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Effect of Soil Textural Classes on the Biological Nitrogen Fixation by *Bradyrhizobium* Measured by ^{15}N Dilution Analysis

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Abstract:
The current study was conductedas a pot experiment to determine the effect of soil texture on biological nitrogen fixation (BNF) of six most efficient local isolates, specified, of *Bradyrhizobium*. Cowpea (*Vignaunguiculata* L.), as a legume host crop, was used as a host crop and ^{15}N dilution analysis was used for accurate determination of the amount of N biologically fixed under experimental parameters specified. Soils used are clay loam, sandy clay loam and sandy loam. Biological Nitrogen Fixation (BNF), in different soil textural classes, was as in the following order: medium texture soil > heavy texture soil > light textured soil. Statistical analysis showed that there is a significant variation in BNF % among six Iraqi isolates in the three soil textural classes. There is a significant variation in the number of the nodules of the six Isolates in one soil texture. However, nodules number does not agree with the BNF% in the same soil for any isolates. Statistical analysis of the data showed that there were significant differences in plant dry weight among the soil textural classes all over local isolates used in this study. Data also showed that there were significant differences in dry weight under different isolates.

Keywords: local Iraqi isolates, Cowpea, BNF, Nodules, dry weight

Introduction:
Soil texture indicates the relative content of particles of various sizes, such as sand, silt and clay in a mass of soil. Texture influences the ease with which soil can be worked the amount of water and air it holds and the rate at which water can enter and move through soil [1]. Accordingly, soil textures are of indirect significant effect on rhizobia and in turn affect biological nitrogen fixation (BNF) [2]. Therefore, several environmental conditions are limiting factors to the growth and activity of the N_2-fixing plants. [3] introduced for the

first time the principle of limiting factors which states that "the level of crop production can be no higher than that allowed by the maximum limiting factor". Therefore, a competitive and persistent rhizobial isolate is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, which are very much depending on soil texture) impose limitations on the vigor of the host legume [2].

[4] reported that successful inoculation of Peanuts and Cowpeas depends on the survival of rhizobia in soils which fluctuate between wide temperature and moisture extremes which both are a function of soil texture. In sterilized soil, the populations of all isolates in moist soil increased during the first 2 weeks, but decreased rapidly when incubated under dry conditions. The populations, however, did not decline under saturated soil conditions.

[5] found that Lupins grow poorly on fine-textured, alkaline or poorly drained soils. Little, however, is understood about which components of these soils affects lupin growth. *Lupinus .sangustifolius*, L. albus and *L. cosentinii* were grown at both an acid and an alkaline soil pH on a sandy clay loam and a sandy with or without additional NH_4NO_3. Furthermore, they observed that plant growth was poor on the fine-textured, alkaline soil where emergence was inhibited and plants were chlorotic. Plant growth was also lower on the acidified fine-textured soil compared to the acid sand.

[6] verify the hypothesis that the population size of introduced bacteria is affected by habitable pore space by varying moisture content and bulk density in sterilized, as well as in natural loamy sand and silt loam. They found that in sterilized soils, the rhizobial numbers were not affected or decreased when water potentials increased from −20 to −5 kPa. In natural soils, the decrease in rhizobial numbers with increasing water potentials was more pronounced. They also found that bulk density had only minor effects on the population sizes of rhizobia or total bacteria.

[7] investigated the Population dynamicsofRhizobiumleguminosarumbiovar trifolii after introduction into loamy sand textured soil using selective plating and immunofluorescence detection techniques. They found that cell numbers declined steadily during the 60 days of the experiment. Upon introductionofR. Leguminosarumbiovartrifoliiinto loamy sand amended with 10% bentonite clay the population size remained constant throughout the incubation.

[8] on the other hand, stressed the fact that the most problematic environments for rhizobia are marginal lands with poor water-holding capacity as that of light textured soils. Populations of Rhizobium and Bradyrhizobium species vary in their tolerance to major environmental factors; consequently, screening for tolerant isolates has been pursued [9]. Biological processes (e.g., N_2 fixation) capable of improving agricultural productivity while minimizing soil loss and meliorating adverse edaphic conditions are essential. Reviewing the literature, however, showed that there is a serious lacking in information concerning the effect of soil textural classes on efficiency of BNF of local Rhizobium isolates. Therefore, the current investigation was conducted to evaluate BNF of local Rhizobium isolate under the three main textural classes of soils in Iraq.

Materials and Methods:

Collection of Nodules

Cowpea root nodules were collected from 20 locations of differentfield sites in 10 governorates representing the most important cowpeaproduction area in Iraq namely Basrah, Dhi-Qar, Mysan, Wasit, Babil, Al Anbar, Baghdad, Salah alden, Suleimanyah, and Ninevah.

Effective and healthy cowpea root nodules were collected from young, healthy and green cowpea plants of 45 to 60 days old. Cowpea plants in specified sites were excavated from cultivated field and adhering soil particles were carefully removed. Nodules reddish to pinkish, healthy and of 0.3 to 0.6 mm diameter were excised from the root along with 0.5-1.0 mm root part on both sides of the nodule attachment. Excised nodules were washed by tap water; shade dried for 1-2 hours and collected in nodule collection vials. The vials were labeled and were stored at 4±1^{o}C for a short-term storage.

Bacterial Isolates

One hundred and ten bacterial isolates were obtained from cowpea root nodules. Nodules were surface sterilized in series of surfactants in laminar air flow cabinet as it had been described by [10]. The sterilized nodules were crashed with sterilized glass rod in petridish containing one mL sterilized distilled water. Furthermore, the nodule suspension was streak inoculated on yeast mannitol agar (YMA) [11] and incubated at 28 ^{o}C. Single colonies were selected and streaked onto YMA slant and kept at 4±1 ^{o}C for short term storage with sub culturing every 4 month. Long term storage was made by storing the culture broth in 10% glycerol at –20 ^{o}C [12].

Authentication of Isolates by Infection Tests

All bacterial isolates, obtained from the cowpea root nodules, were authenticated as the cowpearhizobium, the microsymbiont of cowpea. That is by examining their ability to infect cowpea roots to stimulate the root nodulation. Infection test was performed in sterile sand soil [10] and [13].

HostPlant

Cowpea (*Vignaunguiculata* L.) as a legume crop was used in this study because of its high adaptability to sever climatic conditions, like these prevailing in Iraq. It is a typical legume crop for the arid region, which is Iraq part [14]. Cowpea is also of high tolerance to climatic stress, high capacity in BNF through on symbiosis association with rhizobia. It is in fact receiving most of its nitrogen from the BNF. Due to these properties cowpea is an especially important genetic resource for the region and a source for gens for genetic engineering experiments [14].

Pot Experiment

Efficiency of six isolates of *Bradyrhizobium* spp and controls affected by three soil textural class was evaluated in a greenhouse experiments, using ^{15}N technique, with three replicates in a plastic pots of24 cm diameter and 20 cm height. Therefore, 21 pots are needed for each soil. Samples of five kg of each sterilized soil were transferred in the cleaned pots.

Soils were characterized for relevant chemical and physical properties (Table 1) according to [15].

Table (1) : Properties of the soil in Iraq used in the study

Properties		Unit	Soiltexture		
			Clayloam	Sandyclayloam	Sandyloam
Separations	Sand	gm/Kg	21.5	65.1	75.6
	Silt		37.8	2.40	8.00
	Clay		40. 7	32.5	16.4
Electrical conductivity EC 1:1 soil:solution		dS/m	1.33	1.32	1.28
pH 1:1			7.12	7.18	7.41
Total N		%	0.119	0.105	0.034
Available Phosphorus		Ppm	19.09	18.24	10.04
Organic Carbon O. C		gm/Kg	5.31	4.02	2.45
$CaCO_3$ eq		gm/Kg	30.43	23.84	22.48
Cation exchange capacity CEC		C mol / Kg	22.55	16.21	11.91
Sodium Adsorption Ratio *SAR			3.86	3.30	3.46
Solublecations	Ca	mmo/ Kg	2.32	2.85	2.54
	Mg		1.01	0.98	1.14
	Na		7.03	6.46	6.63
	K		0.63	0.45	0.40
Solubleanions	SO_4		2.18	2.05	1.84
	Cl		9.01	8.75	8.14
	HCO_3		0.81	0.77	0.70

* EC, electrical conductivity. CEC, cation exchange capacity, SAR, sodium absorption ratio

Cowpea was selected as a test legume crop for this study. The healthy seeds of cowpea were surface sterilized with 70% ethanol and 2% sodium hypochlorite for one and five minutes respectively and thoroughly washed with sterilized distilled water for five times. Five surface sterilized seeds were sown per pot. Seeds were inoculated with 7 days old cowpea rhizobium broth and remaining pots were kept un-inoculated (control). Nitrogen, phosphorus and potassium were added at 50, 20, and 50 mg per kg soil respectively[16]. Nitrogen was labeled with stable isotope 15-N at (10 a.a Access Atom). After germination, seedlings were thinned manually to three healthy seedlings in each pot. Proper care and adequate watering on field capacity level was provided to minimize the drought stress. Plants of each pot were cut at the 60^{th} day of growth right at the soil surface using sharp plaid. Plants were dried in an oven at $60^{0}C$ temperature; weight of plant was obtained for further calculations and then grinded to pass 80 mesh sieve. Plant root of each treatment was obtained by removing soil particle by light stream of water. Water on Plant roots was removed by soft tissue and number of nodules was calculated. [17].

Nitrogen fixing efficiency of different Cowpea rhizobium isolates was examined in terms of plant growth, yield performance and nitrogen content by use ^{15}N technique. Nodulation pattern (nodule number per plant), shoot biomass, nitrogen content and yield parameters were recorded [18].

Analysis of data

The data were statistically analyzed with SAS 2001 software using analysis of variance and continued with least significant difference (LSD) for mean comparison.

Results and Discussion: Authentication Test

Total number of 60 isolates out of 110 isolated bacteria were authenticated in nodulating cowpea, their original host plant, confirming their symbiotic status. The results showed that 38 cowpea Rhizobia presented less than10 nodules. Other 8 isolates presented 10 to 20 nodules, number of nodules of 6 isolates out of the 60 authenticated isolates presented 20 to 30 nodules. Nodules number in a range of 30 to 70 was presented by 3 isolates only. Furthermore, 4 isolates namely Isolate-45, Isolate-47, Isolate-48 and Isolate-49 were highly super nodulating.

Soils Used

Physiological and chemical characteristics of the soils used in this study are shown in Table (1). The first soil is clay loam representing the heavy textured soil. The second soil is the sandy clay loam soil which represents the medium textured soil. The third soil is sandy loam soil which represents the light textured soil. These three soils are alluvial soil classified as typical torrifluvent soils. They are the most common agricultural soils in the country. The salinity levels of these three soils are 1.33, 1.32, 1.28 dSm^{-1} for the heavy, medium and light textured soils, respectively are not of harmful effect on plant growth and development [19]. pH of the three soils is in the neutral level which makes the soil adequate for cropping [20]. Total nitrogen is low, however, it is adequate for biological nitrogen fixation process. Plant available phosphorus is sufficient to support good plant growth with the exception of loam soil which is at critical level. For such critical available phosphorus, phosphate fertilizer must be added to obtain good plant growth [20].

Effect of Soil Texture on BNF

Figure 1 shows the effect of soil texture on BNF under the six most affected rhizobium isolates obtained along the climatic change in Iraq.

BNF under isolate -9 is the least among the six isolates in the three soil textural classes. BNF as a percent of total N in plant under this isolate was in the following order: medium texture soil > heavy texture soil > light textured soil. That may be attributed to the fertility level in the three soils which is the following order of magnitude: heavy texture ≥ medium texture > light texture. This is in agreement with [21] who stated that efficiency is the least in soils of poor fertility level. [21], however, concluded that the efficiency of BNF reduced in the soils of good fertility level.

The results also showed that the high BNF (50%) in light textured soils was under isolate-47 which was obtained from the southern provinces.

BNF percentage in medium textured soil was found under isolate-9 and isolate-51.

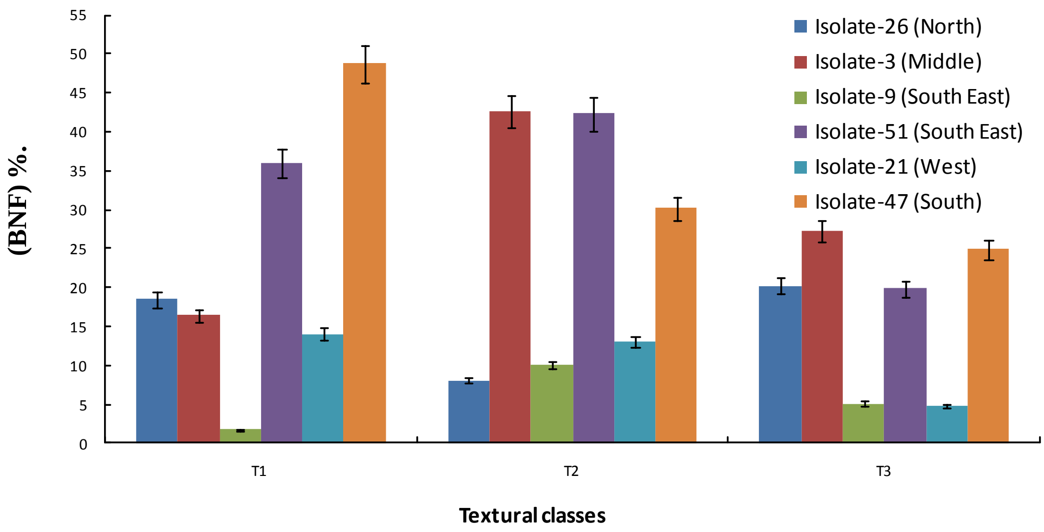

Fig. (1): Effect of soil textural classes on the total BNF percent underlocal isolates *Bradyrhizobium*bacteria.
Where: T1 = Sandy Loam Soil, T2 = Sandy Clay Loam Soil, T3 = ClayLoam Soil

In fact BNF% of both isolates are relatively equal. This finding, however, agrees with [22] who reported that different isolates of rhizobia responded defiantly to soil fertility, soil moisture and aeration.
In heavy textural soil, BNF% was found to be the highest under isolate-47. However BNF% under isolate-47 is very much comparable to that of isolate-3. While BNF% under isolate-9 and isolate-21 is almost equal and that of isolate-26 and isolate-51 is relatively equal. BNF% under isolate-9 and isolate-21 is five times less than that of isolate-26, isolate-3, 4, and isolate-47. These results may coincide with that of [22].
Statistical analysis showed that there is a significant variation at $P \leq (0.01)$ in BNF under different textural classes, being the highest at medium textured soil and the least under heavy textured soils.
Statistical analysis also showed that there is a significant variation in BNF % among different the six Iraqi isolates.

Effect of soil texture on nodules number per plant

Nodules number per plant for the six isolates as affected by soil texture is given in Figure (2).

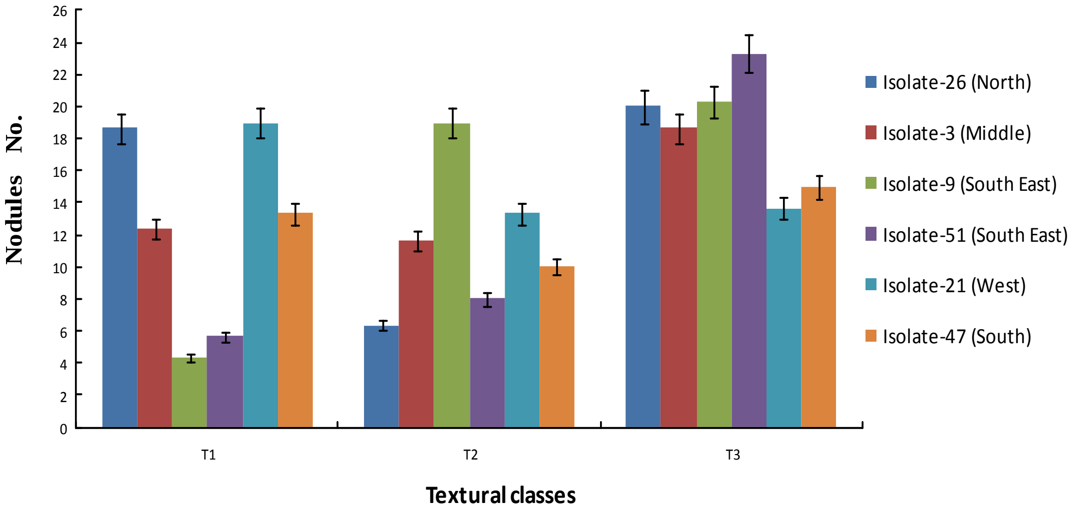

Fig.(2): Effect of soil textural class on cowpea nodulation by specified local*Bradyrhizobium* bacteria
Where: T1= Sandy Loam Soil, T2 = Sandy Clay Loam Soil, T3= Clay Loam Soil

Results clearly show that there is a significant variation in the number of the nodules of the six Isolates. In sandy loam soil (like texture soil), number of nodules is in the following order: Isolate 21 > Isolate 26 > Isolate 47 > isolate 3 > isolate 51 > isolate 9. Clearly, isolates 9 and 51 are the least efficient in nodules formation. However, nodules number is not in agreement with the BNF% in the same soil for any isolates. BNF% in this soil was the highest under isolate 47, while, the highest nodules number was observed under isolate 21. However, least BNF% in this soil was observed under isolate 9 which are very much comparable to the nodules number which is the least under isolate 9. These results are totally in agreement with those of [23] who had indicated that nodule does not necessarily reflect the efficiency of BNF of the same bacterial isolate.

Nodules number in sandy clay loam soil (medium textural soil) is the highest under isolate number 9 and the least was under isolate number 26.Comparing nodules to BNF % showed that in this soil, BNF% is the least under isolate 9. Consequently, this result proves the previous one in light textural soils, that number of nodules does not necessarily reflect the BNF efficiency of the isolate

In heavy textured soils, however, isolates 26, 3, 9 and 51 showed relatively equal nodules number. Isolates 21 and 47 showed the least number of nodules.

The high number of nodules of all isolates in this heavy textured soil may be attributed to the fact that this soil is of the highest clay content, which in return, is of the highest water holding capacity and of sufficiency level of all plant essential elements to support good plant growth. And for that matter, it provides better environmental conditions for livings (plant and microorganisms growth).

Statistical analysis of the data showed that there are significant differences at $P \leq 0.01$ among nodules number of isolates in each soils and in nodules number of isolates in different soils.

Effect of soil textural classes on the plant dry weight

Figure 3 is presenting dry weight of cowpea grown in soils of three textural classes namely (sandy loam, sandy clay loam and clay loam soils). As it reported before that, seeds of cowpea were inoculated with either one of the six local isolates.

Results showed that for all isolates that cowpea dry weight was the highest in the heavy texture soils. This may be attributed to the fact that the plant essential nutrients in this soil are very much higher than the two soils [24].

Plant dry weight in the heavy textured soil was the highest under isolate number 9 and was the least under isolate number 21. Obviously, variations in plant dry weight under different isolates were the least in heavy textured soils.

Dry weight in light textured soils as an average over all isolates was the least among these three soils used in the study. The highest dry weight in light textured soil was under isolate number 47 and the least was under isolate 26 and 3.

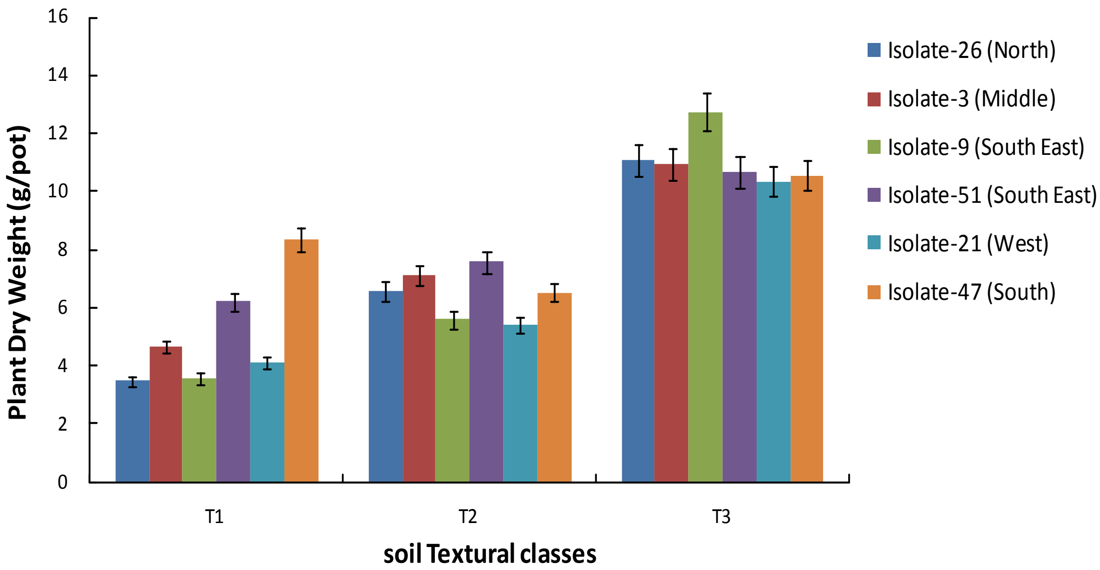

Fig. (3): Effect of local *Bradyrhizobium* isolates on plant dry weight(g/pot) under soil textural classes.
Where: T1 = Sandy Loam Soil, T2 = Sandy Clay Loam Soil, T3 = Clay Loam Soil

Dry weight in medium textured soil was the highest under isolates 51 and 3 while the least was under isolates 9 and 21.

Statistical analysis of the data showed that there are significant differences in plant dry weight among soil textural classes all over local isolates used in this study. Data also showed that there are significant differences in dry weight under different isolates.

The results of dry weight however are similar trend to that of BNF and nodule number.

Effect of soil textural class on the percent of total N in the plant

Total N as a percent in cowpea (N %) as affected by the six local isolates grown in three soils textural is given in Figure (4). In general, under all isolates, the high percent of total N is in silt clay loam (the heavy textural soil). The percent of total N in plant as affected by soil texture is in the following order: heavy textured > medium textured soil >light textured soils.

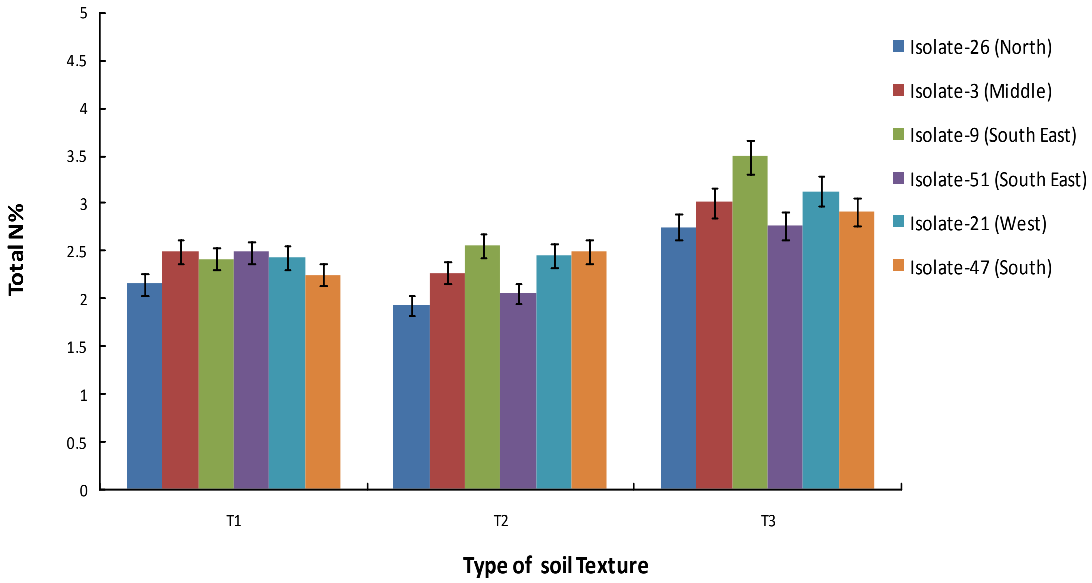

Fig. (4): Effect of soil textural classes on percent of total N in plant underlocal *Bradyrhizobium*bacteria.
Where: T1 = Sandy Loam Soil, T2 = Sandy Clay Loam Soil, T3 = Clay Loam Soil

These results, more or less, are similar to that of plant dry weight (Figure 3) which indicates that plant growing in heavy textured soil is more healthy plant. That may be attributed to the fact that heavy texture soil is of higher cation exchange capacity. Subsequently, it contains more plant available essential elements than other two soils as it stated by [24].

Percent of total N in plant in light textured soil is in the following order under the six isolates: Isolate 3 > Isolate 51 > Isolate 21 > Isolate 47 > isolate 26. Statistical analysis, however, showed that there are no significant differences among isolates. Percent of total nitrogen in cowpea grown in medium textured soil was in the following order: Isolate 9 > Isolate 47 > Isolate 21 > Isolate 3 > isolate 21 > isolate 51.

These results may indicate that isolates are not necessarily of the same activity under different soil textural classes. In heavy textured soil, for example, percent of total N is higher than the other two soils. Isolate efficiency in term of fixed N are much higher in the heavy textured soils than other two soils. These results can be supported by the findings of [25].

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تأثير أصناف نسجة الترب على التثبيت الحيوي للنايتروجين المقاس بنظير النايتروجين- 15 لبكتريا *Bradyrhizobium*

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الخلاصة:

اجريت الدراسة الحالية في تجربة اصص تحت ظروف البيت الزجاجي لتقدير تاثير النسجة على النايتروجين المثبت احيائيا بواسطة العزلات المحلية لبكتريا *Bradyrhizobium*. وقد استخدم نبات اللوبياء (*Vignaunguiculata* L.)وهو محصول بقولي كعائل مضيف للبكتريا واستخدمت طريقة تخفيف نظير النايتروجين- 15 للتقدير الدقيق للنايتروجين المثبت احيائيا . والترب المستخدمة هي تربة طينية مزيجية (ثقيلة) وتربة رملية طينية مزيجية (متوسطة) وترب رملية مزيجية (خفيفة) .وقد كان النتروجين المثبت احيائيا في الترب ذات النسجات المختلفة بالترتيب التالي: التربة المتوسطة النسجة > التربة الثقيلة النسجة > التربة الخفيفة النسجة. وبين التحليل الأحصائي ان هناك فروقات مهمة احصائيا بين النسب المئوية للنايتروجين المثبت احيائيا تحت العزلات المحلية الست وفي الترب ذات النسجات المختلفة كما ان هناك تباين مهم احصائيا في عدد العقد الجذرية للعزلات الست في النسجة الواحدة. وأن عدد العقد لايتوافق مع نسبة النايتروجين المثبت احيائيا في التربة الواحدة ولأي من العزلات.

كما ان نتائج التحليل الأحصائي اظهرت اختلافات مهمة احصائيا في الوزن الجاف للنبات باختلاف نسجات الترب وكمعدل عام لكل العزلات المحلية المستخدمة في هذه الدراسة . واظهرت النتائج ان الوزن الجاف للنبات في التربة الواحدة يختلف باختلاف العزلات المستخدمة .

الكلمات المفتاحية : العزلات المحلية العراقية ، نبات اللوبياء ، العقد الجذرية ، الوزن الجاف للنبات