Influence of *Rhizobia* Inoculation and Rock-Phosphate on Biomass and Nitrogen Content of *Leucaena leucocephala* (Lam.) De Wit Seedlings

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ABSTRACT

The study was carried out in the nursery of the Experimental Station of Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University during the period from 1st May, 2017 to 31st March, 2018. It aimed at evaluating the influence of two races of *Rhizobium* (TAL82 and TAL582) on the overall biomass and the total N content in the leaves of *Leucaena leucocephala* seedlings. In addition, the study aimed at finding out, if any, the impact of rock-phosphate (RF) amendment on the nodulation and growth efficiency of *L. leucocephala* seedlings. Seedlings were inoculated with pure cultures of both strains, then fertilized with rock-phosphate on 7th October, 2017 using 4 levels; 0.0, 0.5, 1.0 and 2.0g / kg soil. Both strains (TAL82 and TAL582) developed typical nodules. However, solubility of RF potential was affected by the strain tested as expressed in terms of growth parameters. The strain TAL582 showed, better and faster growth promoting effect than TAL82 in *L. Leucocephala* seedlings. The fertilization with P induced promoting nodule formation earlier than unamended control. Results showed that fertilization with 1g RF/ kg soil is more effective than the higher tested level (2g RF/ Kg soil). Therefore, it is recommended to inoculate seedlings of *L. Leucocephala* with *Rhizobium* particularly TAL582 strain and to add P as a fertilizer at the rate of 1gRF/Kg soil to achieve the best plant growth in significant short time.

Key words: *Rhizobium*, rock phosphate, *Leucaena leucocephala*, nitrogen fixation, nodulation, PGPR.

INTRODUCTION

Leucaena leucocephala(Lam.) de Wit, often referred to as the "wonder tree", is a widely used woody legume species in the tropical and sub-tropical forests (Orwa *et al.,* 2009) and is an efficient means for sustaining the productivity of agroforestry systems (Shelton, 1998). It is well known that multipurpose legume tree species like Leucaena are important for ecosystem restoration and agroforestry.

Leucaena is a genus of the subfamily Caesalpinioideae, which belongs to the legume family Fabaceae. It contains about 24 species of trees and shrubs, which are commonly known as lead trees (Mabberley 1997). They are native to the Americas, ranging from Texas in the United States south to Peru. The generic name is derived from the Greek word (leukos), meaning "white," referring to the flower's color (Glen and Hugh 2004).

Lead tree is valued as an excellent protein source for cattle fodder, consumed browsed or harvested, mature or immature, green or dry. The nutritive value is equal or superior to alfalfa. Lead tree has gained a favorable reputation in land reclamation, erosion control, water conservation, reforestation and soil improvement programs, and is a good cover and green manure crop.

Leguminous plants play an important role in ecosystem productivity and diversity due to their association with nitrogen (N)-fixing diazotropic soil bacteria called Rhizobium (Thrall *et al*., 2011). This symbiosis can increase plant biomass (Spehn *et al*., 2002) and mitigate land degradation through the use of fast-growing N-fixing trees or shrub "pioneers" in restoration plantations (Miles *et al*., 2006).

Bacteria of the genus *Rhizobium* play a very important role in agriculture by inducing nitrogen-fixing nodules on the roots of many legumes. This symbiosis can relieve the requirements for added nitrogenous fertilizer during their growth.

Many factors, such as cultural practices, weather and soil conditions, may affect the survivability of the carry-over Rhizobium in the soil. Therefore, inoculating the plant is considered as a good practice that the plant will be able to grow to its maximum potential and compete (Deaker *et al.* , 2004; Elkhatib, 2009).

N fixation occurs mostly in root nodules that provide an environment where symbiotic bacteria alter atmospheric nitrogen into ammonia. Plants provide organic-acids as an energy source and in exchange are supplied with fixed N (Miller *et al*., 2007). Variations in plant-rhizobia interactions can affect the successful establishment of legume trees during early developmental stages (i.e., seedlings) (Tharall *et al*., 2011) and can influence the benefit gained from the symbiosis. Such benefits are usually evaluated based on growth parameters and nutrients content, which measured in the plantnotably, total plant biomass, N content in plant organ, nodules (e.g., number of nodules,

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nodule biomass and nodule root ratio) or nodule physiological parameters (e.g., apparent nitrogenase activity in relation to respiration).

Phosphorus (P) enhances the symbiotic nitrogen (N) fixation process in legume crops. Generally, legumes require more P than grasses for root development and energy driven processes. Phosphorus is an essential ingredient for Rhizobium bacteria to convert atmospheric N (N2) into an ammonium (NH4) form useable for plants. Rhizobium are able to synthesize the enzyme nitrogenase, which catalyzes the conversion of N2 to two molecules of ammonia (NH3).The pink color, typical of healthy and effective nodules, is due to the presence of a protein called leghemoglobin. This special protein contains both iron (Fe) and molybedenum (Mo) and is responsible for binding oxygen. This creates a low oxygen environment within the nodule, which allows Rhizobium bacteria to survive and to fix N2. Phosphorus becomes involved as an energy source.

In this study, our aimed were to evaluate the influence of Rhizobium`s races on the overall biomass and the total N content in plant leaves and to find out, if any the impact of rock-phosphate amendment on the nodulation and plant growth efficiency of *L. Leucocephala* seedlings.

MATERIALS AND METHODS

The study was carried out at the nursery of the Experimental Station of Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University during the period from 1stMay, 2017 to 31stMarch, 2018.

Experimental Material

1. Tree species

Fresh certified seeds of *Leucaena leucocephala* trees, collected from Antoniadis Botanical Garden, Alexandria.

2. Soil

Sandy clayey soil was used, obtained from nursery of Experimental Station of the Department, Abies, Alexandria.

3. Fertilizer type

Rock-phosphate (RP), contained 14% P was used in the experiment.

4. *Rhizobium*

Pure cultures of *Rhizobium* of races designated TAL82 and TAL582 were separately blended and added to sterilized peat then kept in refrigerator at 5°C for 5 days till using.

2. Experimental procedure (Methodology)

Seeds of *L. leucocephala* were soaked in hot (90 °C, then kept in cold water for 24 hours as pretreatment to overcome its seed coat dormancy and to accelerate their germination.

The treated seeds were sown on 1stJuly, 2017 in plastic bags of 30cm in height and 20 cm in diameter contained 3.7 kg of sterilized soil. After seed germination, the seedlings were irrigated daily during the first three months, then every 2 days to replenish the water consumption deficits. The average of water amount used in each irrigation was about 250 ml of tap water.

Pure cultures of each of *Rhizobium* of two races designated TAL82 and TAL582 were blended and added to sterilized peat then kept in refrigerator at 5°C for 5 days. Seedlings were inoculated with about 3 g of a sterilized peat containing each one of the two races mentioned above on1stJuli, 2017. Rock-phosphate was applied on 7thOctober, 2017 at four levels; 0.0, 0.5, 1.0 and 2.0g / kg soil.

Control seedlings were also inoculated, but with sterilized (Previously autoclaved) inocula using the same method for the other treatment. Seedling heights were measured just after inoculation to determine growth rate of 2 week intervals until the end of the experiment span, on 31stMarch, 2018.

5. Soil analysis

The main chemical and physical properties of the soil were determined as follows: The electrical conductivity (EC) of the saturated soil paste extract was measured using a conductimeter (GLP 31). Furthermore, pH of the soil was measured for the saturated soil-water suspension using a pH-meter (GLP 21) (Rhoades, 1982).

Water soluble ions were determined in the saturated soil paste extract; calcium and magnesium were determined by versenate method, sodium and potassium by a flame photometer, bicarbonate and carbonate by titration with dilute hydrochloric acid and chloride by silver nitrate method **(Richard,1954)**.

6. Nodulation assessment

Root nodule formation was checked in harvested seedlings. The number of nodules per plant, nodule dry weights and nodule/ root ratio were determined.

7. Growth parameters

Shoot height (cm), shoot growth rate (cm/ month) and dry weight of the seedlings (g) were determined during and at the end of the experiment.

At the end of the experiment, seedlings were extracted carefully from the bags, then roots were washed gently with tap water. Each seedling was divided into root and shoot (leaves and stem) either with nodules or without and their fresh weight were determined then oven-dried at 70°C for 48 hours to reach a constant weight to determine their dry weight.

8. The experimental design

A Complete randomized design (CRD) was used in this experiment. The split plot technique was used in analyzing the data obtained, where the main plot was for fertilization with phosphorus, while the sub plot was for the impact of inoculation with *Rhizobium*. The experimental data was statistically analyzed as it described by Snedecor (1956), using SAS ver. 9.1.3 (2007).

RESULTS

1. Nodulation

1.1. Nodule number (NN)

Examining the inoculated roots, seedlings treated with TAL582 (R2) had displayed the NN higher significantly that the strain TAL82 did (R1), particularly under the higher levels of rock phosphate. Nevertheless, the inoculated seedlings with R2 and fertilized with 1.0g RP/ kg soil brought about the highest NN, since it was 14.0 nodules/ plant, followed by the unfertilized seedlings inoculated with R1(13.25 nodules/ plant) (Table 1).

1.2. Nodule dry weight (NDW) (g)

It was found that the fertilized seedlings with 1.0g RP/ kg (P2) soil and inoculated with R2 of *Rhizobium* had displayed the highest value of NDW, since it was 0.21g**,** followed by thosefertilized seedlings with 0.5g RP/ kg soil and inoculated with R2(0.21g) (Table, 2).

1.3. Nodule root ratio (NRR)

The unfertilized -inoculated seedlings with Race 1 had brought about the highest value of NRR, since it was 0.08, (Table 3).

2. Nodules ultrastructure

The examination of nodules using scanning electron microscope (SEM) has manifested the presence of bacteroid (bacteria enclosed with membrane) in mature infected cell (Fig. 1). In addition, starch granules were found almost colonizing the pranchymatous cells (Fig. 2) of the nodule of the two tested races of *Rhizobium*.

The examination of late senescent infected cell indicated the presence of multiple crystals occupying nodule cell (Fig. 3).

Table 1. Nodule number (NN) (nodule/ plant) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Mean
R1 (TAL82)	13.25	10.5	10.5	8.75	10.75
R2 (TAL582)	10	10.75	14	12.25	11.75
Mean	11.63	10.63	12.25	10.50	

LSD P=------ LSD R=---- LSD PR= 0.5254

P0: 0.00 RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 2. Nodule dry weight (g) in the roots of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Mean
R1 (TAL82)	0.12	0.15	0.1	0.02	0.10
R2 (TAL582)	0.15	0.17	0.21	0.05	0.15
Mean	0.14	0.16	0.16	0.04	

LSD P=------ LSD R=0.014 LSD PR= 0.1201

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 3. Nodule root ratio of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Mean
R1 (TAL82)	0.08	0.05	0.05	0.01	0.0475
R2 (TAL582)	0.04	0.05	0.04	0.01	0.035
Mean	0.06	0.05	0.045	0.01	

LSD P=------ LSD R=0.254 LSD PR=0.0042

P0: 0.00 RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

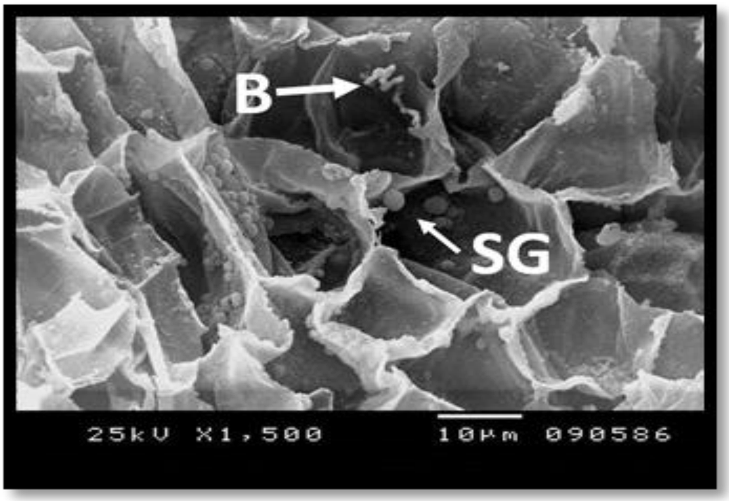

Fig. 1. Mature- infected parenchymateous cells with bacteroid (B), starch granule (SG) in the nodule cell.

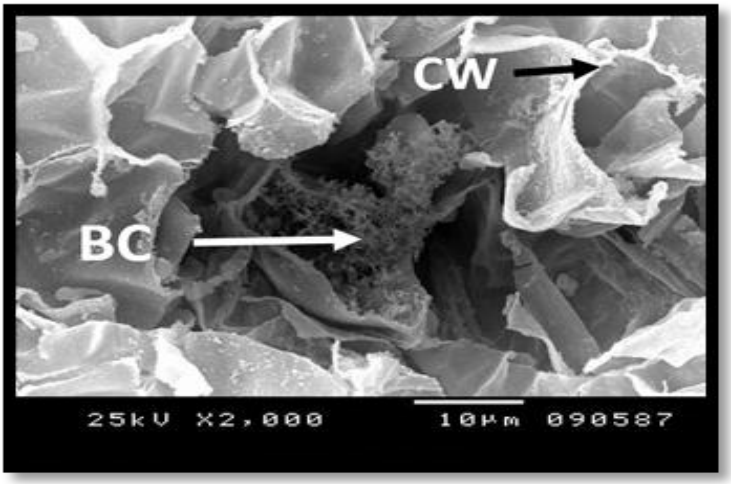

Fig. 2. SEM of an early senescent infected cell of nodule indicated bacterial cell (BC). CW is a cell wall of nodule cell.

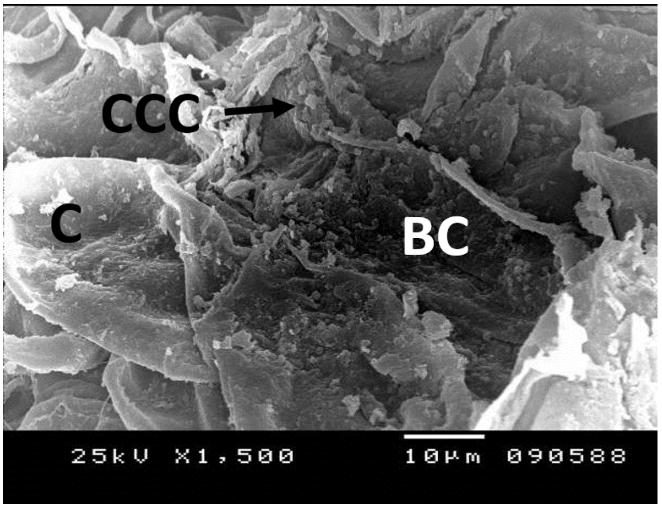

Fig. 3. SEM of late senescent infected cell indicated crystal (C) occupying the cell besides bacterial cells (BC) and chambered cells with crystals (CCC).

3. Growth parameters

3.1. Shoot height (SH)

The analysis of the significant interaction between fertilization with RP and inoculation with *Rhizobium* has revealed that the inoculated seedlings with R 2 and fertilized with1.0g RP\ kg soil displayed the highest SH (98.00cm), yet the uninoculated seedlings (control) which fertilized with the same level of P have exhibited the lowest SH (49.50cm)

3.2. Shoot growth rate (SGR) (cm/ month)

The statistical analysis of variance has revealed that the positive impact of inoculation with the two races of *Rhizobium* was of SGR seedlings.

Regarding the significant interaction between *Rhizobium* and RP fertilizers, it was found that the highest SGR was obtained in the case of inoculated seedlings with R2 and fertilized with 1.0g RP/ kg soil, since it was 7.13 cm/ month, which accounted for more than 2 folds of the uninoculated- fertilized seedlings with 2.0g RP\ kg soil, since it was2.42 cm/ month (Table, 5).

3.4. Root dry weight (RDW) (g).

Considering, the significant interaction between the inoculation with *Rhizobium* and fertilization with phosphorus, the inoculated seedlings with TAL 582 which fertilized with 2g RP exhibited the highest RDW, since it was 5.54g, while the lowest RDW was obtained in the uninoculated –unfertilized seedlings (control) (1.41g) (Table 6).

3.5. Leaf dry weight (LDW) (g)

The fertilized seedlings with 2.0g RP/ Kg soil have brought about the highest LDW, since it was 3.80g. The inoculated seedlings with TAL82and fertilized with 2.0g RP/kg soil had brought about the highest LDW (5.42g)as compared to that uninoculated – fertilized seedlings with 1.0g/kg RP which was(1.71g)(Table, 7).

3.6. Shoot dry weight (SDW) (g)

It was found that the highest SDW was obtained in inoculated seedlings with R2 which fertilized with 1.0g RP/ kg soil, since it was 14.78g, whereas the unfertilized seedlings and inoculated with R1 displayed the lowest SDW since the average was (6.06g) (Table, 8).

Table 4. Shoot height (cm) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phos phate

	P0	P1	P2	P3	Mean
C	55.75	65.00	49.50	44.25	53.63b
R1 (TAL82)	68.50	74.50	59.75	80.00	70.69b
R2 (TAL582)	74.00	91.00	98.00	80.75	85.94a
Mean	66.08	76.83	69.08	68.33	

LSD P=------ LSD R= 4.429 LSD PR= 13.26

P0: 0.00 RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 5. Shoot growth rate (cm/ month) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Avr
C	3.50	3.79	3.04	2.42	3.19b
R1 (TAL82)	5.00	2.79	3.96	6.08	4.46a
R2 (TAL582)	5.33	5.83	7.13	5.96	6.06a
Avr	4.61	4.14	4.71	4.82	

LSD P= ---- LSD R= 1.155 LSD PR=0.211

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 6. Root dry weight (RDW) (g) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Mean
C	1.41	1.54	1.94	0.89	1.45c
R1 (TAL82)	1.45	3.05	2.08	3.22	2.45b
R2 (TAL582)	3.48	3.74	4.77	5.54	4.38a
Mean	2.11b	2.78b	2.93b	3.22a	

LSD P= 0.748 LSD R= 0.859 LSD PR= 1.405

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 7. Leaf dry weight (LDW) (g) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	
C	3.08	3.54	1.71	2.33	2.67b
R1 (TAL82)	2.75	3.38	3.25	5.42	3.70a
R2 (TAL582)	3.07	3.40	3.03	3.65	3.29a
	2.96	3.44	2.66	3.80	

LSD P= ----- LSD R=1.254 LSD PR= 1.248

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

3.7. Total dry weight (TDW) (g)

It is worthy to say that the inoculated seedlings with R2 and fertilized with 1.0g RP/ kg soil displayed the highest TDW (19.55g) as it compared to that of uninoculated and fertilized with 1.0g RP (5.42g) (Table 9).

4. Mineral contents

4.1. Nitrogen content % (N%)

The inoculated seedlings with R2 exhibited the highest N% in the leaves, since it was 3.96%. (Fig. 4).

Upon the significant interaction between the inoculation with *Rhizobium* and fertilization with RP, the fertilized seedlings with 1.0g/ kg soil which inoculated with R2 had displayed the highest N% (4.98%)compared to that of inuninoculated and fertilized ones with 1.0gRF/ kg soil(1.55%)(Table 11).

DISCUSSION

Upon the findings of this experiment, it can be said that the nitrogen fixation efficiency is different between the two tested strains. This might be due to the fact that the growth promoting effect of TAL582 was higher than that of TAL82, and it might also be due to the difference in phosphate solubilizing capabilities between the two strains. Phosphate solubilizing capability was believed to be closely related to the kind of organic acids secreted by different strains.This is in agreement with Zhang *et al.*(2013) who found that phosphate solubilizing capability of various bacteria was significantly different.

Table 8. Shoot dry weight (SDW) (g) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	
C	6.12	5.83	3.48	4.14	4.89c
R1 (TAL82)	6.06	9.41	7.42	9.71	8.15b
R2 (TAL582)	9.70	9.42	14.78	13.62	11.88a
	7.29b	8.22b	8.56b	9.16a	

LSD P= 0.525 LSD R= 1.386 LSD PR= 3.15

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 9. Total dry weight (TDW) (g) of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	
C	7.53	7.36	5.42	5.03	6.33c
R1 (TAL82)	7.51	12.46	9.50	12.93	10.60b
R2 (TAL582)	13.18	13.16	19.55	18.16	16.01a
	9.41d	10.99c	11.49b	12.04a	

LSD P= 0.423 LSD R= 1.7225 LSD PR= 4.101

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

Table 10. Nitrogen content % (N%) in the leaves of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

	P0	P1	P2	P3	Mean
C	1.62	1.55	2.11	2.43	1.93
R1 (TAL82)	3.63	3.25	4.47	3.88	3.81
R2 (TAL582)	3.41	3.76	4.98	3.70	3.96
Mean	2.89	2.85	3.85	3.33	

LSD P= 0.0034 LSD R=0.0074 LSD PR=0.0147

P0: 0.00g RF P1: 0.5gRF P2: 1.0g RF P2: 2.0g RF

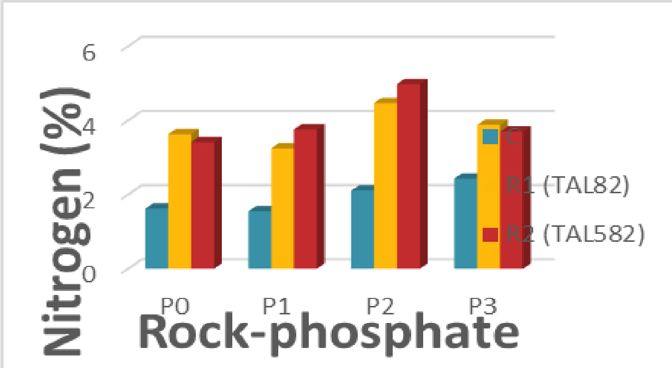

Fig. 4. Nitrogen content % (N%) in the leaves of the inoculated and uninoculated seedlings of *Leucaena leucocephala with Rhizobium* which fertilized and unfertilized with rock-phosphate

Phosphorus has a key role in nodule development through its basic functions in plants as it is the inorganic moiety of adenosine triphosphate; an energy rich source which affects important cell functions; translocation of sugars, photosynthesis, root growth and other functions which directly or indirectly influence significantly N fixation by legume plants. Thus, low levels of phosphorus can affect symbiosis by decreasing the supply of photosynthates necessary to the nodule formation which reduces the rate of bacterial growth and the total population of legume-nodulating microorganisms (Moreira *et al.* 2010). The impact of P on nodule development and the N fixation process by legumes was repeatedly reported(Saber *et al.* 2005). They found that when P was applied to alfalfa, nodules developed earlier. In high P soils, nodules were first noted on alfalfa roots 11 days after seeding. Meanwhile, nodules developed about three days later in low P soils. Nodule number, volume, and dry weight could be increased by treating soils with different levels of P. They mentioned also that nodules became pink earlier, developed more quickly, and became active sooner in response to higher P fertilization. Moreover, Abd-alla 1994 had manifested that even under nonsterile conditions *R. meliloti* strain TAL 1372 was very effective in solubilizing rock phosphate.

Phosphorus increases the yield and nitrogen content in legumes as other studies revealed that P applied to soils with poor level of P could increase the percent of N in legumes, which resulted in greater dry matter. This is believed to be one of the reasons why legumes are dependent on symbiotic N, have a higher P requirement than grasses which depended on amendment N. This is manifested in view of the fact that,16 molecules of adenosine triphosphate (ATP) are converted to adenosine diphosphate (ADP) for each molecule of N2 reduced to NH3 (Better 1999).

Concurrent use of fertilization with RF and inoculation with *Rhizobium* in the nursery may provide typical conditions to produce larger seedlings of high potential to reduce the time needed, thereby help achieve restoration objectives. It is worth notice that the total number of nodules formed depends upon the size and degree of ramification of the root system at the time of inoculation.

The legume-nodulating strains that increased levels of soluble phosphate can improve the efficiency of biological nitrogen fixation, given that nodulated plants require more phosphorus than the plants that use only mineral nitrogen (Silva *et al.* 2006). This also suggests that the efficiency of nitrogen fixation by the strains approved as inoculants may be related to a greater ability to solubilize low soluble phosphates; however, no studies have experimentally demonstrated this point so far.

Furthermore, several investigations noted that a few nodules were formed in unfertilized seedlings with phosphorus and added that it may be due to the endogenetic differences in *Rhizobia* (Li *et al.*, 2009; Zhang *et al.*, 2009).

CONCLUSIONS

Both strains (TAL82 and TAL582) were biotically nitrogen fixing and solubilizing phosphate which resulted in good growth promoting effects. However, strain TAL582 showed better growth promoting effect than TAL82 in *Leucaena* seedlings. Besides, fertilization with P promoted nodule formation earlier than control.

It could also be concluded, however, that the capability of rhizobial strain to solubilize phosphorus in plant-growth media is the key criterion to evaluate the efficiency of nodulation and growth of the partner host plant. Therefore, inoculation with strains of *Rhizobium* and fertilization with 1g RF/Kg soil are recommended to achieve more plant growth in a relatively short time.

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الملخص العربي

تأثير التلقيح بالرايزوبيا وصخر الفوسفات على الكتلة الحيوية والمحتوى النتروجيني لشتلات الليوسينا ليوكوسيفالا

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تمت هذه الدراسة فى مزرعة قسم الغابات وتكنولوجيا الأخشاب فى محطة بحوث التجارب التابعة لكلية الزراعة – جامعة الإسكندرية خلال الفترة من اول يوليو ٢٠١٧ حتي نهاية مارس ٢٠١٨ وذلك لتقييم تأثير سلالتين من الريزوبيوم وهما(R1) TAL 82 و(R2) TAL582 على كل مكونات الكتلة الحيوية والمحتوي النتروجيني الكلي فى أوراق شتلات الليوسينا ليوكوسيفالا. فضلاً عن دراسة ما إذا كان هناك تأثير لإضافة صخر الفوسفات على تكوين العقد البكتيرية المثبتة للنتروجين وكفاءة النمو لشتلات الليوسينا.

تم تلقيح الشتلات بكلتا السلالتين من البكتريا وبعد ثلاثة اشهر من التلقيح فى السابع من اكتوبر تم التسميد بالصخر الفوسفاتي بإستخدام اربعة مستويات منه وهى ٠,٠ و ٠,٥ و١,٠ و ٢,٠ جم لكل كجم تربة وقد أثبتت النتائج أن التلقيح أدى إلى تكوين عقد بكتيرية مثالية. وأوضحت النتائج كذلك أن السلاله R2 اكثر كفاءه من السلاله R1 فى القدرة على اذابة الفوسفات وتكوين العقد وتثبيت النتروجين وعلى مقاييس وسرعة النمو لشتلات الليوسينا، وقد تأكد أيضاً ان التسميد بالفوسفور أدي إلي الاسراع فى تكوين العقد البكتيرية مقارنة بالشتلات غير المسمدة. أظهرت النتائج أن تسميد الشتلات بمعدل ١جم صخر فوسفاتى لكل كجم تربة أكثر تأثيراً من المستوى الأعلى الذى أختبر من صخر الفوسفات (٢جم). لذا يوصي بتلقيح شتلات الليوسينا بالريزوبيوم خاصة السلالة R2 والتسميد بصخر الفوسفات بمعدل ١ جم لكل كجم تربة لهدف الحصول على أفضل صفات لنمو الشتلات.