil by releasing greater amounts of nutrients available for absorption by the roots, expanding the spread of the roots and absorbing water and nutrients, which leads to an increase in the efficiency of the photosynthesis process [17] .This result agrees with [10] in its experiment on the Ranunculus plant when using *Bacillus subtillus* bacteria and with[18] on gladiolus when using *Azospirillum brasilense*. And[19] on the *Catharanthus roseus* plant .From the results in Tables (3-8), it is noted that the agricultural medium A3 (River soil + 5% decomposed horse waste) A2 (River soil + 3% mushroom residues(*Agaricus bisporus*) 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[20] . This result agrees with [21] on *Antirrhinum majus* and with [22] on *Catharanthus roseus* L plant when using mushroom residues, ,[23] when using horse waste on pomegranate trees and with [24] on the plant Chinese Cabbage.

4. CONCLUSIONS

The mixture of B3 bacterial Biofertilizer (Bacillus subtillus + Azospirillium brasilences) achieved the best results by giving it the highest average of vegetative and flowering traits, vase life and the yield of tuberous roots.The agricultural medium A2 (River soil+ 3% mushrooms residues*(Agaricus bisporus*) and A3 River soil + 5% decomposed horse waste)had a significant effect on the trait of vegetative growth, flowering, vase life and the yield of tuberous roots, but the highest average was achieved by the agricultural medium A3 for all the studied traits.

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