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EVALUATION OF THE EFFICIENCY OF A NUMBER OF LOCAL ISOLATES OF RHIZOBIUM BACTERIA AND IRON FOLIAR APPLICATION ON FABA BEAN (*VICIA FABA* L.)

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Abstract

A field experiment was conducted at the winter season (2017-2018) in agricultural research station which belongs to Al-Muhanna University, Agriculture college in AL-Muthanna governorate, AL-Samawah city which located in the Euphrates river to investigated evaluation the efficiency of a number of Local Isolates of rhizobium bacteria and foliar application at different levels from Iron (0, 50, 100) ml Fe L^{-1} as chelated iron (Fe – EDDHA 6%) as a source of Iron on Faba bean growth GRANO Varity, Randomized Complete Block Design(RCBD) was used in this experiment according to Split –plot design, the first factor bacterial pollination was significantly exceeded, especially the second Isolation R2 on all treatment and achieved mean attained 173.4 ml $plant^{-1}$ at increasing rate 11.72% as compared with control treatment on the dry weight attribute of root nodules, R_2 also significantly exceeded and recorded mean attained 34.6 gm $plant^{-1}$ as compared with control treatment R_0 which recorded a lowest mean attained 18.2 gm $plant^{-1}$ at increasing rate 90.10% to the dry weight of plant attribute, R2 was significantly exceeded and achieved a higher mean attained 0.2800* 10^5 CFu gm^{-1} soil as compared with control treatment which recorded a lowest mean attained 0.2000* 10^5 CFu gm^{-1} soil at increasing rate 40% in the total number of bacteria at flowering stage .as for plant content from nitrogen, F_2 was significantly exceeded and recorded a higher mean attained 4.086% as compared with control treatment F_0 which recorded a lowest mean attained 1.715% at increasing rate % 138.25, F_2 also exceeded and recorded a higher mean attained 154.00% as compared with F0 which recorded a lowest mean attained 116.4% at increasing rate 32.30% of plant content from Iron, also the interaction treatment R0F0 was significantly exceeded and recorded a higher mean attained 534.1 gm $plant^{-1}$ and a lowest mean of control treatment R_0F_0 which attained 210.0 gm $plant^{-1}$ at increasing rate 154.33% of biological yield in the harvesting.

Keywords: Rhizobium bacteria, Iron foliar, Faba bean (*Vici faba* L.)

Introduction

Faba bean crop *Vicia faba* L. is a winter crop with seeds contain a high protein content which is estimated at 25-30% (Sabbouh *et al.*, 2011) It also improves soil properties by nitrogen fixation processes in the soil (Shafeek, 2013) the plants of this crop co-exist with bacteria of the genus *Rhizobium leguminosarum* which infects the roots and root nodes formation which it works to convert the atmospheric nitrogen and with help of energy that obtained from the composition of carbohydrates in the photosynthesis process into available nitrogen inside the plant where there are specialized bacteria for each crop (Uchida, 2000). Nitrogen fixed through the symbiotic relationship between Rhizobium and legumes at the global level is estimated at about 80 million tons per year (Adjei *et al*., 2005) and during the recent studies It has been found that the nitrogen-fixing bacteria had a great importance, especially the bacteria that belong to the genus Rhizobium, as these bacteria produce various chemical and hormonal compounds, growth factors and vitamins and it works on the plants emergence stimulation and increase the speed of growth and increase rates of photosynthesis process and it works to reduce the infection of some diseases as it used in the biological resistance process in addition to the process of the atmospheric nitrogen fixation (Dakora, 2003), On the other hand, fertilizer can be added as a foliar application to treating the shortage and increase the availability of the elements (EL-Habbasha *et al*., 2007). The addition of some nutrients including iron which is an important element because it a catalyst of enzymes in the oxidation and reduction processes and it entrance as a essential element as one of the components which contributing in Nitrogenase enzyme synthesis which is responsible of the atmospheric nitrogen fixation process (Bauer *et al*., 2004), Iron is one of the essential nutrients of plant growth which is importance came from its contribution of chlorophyll synthesis and its role in the enzymes synthesis for example NO_3-reductase, NO_2-reductase, Nitrogenase, peroxidase and Catalase, as well as its role in the acquiring and transmission of electrons during the oxidation and reduction processes because it enters in the synthesis of the main components of the plant cell as cytochromes and pheophytin that contributed in the photosynthesis process and constructive breathing and demolition and oxidation and reduction (Havlin *et al*., 1999). Iron in almost agricultural soils especially calcareous soils that accounted about %80 from agricultural lands in Iraq exposed to many reactions as detention (precipitation and adsorption) by metals, carbonate, which reduces the plant's availability in these soils (Al-Uqili *et al*., 2007). In order to treating the shortage that economic growing plants suffering from it in calcareous soils especially legumes as a resulting of lack of Iron availability ,the addition of Iron fertilizers either metal or chelated whether if it was the addition soil or foliar application which is considering from the main styles that used to increase the availability of iron and fill the plant need from it.

Materials and Methods

Experiment Location

A field experiment was conducted at the winter season (2017-2018) in agricultural research station which belongs to AL-Muhanna University, Agriculture college in AL-Muthanna governorate, AL-Samawah city which located in the Euphrates river.

Soil Preparation and Planting Processes

The field soil was prepared by cultivation, softening and leveling and then it was divided into four blocks and block was divided into 9 plots, the area of the single plot was 4 m^2 and then Faba bean seeds (Krano) Variety were planting at 1/11/2017, the plots were divided into five lines and the distance between the line and other 16 cm and the seeds were planted as 4 seeds to each hole then the plants of the single hole was thinning to one plant after two real leaves appeared clearly on each plant, field land was weeding from weeds for more time as it needed, the crop was harvested after it arrives at maturation stage and at 1\4\2018 the crop was harvesting and some measurements were making.

Table 1 : Some physical and chemical properties of field soil's before planting

Properties	Unit	Value
pH	-	7.3
Ec	ds/m^{-1}	3.5
Available nitrogen	Mg Kg $soil^{-1}$	11.21
Available phosphor	Mg Kg $soil^{-1}$	9
Available potassium	Mg Kg $soil^{-1}$	390
Soil texture		
Sand	gm Kg $soil^{-1}$	235
Silt	gm Kg $soil^{-1}$	528
Clay	gm Kg $soil^{-1}$	237
Faba bean rhizobium	CFU g^{-1} soil dry	$1.84*10^5$
Total bacteria	CFu g^{-1} soil dry	$2.67*10^5$

The plant height (cm): The plant height was measured from soil surface until the top of the plant at flowering stage %50 (five plants).

The chlorophyll content: It was measured at flowering stage %50 (five plants).

The number of root nodules of each plant: the plants were pulled carefully so not other plants affected and the plant roots were put in the sieve and it faces a light stream of faucet water and the number of root nodules was calculated of each plant of five plants (Beck *et al*., 1993).

The dry weight of root nodules: After the plant was pulled from the soil and its root washed well it's placed in a paper bags and taken to the laboratory and placed in the oven at a temperature 65 ºC and for 48 hours and then weighted in a delicate balance (Beck *et al*., 1993).

The dry weight of root nodules (mg $plant^{-1}$): It was measured of five plants by using a delicate balance at flowering stage 50% also it was measured at the end of the season.

The dry weight of the vegetative: The samples were taken at the flowering stage of five plants, the plant was pulled completely and carefully then the vegetative part was cut only and put in a paper bags which previous marked by the symbols of treatments and then dried in the oven at temperature 65ºC and for 48 hours and then it's dried weight was measured.

Results and Discussion

The dry weight of root nodules (mg $plant^{-1}$): The results in the table (2) showed the pollination with rhizobium bacteria had a significant effect on the dry weight of the root nodules as R2 (the pollination with rhizobium bacteria) was significantly exceeded on the other treatments and recorded mean attained 173.4 mg $plant^{-1}$ at increasing rate 11.72% as compared with control treatment In contrast, there were no significant differences between R1 Isolation which recorded mean attained 167.2 mg $plant^{-1}$ as compared with control treatment which recorded a lowest mean attained 155.2 mg $plant^{-1}$ the reason may be due to that the pollination with rhizobium bacteria led to increase their numbers in the soil and that make sure that the infection by mention bacteria occurred and formation the root nodules, the results the table (2) indicated there were significant differences in the dry weight of the root nodules where all foliar application of Iron levels gave a significant increasing in the weight of the root nodules as foliar application of iron treatment (F2) was significantly exceeded and gave mean attained 184.2 mg $plant^{-1}$ as compared with control treatment (F0) which recorded mean attained 148.2 mg $plant^{-1}$ at increasing rate 24.29%, whereas (F2) was significantly exceeded on (F1) at increasing rate 11.81%, in the same context (F1) was significantly exceeded on (F0) which recorded mean attained 163.4 mg $plant^{-1}$ as compared with (F0) which recorded the lowest mean attained 148.2 mg $plant^{-1}$ at increasing rate % 10.25 the reason of the increasing of root nodules may be due to needing of the root nodules to Iron as it considering the main part of *nitrogenase* which responsible of nitrogen fixation processes and this agreed with what was found by (Al-Aqeedi, 2002). The results in the table (2) indicated that the interaction between pollination with rhizobium bacteria and Iron foliar application was no significant.

Table 2 : The effect of Rhizobium bacteria and Iron foliar application on the weight of root nodules at the flowering stage (node $plant^{-1}$)

The level of Iron mg L^{-1}				R
	F			
Mean	F_2	F_1	F_0	
155.2	170.1	153.5	141.9	R_0
167.2	187.1	159.5	155.2	R_1
173.4	195.6	177.2	147.5	R_2
	184.2	163.4	148.2	Mean
	R	F	RF	LSD
	13.46	13.46	N.S	

Chlorophyll content (SPAD): The results of the statistical analysis showed that there were significant differences to the pollination with rhizobium bacteria as R2 was significant exceeded and recorded a higher mean attained 50.60 SPAD as compared with R0 which was recorded mean attained 39.46 SPAD at increasing rate 53.31% and it exceeded on R1 at increasing rate %19.31 the reason of increasing the chlorophyll content in the plant may be due to the good supplying of nitrogen which led to increase the synthesis period and make it more active and that led to increased the chlorophyll content in the leaves because of the activity of Rhizobium bacteria (Vaguseviciene *et al.,* 2012). The results also showed that there were significant differences at iron foliar application with certain levels, while F2 was significantly exceeded on F0 and recorded a higher mean attained 50.51 mg $plant^{-1}$ at increasing rate 45.14%, while there was no significant difference between F1 which recorded mean attained 47.16 mg $plant^{-1}$ and F2 ,the reason of increasing of chlorophyll content may be due to that the iron is an essential and necessary element for growth as the

plant can not complete its life cycle without it, it contributed to many activities such as photosynthesis processes, production increasing including weight of pod in the plant and chlorophyll content, the measured of chlorophyll content in the leaves considering from an important attributes to study the response of the crops to the foliar application with micro and macro elements (Al-Naimi, 2000). The results in the table (3) also indicated that the interaction between pollination with rhizobium bacteria and Iron foliar application was no significant on the chlorophyll content of the plant in spite of the apparent differences but the statistical analysis do not refer to it.

Table 3 : The effect of Rhizobium bacteria and Iron foliar application on the chlorophyll content of plant (SPAD)

The level of Iron mg L^{-1}				R
	F			
Mean	F_2	F_1	F_0	
39.46	47.68	41.76	28.93	R_0
42.41	44.47	46.71	36.05	R_1
50.60	59.37	53.01	39.41	R_2
	50.51	47.16	34.80	mean
	R	F	RF	LSD
	4.229	4.229	N.S	

The leaf area (cm^2 $plant^{-1}$): The results of the statistical analysis in table (4) showed that bacterial pollination had a positive and significant effect in the leaf area, as R2 was exceeded and recorded a higher mean attained 80.2 cm^2 $plant^{-1}$ as compared with the control treatment which recorded the lowest mean attained 69.0 cm^2 $plant^{-1}$ at increasing rate % 19.13 and also it exceeded R1 which recorded mean attained 72.5 (cm^2 $plant^{-1}$) at increasing rate %10.62. the results in the table (4) showed that iron foliar application had a positive and significant effect on the leaf area attribute as F2 was significant exceeded and recorded a higher mean attained 82.5 cm^2 $plant^{-1}$ as compared with F0 which recorded mean attained 67.5 cm^2 $plant^{-1}$ at increasing rate 22.22%, also it exceeded on F1 by recorded increasing rate 15.22% and this may be due to that the Iron works to reduces pH and therefore encourages uptake of nitrogen which leads to an increase in the synthesis of essential amino acids which considering necessary for growth and its entrance in protein synthesis and that's leads to encourages cell division and thus increase growth and leaf area and it also led to increasing the activity of the gibberllins within the plant tissue which increase the expansion and elongation of cells and these results correspond to what found by (Salem *et al*.,2014) .the results in the table (4) also showed that the interaction was not significant in the leaf area attribute in spite of the apparent differences.

Table 4 : The effect of Rhizobium bacteria and Iron foliar application on the leaf area (cm^2 $plant^{-1}$)

The level of Iron mg L^{-1}				R
	F			
Mean	F_2	F_1	F_0	
69.0	73.4	68.8	64.9	R_0
72.5	78.8	71.3	67.4	R_1
80.2	95.3	74.8	70.3	R_2
	82.5	71.6	67.5	mean
	R	F	RF	LSD
	7.28	7.28	N.S	

The plant height (cm): The results in the table (5) showed a significant differences between treatments as bacterial pollination recorded a significant differences and R2 recorded a higher mean attained 61.62 cm as compared with R0 which was recorded the lowest mean attained 41.46 cm at increasing rate attained 48.62%, also it exceeded on R1 which recorded mean attained 48.96 cm and at increasing rate attained 25.85%. the reason may be due to that the bacterial pollination with rhizobium led to increasing its numbers in the soil and thus increased its ability to infect roots and formed the root nodules and thus increased their ability to nitrogen fixation which plays an important role on cells division and elongation and that leads to an increase in the growth and height of the plant and this is what referred to it each of (Rikabi, 2012 and Neama, 2011). In addition, the results also showed that iron has a positive and significant role which lead to recorded a significant differences in foliar application at certain levels of iron as F2 was significant exceeded and recorded a higher mean attained 56.81 cm as compared with F0 which recorded the lowest mean attained 40.19 cm at increasing rate 41.35%, while there were no significant differences between the iron foliar application treatments. Al-Hajji (2014) achieved a significant increasing on the plant height when he used iron foliar application on faba bean at concentration (100, 150, 500) mg Fe L^{-1}. as the concentration 100 mg Fe^{-1} gave the highest mean attained 57.6 cm. and these results agreed with results in the table (5) and agreed with what Abdul Karim (2013). the results also showed that the interaction was positive and significant in plant height attribute as R2F2 was exceeded and recorded a higher mean attained 77.25 cm as compared with R0F0 which recorded a lowest mean attained 37.63 cm at increasing rate 105.28% the reason may be due to the activity of Rhizobium bacteria which increase the numbers and weights of the root nodules as well as the importance of iron element which is considered an important element which increases the number of branches and thus increases the height of the plant and vegetative growth.

Table 5 The effect of Rhizobium bacteria and Iron foliar application on the plant height at flowering stage (cm)

The level of Iron mg L^{-1}				R
	F			
Mean	F_2	F_1	F_0	
41.46	44.63	42.13	37.63	R_0
48.96	48.55	55.88	42.45	R_1
61.62	77.25	67.12	40.50	R_2
	56.81	55.04	40.19	mean
	R	F	RF	LSD
	3.853	3.853	6.673	

The number of root nodules (nod $plant^{-1}$): Table (6) showed a significant differences on the number of root nodules as R2 was significantly exceeded and recorded a higher mean attained 26.62 nod $plant^{-1}$ as compared with R0 which recorded a lowest mean attained 16.38 nod $plant^{-1}$ at increasing rate %62.51 also it exceeded on R1 which recorded mean attained 21.42 nod $plant^{-1}$ at increasing rate %24.27 the reason of the bacterial pollination with rhizobium treatments may be due to the increasing of the number of root nodules which leads to increases the number of active Rhizobium bacteria in the soil that are capable of infecting and penetrating the roots of the host plant and that what was

found by (Nooni,2012) when he study about faba bean. The results pointed that the foliar application of iron at several levels led to achieved a significant differences as F2 exceeded and recorded a higher mean attained 29.46 nod plant $^{-1}$ as compared with F0 which recorded the lowest mean attained 13.04 nod plant^{-1} at increasing rate 125.92% and it also exceeded on the F1 which recorded mean attained 21.92 nod plant^{-1} at increasing rate 34.39% the reason may be due to increasing of the number of root nodules and their weight when adding iron and that's return to the importance of Iron in increasing the root branches and root hairs and thus increasing the attacking area by nodules bacteria so the number of root nodules will increase and this agreed with the result of (Al-Aqeedi, 2002). the results also showed that the interaction between bacterial pollination of rhizobium and iron foliar application was significant in the number of root nodules as R2F2 was significant exceeded and recorded a higher mean attained 45.25 nod plant^{-1} as compared with R0F0 which recorded the lowest mean attained 10.25 nod plant^{-1} at increasing rate 341.46%, the reason may be due to that such an increasing in the number of root nodules resulting from the addition of the bacterial pollination addition to iron was obtained by (Yousif and Abd al-Redha, 2000) on alfalfa plant if he noted that addition of chelated iron (Fe-EDDHA) with bacterial pollination helped to root nodules formation much better.

Table 6 : The effect of Rhizobium bacteria and Iron foliar application on the number of root nodules (nod plant^{-1})

The level of Iron mg L^{-1}				R
Mean	F			
	F_2	F_1	F_0	
16.38	19.38	19.50	10.25	R_0
21.42	23.75	25.87	14.62	R_1
26.62	45.25	20.38	14.25	R_2
	29.46	21.92	13.04	mean
	R	F	RF	LSD
	3.444	3.444	5.966	

The dry weight of the plant: The results of the statistical analysis showed significant differences in the dry weight of the vegetative at the bacterial pollination as R2 was exceeded and recorded a higher mean attained 34.6 gm plant^{-1} as compared with R0 which recorded a lowest mean attained 18.2 gm plant^{-1} at increasing rate 90.10% it was also exceeded on R1 which recorded mean attained 20.9 gm plant^{-1} at increasing rate 65.55%, the reason may be due to the increase the number of Rhizobium bacteria when we applied the pollination with Rhizobium isolates and thus increasing the nitrogen fixation processes by Rhizobium bacteria which reflected in the growth of plant and accumulation of the dry matter , as well as increasing the number of plant branches which led to the increase the dry weight of the plant and these result are agreed with (Al-Jader, 2006) and (Neama, 2011) and (Rikabi, 2012). The results in the table (7) showed that there was a significant effect of iron foliar application on the dry weight of the plant as F2 was significant exceeded and recoded a higher mean attained 34.5 gm plant^{-1} as compared with the F0 which recorded the a mean attained 18.5 gm plant^{-1} at increasing rate 86.48% it's also exceeded on F1 which was recorded mean attained 20.7 gm plant^{-1} at increasing rate 66.66%, while there were no significant differences between iron foliar application treatments and these results agreed with each of (Abdul Karim, 2013) and (Faisal *et al*., 2012) and (Salem *et al*., 2014) to the existence the significant differences on the dry weight attribute of Faba bean when they using chelated iron as a foliar application and by different levels. The results of the table (7) showed that the interaction between bacterial pollination and iron foliar application was significant on the dry weight of the plant as R2F2 was significant exceeded and recorded mean attained 57.3 gm plant^{-1} as compared with R0F0 which recorded mean attained 15.8 gm plant^{-1} at increasing rate attained % 262.65 the reason of increase the dry weight of the plant due to the active role of Rhizobium bacteria that works to increase the number of root nodules and thus increase the vegetative and its branches and iron has an important role in increasing the absorption of nutrients and increase its concentration in the vegetative and thus lead to the increase the dry weight of the plant.

Table 7 : The effect of Rhizobium bacteria and Iron foliar application on the dry weight of the plant (gm plant^{-1})

The level of Iron mg L^{-1}				R
Mean	F			
	F_2	F_1	F_0	
16.38	15.8	18.8	19.9	R_0
21.42	16.7	19.5	26.4	R_1
26.62	22.8	23.7	57.3	R_2
	18.5	20. 7	34.5	Mean
Interaction	Iron	Rhizobium Isolates		LSD
10.61	6.13	6.13		

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