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ENHANCING GROWTH OF *LEUCAENA LEUCOCEPHALA* SEEDLINGS BY STIMULATION OF SYMBIOTIC RELATIONSHIP BETWEEN VESICULAR ARBUSCULAR MYCORRHIZAL (VAM) AND NITROGEN FIXING BACTERIA UNDER PHOSPHATE ROCK FERTILIZATION

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ABSTRACT

The woody legume known as Leucaena (*Leucaena leucocephala*) is found all over the globe. Its rapid growth and capacity to fix nitrogen (N_2) with rhizobia are the main contributors to improve its ecological, physiological, and commercial significance. Leucaena is an appropriate species to serve as a model for other woody legumes because of these traits. Therefore, its symbiotic relationship with mycorrhizal fungus has attracted a lot of interest. Plant stress responses can be mediated by vesicular arbuscular mycorrhizal fungal (VAM) symbionts via increasing stress tolerance. The study was carried out in a greenhouse at the private nursery in Itay El-Baroud city and the Laboratories of the Horticulture Department, Faculty of Agriculture, Damanhour University, in collaboration with the Laboratories of the Soil and Water Development Center, Damanhour, El-Beheira Governorate, Egypt, over two consecutive seasons, 2020 and 2021. This study was conducted to determine the effect of dual inoculation with endomycorrhizal fungi (VAM) and *Rhizobium* (R) bacteria on the growth and minerals of the leaves in seedlings of *Leucaena* in the presence and absence of rock phosphate, management of the stimulatory or antagonistic relationship of symbiotic factors (VAM and R). The experimental design was a split -plot system with five replications on randomized complete block design (RCBD).

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Rock phosphate a rate of 1.5 g/1 kg soil fertilization and inoculation treatments with symbiotic-type (ST) (VAM, R, VAM+R and R+VAM) were distributed at random within the main (2 treatments) and sub-plots (5 treatments), respectively. The results showed that, when fertilized seedlings with rock phosphate (RP) as a natural source of phosphurs for plants were compared to unfertilized ones, shoot height, shoot growth rate, chlorophyll content, stem diameter, number of leaves, leaf area, total dry matter, nitrogen, phosphorus, and potassium content increased. In terms of the effect of the symbiotic agents, inoculated seedlings outperformed uninoculated ones in all growth parameters, particularly those dual inoculated with R+VAM. In conclusion, R and VAM inoculation together with rock phosphate had synergistic effects on *Leucaena* growth, especially when it was inoculated with (R)) first then with (VAM), when compared to single inoculation.

Keywords: Mycorrhiza – Rock phosphate - *Leucaena leucocephala* – *Rhizobium.*

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INTRODUCTION

Identification of adapted and efficient legume-rhizobia-fungi tripartite associations in local ecosystems is critical for successful ecosystem restoration, rangeland and forest management, and agricultural sustainability, particularly in unfavorable environmental conditions caused by soil salinity, drought, and heat stress. One of the most important ecological mutualisms is the one between legumes and Rhizobia and arbuscular mycorrhizal fungi (AMF). This three-way symbiotic relationship is extremely important for agriculture and ecology.

Leucaena leucocephala (Lam.) belongs to the family Fabaceae (*leucaena*), due to of its beneficial properties, such as a caloric value of 19.4 kJ/g and a specific weight ranging from 0.50 to 0.59 kg/cm^3, this tree has received a lot of attention in recent decades. These characteristics make it suitable for the production of paper and coal **(Muir *et al.*, 1992)**. Because it is easily machinable, porous to water-soluble preservatives, and non-deformable after drying, it is used to make light structures and containers, as well as various types of fences and furniture such as tables (**Abair *et al.*, 2019 and Dalzell, 2019)**. Also, for regeneration of bare areas, slopes, pastures, and nitrogen fixer in the soil (**Thomas *et al.*, 2001).** Furthermore, *Leucaena* botanical parts have several bioactive compounds, which can act as promising antimicrobial and antioxidant properties **(Elbanoby *et al.,* 2022).**

Nitrogen (N), phosphorus (P), and potassium (K) are the major plant nutrients that play an important role in the balanced use of fertilizer in plant growth. Phosphorus is an essential nutrient for legume growth and the process of atmospheric nitrogen fixation **(Huda *et al.*, 2007)**. It is essential for several physiological and biochemical plant activities such as photosynthesis, sugar to starch conversion, and genetic trait transport **(Soleimanzadeh, 2012)**. One of the major issues with Egyptian soils is that most of them contain a large amount of calcium carbonate ($CaCO_3$), which inhibits phosphorus absorption by plant roots **(Mahdi *et al.*, 2011)**. Due to their regulated release characteristics, phosphate rocks (PRs) are perfect for long-term crops such as plantations and permanent pastures. They may be beneficial even on seasonal crops on extremely acidic soils (**Rajan *et al.*, 1996)**.

Microorganisms have the ability to mineralize and solubilize bound nutrients in soil, making nutrients available in the rhizosphere or inhabitant host, and thus enhancing plant growth **(Hameeda *et al.*, 2008)**. Particularly in drought-prone areas and where the soil has been badly disturbed, like on mine sites, symbiotic root microbes like **mycorrhizae** and *rhizobia* can help trees develop more successfully (**Mengual *et al.*, 2014)**. Known soil microbes called rhizobia infect and colonise the roots of both non-leguminous and leguminous plants. It can enhance nodulation and biological nitrogen (N) fixation (BNF), which in turn can enhance plant survival and performance **(Ahmad *et al.*, 2015 and Zhang *et al.*, 2019)**. ***Rhizobia*** enhances seedlings' capacity to adjust to climatic and environmental change. In arid regions, rhizobia inoculation increased plants' resilience to environmental challenges **(Dekak *et al.*, 2018)**. Also, improved plant nutrition, nodule formation, and N2 fixation rates **(Karthikeyan, 2019).** The most crucial plant helper microorganisms for supplying P nutrient at a good level are biofertilizers such as phosphate solubilizing microorganisms (PSM) and phosphate solubilizing arbuscular mycorrhizal (AM) fungi, known as efficient organisms in this process **(Reyes *et al.*, 1999).** With increased chlorophyll content and improved nitrogen uptake, AM fungus results in a higher rate of photosynthetic activity **(Zhu *et al.*, 2001 and Feng *et al.*, 2002)**. Many physiological processes depend on microorganisms, which are abundant nearby or in the feeder roots of trees. Pathogenicity, symbiosis, and saprophytism are all aspects of this dynamic microbial process. The mycorrhizal relationship between tree feeder roots and root-inhabiting fungus is the most prevalent symbiosis among trees. Non-mycorrhizal trees are the unusual because microbial interaction in natural soil conditions is so widespread **(Marx, 1975).** By causing nitrogen-fixing nodules on the roots of numerous legumes in a symbiotic relationship, bacteria of the genus Rhizobium play a crucial role in agriculture. Throughout its growth, this symbiosis may supply nitrogenous fertilizer. The survival of the carry-over Rhizobium in the soil may be impacted by a variety of elements, including cultural practices, weather patterns, and soil properties **(Deaker *et al.*, 2004)**. Many studies demand three-way symbiosis between legumes, mycorrhiza, and rhizobium.

The goal of this study is to determine the optimal method of dual inoculation with endomycorrhizal fungi (VAM) and Rhizobium (R) bacteria on the growth and mineral content of seedlings *of Leucaena leucocephala* in the presence and absence of rock phosphate, as well as the management of any stimulatory or antagonistic relationships between symbiotic factors (VAM and R).

MATERIALS AND METHODS

This study was carried out in a greenhouse at a private nursery in Itay El-Baroud city and the Laboratories of the Horticulture Department, Faculty of Agriculture, Damanhour University, in collaboration with the Laboratories of the Soil and Water Development Center, Damanhour, El-Beheira Governorate, Egypt, over two consecutive seasons, 2020 and 2021, **Figure 1.**

Fig. 1. soil mixture a. and b., *Leucaena* Seedlings e. and f. and Greenhouse c., where experiment done d., g. and h. at the private nursery in Itay El-Baroud city over two consecutive seasons, 2020 and 2021.

Plant material and growth conditions

Fresh seeds of *Leucaena leucocephala* were collected from certified trees in Antoniadis Botanical Garden, Alexandria Governorate, in 2019. On 1st of February, 2020, the seeds were scarified with sandpaper to break down seed coat dormancy, then soaked in hot water for 48 h to accelerate germination in plastic pots (30 cm in hight and 30 cm in diameter) with 8 kg soil weight capacity using soil mixture of sand, clay and peat with a ratio of (3:1: 0.5 V/V/V) **(Fig.1. a.)**. After germination, **(Fig.1. e.)** and **(Fig.1. f.)** the seedlings received irrigation every day for the first three months after germination, and subsequently every two days after three months. Each irrigation used an average of 300 ml of tap water. On 20 April, 2020, phosphate rock was applied, and on 20 May, 2020, the following treatments were carried out Seedlings used as controls were inoculated, however the inoculate used was sterilized (previously autoclaved).

Following inoculation, measurements of seedling height and stem diameter were taken monthly until the experiment's conclusion on December 30, 2020. The seedlings were measured to the nearest cm for height and mm for diameter. In addition, feeder root samples were obtained after 2 weeks of the inoculation to assess the presence of the symbiotic agents. In the second season, this process has often been repeated (2021). Rock phosphate (0.034 mg available -p.g $^{-1}$ rock P and 71.25 mg total -p.g^{-1} rock-P) was used in the study and obtained from the Agricultural Research Station, El-Beheira Governorate, Egypt.

Glomus fasciculatum, Giga spora, and *Acaulo spora* **fungus** (Each gram contained about 300 survive spores) were used in the study. It was obtained from Land, Water and Environment Unit, Soil, Water and Environment Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation. Rhizobium was used in the study. It was obtained from Biofertilizers Production Unit, Soil, Water and Environment Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation. Soil analysis was conducted at the Laboratories of Soil and Water Development Center, Damanhour, El-Beheira Governorate, Egypt, Table 1.

Experimental design and treatments

The experimental layout was a randomized complete block design (RCBD), with five replicates in a split-plot system arrangement where with rock phosphate or without was assigned allocated in main plots and the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus treatments were assigned as the sub-plots; whereas, the Rhizobium were devoted in sub- sub-plots. Each replicate included ten treatments. All treatment was distributed in the experimental units randomly. All other recommended agricultural practices for commercial *Leucaena* seedlings production were followed. Harvesting was accomplished after 11 months of planting during both seasons.

Table 1. Physical and chemical properties of the soil used during the two seasons.

Soil texture: Sandy clay										
Season	**Sand (%)**	**Clay (%)**		**Peat (%)**	**PH**	**EC (%)**	**$CaCO_3$ %**			
2020	66.7	22.2		11.1	7.6	0.14	1.18			
2021	66.4	22.4		11.2	7.8	0.12	1.23			
Soluble cations and anions (meq/L)					**Available elements (ppm)**					
Season	**HCO_3^-**	**Ca^{++}**	**Mg^{++}**	**K^+**	**Na^+**	**Cl^-**	**SO_4^-**	**N**	**P**	**K**
2020	1.17	0.76	0.47	0.12	0.75	0.78	0.13	13	49	247
2021	1.33	0.69	0.43	0.09	0.77	0.72	0.11	11	53	236

Statistical analysis

All recorded data were statistically analyzed using CoStat computer software (COSTAT, 2005) version 6.4 of cohort software. The revised least significant difference test (RLSD) was applied at 0.05 confidence level to compare means of the different treatments by using the same program.

The chart of treatments of this study can be listed as follows:

No	Treatment
1	Fertilized seedlings of *L. leucocephala* uninoculated.
2	Fertilized seedlings of *L. leucocephala* inoculated with VAM.
3	Fertilized seedlings of *L. leucocephala* inoculated with R.
4	Fertilized seedlings of *L. leucocephala* inoculated with VAM then with R.
5	Fertilized seedlings of *L. leucocephala* inoculated with R then with VAM.
6	Unfertilized seedlings of *L. leucocephala* uninoculated.
7	Unfertilized seedlings of *L. leucocephala* inoculated with VAM.
8	Unfertilized seedlings of *L. leucocephala* inoculated with R.
9	Unfertilized seedlings of *L. leucocephala* inoculated with VAM then with R.
10	Unfertilized seedlings of *L. leucocephala* inoculated with R then with VAM.

Growth parameters and characteristics

Growth parameters including plant height (cm), stem diameter (mm), plant leaf area (cm^2), number of leaves / plants. Each seedling was divided into root and shoot (leaves and stem) in addition to their dry weight was determined, as well as after oven drying at 70°C for 48 h to a constant weight.

Determination of the VAM – infection: Clearing and staining specimen: The method described by **Phillips and Hayman (1970)** was used for preparing root samples for microscopic examination. The degree of root colonization with any structure of VAM viz., hyphae, vesicules or arbuscules was estimated using the slide method (**Giovannetti and Mosse, 1980)** and the values were expressed as percentage of infection or colonization with fungi.

Nodulation assessment: Root nodule formation was checked in harvested seedlings. The number of nodules per plant and their fresh and dry weights (g) were determined.

Determination of total chlorophyll content of the leaves: The total chlorophyll content of the leaves (SPAD index) was nondestructively determined using (Spad-502 Chlorophyll Meter, Japan). The average of three values was taken on different places chosen randomly in each tree for all the treatment was recorded.

Determination of minerals nitrogen content (N %), phosphorus content (P %) and potassium content (K %) of *Leucaena leucocephala* seedlings: Samples of the leaves, stems and roots of the seedlings were dried in oven at 70 °C until constant weight, ground in Wiley mill to a fine powder and stored in plastic bags for analysis according to the method described by **(Cottenie, 1980).**

RESULTS

The following chart illustrates how the inoculation of *leucocephala* seedlings with the vesicular-arbuscular mycorrhizal (VAM) fungus and Rhizobium(R), either individually or dually, under rock phosphate fertilization (RP), altered the growth parameters and mineral contents of the seedlings **Figure 2**.

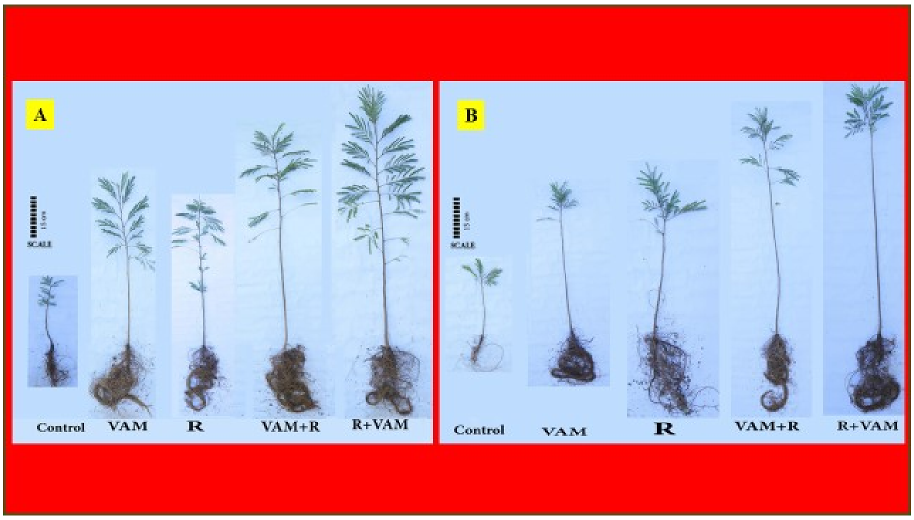

Fig (2): Fertilized seedlings of *Leucaena leucocephala* with rock phosphate (A) and unfertilized ones (B), as it influenced by the inoculation with vesicular arbuscular mycorrhizal (VAM) fungus and *Rhizobium* (R) and its combination, (VAM+R) and (R+VAM).

Growth parameters

Shoot height (SH) (cm)

For the two seasons, the effect of RP and symbiotictype (ST) and their interactions (RP × ST) on shoot height (SH) was significant (Table 2). The RP-fertilized seedlings exhibited significantly higher SH (133.54 and 131.87 cm) than the case of non-RP seedlings (128.33 and 125.18 cm) for the first and the second seasons, respectively.

As for the effect of ST treatments, for the two seasons, all ST treatments significantly increased SH over the control. Among all symbiotic-types, the inoculation with R followed by VAM (R+VAM) treatment has displayed the highest SH (170.43 and 153.68 cm, for the first and second seasons, respectively). On the other hand, the lowest SH was recorded for the control (64.06 and 62.77 cm, for the first and second season, respectively) (Table 2).

Table (2): Means of shoot height (SH), shoot growth rate (SGR) and stem diameter (SD) of *Leucaena leucocephala* seedlings as it influenced by the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus, Rhizobium and their combinations with or without rock phosphate fertilization in the two seasons (2020 and 2021).

Symbiosis type (ST)	Rock phosphate (RP) (1.5 g Kg $^{-1}$ soil)					
	RP	Non-RP	Mean	RP	Non-RP	Mean
	First season			Second season		
	Shoot height (cm)					
C	63.44 g	64.68 g	**64.06 d**	68.5 i	57.04 j	**62.77 d**
VAM	134.38 d	122.88 f	**128.63 c**	141.9 e	132.1 h	**137.00 c**
R	127.84 e	127.56 e	**127.7 c**	139.04 f	136.18 g	**137.61 c**
VAM + R	167.34 b	160.36 c	**163.85 b**	153.56 b	149.56 d	**151.56 b**
R + VAM	174.7 a	166.16 b	**170.43 a**	156.34 a	151.02 c	**153.68 a**
Mean	**133.54 [a]**	**128.328 [b]**	-------	**131.87 [a]**	**125.18 [b]**	-------
LSD (0.05)	**RP= 0.967 ST= 1.056 RP*ST= 1.493**			**RP= 0.485 ST= 0.748 RP*ST= 1.057**		
	Shoot growth rate (cm/month)					
C	3.726 g	3.878 g	**3.802 d**	5.408 i	3.976 j	**4.692 d**
VAM	12.508 d	11.072 f	**11.79 c**	14.79 g	13.536 h	**14.163 c**
R	11.648 e	11.746 e	**11.697 c**	14.374 e	14.138 f	**14.256 c**
VAM + R	16.756 b	15.756 c	**16.256 b**	16.336 b	15.476 d	**15.906 b**
R + VAM	17.572 a	16.634 b	**17.103 a**	16.582 a	16.038 c	**16.310 a**
Mean	**12.442 a**	**11.817 b**	-------	**13.498 a**	**12.633 b**	-------
LSD (0.05)	**RP= 0.084 ST= 0.131 RP*ST= 0.186**			**RP= 0.096 ST= 0.126 RP*ST= 0.178**		
	Stem diameter (mm)					
C	2.264 d	2.22 d	**2.242 c**	2.362 g	2.138 h	**2.250 e**
VAM	4.734 bc	4.48 c	**4.607 b**	4.234 de	3.616 f	**3.925 d**
R	4.604 c	4.734 bc	**4.669 b**	4.508 c	4.126 e	**4.317 c**
VAM + R	5.31 a	4.88 abc	**5.095 a**	4.968 b	4.244 de	**4.606 b**
R + VAM	5.156 ab	5.024 abc	**5.09 a**	5.240 a	4.450 cd	**4.845 a**
Mean	**4.4136 a**	**4.2676 a**	-------	**4.262 a**	**3.715 b**	-------
LSD (0.05)	**RP= 0.155 ST= 0.262 RP*ST= 0.371**			**RP= 0.058 ST= 0.128 RP*ST= 0.181**		

Means with the same superscript letter are not significant at a level of probability of 0.05, for each parameter. Where, ST, Symbiosis type; RP, rook phosphate; Non-RP, non-fertilized with RP; C, control; VAM, inoculated with vesicular arbuscular mycorrhiza; R, inoculated with Rhizobium; VAM+R, inoculated with vesicular arbuscular mycorrhiza then with inoculated with Rhizobium; R+VAM, inoculated with Rhizobium then inoculated with vesicular arbuscular mycorrhiza.

Regarding the interaction (RP × ST), both RP-fertilized soil and non-fertilized soil, for the two seasons, the SH was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and their combinations. Among all treatments, the RP-fertilized and dually inoculated with R+VAM have displayed the highest SH (174.7 and 156.34 cm, for the first and second seasons, respectively). However, the control treatment has displayed the lowest SH (63.44 and 57.04 cm, for the first and second seasons, respectively) (Table 2).

Shoot growth rate (SGR) (cm/month):

Data in Table (2), it can be seen in both seasons that the effect of RP and symbiotic-type (ST) and their interactions (RP × ST) on shoot growth rate (SGR) was significant. The RP-fertilized seedlings exhibited significantly higher SGR (12.44 and13.50 cm/month) than that obtained in case of non-RP seedlings (11.82 and 12.63cm/month) for the first and second seasons, respectively.

The effect of ST treatments, for the two seasons, all ST treatments significantly increased SGR over the control. Among all symbiotic-types, the inoculation with R followed by VAM (R+VAM) treatment has displayed the highest SGR (17.10and 16.31cm/month, for the first and second seasons, respectively), whilst, the lowest SGR was obtained in the control (3.80 and 4.69 cm/month, for the first and second seasons) (Table 2).

Regarding to the interaction (RP × ST), in both RP-fertilized soil and non-fertilized soil, for the two seasons, the SGR was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and their combinations. Among all treatments, the RP-fertilized and that dually inoculated with R+VAM have displayed the highest SGR (17.57 and 16.58, for the first and second seasons, respectively). However, the control treatment has displayed the lowest SGR (3.73 and 3.98, for the first and second seasons, respectively) (Table 2).

Stem diameter (SD) (mm)

Data in table (2) showed that the effect of RP on the SD was significant only in the second season (Table 2). For the two seasons, the effect of symbiotic-type (ST) and interaction between RP × ST on SD was significant (Table 2). In the second season, the RP-fertilized seedlings exhibited significantly higher SD (4.26 mm) than that obtained in case of non-RP seedlings (3.72 mm).

For the effect of ST treatments, in the two seasons, all ST treatments significantly increased SD over the control. In the first season, among all symbiotic-types, both VAM + R and R+VAM treated seedlings have shown the highest similar SD (5.09 mm), while the value was detected in the control (2.24 mm). In the second season, VAM + R treated seedlings have shown the highest SD (4.84 mm) while the lowest SD was observed in the control (2.25 mm) (Table 2).

Regarding to the interaction (RP × ST), in both RP-fertilized soil and non-fertilized soil, for the two seasons, the SD was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and its combinations. In the first season, both VAM + R and R+ VAM treatments in the PR and non-RP attained a significantly higher and similar SD (4.88 and 5.31 mm) over the control (2.22 and 2.26 mm). In the second season, RP-fertilized seedlings and R+VAM treatment recorded the highest SD (5.24 mm), while the lowest SD was observed for the control (2.13 mm) (Table 2).

Leaf area (LA) (cm^2)

Observing the data in Table (3), it can be seen in both seasons that the effect of RP and symbiotic-type (ST) and their interactions (RP × ST) on the leaf area (LA) was significant. The RP-fertilized seedlings exhibited significantly higher LA (62.25 and 57.33 cm^2) than those obtained in case of non-RP seedlings (61.08 and 54.22 cm^2) for the first and second seasons, respectively). AS for the effect of ST treatments, for both seasons, all ST treatments significantly increased LA compared to the control. Among all symbiotic-types, the inoculation with R followed by VAM (R+VAM) has induced the highest LA (70.71 cm^2) for the first season, while in the second one, the inoculation with R treatment has induced the highest LA (61.88 cm^2). On the other side, the lowest LA was recorded in the control (39.18 and 33.82 cm^2, for the first and second season, respectively) (Table 3).

Table (3): Means of leaf area (LA), Leaves number (LN) and Chlorophyll content (CC) of *Leucaena leucocephala* seedlings as it influenced by the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus, Rhizobium and their combinations with or without rock phosphate fertilization in the two seasons (2020 and 2021).

Symbiosis type (ST))	Rock phosphate (RP) (1.5 g/Kg $^{-1}$ soil)					
	RP	Non-RP	Mean	RP	Non-RP	Mean
	First season			Second season		
Leaf area (cm^2)						
C	39.56 e	38.8 e	**39.18 d**	33.56 e	34.088 e	**33.824 c**
VAM	65.28 cd	63 d	**64.14 c**	63.43 ab	57.148 d	**60.289 b**
R	67.64 bc	66.2 c	**66.92 b**	61.55 b	62.184 b	**61.867 a**
VAM + R	69.74 b	65 cd	**67.37 b**	63.232 ab	59.786 c	**61.509 ab**
R + VAM	69.04 b	72.38 a	**70.71 a**	64.86 a	57.87 d	**61.365 ab**
Mean	**62.252 a**	**61.076 b**	-------	**57.326 a**	**54.215 b**	-------
LSD (0.05)	**RP= 1.144 ST= 1.612 RP*ST= 2.280**			**RP= 0.507 ST= 1.060 RP*ST= 1.499**		
Number of leaves						
C	12.6 e	13 e	**12.8 d**	13.2 e	21.4 cd	**17.3 c**
VAM	17.2 d	15.4 d	**16.3 c**	22.8 bc	20.2 d	**21.5 b**
R	16.4 d	17.2 d	**16.8 c**	24.4 ab	25.2 a	**24.8 a**
VAM + R	22.8 b	20.4 c	**21.6 b**	22.8 bc	20 d	**21.4 b**
R + VAM	25 a	21 c	**23 a**	22 cd	22.6 bc	**22.3 b**
Mean	**18.8 a**	**17.4 b**	-------	**21.04 a**	**21.88 a**	-------
LSD (0.05)	**RP= 0.176 ST= 1.024 RP*ST= 1.448**			**RP= 1.259 ST= 1.083 RP*ST= 1.531**		
Chlorophyll content (Spad unit)						
C	15.78 g	15.11 g	**15.449 d**	15.24 g	16.88 f	**16.06 d**
VAM	58.804 bc	50.06 e	**54.434 b**	56.32 d	52.184 e	**54.252 c**
R	58.80 bc	47.2 f	**53 c**	58.58 c	59.82 b	**59.2 b**
VAM + R	58.22 c	51.5 d	**54.86 b**	63.76 a	56.68 d	**60.22 a**
R + VAM	63.04 a	59.62 b	**61.33 a**	63.224 a	56.6 d	**59.912 ab**
Mean	**50.930 a**	**44.610 b**	-------	**51.425**	**48.433**	-------
LSD (0.05)	**RP= 0.621 ST= 0.739 RP*ST= 1.045**			**RP= 1.491 ST= 0.780 RP*ST= 1.103**		

Means with the same superscript letter are not significant at level of probability of 0.05, for each parameter. Where, ST, Symbiosis type; RP, rook phosphate; Non-RP, non-fertilized with RP; C, control; VAM, inoculated with vesicular arbuscular mycorrhiza; R, inoculated with Rhizobium; VAM+R, inoculated with vesicular arbuscular mycorrhiza then with inoculated with Rhizobium; R+VAM, inoculated with Rhizobium then inoculated with vesicular arbuscular mycorrhiza.

Regarding to the interaction (RP × ST), in both RP-fertilized soil and nonfertilized soil, for the two seasons, the SH was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and by its combination. Among all treatments, the non-RP and dually inoculated seedling with R+VAM have displayed the highest LA (72.38 cm^2) for the first season, but in the second season, the highest LA (64.86 cm^2) was obtained the in RP-fertilized and dually inoculated with R+VAM seedlings. However, the control treatment has displayed the lowest LA (38 and 33.56 cm^2, for the first and second seasons, respectively) (Table 3).

Leaf number (LN)

Data in Table (3) revealed that the effect of RP on the leaf number (LN) was significant only in the first season (Table 2). For the two seasons, the effect of symbiotic-type (ST) and interaction between RP × ST on SD (LN) was significant (Table 2). In the first season, the RP-fertilized seedlings exhibited LN (18.8) significantly higher than that obtained in case of non-RP ones (17.4).

According to the effect of ST treatments, for the two seasons, all ST treatments significantly induced LN higher than that of the control. In the first season, among all symbiotic-types, seedlings inoculated with R+VAM have exhibited the highest LN (23), while the lowest LN was observed in that of the control (12.8). In the second season, R showed the highest LN (24.8), while the lowest LN was observed in the control (17.3) (Table 3).

Regarding to the interaction between RP and ST, in both RP-fertilized soil and non-fertilized one, for the two seasons, the LN was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and its interaction. In the first season, among all treatments, the RP-fertilized and dually inoculated seedlings with R+VAM have displayed the highest LN (25) for the first season, but for the second one, the highest LA (25.2) was obtained in the RP-fertilized and inoculated seedlings with R, while the lowest LN was observed in that of the control (17.3) (Table 3).

Chlorophyll content (CC) (SPAD unit)

From data set remove in Table (3), in both seasons, the effect of the rook phosphate (RP) and symbiotic-type (ST) and its interactions (RP × ST) on chlorophyll content (CC) was significant. The RP-fertilized seedlings exhibited CC (50.93 and 51.43) higher significantly than that obtained in the case of non-RP treated seedlings (44.61 and 48.43) for the first and second season, respectively.

The effect of ST treatments, for both seasons, all ST treatments have induced CC significantly higher than that of the control. In the first season, among all symbiotic-types, seedlings inoculated with R+VAM indicated the highest LN (61.33) while the lowest value was observed in the control (15.45). In the second season, seedling treated with VAM+R have showed the highest CC (60.22), while the lowest LN was observed in the control (16.06) (Table 3).

Upon the interaction between RP and ST, in both RP-fertilized soil and non-fertilized one, for the two seasons, the CC was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and their combinations. In the first season, out of all treatments, RP-fertilized seedlings and inoculated with R+VAM have showed the highest CC (63.04), while the lowest CC was observed in that of the control (15.11). In the second season, both VAM + R and R+ VAM treatments in the RP-fertilized seedlings displayed CC (63.76 and 63.22) higher significantly than those obtained in the control (15.78 and 15.11) (Table 3).

Shoot dry weight (SDW) (g)

For the two seasons, the effect of RP and symbiotic-type (ST) and their interactions (RP × ST) on shoot dry weight (SDW) was significant (Table 4). The RP-fertilized seedlings Exhibited significantly higher SDW (44.32 and 43.72 g) than those obtained in case of non-RP seedlings (42.20 and 39.89 g for the first and second seasons, respectively).

Table (4): Means of shoot dry weight (SDW), root dry weight (RDW), shoot / root ratio (SRR) and total dry weight (TDW) of *Leucaena leucocephala* seedlings as it influenced by the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus, Rhizobium and its combinations with or without rock phosphate fertilization in the two seasons (2020 and 2021).

Symbiosis type (ST)	Rock phosphate (RP) (1.5 g/Kg $^{-1}$ soil)					
	RP	Non-RP	Mean	RP	Non-RP	Mean
	First season			Second season		
	Shoot dry weight (g)					
C	22.13 g	21.93 g	**22.03 d**	20.74 f	19.332 f	**20.036 e**
VAM	46.372 d	42.902 f	**44.637 c**	47.128 c	41.88 e	**44.504 d**
R	44.582 e	43.982 e	**44.282 c**	47.58 c	44.22 d	**45.9 c**
VAM + R	52.406 b	50.784 c	**51.595 b**	49.8 b	46.122 c	**47.961 b**
R + VAM	56.11 a	51.384 c	**53.747 a**	53.37 a	47.9 c	**50.635 a**
Mean	**44.32 a**	**42.196 b**		**43.724 a**	**39.891 b**	
LSD (0.05)	**RP= 0.622 ST= 0.716 RP*ST= 1.013**			**RP= 0.199 ST= 1.175 RP*ST= 1.661**		
	Root dry weight (g)					
C	15.14 f	15.03 f	**15.09 c**	12.174 e	13.48 e	**12.827 d**
VAM	44.54 d	40.94 e	**42.74 b**	46.8 ab	45.062 bc	**45.931 b**
R	41.2 e	43.32 d	**42.26 b**	44.38 c	41.9 d	**43.14 c**
VAM + R	53.9 a	48.96 c	**51.43 a**	46.962 ab	48.56 a	**47.761 a**
R + VAM	50.14 bc	51.08 b	**50.61 a**	48.922 a	45.16 bc	**47.041 ab**
Mean	**40.986 a**	**39.866 b**	-------	**39.848 a**	**38.832 b**	-------
LSD (0.05)	**RP= 0.724 ST= 0.999 RP*ST= 1.413**			**RP= 0.790 ST= 1.223 RP*ST= 1.730**		
	Shoot / root ratio					
C	1.462 a	1.46 a	**1.461 a**	1.712 a	1.44 b	**1.576 a**
VAM	1.04 cd	1.046 cd	**1.043 b**	1.008 c	0.93 c	**0.969 b**
R	1.084 bc	1.016 de	**1.05 b**	1.072 c	1.058 c	**1.065 b**
VAM + R	0.972 e	1.04 cd	**1.006 c**	1.062 c	0.95 c	**1.006 b**
R + VAM	1.12 b	1.006 de	**1.063 b**	1.092 c	1.062 c	**1.077 b**
Mean	**1.136 a**	**1.114 b**	-------	**1.189 a**	**1.088 b**	-------
LSD (0.05)	**RP= 0.018 ST= 0.029 RP*ST= 0.041**			**RP= 0.031 ST= 0.082 RP*ST= 0.117**		
	Total dry weight (g)					
C	37.272 g	36.954 g	**37.11 d**	32.914 e	32.812 e	**32.863 d**

VAM	90.916 d	83.85 f	**87.38 c**	93.928 c	86.942 d	**90.435 c**
R	85.782 e	87.3 e	**86.54 c**	91.96 c	86.12 d	**89.04 c**
VAM + R	106.306 a	99.744 c	**103.03 b**	96.762 b	94.682 bc	**95.722 b**
R + VAM	106.25 a	102.464 b	**104.36 a**	102.292 a	93.06 c	**97.676 a**
Mean	**85.305 a**	**82.062 b**	-------	**83.571 a**	**78.723 b**	-------
LSD (0.05)	**RP= 1.018 ST= 1.278 RP*ST= 1.808**			**RP= 0.892 ST= 1.589 RP*ST= 2.247**		

Means with the same superscript letter are not significant at level of probability of 0.05, for each parameter. Where, ST, Symbiosis type; RP, rook phosphate; Non-RP, non-fertilized with RP; C, control; VAM, inoculated with vesicular arbuscular mycorrhiza; R, inoculated with Rhizobium; VAM+R, inoculated with vesicular arbuscular mycorrhiza then with inoculated with Rhizobium; R+VAM, inoculated with Rhizobium then inoculated with vesicular arbuscular mycorrhiza.

The effect of ST treatments, in the two seasons showed that, all ST treatments significantly increased SDW over the control. Within all symbiotic types, the inoculation with R followed by VAM (R+VAM) has brought about the highest SDW (53.75 g and 50.64 g, for the first and second seasons, respectively). On the other hand, the lowest SDW was recorded in that of the control (22.03 g and 20.04 g, for the first and second seasons) (Table 4).

Upon the significant interaction between RP and ST, in both RP-fertilized and non-fertilized soil, for the two seasons, the SDW was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and its combinations. Out of all treatments, the RP-fertilized and dually inoculated (R+VAM) seedlings have shown the highest SDW (56.11 g and 53.37 g, for the first and second season, respectively). However, the control treatment has displayed the lowest SDW (21.93 g and 19.33 g, for the first and second season, respectively) (Table 4).

Root dry weight (RDW) (g):

As reported in Table (4), it can be seen in both seasons that the effect of RP and symbiotic-type (ST) and their interactions (RP × ST) on root dry weight (RDW) was significant. The RP-fertilized seedlings exhibited RDW (40.99 g and 39.85 g) higher significantly than that obtained in non-RP ones (39.87 g and 38.83 g for the first and second season, respectively). According to the effect of ST treatments, for the two seasons, all ST treatments significantly increased RDW as it compared to control. In the first season, within all symbiotic-types, both VAM + R and R+VAM displayed the highest RDW (51.43 g and 50.61 g, respectively), with no significant differences between each other, while the lowest RDW was observed in the control (15.09 g). In the second season, VAM + R treated seedlings have the highest RDW (47.76 g), while the lowest value was observed in the control (12.83 g) Table 2. Based on the significant interaction between RP and ST, in both RP-fertilized and non-fertilized soil, for the two seasons, the RDW was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and their combinations. For all treatments, the RP-fertilized seedlings which dually inoculated with VAM+R have displayed the highest RDW (53.9 g) while the lowest RDW was observed in the control (15.03 g), for the first season. In the second season, among all symbiotic-types, both the RP-fertilized which dually inoculated with R+VAM and non-RP ones that dually inoculated with VAM+R attained the highest similar RDW (48.92 g and 48.56 g, respectively), however, the control treatment recorded the lowest RDW (12.17 g) (Table 2).

Shoot / root ratio (SRR)

For the two seasons, the effect of RP and symbiotic-type (ST) and its interactions (RP × ST) on SRR was significant (Table 2). The RP-fertilized seedlings exhibited the highest SRR (1.136 and 1.189) as compared with that obtained in non-RP ones (1.114 and 1.088) for the first and second season, respectively. For the effect of ST, in the first season, between all symbiotic-types, it noticed that there were no significant influences on SRR, where the highest SRR (1.576) was obtained in the control. In the second season, the highest SRR (1.461) was obtained in the control, but among all symbiotic-types, seedlings inoculated with VAM then inoculated with R (VAM+R) showed the lowest value (1.006) as compared to those obtained in other treatments (Table 2).

Regarding to the interaction between RP and ST, in both RP-fertilized and non-fertilized soil, for both seasons, the SRR was significantly affected by the inoculation with VAM fungus, Rhizobium

(R) and its interaction. In the first season, out of symbiotic-types, the highest SRR (1.46) was obtained in the control, while non-RP seedlings that were inoculated with VAM+R have showed the lowest SRR (0.972). In the second season among all treatments, the control has displayed the highest SRR (1.46). However, there were no significant differences between other treatments (Table 4).

Total dry weight (TDW) (g)

The results arranged in Table (4) showed that the effect of RP and symbiotic-type (ST) and its interactions (RP × ST) on TDW was significant for both seasons (Table 4). The RP-fertilized seedlings exhibited TDW (85.31 g and 83.57 g) higher significantly than that obtained in non-RP ones (82.06 g and 78.72 g) for the first and second season, respectively. According to the effect of ST, for both seasons, all ST treatments significantly induced TDW higher than that of the control. Among all symbiotic-types, seedlings inoculated with R followed by VAM (R+VAM) have showed the highest TDW (104.36 g and 97.68 g, for the first and second season, respectively). On the other hand, the lowest value was obtained in the control (37.11 g and 32.86 g, for the first and second season, respectively) (Table 4).

It should be noted here that there was no significant difference between seedlings inoculated with VAM only or inoculated with R only for both seasons (Table 4). Upon the interaction between RP and ST, in both RP-fertilized soil and non-fertilized one, for both seasons, the RDW was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and its combinations. In the first season, out of all symbiotic-types, both the RP-fertilized seedlings which dually inoculated with R+VAM and non-RP ones which dually inoculated with VAM+R have displayed the highest RDW (106.25g and 106.31 g, respectively), However, the control has displayed the lowest value (36.95 g). For the second one, out of all treatments, the RP-fertilized seedlings which dually inoculated with R+VAM have exhibited the highest RDW (102.29 g) whilst the lowest value was observed in that of the control (32.81 g) (Table 2).

Nodule number (NN)

Table (5) showed that the effect of RP on the nodule number (NN) was significant only in the first season. Whilst the effect of symbiotic-type (ST) and interaction between RP and ST on SD was significant for both seasons. In the first season, the RP-fertilized seedlings exhibited NN (8.12) higher significantly than that obtained in non-RP ones (7.2). For the effect of ST treatments, in both seasons, there was significantly effect on NN, out of all symbiotic-types, in the 1st season; seedlings inoculated with R only have displayed the highest NN (14.1). However, there were no significant differences between each of seedlings inoculated with VAM+R and those inoculated with R+VAM. In the second season, seedlings inoculated with R+VAM have showed the highest NN (13.9). However, there were no significant differences between each of seedlings inoculated with VAM+R and those inoculated with R only (Table 5).

Regarding the interaction between RP and ST, in both RP-fertilized soil and non-fertilized one, for both seasons, the NN was significantly affected by the inoculation with VAM fungus, Rhizobium (R) and its combinations. In the first season, out of all treatments, the RP fertilized and inoculated seedlings with R have displayed the highest NN (14.1), whilst, the lowest value (10.8) was obtained in non-RP ones and dually inoculated with R+VAM. In the second season, both RP-fertilized seedlings and inoculated with R+VAM and non-RP ones and inoculated with VAM+R have displayed the highest NN (15.2 and 14.4, respectively)

with nonsignificant differences between each other, whilst the lowest value was observed in both, RP-fertilized and inoculated seedlings with VAM+R and non-RP inoculated with R only (9.4 and 10.4, respectively) with non-significant differences between each other (Table 5).

Table (5): Means of nodule number, nodule dry weight (mg) and nodule/root ratio in *Leucaena leucocephala* seedlings as it influenced by the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus, Rhizobium and their combinations with or without rock phosphate fertilization in the two seasons (2020 and 2021).

Symbiosis type (ST)	**Rock phosphate (RP)** (1.5 g/Kg $^{-1}$ soil)					
	RP	**Non-RP**	**Mean**	**RP**	**Non-RP**	**Mean**
	First season			**Second season**		
	Nodule number					
C	0	0	**0**	0	0	**0**
VAM	0	0	**0**	0	0	**0**
R	14.8 a	13.4 b	**14.1 a**	12.2 b	10.4 c	**11.3 b**
VAM + R	13 bc	11.8 cd	**12.4 b**	9.4 c	14.4 a	**11.9 b**
R + VAM	12.8 bc	10.8 d	**11.8 b**	15.2 a	12.6 b	**13.9 a**
Mean	**8.12 a**	**7.2 b**	**-------**	**7.36 a**	**7.48 a**	**-------**
LSD (0.05)	**RP= 0.283 ST= 0.830 RP*ST= 1.174**			**RP= 0.377 ST= 0.846 RP*ST= 1.196**		
	Nodule dry weight (mg)					
C	0	0	**0**	0	0	**0**
VAM	0	0	**0**	0	0	**0**
R	394.24 c	365.12 d	**379.68 b**	444.6 bc	346.2 e	**395.4 c**
VAM + R	348.32 e	505.68 a	**427 a**	462 b	375 d	**418.5 b**
R + VAM	424.48 b	430.64 b	**427.56 a**	507.6 a	424.8 c	**466.2 a**
Mean	**233.408 b**	**260.288 a**	**-------**	**282.84 a**	**229.2 b**	**-------**
LSD (0.05)	**RP= 4.533 ST= 4.833 RP*ST= 6.835**			**RP= 3.784 ST= 16.999 RP*ST= 24.040**		
	Nodule/root ratio					
C	0	0	**0**	0	0	**0**
VAM	0	0	**0**	0	0	**0**
R	0.00958 b	0.00844 c	**0.00901 a**	0.01002 ab	0.00825 c	**0.0091 b**
VAM + R	0.00646 d	0.01034 a	**0.00840 b**	0.00984 ab	0.00772 d	**0.00878 c**
R + VAM	0.00847 c	0.00844 c	**0.00845 b**	0.01038 a	0.00943 b	**0.00991 a**
Mean	**0.00490 b**	**0.00545 a**	**-------**	**0.00605 a**	**0.00508 b**	**-------**
LSD (0.05)	**RP= 1.248 ST= 2.202 RP*ST= 3.114**			**RP= 1.923 ST= 3.458 RP*ST= 4.891**		

Means with the same superscript letter are not significant at a level of probability of 0.05, for each parameter. Where, ST, Symbiosis type; RP, rook phosphate; Non-RP, non-fertilized with RP; C, control; VAM, inoculated with vesicular arbuscular mycorrhiza; R, inoculated with Rhizobium; VAM+R, inoculated with vesicular arbuscular mycorrhiza then with inoculated with Rhizobium; R+VAM, inoculated with Rhizobium then inoculated with vesicular arbuscular mycorrhiza.

Nodule dry weight (NDW) (mg)

Data set out in Table (5) showed that the effect of RP, ST and its interaction on the NDW was significant for both seasons. In the first season, the non-RP-fertilized seedlings exhibited NDW (233.41) higher significant than that obtained in RP-fertilized ones (260.29 g), whilst in the second one, the RP-fertilized seedlings exhibited NDW (282.84 g) higher significant than that obtained in non-RP ones (229.2 g).

Based on the effect of ST treatments, in both seasons, there was significantly impact on all treatments. In the first season, VAM+R and R+VAM treated seedlings have showed the highest NDW (427.0 g and 427.56 g, respectively) with no significant differences between them. On the other hand, in the second season, the highest NDW (466.2 g) was obtained in R+VAM inoculated seedlings, whilst, the lowest value (395.4 g) was detected in R inoculated treatment (Table 5).

Upon the interaction between RP and ST, in both RP-fertilized soil and non-fertilized one, for both seasons, the NDW was significantly affected by the inoculation with VAM, R and its combinations. In the first season, non-RP-fertilized and dually inoculated seedlings with VAM+R have showed the highest NDW (505.68 g), while, the lowest value (348.32 g) was obtained in RP ones and inoculated with R+VAM. But in the second one, RP-fertilized and dually inoculated seedlings with R+VAM have displayed the highest NDW (507.6 g), whilst, the lowest value (346.2 g) was obtained in non-RP treatment and inoculated only with R (Table 5).

Nodule/root ratio (NRR)

Observing the data in Table (3), the effect of RP, ST and its interaction on the NRR was significant for both seasons. In the first season, the non-RP-fertilized seedlings exhibited NDW (0.00545) higher significant than that obtained in RP-fertilized ones (0.00490), while in the second one, the RP-fertilized seedlings exhibited NDW (0.00605) higher significant than that obtained in non-RP-fertilized ones (0.00508).

Upon the effect of ST treatments, for both seasons, there was a significantly effect on NN, among all symbiotic-types, in the first season, inoculated seedlings with R have displayed the highest NRR (0.00901). however, there were no significant differences between VAM+R and R+VAM treated seedlings. In the second season, R+VAM treated seedlings have shown the highest NRR (0.00991), while, the lowest value (0.00878) was detected in VAM+R treated (Table 5).

Based on the significant interaction between RP and ST, in the first season, out of all treatments, the non-RP-fertilized and inoculated seedlings with VAM+R have displayed the highest NRR (0.01034), whilst, the lowest value (0.00646) was detected in non-RP treatment and inoculated with R+VAM. In the second season, the RP-fertilized and inoculated seedlings with R+VAM have displayed the highest NRR (0.01038), whilst, the lowest value (0.00772) was obtained in non-RP ones and inoculated with VAM+R (Table 5).

Chemical Content:

Nitrogen content (N %)

From data set in Table (6), it can be seen that in both seasons, the effect of RP, ST and its interactions on the N % was significant. RP fertilized seedlings exhibited a significantly higher percentage (3.683% and 3.738%) than that obtained in the case of non-RP seedlings (3.521% and 3.738 % for the first and second seasons, respectively).

As for the effect of ST treatments, for both seasons, all ST treatments significantly induced N% higher than that of the control. among all symbiotic-types, seedlings inoculated with R+VAM have exhibited the highest N% (4.008%) for the first season, but for the second one, each of R, VAM+R and R+VAM treated ones have displayed the highest N% (3.896%, 3.842% and 3.851 %, respectively) with non-significant differences between them. However, the lowest value (2.739% and 2.723 %) was obtained in the control, for the first and second season, respectively) (Table 6).

Table (6): Means of nitrogen content (N %), phosphorus content (P %) and potassium content (K %) in seedlings of *Leucaena leucocephala* seedlings as it influenced by the inoculated with vesicular arbuscular mycorrhiza (VAM) fungus, Rhizobium and their combinations with or without rock phosphate fertilization in the two seasons (2020 and 2021).

Symbiosis type (ST)	Rock phosphate (RP) (1.5 g/Kg $^{-1}$ soil)					
	RP	**Non-RP**	**Mean**	**RP**	**Non-RP**	**Mean**
	First season			**Second season**		
	Nitrogen content (N %)					
C	2.932 e	2.545 f	**2.739 c**	2.766 e	2.68 e	**2.723 c**
VAM	3.868 bc	3.532 d	**3.7 b**	3.744 b	3.294 d	**3.519 b**
R	3.908 bc	3.7 cd	**3.804 b**	3.878 b	3.914 b	**3.896 a**
VAM + R	3.502 d	4.014 b	**3.758 b**	4.116 a	3.568 c	**3.842 a**
R + VAM	4.204 a	3.812 bc	**4.008 a**	4.188 a	3.514 c	**3.851 a**
Mean	**3.683 a**	**3.521 b**	-------	**3.738 a**	**3.394 b**	-------
LSD (0.05)	**RP= 0.069 ST= 0.118 RP*ST= 0.167**			**RP= 0.028 ST= 0.121 RP*ST= 0.171**		
	Phosphorus content (P %)					
C	0.3244 e	0.3078 e	**0.3161 d**	0.2836 d	0.3126 cd	**0.2981 b**
VAM	0.549 a	0.4274 d	**0.4882 b**	0.5148 a	0.4218 ab	**0.4683 a**
R	0.516 b	0.3162 e	**0.4161 c**	0.4616 ab	0.3732 bc	**0.4174 a**
VAM + R	0.572 a	0.4128 d	**0.4924 b**	0.4838 a	0.4472 ab	**0.4655 a**
R + VAM	0.5526 a	0.4868 c	**0.5197 a**	0.522 a	0.4254 ab	**0.4737 a**
Mean	**0.5028 a**	**0.3902 b**	-------	**0.4532 a**	**0.3960 b**	-------
LSD (0.05)	**RP= 0.015 ST= 0.018 RP*ST= 0.025**			**RP= 0.030 ST= 0.046 RP*ST= 0.065**		
	Potassium content (K %)					
C	1.860 f	1.685 g	**1.773 c**	1.75 e	1.788 e	**1.769 c**
VAM	2.449 a	2.013 def	**2.231 a**	2.714 a	2.142 d	**2.428 a**
R	2.522 a	1.890 ef	**2.206 a**	2.43 b	2.254 cd	**2.342 a**
VAM + R	2.122 bcd	2.066 cde	**2.094 b**	2.382 bc	1.572 f	**1.977 b**
R + VAM	2.288 b	2.232 bc	**2.260 a**	2.454 b	2.188 d	**2.321 a**
Mean	**2.248 a**	**1.977 b**	-------	**2.346 a**	**1.989 b**	-------
LSD (0.05)	**RP= 0.061 ST= 0.109 RP*ST= 0.155**			**RP= 0.065 ST= 0.098 RP*ST= 0.139**		

Means with the same superscript letter are not significant at level of probability of 0.05, for each parameter. Where, ST, Symbiosis type; RP, rook phosphate; Non-RP, non-fertilized with RP; C, control; VAM, inoculated with vesicular arbuscular mycorrhiza; R, inoculated with Rhizobium; VAM+R, inoculated with vesicular arbuscular mycorrhiza then with inoculated with Rhizobium; R+VAM, inoculated with Rhizobium then inoculated with vesicular arbuscular mycorrhiza.

Regarding to the significant interaction between RP and ST. In the first season, within all treatments, the RP-fertilized and inoculated seedlings with R+VAM exhibited N% (2.204%) higher significant than that of the control (2.545 %), whilst, in the second one, both R+VAM and VAM+R treated seedlings and fertilized with RP have displayed N% (4.188% - 4.116 %, respectively) higher significant than that of the control (2.68 %).

Phosphorus content (P %)

Data in Table (6) revealed that in both seasons, the effect of RP and ST and its interactions on P % was significant. The RP-fertilized seedlings exhibited P % (0.50% and 0.45%) more significant than those obtained in non-RP seedlings (0.39% and 0.4 % for the first and second seasons, respectively).

Upon the effect of ST treatments, for both seasons, all ST treatments significantly induced P % higher than that of the control. In the first season, R+VAM treatment has displayed the highest P % (0.5197 %) whilst, the lowest value was detected in the control (0.3161 %), but in the second season, R, VAM, VAM + R and R+VAM treated seedlings have displayed the highest P % (0.4683%, 0.4174%, 0.4655% and 0.4737 %, respectively) with non-significant differences between them, whilst, the lowest value was detected in the control (0.2981 %) (Table 6).

According to the significant interaction between RP and ST, in both RP-fertilized and non-fertilized soil, in both seasons. Out of all treatments, the RP-fertilized and inoculated seedlings with R only, VAM+R and R+VAM have displayed the highest P % (0.549%, 0.572% and 0.5526% respectively), with non-significant differences between them, whilst, the lowest value recorded in the control (0.3078 %). In the first season, were to great extent, similar to that has been recorded in the first season (Table 6).

Potassium content (K %)

Observing the data in Table (6), the effect of RP, ST and its interaction on the K % was significant in both seasons. The RP fertilized seedlings have displayed a significantly higher K% (2.248% and 2.346%) than that obtained in non-RP seeds (1.977% and 1.989 % for the first and second seasons, respectively).

Regarding to the effect of ST treatments, in both seasons, all ST treatments significantly induced P % higher than that of the control. In the first season, out of all treatments, VAM, R and R+VAM treated seedlings have displayed the highest K% (2.231%, 2.206% and 2.260% respectively), with nonsignificant differences between each other, meanwhile, the lowest value recorded in the control (1.773 %). In the second season, were to great extent, similar to that has been recorded in the first one (Table 6).

Based on the significant interaction between RP and ST, in both RP-fertilized soil and non-fertilized soil, for the two seasons, the K% was significantly affected by the inoculation with VAM, R and its combinations. In the first season, both the RP-fertilized seedlings and inoculated with R only or VAM only have displayed the highest N% (2.449% and 2.522 % respectively), with non-significant differences between them, while, the lowest value was recorded in the control (1.685 %). Additionally, in the second season, the highest K% (2.714%) was obtained in RP-fertilized seedlings inoculated with VAM only, whilst, the lowest value was recorded in the control (1.75 %) (Table 6).

DISCUSSION

In the current study the inoculation with symbiotic-type (ST), vesicular arbuscular mycorrhiza (VAM), Rhizobium (R), and its interaction (VAM+R and R+VAM) and fertilization with rock phosphate (RP) have enhanced vegetative growth parameters of *Leucaena leucocephala* seedlings such as shoot height, shoot growth rate, stem diameter, chlorophyll content, shoot dry weight, root dry weight, shoot / root ratio and total dry weigh, and P uptake as well. Our results were in agreement with the findings of (**Marcelo et *al*., 2020; Dash and Gupta, 2021 and Ibrahim, 2021)** in terms of positive impact of VAM, they reported that Arbuscular mycorrhizal fungi can improve growth by increasing the uptake of nutrients from soils in

Anadenanthera peregrine and *Acacia nilotica*, since the extraradical mycelium of the AM fungi serves as an extension of plant roots that extend beyond the root depletion zone for exploitation of mineral nutrients (**Frahat and Shehata 2021)**. **Ibrahim (2021)** reported that the effective AMF can greatly assist nodulation and N_2 fixation of legumes. For systems of intercropping legumes and non-legume crops in sustainable agriculture, the overall AMF contributions to N transfer are seen as being of major significance AMF increases nutrient uptake, and increases nutrient mineral transporters in plant roots, while rooted nodules fix N_2 **(Pedro Henrigue *et al.,* 2019; Marcelo *et al.,* 2020 and Luluk *et al.,* 2020).** Furthermore, **(He *et al.,* 2017)** discovered that AM fungi colonization of Black Locust increased the net rate of photosynthesis by increasing the concentration of photosynthetic pigments, improving photochemistry efficiency, carbohydrate synthesis and increasing stomatal conductance and transpiration rate, regardless of watering regimes. **Soleimanzadeh *et al.* (2012)** reported that the VAM fungus may produce secondary metabolites (as an antibiotic and plant hormones), which improve the physiological processes such as water absorption capacity of plants by increasing root hydraulic conductivity and increasing the uptake of essential macro- and micronutrients, which in turn improves the plants growth.

The beneficial effect of mycorrhizal symbiosis on photosynthetic CO_2-fixation can be described as a facilitation effect mediated by increased carbohydrate sink strength by mycorrhizal roots and enhancing N supply by N2-fixing symbiosis with Rhizobia, mycorrhizal symbioses can provide *Robinia pseudoacacia L.* (Black Locust) with additional sources of P, C and water **(Du *et al*., 2019)**.

Karthikeyan and Arunprasad (2021) reported that indigenous microbial symbionts, arbuscular mycorrhizal fungi (Glomus fasciculatum, Glomus geosporum and Glomus aggregatum) and N_2-fixing bacteria (*Rhizobium aegyptiacum*) increased the growth and biomass of *P. santalinus* seedlings by providing essential levels of phosphorous nitrogen under nursery conditions. **(Ashwin *et al.,* 2022)** proved that all the growth parameters studied under stress with dual inoculation signify the importance of rhizobia and mycorrhizal inoculation for imparting stress tolerance in soybean. **(Zhenshan *et al.*, 2020)** reported that double inoculation of Black Locust with AM fungi and rhizobia increases nodule number and dry weight compared to single inoculation with rhizobia.

The increase N, P, and K in the inoculated seedlings may be due to several factors, including the effects of the tripartite symbiotic association (legume + R. aegyptiacum + AM fungi), attributed to the combination of arbuscular mycorrhizal fungi and R. aegyptiacum as significantly increasing N content, and AM fungi increasing P content **(Muthukumar and Udaiyan 2018).** Increased levels of K in seedlings inoculated with microbial symbionts showed that potassium may act as an enzyme activator and may also stimulate nodule activity **(Karthikeyan and Muthukumar 2006).** It is assumed that AM fungi are able to increase nodulation and N fixation by supplying high levels of P to the nodules **(Ding *et al.*, 2016 and Luluk *et al.*, 2020).**

Debadatta *et al.* (2021) stated that the nitrogen concentration and uptake were higher in the saplings inoculated with Rhizobium. It was due to the ability of Rhizobium to fix atmospheric N_2 that improved plant growth **(Püschel *et al.*, 2017).** On Acacia cyanophylla L., the plant's nutrition and growth are enhanced by the rhizosphere microbial populations **(Dounas *et al.*, 2022)**. It was suggested that AMF and Rh might cooperatively enhance one other's root colonization, further improving plant growth and N uptake. Our results revealed that, the dual inoculation increased growth of *L. leucocephala*, compared to the single inoculation and inoculation with Rhizobium then VAM induced best results, as previously reported in a number of different plant species **(Marcelo *et al.*, 2020; Luluk *et al.*, 2020**; **Ibrahim 2021).**

CONCLUSION

Rhizobia and vesicular arbuscular mycorrhiza (VAM) inoculation together with rock phosphate had synergistic effects on *Leucaena* growth parameters and mineral content, especially when it was inoculated with R first then with VAM, when compared to single inoculation.

REFERENCES

Abair, A., Hughes, C.E. and Bailey, C.D. (2019). The evolutionary history of Leucaena: Recent research, new genomic resources and future directions. Tropical Grasslands-Forrajes Tropicales, Vol. 7(2):65–73. doi.org%2F10.17138%2FTGFT(7)65-73

Ahmad, M.T.; Asghar, H.N.; Saleem, M.; Khan, M.Y.; Zahir, Z.A. (2015). Synergistic effect of rhizobia and biochar on growth and physiology of maize. Agron. J. 107, 2327–2334. DOI: 10.2134/aj15.0212

Ashwin, R., Bagyaraj, D.J., Raju, B.M. (2022). Dual inoculation with rhizobia and arbuscular mycorrhizal fungus improves water stress tolerance and productivity in soybean Plant Stress 4, 100084: 1-9. https://doi.org/10.1016/j.stress.2022.100084

Cottenie, A. (1980). Soil and Plant Testing as a Basis of Fertilizer Recommendations. F.A.O. No. (38/2). Methods of Plant Analysis. Wet Destruction, 94-95.

Dalzell, S.A. (2019). Leucaena cultivars – current releases and future opportunities. Tropical Grasslands-Forrajes Tropicales. Vol. 7(2):56–64. http://dx.doi.org/10.17138/TGFT(7)56-64

Dash, S. and Gupta, N. (2021). Growth promoting effect of microbial inoculants on *Acacia nilotica* grown under nursery conditions. Studies in Fungi 6(1): 401–407 www.studiesinfungi.org ISSN 2465-4973, Doi 10.5943/sif/6/1/30.

Deaker, R.; Roughley, R.J. and Kenneedy, I.R. (2004). Legume seed inoculation technology — a review. Soil B tology&Biochemistry, 36: 1275-1288. DOI: 10.1016/j.soilbio.2004.04.009

Debadatta, S.; Sachidananda, S.; Vishnu, D.R.; Konathala, K.; Tapas R.S.; Dash, S.; Satyabrata, M.; Dhirendra, K.N.; Sushanta, K.P.; Tatiana, M.; Alexey, P.G. and Valery, P.K. (2021). Exploring the Role of Mycorrhizal and Rhizobium Inoculation with Organic and Inorganic Fertilizers on the Nutrient Uptak and Growth of *Acacia mangium* Saplings in Acidic Soil. Forests 2021, 12, 1657. **https://doi.org/10.3390/f12121657**

Dekak, A.; Chabi, R.; Menasria, T.; Benhizia, Y. (2018). Phenotypic characterization of rhizobia nodulating legumes *Genista microcephala* and *Argyrolobium uniflorum* growing under arid conditions. J. Adv. Res. 14, 35–42. https://doi.org/10.1016/j.jare.2018.06.001

Ding, X.; Zhang, S.; Wang, R.; Li, S. and Liao, X. (2016). AM fungi and rhizobium regulate nodule growth, phosphorous (P) uptake, and soluble sugar concentration of soybeans experiencing P deficiency. J Plant Nutr. 39 (13):1915-1925. https://doi.org/10.1080/01904167.2016.1201104

Dounas H, Bourhia M, Outamamat E, Bouskout M, Nafidi H-A, El-Sheikh MA, Al-Abbadi GA and Ouahmane L (2022) Effects of Dual Symbiotic Interactions Performed by the Exotic Tree Golden Wreath Wattle (*Acacia cyanophylla* Lindl.) on Soil Fertility in a Costal Sand Dune Ecosystem. Front. Environ. Sci. 10:895462. doi: 10.3389/fenvs.2022.895462

Du, B.; Pang, J.; Hu, B.; Allen, D.E.; Bell, T.L.; Pfautsch, S.; Netzer, F.; Dannenmann, M.; Zhang, S. and Rennenberg, H., (2019). Intercropping with N_2-fixing black locust improves soil fertility and tree nutrition upon afforestation on the vulnerable semi-arid region of the Loess Plateau, China. Science of the Total Environment 688, 333–345. DOI: 10.1016/j.scitotenv.2019.06.245.

Elbanoby, N.E.; El-Settawy, A.A.A.; Mohamed, A.A. and Salem, M.Z.M. (2022). Phytochemicals derived from *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) biomass and their antimicrobial and antioxidant activities: HPLC analysis of extracts. *Biomass Conv. Bioref.* . **https://doi.org/10.1007/s13399-022-03420-1**

Feng, G.; Zhang, F.S.; Li, X.L.; Tian, C.Y.; Tang, C. and Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza., 12: 185–190. https://doi.org/10.1007/s00572-002-0170-0

Frahat, M.G.S. and Shehata, M.R.A. (2021). Effect of Vesicular Arbuscular Mycorrhizal (VAM) Fungus and Rock- Phosphate Application on the Growth and Biomass of *Moringa oleifera* Lam. Seedlings under Salinity Stress. Alexandria science exchange journal, 42 (2): 307-325. DOI:10.21608/asejaiqjsae.2021.167455

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist, 84:489-500. https://www.jstor.org/stable/2432123

Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P., Reddy, G. (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts macrofauna Microbiol Res 163 (2): 234–242. https://doi.org/10.1016/j.micres.2006.05.009

He, F.; Sheng, M. and Tang, M. (2017). Effects of *Rhizophagus irregularis* on photosynthesis and antioxidative enzymatic system in *Robinia pseudoacacia* L. under drought stress. Frontiers of Plant Science 8, 183.

Huda S.M.S., Sujauddin M., Shafinat S., Uddin M.S. (2007). Effects of phosphorus potassium addition on growth nodulation of *Dalbergia sissoo* in the nursery J For Res 18 (4): 279–282. https://doi.org/10.1007/s11676-007-0056-2

Ibrahim, M. (2021). Role of Arbuscular Mycorrhizal Fungi in Biological Nitrogen Fixation and Nitrogen Transfer from Legume to Companion Species**.** Journal of Stress Physiology & Biochemistry, Vol. 17, No. 2, 2021, pp. 121-134 ISSN 1997-0838. DOI: 10.12692/ijb/7.4.31-46

Karthikeyan, A. (2019). Effect of *arbuscular mycorrhizal* fungi and rhizobium on photosynthetic activity and growth response in *Acacia auriculiflormis* seedlings under elevated CO2. J. Trop. For. Sci., 31, 398–403. https://doi.org/10.26525/jtfs2019.31.4.398

Karthikeyan, A. and Muthukumar, T. (2006). Growth response of *Acacia planifrons* W. et. A to arbuscular mycorrhizal fungi as nitro- gen fixing bacteria under nursery conditions. For Tree Live 16:269–275. DOI:10.1080/14728028.2006.9752564

Karthikeyan, A., and Arunprasad, T. (2021). Growth response of *Pterocarpus santalinus* seedlings to native microbial symbionts (*arbuscular mycorrhizal* fungi and *Rhizobium aegyptiacum*) under nursery conditions. *J. For. Res.* **32**, 225–231. https://doi.org/10.1007/s11676-019-01072-y

Luluk, S.; Firli, A.D. and Arum, S.W. (2020). Arbuscular mycorrhizal fungi and Rhizobium enhance the growth of *Samanea saman* (trembesi) planted on gold-mine tailings in Pongkor, West Java, Indonesia. BIODIVERSITAS, Volume 21, Number 2, Pages: 611-616. DOI: 10.13057/biodiv/d210224

Mahdi, S.S.; Hassan, G.I.; Hussain, A. and Rasool. F. (2011). Phosphorus availability issue- its fixation and role of phosphate solubilizing bacteria in phosphate solubilization. Research Journal of Agricultural Sciences, 2(1):174-179**. www.rjas.info**

Marcelo, P.G., Raizza, Z.M.; Clésia, C.N. and Maria, R.S. (2020). Synergistic effects between arbuscular mycorrhizal fungi and rhizobium isolated from As- contaminated soils on the As-phytoremediation capacity of the tropical woody legume *Anadenanthera peregrine*. International Journal of Phytoremediation, ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bijp20.

Marx, D.H. (1975). Mycorrhizae and Establishment of Trees on Strip-Mined Land. The Ohio Journal of Science. v75, n6 (November, 1975), 288-297. http://hdl.handle.net/1811/22328

Mengual, C.; Schoebitz, M.; Azcón, R.; Roldán, A. (2014). Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. J. Environ. Manag., 134, 1–7. DOI: 10.1016/j.jenvman.2014.01.008

Muir, J.P.; Massaete, E. S. and Tsombe, H. N. (1992). Effect of *Leucaena leucocephala* and Brassica napes on growth of pigs fed wheat bran diets. Livestock Research for Rural Development.4 (2). http://www.lrrd.org/lrrd4/2/mozam.htm

Muthukumar, T. and Udaiyan, K. (2018). Coinoculation of bioinoculants improve *Acacia auriculiformis* seedling growth and quality in a tropical Alfisol soil. J For Res 29(3):663–673. DOI: 10.1007/s11676-017-0497-1

Pedro, H.R.M.; Glaciela, K.; Etienne, W.; Celso, G.A. and Antônio, R.H. (2019). Rhizobial inoculation in black wattle plantation (*Acacia mearnsii* De Wild.) in production systems of southern Brazil. Brazilian Journal of Microbiology, 50:989–998 . **https://doi.org/10.1007/s42770-019-00148-5**

Phillips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing and staining parasitic and vesicular- arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br, Mycol. Soc., 55: 158-161. https://doi.org/10.1016/S0007-1536(70)80110-3

Püschel, D.; Janoušková, M.; Vorˇíšková, A.; Gryndlerová, H.; Vosátka, M. and Jansa, J. (2017). Arbuscular Mycorrhiza Stimulates Biological Nitrogen Fixation in Two Medicago spp. through Improved Phosphorus Acquisition. Front. Plant Sci., 8, 390. DOI: 10.3389/fpls.2017.00390

Rajan, S.S.S.; Watkinson, J.H. and Sinclair, A.G. (1996). Phosphate Rocks for Direct Application to Soils. Advances in Agronomy, 57:77-159. https://doi.org/10.3389/fpls.2017.00390

Reyes, I.; Bernier, L.; Simard, R.R. and Antoun, H. (1999). Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV induced mutants, FEMS Micobiol. Ecol., 28:281–290. https://doi.org/10.1111/j.1574-6941.1999.tb00583.x

Soleimanzadeh, H. (2012). Response of Sunflower (*Helianthus annuus* L.) to inoculation with mycorrhiza under different phosphorus levels.Am-Euras. J. Agric. Environ. Sci., 12(3): 337-341. DOI: 10.2174/1874331501812010123

Thomas, R.B., Bashkin, M.A., and Richter, D.D. (2001). Nitrogen inhibition of nodulationand N $_2$ fixation of a tropical N 2-fixing tree (*Gliricidia sepium*) grown in elevatedatmospheric CO_2. New Phytologist, 145, 233–243 **https://doi.org/10.1046/j.1469-8137.2000.00577.x**

Zhang, P.; Dumroese, R.K.; Pinto, J.R. (2019). Organic or inorganic nitrogen and rhizobia inoculation provide synergistic growth response of a leguminous forb and tree. Front. Plant Sci., 10, 1308. DOI: 10.3389/fpls.2019.01308

Zhenshan, L., Bin, H., Tina, L. B., Emmanouil, F., Heinz, R. (2020). Significance of mycorrhizal associations for the performance of N_2-fixing Black Locust (*Robinia pseudoacacia* L.). Soil Biology and Biochemistry, Volume 145, 107776, ISSN 0038-0717, **https://doi.org/10.1016/j.soilbio.2020.107776**

Zhu, Y.G.; Christie, P. and Laidlaw, A.S. (2001). Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil.

Chemosphere., 42: 193–199. DOI: 10.1016/s0045-6535(00)00125-9

الملخص العربي

تعزيز نمو شتلات ليوسينا *Leucaena leucocephala* عن طريق تحفيز العلاقة التكافلية بين فطريات الميكوريزا الداخلية (VAM) وبكتيريا المثبتة للنيتروجين تحت التسميد بالفوسفات الصخري

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تعتبر أشجار الليوسينا *Leucaena leucocephala* أحد الأشجار البقولية الأكثر انتشارا في جميع أنحاء العالم نظرا لسرعة نموها وقدرتها على تثبيت النيتروجين الجوى تكافليا مع بكتيريا الرايزوبيوم مما يساهم في زيادة أهميتها البيئية والفسيولوجية والتجارية. علاوة على ذلك تتميز بعلاقتها التكافلية مع فطريات الميكوريزا التي تساعدها في تحسن النمو وتحمل الإجهادات البيئية. أجريت هذه التجربة بصوبة زراعية بالتعاون بين أحد المشاتل الخاصة بمدينة إيتاى البارود ومعمل قسم البساتين – كلية الزراعة – جامعة دمنهور بمحافظة البحيرة – جمهورية مصر العربية وذلك خلال موسمين متتاليين 2020 ، 2021 ، بهدف دراسة تأثير التلقيح بكل من فطريات الميكوريزا الداخلية وبكتيريا الريزوبيوم تحت ظروف التسميد بالفوسفات الصخري أو عدمه علي النمو الخضري والمحتوي من العناصر المعدنية لشتلات الليوسينا. حيث تم التسميد بصخر الفوسفات بعد ثلاثة أشهر من إنبات الشتلات وذلك بمعدل 1,5 جم فوسفات صخري/كجم تربة وذلك لنصف عدد المعاملات فقط. ثم إجراء التلقيح بكل من فطريات الميكوريزا وبكتيريا الرايزوبيوم بعد أسبوع من التسميد بالفوسفات الصخرى مع تسجيل إرتفاع النباتات وقت التلقيح ثم بعد ذلك كل شهر حتي نهاية التجربة. وكان التصميم الإحصائي المستخدم عبارة قطاعات كاملة العشوائية (RCBD) وتم تحليل البيانات المتحصل عليها بنظام القطع المنشقة مرة واحدة (Split-plot) حيث كانت القطع الرئيسية مخصصة للسماد الفوسفاتي المستخدم (صخر الفوسفات) أما التحت رئيسية كانت لنظام التلقيح (VAM,

R, VAM+R, R+VAM). ومن هنا نجد أن وحدات المعاملات شملت كل من: صخر الفوسفات المستخدم (معاملتان هما مسمد وغير مسمد) ونظام التلقيح (5 معاملات) مع إستخدام خمسة مكررات لكل معاملة. أظهرت النتائج أنه هناك زيادة معنوية ملحوظة للنباتات المسمدة بصخر الفوسفات في إرتفاع النبات والوزن الجاف للمجموع الخضري وكذلك المجموع الجذري والوزن الجاف الكلي وقطر الساق وكذلك نسبة المجموع الجذري للمجموع الخضري. كما أظهرت النتائج أيضا زيادة ملحوظة في المحتوي المعدني للنبات من النيتروجين والفوسفور والبوتاسيوم. ومن حيث تأثير العوامل التكافلية ، تفوقت الشتلات الملقحة على الشتلات غير الملقحة في جميع معايير النمو خاصة في حالة التلقيح الثنائي وبالأخص عندما تم التلقيح بالريزوبيوم أولا ثم بالميكوريزا. و بناءا علي النتائج المتحصل عليها، يمكن التوصية بإستخدام التلقيح الثنائي بفطريات الميكوريزا الداخلية وبكتيريا الرايزوبيوم في وجود الفوسفات الصخري كمصدر طبيعي من الفوسفور لتغذية شتلات أشجار الليوسينا *Leucaena leucocephala* حيث يؤدي إلي تحسين وزيادة النمو والمحتوى المعدني بشكل كبير جدا وخاصة في حالة التلقيح ببكتيريا الرايزوبيوم أولا ثم التلقيح بفطريات الميكوريزا ، وذلك عند مقارنته بالتلقيح الفردي لكل منهما.