European Journal of Biology and Medical Science Research

Vol.3, No.3, pp.64-72, June 2015

Published by European Centre for Research Training and Development Studies (www.eajournals.org)

EFFECT ADDING OF FE-EDTA ON EFFICIENCY OF (*SINORHIZOBIUM MELILOTI),* ISOLATED LOCALLY IN NITROGEN FIXIATION WITH ALFAFA (*MEDICAGO SATIVA*) PLANTS.

Mohammed Abd AL-Jaleel
Al-Mustansiryah University
College of Sciences / Dept. Biology
Iraq - Baghdad

ABSTRACT: *This experiment studied the effect of various concentrations of Fe-chealate (Fe-EDTA) in the efficacy of four strains of Sinorhizobium melioti, isolated locally from root nodules of alfafa plants (Medico sativa), these plants garthing from different location in Iraq (Baghdad, and Babylon). The rhizome strains were named (S1, S2, S3, S4).This stady divided in two parts, alabrotary experiment meased the colony forming unit (CFU) for all isolated strains under 3 concentrations of Fe-EDTA (2mg, 4mg, 8mg)/ L, through incubation of 48, 72 hours. The results of this experiment showed a significant increasing in a count of (CFU) ($P \leq 0.05$) for all strains, specially and (4mg, 8mg,) Fe/L.The farmer experiment in plastic pots size (5kg) soil putted in the green house, studied some growth parameters of alfafa plants such as: the length of shoot, dry weight, numbers of root nodules, and the concentration of nitrogen and protein in plants. The results shoed that the effect of (Fe-EDTA) was very effective in the effecinacy of all four Sinorhizobium strains under study, in nitrogen fixation process, and increasing there ability for fixing nitrogen when issociation with Medico sativa plants. The results present a significant increasing in the growth parameters were mentioned. These parameters were increased when increased the Fe-EDTA concentrations. The treatment (bacteria + 0.6mg Fe) showed the best results comparing with other treatments for all strains. The strain S3 present the best results comparing with other strains but S2 strain present the less significant results in this study.*

KEYWORDS: Sinorhizobium, Fe-EDTA, Alfafa plants

INTRODUCTION

Biological nitrogen fixation by *Rhizobium* bacteria is avery important nitrogen source for plants, because it's requires less energy and causes less environment pollution. [1] .Biofertilizing technology by using microorganisms like *Rhizobium* bacteria with legume crops is an important way for fixing nitrogen in legume plants, and this technology used a wildly range in different conteries, also this technology add nutrients to the soil, and stimulating plant growth through synthesis of growth – promoting substantanes [2].Biofertilizers by inoculation with rhizobium bacteria has an extera benefit of nitrogen addition to soil and to the legums plants, also this way known as eco-friendly which avoiding the environmental pollution also it's cost-effective relative to chemincal fertilizers. [3] and [4].

The rhizobium bacteria required iron for nitrogen fixation with legums, many researches present that the Fe element enhancing the bacterial activity in fixing nitrogen in plants. Deficiency in iron can affect the nodulation process, initiation and development of the nodules on roots, because iron is necessary for synthesis of iron-containing proteins in host plant,

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

including (Legheamoglobin), and in bacteriods for nitrogenase and cytochroms of the electrons transport chains, which are moving the nitrogen fixiation process. [5].

MATERIAL AND METHODS:

Isolation of rhizobium strains.

Four isolates were isolation and identification locally, the isolates were for *Sinorhizobium meliloti* isolated from root nodules of alfafa plant (***Medicage sativa***) that are cultured in different regions in Baghdad and Babylon. The isolates named (S1, S2, S3, S4). Alfafa plants growing in good from were selected and taken from adifferent area, the soil was non fertilizer, for isolation of nodules bacteria. The roots whashed well by water, than root nodules that are close to the main root were removed, wash with distal water many times and sterilized according to the [6] the root nodules were washed with sterilized distalled water, then ethyl alcohol (95%) for 5 min, wash with sterilized distal water, then with (0.1%) of acidifed HgCl2 for 3-6 min, then planed in glass test tube with (3ml) of Nacl (0.85%) with good scratch. Taking (1m) from bacterial suspension and cultured on manitol salt yeast extract medium (MSY) and incubated at 27°c for 2-3 day until appearance of colonies. The bacteria isolated were identification by using gram stain and with successful infected for alfafa plants again.

The purification of Islolates.

After appearance of white mucous coloies, a loopfull were taken from the colonies and streaking on (MSY) and incubated for 3 days. This experiment was repeated for many times unit oblain of pure cultures. Four dilutions of bacterial suspension were done by using normal saline (0.85%), and the diluted bacteria were cultured by spread (0.1)ml from last dilution on MSY in petri disc and incubated at 28°c until appearance of colonies.

The measurement of bacterial colonies (colony forming unit CFU) under affecting of Fe-EDTA concentrations.

Prepared a broth culture of (MSY) media, and inoculumed with isolates (under study), then incubated in shaker incubator (100 rpm/sec) with 28°c [7]. After 24h of incubation, taking 1 ml from sample and inoculated with 25 ml broth culture of (MSY) media in conical flask, then incubate in a shaker incubator (100 rpm/sec) under 28°c., taking (1ml) from bacterial culture after 48h and 72h respectively. After serial dilutions until (10^{-6}), spread 1 ml broth soild culture media that contain on concentrations of Fe-EDTA (0.1mg, 0.3mg, 0.6mg)/L, and incubated for 2-3 days under 28°c. the bacterial colonies were count for three times with control sample for each growth period.

Host plant used.

The local variety seed of *Medicago sativa* were used. Which obtain from seed certificate center (college of Agriculture).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Soil analysis and preparation.

The soil used in the farmer experiment was analysised to determine physical and chemical properties befor using.

Silt	Clay	Sand	Organic matter	Mixture soil	pH	EC (ds/m)	CaCo3
S10/g soil	185/g soil	245/g soil	905/g soil	Loamy soil	7.5	2.30	21.3%

In this experiment we used a plastic post size (5kgm) and sterilization well by using sodium hyopchloride, and each pot filled with constant quantity (4kgm) of loamy soil, the soil was sieved with diameter (2mm) to performe homogenate and remove impure from it.
The soil was sterilized by autoclave. The concentration of Fe-EDTA was counted on the basis soil weigh (4kgm) (0.1, 0.3, 0.6gm/4kgm) and mized with soil by good form to distribution of element to all parts of soil. The seeds of *Medicago sative* were used (10) seeds in each pot. The pots put in green house under 20-25°c, and irrigated rotator.

Inculation the soil by Sinorhizobium stains.

A loop full from all *Sinorhizbium* isolates (under study) were taking and grown in (10ml) of (MSY) broth, and incubated in shaker inocubator (100rpm/sec) in 28°c for 24h., then (2ml) from incubated in shaker incubator (100rpm/sec) in 28°c for 48h, the this bacteria browth was centrifuged in (3000rpm/sec) for 5 min, then susppensitioned the bacterial precipitate by normal saline (0.85%), and complete the volume to (50ml), then added to the pot after 3 days of agriculturing.

The harvest of plants.

The plants were harvested in march/2014, these plants harvesred with roots, the roots washed with water to remove the soil, then the plant's samples were transport to the labrotary and recorded the following data:

1. The length of shoot system.
2. The dry weight of plant.
3. The number of root nodules.

Estimation of nitrogen and protein ratio in plants.

The known weight of dried plant are grounded with good form according to [8] and then, estimation of N2 ration in plants according to [9]. The estimation of protein percentage was according to [10].

Statistical analysis.

The statistical analysis system, [11] was used to effect of isolates and concentrations in study parameters. Least significant difference (LSD) test was used to significant compare between means in this study.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

RESULT AND DISCUSSIONS

Bacterial colonies account.

Table (1) showed that all four strains were effected by high concentrations of Fe-EDTA under study. There were a significant increasing in the number of colonies ($P \leq 0.05$) comparing with control (without Fe-EDTA), after 48 and 72 hours from growth.Bacteria grow up with (4mg) and (8mg) /L present a significant result comparing with control. Strain (S3) present higher significant, and strain (S2) present the less significant increasing comparing with other strains. These results agree with anthor study [12] which was showed an increasing in account of rhizobium bacteria assiociated with *Vigna radiate* plants, under different concentration of Fe-EDTA, and supported with anthor study [13] which was present a significant increasing in account of *Sinorhizobium meliloti* colonies under effect of (2,4,6mg Fe)/L, anthor study [14] showed that an enhaced growthe of rhizobium colonies isolated from (pigeopea) under effect of (0.1% to 0.3%) concentrations of (Fe) added with growth media. The result in this study maybe related to that (Fe) is nessary and essiential for synsthesis of many proteins like (cytochromes) which important in electrons transport chains in the bacteria. [5]

The effect of Fe-EDTA concentrations and rhizobium strains on growth parameyers of alfafa plants.

The famer experiment showed that the interaction between Sinorhizobium strains and Fe-EDTA was very effective in increasing and promoting the growth of plants, and the adding of (Fe-EDTA) to the soil enhancing the bacterial activity in nitrogen fixiation in the plant growup with rhizobium bacteria and Fe-EDTA comparing with control plants which were (0 bacteria + 0 Fe), and (bacteria + 0 Fe) (plants inoculumed byrhizobium strain without adding Fe-EDTA).

The strain S3 present the highest significant increasing for all growth parameaters, and S2 present the less increasing in all growth parameters comparing with other strains.

Plant legth, and dry weight.

Table [2], showed asigninfcant increasing in the length of alfafa plants that inoculumed by Sinorhizobium strains, comparing with control plants, also the table showed no significant increasing in length of plants growth with (0.1gm Fe/4kg pot soil), but a simple increasing presented in plants grown with (0.3gm Fe) and (0.6gm Fe)/4kg pot soil (without bacteria) comparing with control groups (0 bacteria + 0 Fe). A significant increasing in the lengths of plants that inoculumed by *Sinorhizobium* bacteria and the Fe-EDTA, spically the concentiations (0.3gm Fe) and (0.6gm Fe)/4kg pot soil.

Table (3) also showed the same significant increasing in dry weight of alfafa plant that inoculumed by *Sinorhizobium* and (0.1gm, 0.3gm, 0.6gm) Fe/4kg pot, comparing with the control groups which were (0 bacteria + 0 Fe), and (bacteria + 0 Fe).

These results support with other study [15]. [16]

The number of nodules.

Table (4) showed asignificant increasing in numbers of root nodules in the alfafa plants inculumed by Sinorhizobium strain spically plants grown with (0.3gm) and (0.6gm) Fe/4kg pot.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Comparing with control groups (bacteria + 0 Fe). These results may be related to the (Fe)is an essential element for nodution process, and necessary for synthesis of leyheamoglobiun, and nitrogenase enzyme. [5] [17].

The ratio of nitrogen and protein in plants.

Table (4), and table (5) showed the alfafa plants which were grown up with *Sinorhizobium* strains and Fe-EDTA concentrations gives the best results in ratio of nitrogen and protin comparing with control groups (0 bactria + 0 Fe) and (bacteria + 0 Fe).
The plants grown with all *Sinorhizobium* stain and with (0.1gm Fe / 4kg pot) present no significant increasing, the plants grown with (0.3gm) and (0.6gm) Fe/4kg pot, present significant increasing in ratio of nitrogen and protein for all strains, comparing with control groups. These results supported and agree with anthor studies [13] [18] [19] and [20] these experiments presented a significant increasing in numbers of root nodules, and in concentrations of nitrogen and protein (Symbiotic nitrogen fixation) in leguns plants when added a different concentrations of (Fe-EDTA) to the soil of agriculte with the plants.

Table (1). Effect of Fe concentrations and *Sinorhizobium* isolates in **Number of cfu**

Isolate	Concentration Fe (mg/L)				LSD value
	0	2	4	8	
S1	7 ± 0.35	8 ± 0.19	21 ± 0.13	33 ± 0.40	6.592 *
S2	9 ± 0.50	11 ± 0.36	28 ± 0.72	35 ± 0.29	5.713 *
S3	12 ± 0.42	19 ± 0.64	30 ± 0.53	43 ± 0.75	6.985 *
S4	11 ± 0.56	13 ± 0.39	24 ± 0.44	38 ± 0.38	6.746 *
LSD value	3.188 *	3.752 *	4.067 *	4.974 *	----
* (P<0.05).					

Control (0/0)without bacteria and without Fe.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Table (2). Effect of Fe concentrations and *Sinorhizobium* isolates in
Plant length (cm)

Isolate	Concentration of Fe (gm/4kg soil)				LSD value
	0	0.1	0.3	0.6	
0	16.80 ± 0.38	20.33 ± 0.43	24.66 ± 0.63	31.33 ± 0.74	6.294 *
S1	24.50 ± 0.93	32.33 ± 0.77	46.33 ± 1.28	56.33 ± 1.19	6.704 *
S2	18.83 ± 0.62	25.00 ± 0.46	37.00 ± 0.95	43.33 ± 0.85	5.926 *
S3	31.66 ± 0.59	41.66 ± 1.15	58.33 ± 2.09	67.33 ± 2.37	7.016 *
S4	28.66 ± 0.48	32.00 ± 0.94	48.33 ± 1.26	59.33 ± 1.93	6.833 *
LSD value	5.179 *	5.883 *	6.074 *	5.821 *	----
* (P<0.05).					

Control (0/0)without bacteria and without Fe.

Table (3). Effect of Fe concentrations and *Sinorhizobium* isolates in
Dry weight (gm)

Isolate	Concentration of Fe (gm/4kg soil)				LSD value
	0	0.1	0.3	0.6	
0	3.13 ± 0.07	4.10 ± 0.15	5.33 ± 0.09	6.32 ± 0.25	1.644 *
S1	5.80 ± 0.12	7.36 ± 0.19	9.45 ± 0.23	11.46 ± 0.44	2.497 *
S2	4.10 ± 0.07	5.20 ± 0.08	6.73 ± 0.12	7.77 ± 0.30	1.426 *
S3	6.50 ± 0.04	8.30 ± 0.11	10.53 ± 0.42	13.33 ± 0.57	2.052 *
S4	6.11 ± 0.18	7.76 ± 0.29	9.50 ± 0.39	12.23 ± 0.31	2.869 *
LSD value	1.842 *	1.796 *	2.053 *	2.916 *	----
* (P<0.05).					

Control (0/0)without bacteria and without Fe.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Table (4). Effect of Fe concentrations and *Sinorhizobium* isolates in **Number of roots nodules**

Isolate	Concentration of Fe (gm/4kg soil)				LSD value
	0	0.1	0.3	0.6	
0	8 ± 0.15	11 ± 0.37	17 ± 0.42	22 ± 0.61	5.409 *
S1	33 ± 1.46	48 ± 2.52	67 ± 1.79	82 ± 2.68	7.629 *
S2	12 ± 0.42	20 ± 1.25	30 ± 1.04	40 ± 1.19	7.842 *
S3	46 ± 2.09	55 ± 1.84	75 ± 3.62	93 ± 3.73	9.256 *
S4	40 ± 1.37	54 ± 2.06	70 ± 2.59	86 ± 2.16	7.883 *
LSD value	6.724 *	8.629 *	8.733 *	10.315 *	----
* (P<0.05).					

Control (0/0)without bacteria and without Fe.

Table (5). Effect of Fe concentrations and *Sinorhizobium* isolates in **Nitrogen percentage (%)**

Isolate	Concentration of Fe (gm/4kg soil)				LSD value
	0	0.1	0.3	0.6	
0	3.50 ± 0.14	4.20 ± 0.07	5.10 ± 0.19	6.20 ± 0.35	2.275 *
S1	6.96 ± 0.22	9.20 ± 0.52	11.76 ± 0.48	14.60 ± 0.74	3.416 *
S2	5.70 ± 0.09	7.66 ± 0.16	9.46 ± 0.33	11.13 ± 0.52	2.861 *
S3	7.70 ± 0.36	10.46 ± 0.47	12.93 ± 0.59	16.43 ± 0.64	4.094 *
S4	7.33 ± 0.41	10.11 ± 0.52	12.16 ± 0.41	15.10 ± 0.56	3.417 *
LSD value	2.072 *	2.556 *	3.163 *	2.945 *	----
* (P<0.05).					

Control (0/0)without bacteria and without Fe.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Table (6). Effect of Fe concentrations and *Sinorhizobium* isolates in **Protein percentage (%)**

Isolate	Concentration of Fe (gm/4kg soil)				LSD value
	0	0.1	0.3	0.6	
0	22.75 ± 0.84	27.30 ± 1.03	33.15 ± 1.72	40.30 ± 1.89	8.015 *
S1	45.24 ± 1.78	59.80 ± 2.52	76.44 ± 3.08	94.90 ± 4.16	7.442 *
S2	37.05 ± 1.09	49.79 ± 1.66	61.49 ± 2.53	72.34 ± 2.85	9.671 *
S3	50.05 ± 2.37	67.99 ± 2.48	84.04 ± 3.94	106.79 ±4.69	11.825 *
S4	47.64 ± 1.85	65.71 ± 2.51	79.04 ± 3.07	98.15 ± 3.86	9.558 *
LSD value	7.573 *	9.187 *	7.662 *	7.902 *	----
* ($P<0.05$).					

Control (0/0)without bacteria and without Fe.

References:

1. Erman,Murat ; Yildrim,Bunyamin ; Togy,Necat , and Cig ,Fatih. (2009). Effect of phosphorus application and *Rhizobium* inoculation on the yield , nodulation and nutrient up take in field pea (*Pisum sativam sp. arvens L.*). Journal of Animal and Veterinary Advance. 8(2):301-304.
2. Mia, Baset MA., and Shamsuddin ZH. (2006).*Rhizobium* as a crop enhacer and biofertilizer for increased ceral production . African J. of Biotecnology V.9. No(37).
3. Vessey,J.K. (2003). Plant growth promoting bacteria as a bio-fertilizers. Plant soil 255, 571-586.
4. Khalequzaman, K.M. , and Hossain,I. (2008). Effect of seed treatment with *Rhizobum* strain and biofertilizers on foot/root rot and yield of busbean in *Fusarium oxysporum* infested soil.J. Agric . Res. 26(1).
5. Brear, Ella.M. ; Day,David A. ,and Smith,P.M.C..(2013). Iron : anessential micronutrient for the legume-rhizobum symbiosis . Front plant Sci. V.(4):359.
6. Vincent, J.M. (1970). A manual for partical study of the root nodule bacteria I.B.P. handbook, No.15. Black wellscientific publication Ltd.Oxrord.
7. Hussein , I.A. (2000) . Genetic and biochemical studies on strees tolerance in *Rhizobium*. Ph.D. Thesis. Indian Institute of Technology-Roorkee(I.I.T-Roorkee).India.
8. Gresser,M.S. , and Parsons,J.W. (1979). Sulpheric perchloric digestion of plant material for the determination of nitrogen phosphorus , calisum and magnesium . Anal.Chem.Acat. , 109:431-436.
9. Chapman, H.D. and Pratt,F.P. (1961) Methods of analysis for soils plant and water . Univ. Calif.Dir. Agric.Sci.

10. Schaffelen, A.G.A. and Vanschawenbury, J.C.H. (1960). Quick tests for soil and plant analysis used by small laboratories. Neth.J.Agric.Sci., 9:2-16.
11. SAS. (2012). Staistical Analysis System , users guide statistical. Version 9.1th ed . SAS. Inst.Ins.Cary. N.C.USA.
12. AL-Saidy ,Ali Sadoon .(2001) . Effect adding phosphorus and iron on efficiency of *Rhizobum* ,growth and yield of (*Vigna radiate*). M.Sc. thesis. Colloge of Agriculture.Univ. Baghdad.Iraq.
13. Huthily,Kadhim Hassan. (2005). Effect of iron and molybdenum on (*Sinorhizobium meliloti*) effecincy and on growth and yield of Alfafa (Medicago sativa L.) Ph.D. thesis. Colloge of Agriculture . Univ. Basrah. Iraq.
14. Paliya S.; Tikle,A.N. , and Thoms,T. (2014). Efficincy of micronutrient in influencing growth behavior of *Rhizobium* of Pigeonpea (*Cajanus cajan L*. [Millsp.]). Orient J. of chemistry. V.30(2).
15. Yazdanpanah ,Hamid R.; Daneshian,Jahanfa r; Pourseyedi ,Shahram, and Shirannirad, A.H. (2014). Effect of *Rhizobium* and iron and zinc on quantitative and qualitative traits of Alfafa . Trend in life science. V.3 (5).
16. Tang,C. (1991). The role of iron in nodulation andsymbiotic nitrogen fixation by legumes . Ph.D. thesis, the Univ. of Western Australia.
17. Todd,Jonthan D. ; Sawers,Gray ; Roddionov,Dmitry A. , and Johnston,Andrew W.B. (2006). The *Rhizobium leguminosarum* regulater IrrA affects the transcription of wide rang of genes in response to Fe availability. Mol Gen Genomics 275: 564-577.
18. Tang ,C. (1995) . Iron in symbiotic nitrogen fixation in legumes . In : Advance in nutrition reasch . Hemantaranjan . A.(ed). Pp : 145-175. Scientific puplishers , Jodhpur.
19. Al-Kartany,Raheem Hadi. (2005). Effect of iron and phosphorus in effecincy of *Rhizobium* and in growth, and yield of chickpea (Cicer arietinum). M.Sc. thesis. Colloge of Agriculture. Univ. of Baghdad.Iraq.
20. Paudyal,S.P. ; Aryal,Rishi R.; Chauhanan,S.V.S., and Maheshwari,D.K. (2007). Effect of heavy metals on growth of *Rhizobium* strains and symbiotic efficiency of two spices of tropical legums . Scientific World J. V.(5) 5:27-32.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)