Effect the different levels of zinc element in efficiency of Sinorhizobium meliloti atmosphere Nitrogen fixing isolated from Medicago sativa and some plant characteristics inoculums with it laboratory and formerly

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Abstract

Environmental pollutants like heavy metals at lowe concentrations are required for various metabolic activities of microbes including *Rhizobia* and legume crops on one hand, the excessive metal concentrations on the other hand cause undeniable damage to *Rhizobia*, legumes and their symbiosis. This study deals with the effect of different levels of zinc element in the efficiency and growth of *Sinorhizobium meliloti* that infect Alfa alfa plant(*Medicago sativa*) laborator. Symbiotic characteristics of these bacteria and some characteristic of Alfa alfa plants were studied by treating them with zinc element under normal conditions. Four isolates of *S. meliloti* have been isolated and identified from root nodules of *Medicago sativa* plants. These plants were gathered from different agricultural locations in Baghdad and Diyla provinces. Symbols have been given for each isolate (AN-1,AN-2 AN-3 and AN-4).The viable bacterial count have been measured (Cfu) laboratory under the effect of three concentrations of zinc element(0.1, 0.2, 0.3 g/L) through incubation for 48, 72 hours from the beginning of bacterial growth. The results showed significant decrease ($P \leq 0.05$) in viable bacterial count and for all zinc concentrations that are used compared with control groups. Increasing zinc concentrations leads to decrease .Also, there

were differences between isolates since AN-3, AN-4 isolates were the more sensitive for zinc element than other isolates. The alfa-alfa plants were infected with (AN-1, AN-2, AN-3 and AN-4) isolates. These plants were cultured in soil treated with (0.13, 0.2, 0.52 g /3Kg) concentrations of zinc under normal conditions. Some characteristic of alfa-alfa plants were studied; such as the measurement of the length of shoot and root net, measurement of the dry and wet weight of the plants, number of root nodules, percentage of nitrogen and the content of protein in plants. Also the results showed ahighly significant decrease of ($P \leq 0.05$) for most plan characteristics that are under study, and an apparent decrease in the nodulation process of the plants in comparision with the control groups. The most negatively effected isolates were (AN-3, AN-4) and then (AN-1, AN-2).

Keywords: Nitrogen fixation, Alfa alfa, *Sinorhizobium meliloti*, zinc element

تاثير مستويات مختلفة من عنصر الزنك في كفاءة بكتريا *Sinorhizobium meliloti* المثبتة للنتروجين الجوي والمعزولة من نبات الجت *Medicago sativa* وبعض صفات النبات الملقح بها مختبريا وحقليا

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ألخلاصة

تناولت هذه الدراسة تأثير تراكيزمختلفة من عنصر الزنك في نمو و كفاءة بكتريا *Sinorhizobium meliloti* التي تصيب نبات الجت *Medicago sativa* مختبريا, كذلك دراسة الصفات التكافلية للبكتريا و بعض صفات نبات الجت الملقح بها حقليا تحت تاثير عنصر الزنك و في الظروف الطبيعية. تم عزل و تشخيص اربع عزلات من بكتريا *S. meliloti* من العقد الجذرية لنبات *Medicago sativa* من مناطق زراعية مختلفة في محافظتي بغداد و ديالى واعطي رمز لكل عزلة(AN-1, AN-2, AN-3, AN-4) مختبريا تحت تاثير ثلاث تراكيز .تم حساب اعداد البكتريا الحيه(Cfu)

Effect the different levels of zinc element in efficiency of Sinorhizobium meliloti atmosphere Nitrogen fixing isolated from Medicago sativa and some plant characteristics inoculums with it laboratory and farmerly

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من عنصر الزنك(0.1,0.2,0.3 g/L) خلال 48, 72 ساعة حضانة من بداية تنمية البكتريا واظهرت النتائج وجود انخفاض معنوي (P≤0.05) في اعداد البكتريا الحية و لجميع التراكيز المستخدمة من الزنك مقارنة بمجاميع السيطرة, ويزداد هذا الانخفاض كلما ازداد تركيز الزك, كذلك وجود تفاوت بين العزلات اذ كانت العزلتان (AN-3, AN-4) اكثر حساسية للزنك من بقية العزلات . تم اصابة نبات الجت بالعزلات (AN-1,AN-2, AN-3, AN-4)و المزروع في تربة ملوثة بالزنك بثلاث تراكيز (0.13,0.26,0.52 g /3kg) وفي الظروف الطبيعية ودراسة بعض صفات نبات الجت كقياس طول المجموع الخضري والجذري وقياس الوزن الطري و الجاف للنبات و عدد العقد الجذرية ونسبة النتروجين والمحتوى البروتيني في النبات و اظهرت النتائج انخفاضا معنويا عاليا (P≤0.05) لاغلب صفات النبات المدروسة وانخفاض ملحوظ في عملية التعقيد في النبات مقارنة بمجاميع السيطرة وكانت اكثر العزلات تاثرا بشكل سلبي (AN-3,AN-4) ثم العزلتين (AN-1, AN-2).

الكلمات المفتاحية: تثبيت النتروجين ، نبات الجت ، بكتريا الرايزوبيا ، عنصر الزنك

Introduction

Biological nitrogen fixation represents the major source of nitrogen input in agriculture soils including those in arid regions, the major N2- fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of low-N soils [1, 2]. *Rhizobia* among soil bacteria have been the organism of great interest for agronomist in general and legume growers in particular primarily due to their ability to provide N to plants. Considering the benefits of *Rhizobia* in N economy and the role of legumes in animal and human health, attention in recent times has been paid onto understanding how metals could affect the very survival of *Rhizobia* either present as free-living organism or when they are in intimate relationship(symbiosis) with legumes.[3,4]. Most of the *Rhizobia* are very sensitive to soil environmental factors, which affect their dinitrogen fixation capacity and hence the productivity of legumes , Most of the cultivated legumes are exposed to agrochemicals such as plant protecting agrochemicals and fertilizers which not only contain essential nutrients but also comprise of contaminants such as heavy metals. Due to their toxic nature many of the chemicals cause a threat to symbiotic nitrogen fixation.

Elevated amounts of heavy metals could be a result of the use of contaminated inorganic or organic fertilizers or contamination by aerial deposition and are deleterious to both the soil micro organisms and plant beneficial microbial processes [5, 6]. Most of the metals are non essential, have no nutritive value and are potentially toxic to micro organisms. Zinc, adivalent heavy metal with saturated d – orbital favour tetrahedral coordination for stable metallo enzyme complexes and there by regulate the various process of cell metabolism. It acts as micronutrient on one hand and environmental toxic factor on the other hand and is known to affect nodulation and dinitrogen fixation [7, 8]. There are numerous reports where elevated amounts of heavy metals have been found to limit the rhizobial growth and their host legumes and concomitantly reduce the crop yields for example, a single strain of *Rhizobium leguminosarum* could survive well in the metal contaminated plots, but this strain did not fix N with white clover (*Trifolium repens* L.), although it resulted in N formation with *Trifolium subterraneum* .In a similar manner, a profound toxic effect of metal on N2 fixing ability of culture inoculated white clover was observed In other reports, when sludge was applied for field trials in Braunschweig, it was found that the increasing sludge rates reduced the number of indigenous populations of *R. leguminosarum* bv. *trifolii* to low or undetectable levels[9,10]. Similarly, adverse effect of sludge application on N2 fixation in faba bean is reported. The reduction in growth and symbiosis in white clover were due to cadmium, lead and zinc, when plants were grown in soils highly contaminated with these metals [11, 12]. The aim of the present study was known the growth and efficiency of *Sinorhizobium meliloti* and Alfa alfa plant that inoculums with it in atmosphere nitrogen fixation under the effect of zinc concentrations.

Materials and Methods

Bacterial isolates sources:

The isolation and identification of four local isolates were done for *Sinorhizobium meliloti* from root nodules of Alfa alfa plant (*Medicago sativa*) that are cultured in different area in (Baghdad and Diyla). The specific symbols were given for each isolates (AN-1, AN-2, AN-3, AN-4). Numbers of Alfa alfa plants that grow in good form were selected in soil (non

fertilizer)from four different area for isolation of nodules bacteria. The soil was wetting with water for 3-6 hr before begging with plant uproted for obtain non-infected roots, the root nodules that are close to the main root were removed , wash with distal water for many time and sterilized according to the [13].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 acidified HgCl2 for 3-6 min, then plmed in glass test tube with 3 ml of NaCl(0.85%) with good scratch.

Taking 1 ml from bacterial suspension and cultured on mannitol salt yeast extract medium (MSY) and incubated at 28°c for 2-4 days until appearance of colonies. The bacterial isolates were identification by using gram stain and with successful infected for Alfa alfa plant for twice.

Host plant used:

The local variaty seed of *Medicago sativa* were used, which obtain from seed certificate center (Ministry of Agricultutre)

The purification of root nodules bacterial isolates:

After appearance of mucoid white colonies, were taking from it with loopful and streaking on (MSY) for 3 days. The experiment was repeated for many time until obtain of pure culture. four dilutions of bacterial suspension were done by using normal saline (0.85%), and the dilute bacterial suspension were cultured by spread(0.1) ml from last dilution on MSY in Petri dish and incubated at 28°c until appearance of colonies.

Measurement of viable bacterial count under affecting of zinc element:

Broth culture of bacterial were done and inoculums with isolates (under studied), then incubated in vortex incubator (100 rpm/sec) with 28°c [14]. After 24hr of incubation, taking 1 ml from sample and inoculated with 25ml from broth culture media (MSY) in conical flask, then the conical flask were incubate with vortex incubator with (100 sec/min) under 28°c.

Taking 0.1 ml from bacterial culture after 48hr and 72 hr respectively. After serial dilution were done until (10^{-6}), spread on solid culture media that contain on concentration of zinc sulfate (0.1, 0.2, 0.3 gm/L), and incubate under 28°c for 2-4 days. The bacterial colonies were count for three times with control sample for each growth period. The effect of Zinc on the bacteria under study and on some characterstic of *Medicago sativa* that are inoculums with zinc element.

This experiment was performed in glass house by using three concentration of zinc element rather control and the effect range in the growth of *Medicago sativa* and formation of root nodules.

Soil analysis:

Some of these analysis was performed to determine physical and chemical properties for soil that are used in experiment.

Silt	Clay	Sand	Organic matter	Mixture soil	pH	EC (dS/m)	CaCO3
510/g soil	185/g soil	245/g soil	9.5/g soil	Loamy soil	7.5	2.30	21.3%

Soil preparation:

In this experiment, the plastic pots were used (5Kgm) and sterilization well by using sodium hyopchloride and each pots were filled with constant quantity(3Kgm) of Loamy soil, after the soil was sieved with diameter(2mm)to performe homogenate and remove impure from it.

The soil was sterilized by Autoclave with 121°c and pressure 1 psi. The concentration of zinc elements was counted on the basis soil weigh (3Kgm) (0.13, 0.26, 0.52 gm/ 3Kg) and then mixed with soil by good form to ensure distribution of element to all parts of soil in homogenate form.

The seed of *Medicago sativus* was used that are obtained from seed certificate center /Ministry of agriculture and sterilized according to [13].

Agriculture and Irregated:

The cultured was performed through March /2013.Taking loopful from four Rhizobium isolates and grown on broth MSY , incubate in vortex incubator with speed 100 rpm/sec with temperature 28°c . Taking 2 ml($40x\ 10^6$) bacterial cell/ ml, then add to MSY broth to complete the volume into 50ml, put it in vortex for 2 days. After that the seed of Alfa alfa plant were add to the cultured soil (10 seed to each pot) and three time for each zinc element in addition to the control sample with bacteria and with out bacteria. The pots was put in green house with temperature between 20-25°c, all the pots were Irregated rotatory.

The harvest of plants:

The plants were harvested on May/2013 , the plants were harvested completely with roots, the roots were washed with water to remove the residues of soil that are found in root systems,then the plant sample were transport to the laboratory and the following data were recorded:

1-The length of shoot systems
2-The length of root systems
3-The number of nodules.
4-Fresh weight of plant
5-Dry weight of plant

The plants were dried in oven with temperature at 60°c for 48hr.

Estimation the quantity of N2 and protein in plant:

The known weight of dried plant are grounded with good form according to [15] and then, estimation of N2 in plants was done according to [16].The estimation of percent protein according to [17].

Statistical analysis:

The obtained results were statistically analyzed using analysis of variance test ANOVA test (one way) which was applied to differentiate between the various means of treatments followed by least significant differences (LSD), this was done by using statistical package of social science (SPSS). The experimental design was completely randomized block design.[18].

Results and Discussion

Bacterial count (AN-1,AN-2,AN-3 and AN-4) which effected with different concentrations of zinc were measured laboratory.

Table(1) showed the effect of zinc concentration in bacterial count for AN-1 isolate, since, there were significant decreased($P \leq 0.05$) in bacterial count compared with control groups after 48 and 72 hr from growth for all three concentrations, and the viable bacterial count were decreased when the concentrations of zinc element were increased.

For AN-2 bacterial isolate; there were significant decreased ($P \leq 0.05$) in concentration (0.2)gm/ml compared with control groups at 48hr and significant decreased ($P \leq 0.05$) in bacterial count at concentration (0.3)gm/ml compared with control groups (table 2).

The results of present study also showed that there were highly significant decreased ($P \leq 0.05$) in viable bacterial count for AN-3 isolate and in all concentrations of zinc element compared with control group at 48 and 27 hr respectively. The viable cell number were ($18x\ 10^6$) cell/ml and ($21x\ 10^6$) cell/ml compared with control groups ($52x\ 10^6$) cell/ml and ($43x\ 10^6$) cell/ ml at 48 and 72 hr respectively (table 3).

For AN-4 bacterial isolate, the results showed highly significant decreased ($P \leq 0.05$) in viable bacterial count especially of concentration (0.3)gm/ml at 48 and 72 hr,the viable bacterial number were ($22x\ 10^6$) and ($18x10^6$) cell/ml respectively (table 4).

According to the results of the present study and statistical analysis, the results showed that zinc element were effect in significant form in bacterial growth but there were differences

in effects intensity, since the bacterial number decreased when concentrations of zinc were increased and the effect was sever on AN-4 and AN-3 bacterial isolates.

Table 1: Effect concentrations of zinc element in bacterial number (10^6)cell/ml for isolates *S.meliloti* AN-1

Time	Control	Concentration of Zinc			LSD
		0.1 g/ L	0.2 g/ L	0.3 g/ L	
48h	22.00 d	18.33 c	12.67 b	7.67 a	1.970
72h	14.00 c	12.00 bc	10.00 b	4.67 a	2.766

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 2: Effect concentrations of zinc element in bacterial number (10^6)cell/ml for isolates *S. meliloti* AN-2

Time	Control	Concentration of Zinc			LSD
		0.1 g/ L	0.2 g/ L	0.3 g/ L	
48h	17.00 b	16.67 b	9.33 a	7.67 a	1.970
72h	10.00 c	7.67 bc	6.33 ab	3.67 a	2.746

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 3: Effect concentrations of zinc element in bacterial number (10^6)cell/ml for isolates *S. meliloti* AN-3

Time	Control	Concentration of Zinc			LSD
		0.1 g/ L	0.2 g/ L	0.3 g/ L	
48h	52.67 d	42.00 c	32.00 b	18.00 a	2.079
72h	43.67 d	39.00 c	29.00 b	21.00 a	3.211

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 4: Effect concentrations of zinc element in bacterial number (10^6)cell/ml for isolates *S. meliloti* AN-4

Time	Control	Concentration of Zinc			LSD
		0.1 g/ L	0.2 g/ L	0.3 g/ L	
48h	42.00 d	39.00 c	31.67 b	22.00 a	1.998
72h	32.67 d	27.33 c	21.67 b	18.67 a	1.526

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

The results of the present study were support by another study. Singh *etal.* revealed that isolates showed maximum growth up to 50 mg/ml of zinc concentration, were grouped as zinc tolerant strain and others were zinc sensitive isolates which shows minimum Cfu under

different treatment at 24 hr incubation period [9]. Stressors like heavy metal have also been reported to convert the viable bacterial cells to non-culture able form (Therefore, once the soil is destructed by heavy metals, metals found naturally within soil or accumulated as a result of anthropogenic activities [19]. Another study revealed that cell viability was maintained throughout the growth period. It appears that *Rhizobium* isolates tested to demonstrate an elevated tolerance may be important for the survival in the metal contaminated conditions. Microorganisms tolerant to metal contaminants are known to have two mechanisms by which they protect themselves from the toxic effects of heavy metals. Firstly, Exclusion, as mediated by the extra polysaccharide capsule around the cell, has been shown to prevent cellular uptake of metals ,The polysaccharide capsule around most species of *Rhizobia* is quite thick and therefore should provide adequate protection against most metals. Secondly, several genera of soil microbes are known to produce phytochelatins, which may internally bind metals and thus effectively lower activity with in the cell [20, 21]. Another study also found that motile strains of *Rhizobium trifolii* formed approximately five times more nodule than non motile strains and suggesting that motility is a factor in competition for nodule formation of zinc on nodulation may perhaps be as much due to inhibition of motility as to direct toxicity especially at low concentrations. The study concludes that under zinc stress conditions, tolerant strains successfully overcome the stressful environmental conditions by maintaining the factors, essential for nodulation like lucan, lipopolysaccharide, exopolysaccharide and motolity in an ideal system. In legume *Rhizobium* symbiosis zinc is essential as a micronutrient on the one hand and become toxic, when agricultural fields are get polluted due to industrialization [22].

The result of farmer experiment that were aimed to study the effect of zinc element on *S. meleloti* farmerly and on the ability to infect the Alfa alfa plant. These were done by culture Alfa alfa plant that inoculum with bacteria in contaminated soil with zinc element, then some parameter were performed ex. the length of shoot and root net, dry and wet weight of plant, count the number of root nodules, count the percentage and the quantity of protein in the plant.

Table (5) showed the effect of zinc element in some characteristic of alfa-alfa plant that inoculated with AN-1 isolate in soil. The results showed that asignificant decreased (P≤0.05) in the length rate of shoot ,root net and the rate of dry and wet weight of plants especially in the high concentration of zinc element(0.250 gm/3kg) compared with control groups as shown in(Fig. 4-1).Also, there were significant decreased (P≤0.05) in the number of root nodules and the small size of nodules, (Fig.5). Also, there were significant decreased(P≤0.05) in the percentage of nitrogen and quantity of protein in the plant compared with control groups especially in the concentration (0.25 gm/3kg) (Fig.6,7).

Table (6) showed there were significant decreased (P≤0.05) in the length rate of shoot net of plant, while there were non significant decreased in the length rate of root net, dry and wet weight of plant compared with control groups for AN-2 (Fig. 4-1).The result of this study showed, there were significant decreased (P≤0.05) in the number of root nodules compared with control groups (Fig.5); Also, there were significant decreased (P≤0.05) in the percentage of nitrogen and protein in the high concentration (0.52 gm/3kg) , while there were non significant decreased in the concentrations (0.13, 0.26 gm/3kg) compared with control groups (Fig.6,7).

About AN-3 isolate, table (7) showed there were highly significant decreased (P≤0.05) in the length rate of root , shoot net and the rate of dry and wet weight of plant for all concentration that are used of zinc element (Fig. 4-1).Also, there were highly significant decreased in the number of root nodules, the number of root nodules(9) in the high concentration (0.52 gm/3kg) compared with control groups(48) nodules (Fig. 5).Also, there were highly significant decreased (P≤0.05) in the percentage of nitrogen and protein in the plant compared with control groups (Fig. 6,7).

For AN-4 isolate, table (8) showed there were highly significant differences (P≤0.05) in the length rate shoot and root net, dry and wet weight in the high concentration of zinc (0.52 gm/3kg) compared with control groups, while in the concentration(0.13, 0.26 gm/3kg) , there were non significant differences compared with control groups (Fig.4-1). Also, there were highly significant differences (P≤0.05) in the number of root nodules and the small size of

nodules, the number were reached to (10) in the high concentration(0.52 gm/3kg)compared with control groups(28)) nodules (Fig.5).

There were non significant differences in the percentage of nitrogen and protein in the plant in the concentrations (0.13, 0.26 gm/3kg), while there were significant differences ($P \leq 0.05$) in the high concentrations of zinc (0.52 gm/3kg) compared with control groups (Fig. 6,7).

According to the above results and statistical analysis, the zinc element has negatively effect on Alfa alfa plant and process of nodulation for four isolates under studies but with different degrees. Since, the AN-3, AN-4 isolates were more effect with zinc concentration .When the concentrations of zinc were increased , the negatively effect were increased on the plant and formation of nodules and the percentage of nitrogen, protein and also effect on plant net.

Table 5: Effect the concentrations of zinc element in some characteristics of Alfa alfa plant that inoculume with *S.meliloti* AN-1

Properties studies	Control	Control Without Bacteria	Concentration of Zinc			LSD
			0.13 g/3Kg	0.26 g/3Kg	0.52 g/3Kg	
Length of shoot (cm)	32.00 c	31.00 c	27.67 b	25.33 ab	25.33 a	2.406
Length of root (cm)	21.67 c	20.00 c	17.33 b	17.00 b	14.67 a	1.819
Fresh weight (g/plant)	16.53 c	15.37 c	13.53 b	12.73 b	9.80 a	1.545
Dry weight (g/plant)	4.467 d	3.533 c	3.300 bc	3.067 ab	2.667 a	0.4300
Number of nodules	37.33 e	0.00 a	34.33 d	30.33 c	24.00 b	2.977
Percent of N %	4.180 c	3.580 ab	3.940 bc	3.840 bc	3.407 a	0.3691
Percent of Protein %	26.12 c	22.37 ab	24.62 bc	24.00 bc	21.29 a	2.306

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 6: Effect the concentrations of zinc element in some characteristics of Alfa alfa plant that inoculume with *S.meliloti* AN-2

Properties studies	Control	Control Without Bacteria	Concentration of Zinc			LSD
			0.13 g/3Kg	0.26 g/3Kg	0.52 g/3Kg	
Length of shoot (cm)	31.67 c	31.00 c	28.67 b	25.67 a	24.67 a	2.319
Length of root (cm)	21.33 c	20.00 c	16.67 b	15.67 ab	14.67 a	1.945
Fresh weight (g/plant)	16.07 c	15.37 bc	15.13 bc	14.20 ab	13.40 a	1.198
Dry weight (g/plant)	4.367 d	3.533 c	3.000 b	2.900 b	2.233 a	0.3386
Number of nodules	29.00 d	0.00 a	27.67 d	24.00 c	19.67 b	1.786
Percent of N %	4.773 c	3.580 b	3.457 b	3.150 ab	2.747 a	0.4337
Percent of Protein %	29.83 c	22.37 b	21.60 b	19.68 ab	17.16 a	2.710

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 7: Effect the concentrations of zinc element in some characteristics of Alfa alfa plant that inoculume with *S.meliloti* AN-3

Properties studies	Control	Control Without Bacteria	Concentration of Zinc			LSD
			0.13 g/3Kg	0.26 g/3Kg	0.52 g/3Kg	
Length of shoot (cm)	33.00 d	31.00 c	24.00 b	21.00 a	19.67 a	1.753
Length of root (cm)	23.00 e	20.00 d	15.00 c	12.67 b	10.00 a	1.898
Fresh weight (g/plant)	17.67 e	15.37 d	13.23 c	10.07 b	7.93 a	1.214
Dry weight (g/plant)	5.400 d	3.533 c	2.233 b	1.867 ab	1.500 a	0.4009
Number of nodules	48.67 e	0.00 a	24.00 d	17.67 c	9.67 b	4.016
Percent of N %	4.160 e	3.580 d	3.157 c	2.627 b	1.963 a	0.3791
Percent of Protein %	26.00 e	22.37 d	19.73 c	16.41 b	12.27 a	2.368

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

Table 8: Effect the concentrations of zinc element in some characteristics of Alfa alfa plant that inoculume with *S.meliloti* AN-4

Properties studies	Control	Control Without Bacteria	Concentration of Zinc			LSD
			0.13 g/3Kg	0.26 g/3Kg	0.52 g/3Kg	
Length of shoot (cm)	30.33 cd	31.00 d	29.00 c	27.00 b	24.00 a	1.594
Length of root (cm)	20.33 c	20.00 c	17.67 b	16.00 b	12.00 a	2.119
Fresh weight (g/plant)	16.23 d	15.37 cd	14.80 c	13.37 b	11.90 a	1.084
Dry weight (g/plant)	4.200 d	3.533 c	2.833 b	2.633 b	1.967 a	0.4422
Number of nodules	28.67 e	0.00 a	22.67 d	17.33 c	10.00 b	1.867
Percent of N %	4.657 c	3.580 b	3.177 b	3.220 b	2.590 a	0.5179
Percent of Protein %	29.10 c	22.37 b	19.85 b	20.12 b	16.19 a	3.236

There was no significant differences between means with the same letters for the same raw at (5% level).

Least significant differences of means (5% level).

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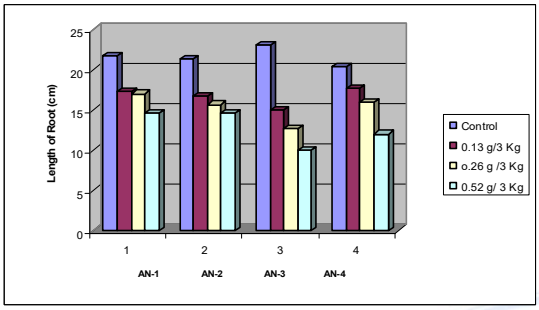

Fig.2: Effect of zinc element in characteristic of Alfa alfa plant inoculums with *S. meliloti*

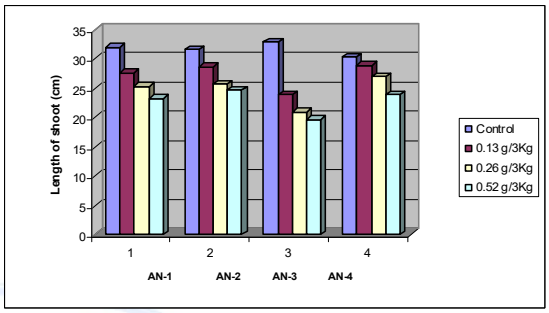

Fig.1:Effect of zinc element in root length for characteristic of shoot length for Alfa alfa plant inoculums with *S. meliloti*

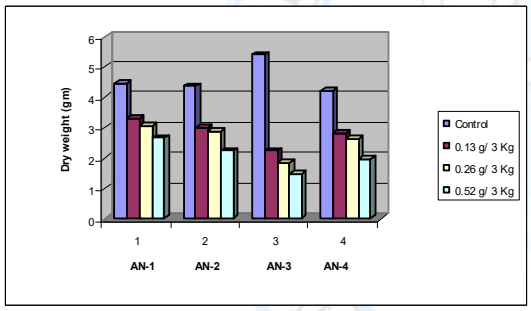

Fig.4: Effect of zinc element in characteristic of fres Dry weight for Alfa alfa plant inoculums with *S. meliloti*

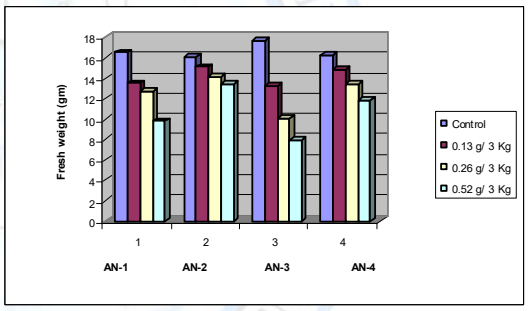

Fig.3:Effect of zinc element in characteristic of weight for Alfa alfa plant inoculums with *S. meliloti*

Effect the different levels of zinc element in efficiency of Sinorhizobium meliloti atmosphere Nitrogen fixing isolated from Medicago sativa and some plant characteristics inoculums with it laboratory and farmerly Lecturer. Anmar Saadi Aboud

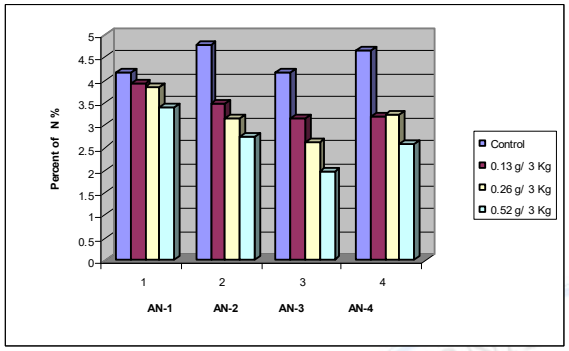

Fig.6: Effect of zinc element in characteristic of characteristic of nitrogen percentage for Alfa alfa plant inoculums Inoculums with *S. meliloti*

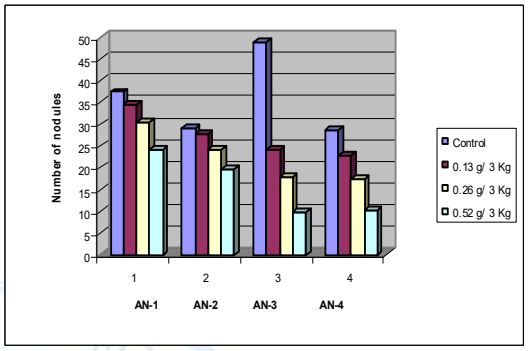

Fig.5:Effect of zinc element in nodules number for Alfa alfa plant with *S. meliloti*

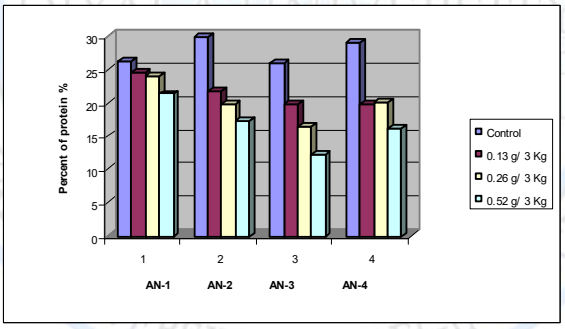

Fig.7: Effect of zinc element in characteristic of protein percentage for Alfa alfa plant inoculums with *S. meliloti*

The results of the present study were support by another study ;One study in a long-term field trial reported a decrease in two agriculturally important species of *Rhizobia, R. leguminosarum* bv. viciae and *R. leguminosarum* bv. trifolii, in soils, which were irrigated with sewage sludge containing Zn or Cu or mixture of Zn and Cu. Besides the potential toxicity of heavy metals on the growth and survival of *Rhizobia*, nodulation in legumes is also

considerably affected .In sludge-treated soils, even though the nodulation on the root systems of clover was observed, the nodules were ineffective [23,24]. However, when grown in soils treated intentionally with heavy metals for experimental purpose or in soils already contaminated with heavy metals mainly due to contaminated agrochemicals and sewage sludge, most legume crops are not safe and affected negatively. The deleterious effects of heavy metals on nodulation and N2 fixation of *Rhizobium*–legume symbiosis are probably due to their inhibitory effects on the growth and activity of both symbionts[12]. The abnormally higher concentrations of metal also limit the uptake of water and nutrients by plants and concomitantly the health of plants. However, when a single or mixture of metals get a chance to enter within plant tissues and are translocated subsequently to various plant organs, they can interact directly with cellular components and disrupt the metabolic activities, causing cellular injuries and in some cases even may lead to the death of the plants [25].

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