Effect of halophytes wild plants on the microbial community in the rhizosphere

Saad Ali Hussein Al-Akabi*, Najim A. Jumah Al-Zubaidy[1] and Faris Mohammad Suhail[2]

[1]*College of Education for Pure Sciences, University of Diyala, Iraq*
[2]*College of Agriculture, University of Diyala, Iraq*
**Email:* d.saadalihussen@gmail.com

ABSTRACT: A factorial experiment was carried out according to the randomized complete block design (RCBD) during the season of 2020 to study the effect of three factors are location, plant type, and growth stages of wild halophytic plants in the microbial community. The first factor is the location, included three regions (Diyala University, Muqdadiya District, and Baladrooz District), the second factor is a plant type, included (the rhizosphere of *Schanginia aeguptiace* plant, the rhizosphere of *Atriplex halimus* plant, and the comparison soil far from the effect of the roots) and the third factor is the stages of plant growth, included (germination stage, vegetative growth stage, and stage of post flowering). The results showed the location of Al-Muqdadiya district was significantly superior in most of the traits compared to the other locations such as Diyala University and Baladrooz district, where the total number of bacteria in the rhizosphere, amounted to (2.250 x 10^7 cfu g dry soil^{-1}) in the location of Al-Muqdadiya district with significantly different from the total number of bacteria (2.018×10^7 cfu gm dry soil^{-1}) in the location of Diyala university. The treatment of the plant type (*Atriplex halimus* plant) was significantly superior in most of the studied traits compared to (*Schanginia aeguptiace* plant and the comparison soil), the roots of *Atriplex halimus* plant had an encouraging and significant effect on the total number of bacteria in the rhizosphere and fungi in the rhizosphere and the amount of CO_2 released, which amounted (3.141 x 10^7 cfu g dry soil^{-1}, 2.43 x 10^3 cfu g dry soil^{-1} and 70.26 mg CO_2.100 g soil^{-1}.14 day^{-1}) respectively. The growth stages of halophytic wild plants (germination stage) had a significant effect on the total number of bacteria in the rhizosphere, and fungi in the rhizosphere, and the amount of CO_2 released, which amounted (2.84 x 10^7 cfu g dry soil^{-1}, 2.296 x 10^3 cfu g dry soil^{-1} and 63,572 mg CO_2.100 g soil^{-1}. 14 day^{-1}) respectively. The binary and triple interaction had a significant effect in most of the studied traits.

Keywords: *Schanginia aeguptiace, Atriplex halimus*

الملخص:

تأثير النباتات البرية الملحية على المجتمع الميكروبي في منطقة الجذور

أجريت تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة (RCBD) خلال موسم 2020 لدراسة تأثير ثلاثة عوامل هي الموقع ونوع النبات ومراحل نمو النباتات الملحية البرية في المجتمع الميكروبي.

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العامل الأول هو الموقع، وشمل ثلاث مناطق (جامعة ديالى، قضاء المقدادية، قضاء بلدروز)، والعامل الثاني هو نوع النبات، وشمل (جذور نبات شانجينيا ايجوبتياس، وجذور نبات أتريبليكس هاليموس، والتربة المقارنة). بعيد عن تأثير الجذور) والعامل الثالث هو مراحل نمو النبات وتشمل (مرحلة الإنبات، مرحلة النمو الخضري، ومرحلة ما بعد التزهير). وأظهرت النتائج تفوق موقع قضاء المقدادية معنويا في معظم الصفات مقارنة بالمواقع الاخرى مثل جامعة ديالى وقضاء بلدروز حيث بلغ العدد الكلي للبكتيريا في منطقة الجذور (2.250g cfu x107 جاف). التربة-1) في موقع قضاء المقدادية مع اختلاف معنوي عن العدد الكلي للبكتيريا (2.018×107 cfu غم تربة جافة-1) في موقع جامعة ديالى. تفوقت معاملة نوع النبات (Atriplex halimus plant) معنوياً في معظم الصفات المدروسة مقارنة بنبات (Schanginia aeguptiace وتربة المقارنة)، وكان لجذور نبات Atriplex halimus تأثير مشجع ومعنوي في العدد الكلي للبكتيريا. في منطقة الجذور والفطريات في منطقة الجذور وكمية ثاني أكسيد الكربون المنطلقة والتي بلغت (3.141cfu x107 جرام تربة جافة-1، 2.43cfu x103 جرام تربة جافة-1 و 70.26 ملجم CO2.100 جرام تربة-1.14 يوم-1)) على التوالى. كان لمراحل نمو النباتات البرية الملحية (مرحلة الإنبات) تأثير كبير على إجمالي عدد البكتيريا في منطقة الجذور، والفطريات في منطقة الجذور، وكمية ثاني أكسيد الكربون المنطلقة والتي بلغت (2.84×107 قدم مكعب تربة جافة-1) ، 2.296 × 103 جم تربة جافة -1 و 63.572 ملجم ثاني أكسيد الكربون.100 جم تربة -1 14 يوم -1) على التوالي. وكان للتفاعل الثنائي والثلاثي تأثير معنوي في معظم الصفات المدروسة.

The region of soil affected by the roots of plants is called the rhizosphere, which extends a few millimeters from the surface of the roots, in which the number of microorganisms are affected quantitatively and qualitatively by the existence of plant roots as a result of the influence of these organisms by the vital processes of the plant (Al-Hammadi 2014). It contains many organic compounds resulting from the secretions of the roots and the decomposition of dead root hairs and sloughed cells, which are a source of carbon and nitrogen in this region (Marschnar 2007). The interactions between microorganisms and the root system in higher plants are very complex due to the nature of the produced compounds by the plants roots, and the number of microorganisms increases due to the increase in the concentration of organic matter as a result of the root secretions (Dahir and Tahir 2016), The number of microorganisms with different nutrition increases in the region around the roots compared to the far region (Al-Hammadi 2014, Al-Adwani and Al-Hayali 2018 b), this increase was attributed to the secretion of mucigel that surround the root surface, as well as the presence of organic compounds, decaying tissues and scratched roots, which release organic acids, carbohydrates, amino acids and hormones , which improve the growth and reproduction of microorganisms in the rhizosphere (Al-Azzawi and Qassem 2018, Al-Adwani and Al-Hayali 2018 a).The coexistence between microorganisms and the root system in the soil provides a perfect environment for the growth of microorganisms and is the main key for bio-fertilizers techniques and its important role in improving plant production in modern agriculture (Al-Samarrai and Al-Tamimi, 2018). Microorganisms in this area improve the nutritional status of the plant through their secretion of plant growth-regulating hormones such as auxins, gibberellins and cytokines, and the

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production of a group of vitamins B2, B12, Folic acid, Thiami, Niacin, Pantothenic acid, Riboflavin and sidrophores (Mahdi et al 2010, Jnawali et al 2015). In addition to its ability to reduce the spread of plant diseases (Kang and Mills 2004), as well as the role of many types of free-living bacteria, such as nitrogen-fixing azotobacter and azospirillum, which spread in all types of neutral and basic soils, and their presence is less in acidic soils, where they fix Nitrogen is not symbiotic and released in the form of ammonium NH4 in the soil (Rajaram et al 2013), as well as its high antagonism against plant pathogens. *Schanginia aeguptiace* is one of the annual plants, it grows in saline clay soils rich in nitrogen. Its roots penetrate into the soil for distances to absorb water and nutrients, as it contributes to attracting microorganisms in the soil and increasing its numbers through what it presents of secretions, sloughed cells and gelatinous materials to the growing tops of the root branches. (Al-Khashin 1985). *Atriplex haliums* is one of the salt-loving plants of halophyte, it is a perennial plant that needs water with small quantities (El-Shaer 2010). This plant contains high levels of salts and oxalates in the leaves, which makes it less palatable (Hassan 2009), as well as for its high content of crude proteins (Aganga et al 2003). Al-Rashidi and Jabbar (2015) confirmed that both roots and microorganisms consume organic matter in the roots of plants, thus co_2 is produced in certain quantities in the rhizosphere, as carbonic acid is formed, which affects on the degree of soil interaction, which helps in the growth and reproduction of microbes, where this emitted gas from the soil is used as a guide for the activity and effectiveness of soil organisms, its concentration is a sensitive measure of the soil environment (Nielsenm et al 2002). Due to the lack of studies on the effect of the root environment of *Schanginia aeguptiace* and *Atriplex halimus* plants on the microbial community compared to the comparison soil (far from the influence of roots), this study was carried out.

MATERIALS AND METHODS

A Factorial experiment was carried out according to a randomized complete block design (RCBD), the experiment included 27 treatments and three replications and resulted from the interaction of three factors (the location, plant type, growth stage), with the aim of studying the microbial community (bacteria and fungi) in the rhizosphere of *Schanginia aeguptiace* and *Atriplex halimus* plants, and the comparison soil far from the influence of roots, and study of microbial activity through the release of carbon dioxide (CO_2). The first factor is the location, included three regions (Diyala University, Muqdadiya District, and Baladrooz District), the second factor is a plant type, included (the rhizosphere of *Schanginia aeguptiace* plant, the rhizosphere of *Atriplex halimus* plant, and the comparison soil far from the effect of the roots), and the third factor is the stages of plant growth, included (germination stage, vegetative growth stage, and stage of post flowering).

The studied traits

1- Total numbers of bacteria cells in the rhizosphere of halophytes: The number of bacteria was estimated in the rhizosphere soil of *Schanginia aeguptiace* and *Atriplex halimus* plants, which its diameter is not more than 1 cm around the roots of the plants (Whitelaw 2000), the roots were carefully removed from the soil, then placed in sterile polyethylene bags for each region and growth stage of the study, 10 g of the studied plants rhizosphere and the comparison soil was taken separately and placed in a glass beaker containing 90 ml of sterile distilled water, mixed well and left for 5 minutes, 10 ml of the suspension was taken and transferred to another sterile glass beaker containing 90 ml of sterile distilled water to obtain a dilution of 10^{-2}, this process was repeated until reaching 10^{-8}, then 1 ml of the last dilutions were transferred to sterile petri dishes after pouring the Nutrient Ager medium, which consists of (Beef extract 3.0 g. l^{-1}, Peptone 5.0 g. l^{-1}, Ager-Ager 20 %, H2O 990 ml), which was sterilized with autoclave at a temperature of 121°C and a pressure of 15 pounds/ing^2 for a period of 20 minutes, 1 ml of each dilution was poured on the nutrient medium with three replicates for each dilution and a moving to the right and left in order to spread the soil suspension, the petri dishes were incubated in the incubator at a temperature of 28 ° C for a period of 3-7 days according to the method (Black 1965).

2- Total numbers of fungi in the rhizosphere of halophytes: Same protocol of total numbers of bacteria cells in the rhizosphere of halophytes was followed as mentioned in (Black, 1965), but the only difference is using the Martin Ager medium instead of Nutrient Ager medium and dilutions series of the soil samples were prepared until 10^{-5} instead of 10^{-8}**.**

3- Measuring the activity of microorganisms through the amount of CO2 released from the rhizosphere soil samples of halophytes: The amount of CO2 was measured from soil samples of the rhizosphere of *Schanginia aeguptiace* and *Atriplex halimus* plants and the comparison soil far from the influence of the roots for all study regions and for each stage of plant growth**,** soil samples were incubated for 14 days, according to the method (Janzen 1987), 100 g of soil was placed separately according to the treatments in glass bottles 1 liters, then a beaker100 ml containing 10 ml of sodium hydroxide (M1) was placed inside the glass bottles and covered tightly to prevent any loss of released CO2 gas, after the sodium hydroxide solution absorbs gas CO2 from the trapped air in the bottle, it will react with it, and producing turbid sodium carbonate, which is precipitated by adding 5 ml of barium chloride (N1), as in the following equation.

$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$

$Na_2CO_3 + BaCl_2 \rightarrow 2NaCl\downarrow + BaCO_3$

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Drops of Phenolphthalein dye were added to show the end point of the reaction and placed in the Burette, opposite (N0.5) of hydrochloric acid, the amount of CO2 released during 14 days of incubation was estimated according to the following equation.

mg CO_2 /100 gm soil = (B-V) NE (Janzen 1987)

B: volume of acid consumed in the comparison sample.

V: volume of acid consumed in the treatment sample.

N: Normality of hydrochloric acid (N 0.5).

E: The equivalent weight of CO2 gas is 22.

The experimental design: The results were analyzed using the SAS statistical program and the averages were compared according to Duncan's test at a probability level of 0.05 (Al-Rawi and Khalaf Allah 2000).

RESULTS AND DISCUSSION

The results of Table (1) showed that significantly superior in the total number of bacteria cells in Al-Muqdadiya region 2.256×10^{7} cfu g^{-1} dry soil compared to their numbers in Diyala University region, which recorded 2.018×10^{7} cfu g^{-1} dry soil, while the total number of bacteria cells in Baladrooz region reached 2.082×10^{7} cfu g^{-1} dry soil, which did not differ significantly from Diyala University and Muqdadiya district. The reason is the study regions of Diyala University, Muqdadiya District, and Baladruz District are under the influence of the same influencing environmental factors in the growth and spread of microorganisms in soil (Al-Hammadi 2014, Alexander 1982).

There were significant differences between the treatments under the influence of the plant type, the rhizosphere of *Atriplex halimus* plant was significantly superior in the total number of bacteria cells 3.141×10^{7}cfu g^{-1} dry soil compared to rhizosphere of *Schanginia aeguptiace* plant and rhizosphere of the comparison soil, which amounted 2.673×10^{7} cfu g^{-1} dry soil and 0.542×10^{7} cfu g^{-1} dry soil respectively.This may be due to difference of plant type *Schanginia aeguptiace* and *Atriplex halimus* in nature and quantity the secreted substances from the root that affect in the rhizosphere environment, as they are sources of carbohydrates and nitrogen, which caused an increase in their numbers and activity (Qasim and Ali 1989).

There were significant differences during the growth stages of halophytic wild plants, the germination stage was significantly superior by giving the highest average number of the total number of bacteria cells amounted to 2.845 x 107 cfu g-1 dry soil, while the vegetative growth stage recorded the lowest average in the total number of bacteria cells amounted to 1.551 x 107 cfu g-1 dry soil. The reason is the great response of bacteria to the secretions of the roots during the germination stage more than its response to dead or decaying plant tissues during the age of the plant, where it contributes to increasing the density of the microbial community, and at the end of the growth stage, as the roots grow, root hairs degenerate, and carbohydrates are rapidly consumed, resulting in a decrease in total bacterial cells (Al-Adwani and Al-Hayali 2018 a).

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Regarding the binary interaction between the region and the plant type, the results showed that the treatment (Muqdadiya + rhizosphere of *Atriplex halimus* plant) was significantly superior by recording the highest average in the total number of bacterial cells amounted to 3.311 x 107 cfu g-1 dry soil followed by (Al-Muqdadiya + rhizosphere of *Schanginia aeguptiace* plant) which recorded 3.108 x 107 cfu g-1 dry soil, while the treatment (Diyala University + rhizosphere of the comparison soil) gave the lowest average in the total number of bacterial cells 0.208 x 107 cfu g-1 dry soil. This difference in the total number of bacterial cells is attributed to the difference in the soil content of nutrients, organic matter and soil texture, which encouraged the growth of plant roots and increased their secretions, which reflected positively on the activity of microorganisms, as well as the nature of the plant, where *Atriplex halimus* plant is a perennial plant, as the roots and its branches extend for great distances, which increases the aeration of the soil, and this provides an ideal environment for the growth and spread of the total bacteria cells (Ali and Majid 2016).

The effect of the binary interaction between the location and the growth stages was significant on the total number of bacterial cells, as the treatment (Al-Muqdadiya + germination stage) achieved the highest average in the total number of bacterial cells 3.168 x 10^7 cfu g^{-1} in dry soil, which was significantly superior on all treatments, while the lowest average was recorded at treatment (Muqdadiya + vegetative growth stage) 1.540 x 10^7 cfu g^{-1} dry soil. Whereas the binary interaction between plant type and growth stages was significant on the total number of bacterial cells, where (rhizosphere of *Atriplex halimus* plant + germination stage) was significantly superior by giving the highest average amounted 4.423×10^7 cfu g^{-1} dry soil, while the treatment (the comparison soil + vegetative growth stage) achieved the lowest average 0.366×10^7 cfu g^{-1} dry soil. As for the triple interaction, the treatments (Diyala University+ rhizosphere of *Atriplex halimus* plant+ germination stage) and (Muqdadiya+ rhizosphere of *Atriplex halimus* plant+ germination stage) were significantly superior in the total number of bacterial cells on all treatments, which amounted 5.035×10^7 cfug^{-1} dry soil and 4.810×10^7 cfug^{-1} in dry soil respectively, while the treatment (Muqdadiya + rhizosphere of the comparison soil + the stage of vegetative growth) was recorded the lowest average reached 0.115 x 10^7 cfu g^{-1} dry soil. This is consistent with the findings of Al-Adwani and Al-Hayali, (2018b) who reported that an increase in the number of bacteria in the rhizosphere soil (RS) compared to their numbers in the comparison soil far from the influence of roots (BS). The numbers of microorganisms in (RS) are more prevalent than the numbers of microorganisms in (BS), where it reaches approximately 100 times due to the availability of carbon and energy sources (Radical secretions) (Al-Azzawi 2012), the figure (1) shows the bacterial colonies developing in the nutrient media.

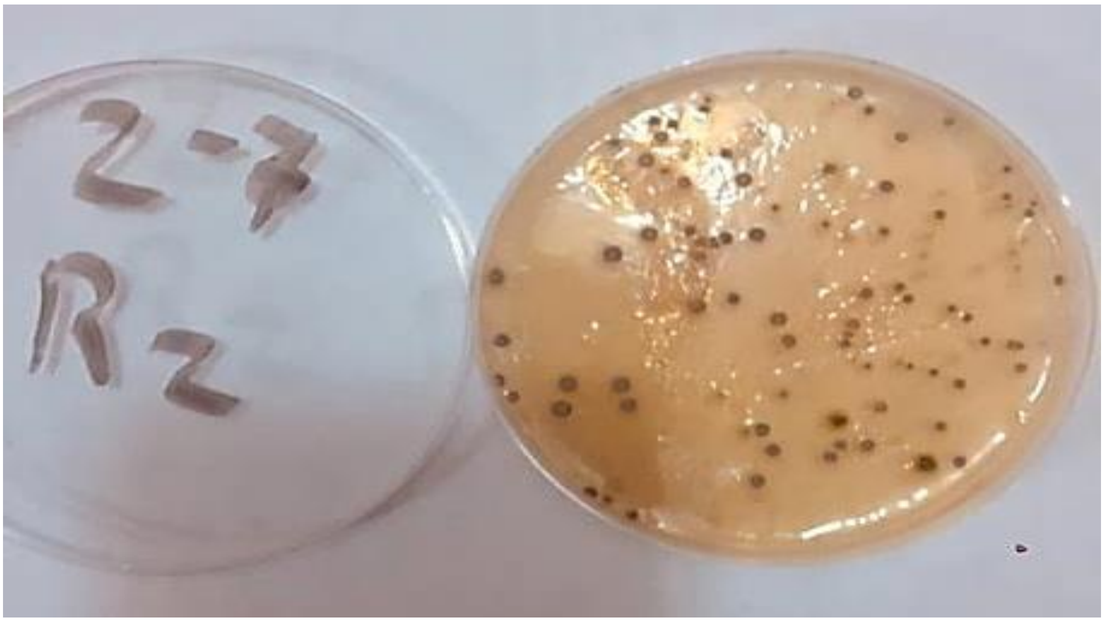

Figure 1. The isolated bacterial colonies from the rhizosphere of *Atriplex halimus* plants during the vegetative growth stage

Table 1. Effect of growth stages of halophytic wild plants and study locations on the total number of bacteria cells in the rhizosphere of plants (cfu g^{-1} dry soil*10^7)

Study location	Plant type	Plant growth stages			The interaction between location * plant type	Averages of study location	Averages of plant type
		Germination stage	Vegetative growth stage	Stage of post flowering			
Diyala University	rhizosphere of *Schanginia aeguptiace*	2.805 cde	1.920 fgh	2.555 defg	2.426 b		
	rhizosphere of *Atriplex halimus*	5.035 a	2.640 def	2.585 defg	3.420 a		
	rhizosphere of the comparison soil	0.360 jk	0.125 k	1.400 k	0.208 d		
Muqdadiya	rhizosphere of *Schanginia*	4.060 b	2.295 defg	2.970 cde	3.108 a		

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District	*aeguptiace*						
	rhizosphere of *Atriplex halimus*	4.810 a	2.210 defg	2.915 cde	3.311 a		
	rhizosphere of the comparison soil	0.635 jk	0.115 k	0.295 jk	0.348 d		
Baladrooz District	rhizosphere of *Schanginia aeguptiace*	3.020 cd	1.865 gh	2.565 defg	2.486 b		
	rhizosphere of *Atriplex halimus*	3.425 bc	1.930 fge	2.720 cde	2.691 b		
	rhizosphere of the comparison soil	1.460 hi	0.860 ijk	0.890 ij	1.070 b		
The interaction between locations and growth stages	Diyala University	2.733 b	1.561 d	1.760 cd		2.018 b	
	Muqdadiya District	3.168 a	1.540 d	2.060 c		2.256 a	
	Baladrooz District	2.635 b	1.551 d	2.061 c		2.082 ab	
The interaction between the plant type and growth stages	rhizosphere of *Schanginia aeguptiace*	3.295 b	2.026 d	2.700 c			2.673 b
	rhizosphere of *Atriplex halimus*	4.423 a	2.260 d	2.740 c			3.141 a
	rhizosphere of the comparison soil	0.818 e	0.366 f	0.441 ef			0.542 c
averages of growth stages		2.845 a	1.551 c	1.960 b			

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The results of Table (2) showed that there was no significant difference between the treatments under the influence of the location factor, while the effect of the plant type was significant among all treatments, as the treatment (rhizosphere of *Atriplex halimus* plant) was significantly superior to all the treatments under the influence of the plant type by giving the highest average in total number of fungi reached 2.438 x 10^3 cfu g^{-1} dry soil, while the treatment (rhizosphere of the comparison soil) gave the lowest average 0.430 x 10^3 cfu g^{-1} dry soil. This may be attributed to the encouraging role of root secretions in the germination of the dormant phases of many fungi, therefore the chlamydial spores of Fusarium, spores of Pythium, conidia of Verticillium, and Sclerotia of Sclerotium can germinate when they are close to the roots, where the secretions of the roots are used as sources of energy, which encourages the germination of many fungi that are not able to compete strongly with soil microbes, as they remain in dormant phases either for lack of food or as a result of a phenomenon of fungal stagnation (Firew Elias et al 2016).
The treatment of the germination stage was significantly superior to the other of the stages, which recorded the highest average in total number of fungi 2.296×10^3 cfu g^{-1} dry soil, while the treatment stage of post flowering recorded the lowest average amounted 1.283×10^3 cfu g^{-1} dry soil. As for the effect of the binary interaction between the location and the plant type, the treatment (Baladrooz + rhizosphere of *Atriplex halimus* plant) was significantly superior to the other treatments by giving the highest average in total number of fungi 3.000×10^3 cfu g^{-1} dry soil, while the treatment (Baladrooz + rhizosphere of the comparison soil) recorded the lowest average amounted 0.323×10^3 cfu g^{-1} in dry soil. There was a significant difference between the treatments of binary interaction (plant type + plant growth stages), as the treatment (rhizosphere of Schanginia aeguptiace + germination stage) achieved the highest average in total number of fungi, which amounted to 3.166×103 cfu g-1 dry soil, while the lowest average was recorded in the treatment (rhizosphere of the comparison soil + vegetative growth stage) amounted 0.283×103 cfu g-1 dry soil. The fungi, like the rest of the microorganisms in the soil are affected by seasonal factors, as they are active in the spring and autumn and their numbers decrease in the summer and winter, in addition to the encouraging role of plant roots in supplying fungi with sources of energy, food and moisture, and a decrease in the pH value of the rhizosphere soil as a result of organic acids secretions and carbonic acid formation, which provides a suitable environment for the growth of many types of fungi (Elif et al 2018).
The effect of the triple interaction was significant in the treatment (Baladrooz district + rhizosphere of *Atriplex halimus* plant + germination stage), which superior on most of the treatments of the triple interaction by giving the highest average in total number of fungi amounted 4.000×10^3 cfu g^{-1} dry soil, while the treatment (Diyala University + rhizosphere of the comparison soi + stage of post flowering) was recorded the lowest average reached 0.15×10^3 cfu g^{-1} dry soil.

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These results are consistent with what was found by (Morgan et al 2005, Brimecombe et al 2007, Elif et al 2018), it is noted that the numbers of fungi in the rhizosphere increased compared to the comparison soil far from the influence of roots during the germination stages and decreased in the advanced stages of the age of the plant and this is due to the lack of root secretions and the change in environmental conditions (Al-Adwani and Al-Hayali 2018 b) and Figure (2) shows the colonies of fungi developing on PDA and Martin medium.

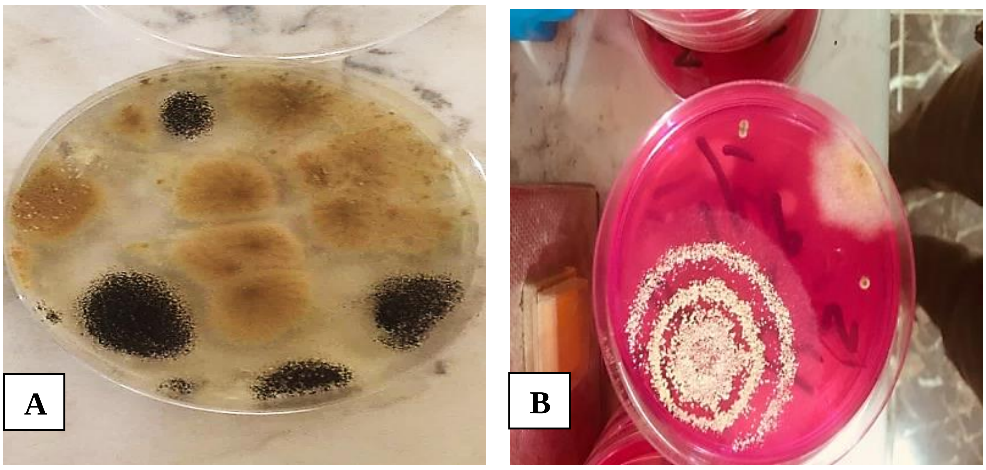

Figure 2. A- Fungal colonies on PDA medium, B- fungal colonies on Martin's medium

Table 2. Effect of growth stages of halophytic wild plants and study locations on the total number of fungi in the rhizosphere of plants (cfu g^{-1} dry soil*10^7)

Study location	Plant type	Plant growth stages			The interaction between location * plant type	Averages of study location	Averages of plant type
		Germination stage	Vegetative growth stage	Stage of post flowering			
Diyala University	rhizosphere of *Schanginia aeguptiace*	3.450 a	1.500 ef	1.700 cde	2.216 b		
	rhizosphere of *Atriplex halimus*	2.600 b	2.000 bcde	1.700 cde	2.100 b		
	rhizosphere	0.700	0.250	0.150	0.366		

	of the comparison soil	ghi	i	i	c		
Muqdadiya District	rhizosphere of *Schanginia aeguptiace*	3.600 a	1.700 cde	1.450 efg	2.250 b		
	rhizosphere of *Atriplex halimus*	2.600 b	2.450 bc	1.600 def	2.216 b		
	rhizosphere of the comparison soil	0.850 fghi	0.300 i	0.650 hi	0.600 c		
Baladrooz District	rhizosphere of *Schanginia aeguptiace*	2.450 bc	1.350 efgh	1.450 efg	1.816 b		
	rhizosphere of *Atriplex halimus*	4.000 a	2.600 b	2.400 bcd	3.000 a		
	rhizosphere of the comparison soil	0.420 i	0.300 i	0.250 i	0.323 c		
The interaction between locations and growth stages	Diyala University	2.250 a	1.250 b	1.183 b		1.561 a	
	Muqdadiya District	2.350 a	1.483 b	1.233 b		1.688 a	
	Baladrooz District	2.290 a	1.416 b	1.433 b		1.713 a	
The interaction between the plant type and growth stages	rhizosphere of *Schanginia aeguptiace*	3.166 a	1.516 c	1.600 c			2.094 b
	rhizosphere of *Atriplex halimus*	3.066 a	2.350 b	1.900 c			2.438 a
	rhizosphere of the	0.656 d	0.283 d	0.350 d			0.430 c

	comparison soil						
averages of growth stages		2.296 a	1.383 b	1.283 b			

The results of Table (3) showed that there was significant difference between the treatments under the influence of the location factor, soil of Diyala University was superior by giving the highest average in amount of CO2 released, which amounted (62.305 mg CO2,100 g soil^{-1}. 14 day^{-1}), while the soil of Baladrooz district recorded the lowest average in amount of CO2 released (54.716 mg CO2,100 g soil^{-1}. 14 day^{-1}). This difference in the amount of CO2 released can be attributed to the activity of those organisms in these regions, as they contain varying amounts of energy, food, water and salt sources, which led to a difference in the microbial community quantitatively and qualitatively, as well as the difference of the locations soils in their chemical and physical characteristics, which encouraged the growth and spread Microbiology (Jarallah 2014).

As for the effect of the plant type, the treatment (rhizosphere of *Atriplex halimus* plant achieved the highest average in amount of CO2 released, which reached (70,261 mg CO2,100 g soil^{-1}.14 day^{-1}), while the treatment (rhizosphere of the comparison soil) gave the lowest average in amount of CO2 released, which amounted (46,500 mg CO2. 100 g soil^{-1}.14 day^{-1}). It can be attributed to the difference in the density of the microbial community and its activity according to the different plant type (*Schanginia aeguptiace* and *Atriplex halimus*). The biological community in rhizosphere of *Atriplex halimus* plant may be more dense than the biological community in rhizosphere of *Schanginia aeguptiace* plant, as these regions provide sources of carbohydrates, nitrogen, vitamins and growth regulators, which encourages the growth and activity of organisms compared to the activity of these organisms in soils far from the influence of roots (Nelson and Mele 2007). The stages of plant growth had a significant effect among the treatments, as the treatment (germination stage) achieved the highest average in an amount of CO2 released (63,672 mg CO2,100 g soil^{-1}.14 day^{-1}), while the microbial activity decreased when the treatment (vegetative growth stage), which the average in an amount of CO2 released was (56.016 mg CO2,100 g soil^{-1}.14 day^{-1}. It can be attributed to the influence of environmental factors in determining the activity of microorganisms in the soil.

Whereas, the binary interaction treatment between the location and plant type (Diyala University+ rhizosphere of *Atriplex halimus* plant) achieved the highest average in an amount of CO2 released, which reached (74.400 mg CO2,100 g soil^{-1}.14 day^{-1}), while the treatment (Baladrooz district + the comparison soil) recorded the lowest average in an amount of CO2 released (42.450 mg CO2,100 g soil^{-1}.14 day^{-1}).

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The variation of locations in the amount of CO2 released as a result of the activity and interaction of soil organisms among them, may be attributed to the variation in the origin of soil formation (parent materials) and soil topography, which contributed to construction of good soil through the formation of the main soil particles sand, silt and clay, in addition to the release of nutrients and oxides of iron, aluminum and silica. In addition to the role of the plant type in providing these organisms with sources of carbon and nitrogen and aerating the soil through the penetration of roots and the addition of organic matter, which positively affected on the activity of these organisms in those soils (Alexander 1981, Al-Khazraji 2012).

As for the effect of the binary interaction between the plant type and the growth stages, as the treatment of (rhizosphere of *Atriplex halimus* plant + germination stage) recorded the highest average in an amount of CO2 released, which amounted (73,783 mg CO2 gm soil^{-1}.14 day^{-1}), while the lowest average of this trait was recorded in the treatment (rhizosphere of the comparison soil + vegetative growth stage), which amounted (42,852 mg 100. CO2 g soil^{-1}. 14 day^{-1}). The binary interaction treatment (Diyala University + germination stage) was significantly superior to all treatments, as it recorded the highest average in an amount of CO2 released (66,916 mg 100. CO2 g soil^{-1}.14 day^{-1}), while the treatment (Baladrooz district + vegetative growth stage) recorded the lowest average amounted 50.383 mg 100. CO2 g soil^{-1}.14 day^{-1}).

The triple interaction between locations, plant type and growth stages had a clear and significant effect on the treatment (Diyala University + rhizosphere of *Atriplex halimus* plant + germination stage), which recorded the highest average in an amount of CO2 released amounted (78.90 mg 100. CO2 g soil^{-1}. 14 day^{-1}), while the lowest average for this trait was recorded in the treatment (Baladrooz district + rhizosphere of the comparison soil + vegetative growth stage) which amounted to (38.600 mg/100.CO2 g soil^{-1}.14 day^{-1}), this is due to the role of various factors in providing the best environment for the growth of organisms, as organisms prefer soils that contain balanced proportions of clay, sand and silt, in addition to providing a good air moisture system that allows the growth of microorganisms, as well as the role of the plant type in preparing different sources of carbohydrates, organic substances and amino acids, as well as the role of the plant growth stages and the moderation of the climate during the germination stage, which contributed to the flourishing of microorganisms and their various metabolic processes such as the analysis of complex substances, the processes of nitrification, nitration and transformations of other elements, which in turn release larger amounts of CO2 into the rhizosphere environment (Mahdi et al 2010, Michra et al 2013) and these results are consistent with the findings of Al-Azzawi and Qassem (2018), Jarallah (2014).

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CONCLUSION

The plant type (*Schanginia aeguptiace* and *Atriplex halimus*) had a clear effect on the microbial community, as the roots of the *Atriplex halimus* plant had an encouraging role in increasing the numbers of (bacteria and fungi) in the rhizosphere compared to their numbers in the comparison soil far from the influence of the roots, while the stages of plant growth had a significant effect on the numbers of bacteria and fungi, where the highest numerical density was achieved during the germination stage, then their numbers decreased in the stage of post flowering.

Table 3. Effect of growth stages of halophytic wild plants and study sites on the amount of CO2 released, mg CO2. 100 g soil^{-1} 14 day^{-1}

Study location	Plant type	Plant growth stages			The interaction between location * plant type	Averages of study location	Averages of plant type
		Germination stage	Vegetative growth stage	Stage of post flowering			
Diyala University	rhizosphere of *Schanginia aeguptiace*	69.300 cd	60.600 gh	61.400 gh	63.766 d		
	rhizosphere of *Atriplex halimus*	78.900 a	70.850 bc	73.450 b	74.400 a		
	rhizosphere of the comparison soil	52.550 ij	44.70 m	49.00 jkl	48.750 f		
Muqdadiya District	rhizosphere of *Schanginia aeguptiace*	71.600 bc	65.200 ef	73.450 b	66.100 c		
	rhizosphere of *Atriplex halimus*	70.500 bc	66.300 de	70.05 bc	68.95 b		
	rhizosphere of the comparison soil	51.700 ij	45.350 lm	47.850 klm	48.300 f		

Baladrooz District	rhizosphere of *Schanginia aeguptiace*	57.800 h	50.800 ijk	54.200 i	54.266 e		
	rhizosphere of *Atriplex halimus*	71.950 bc	61.750 fg	68.600 cde	67.433 bc		
	rhizosphere of the comparison soil	47.850 klm	38.600 n	40.900 n	42.450 g		
The interaction between locations and growth stages	Diyala University	66.916 a	58.716 d	61.28 c		62.305 a	
	Muqdadiya District	64.600 b	58.950 d	59.800 cd		61.116 b	
	Baladrooz District	59.200 cd	50.383 f	54.566 e		54.716 c	
The interaction between the plant type and growth stages	rhizosphere of *Schanginia aeguptiace*	66.233 c	58.866 d	59.033 d			61.317 b
	rhizosphere of *Atriplex halimus*	73.783 a	66.300 c	70.700 b			70.261 a
	rhizosphere of the comparison soil	50.700 e	42.883 g	45.916 f			46.500 c
averages of growth stages		63.572 a	56.016 c	58.550 b			

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