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Response of Some Timber Tree Seedlings Grown in Calcareous Soil to Mycorrhizal and Phosphate Dissolving Bacteria Inoculation

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Abstract: The present investigation was carried out at the experimental farm of Horticulture Research Station of El-Kanater El-Khayria, Qaluobeia Governorate, Egypt, along two successive years (30 months starting from March 2018 to August 2020), aimed to study the effects of Vesicular-arbuscular mycorrhiza (VAM) and the phosphate dissolving bacteria *Bacillus megatherium* (B.M.) and *Bacillus polymexa* (B.P.) inoculationon seedlings of *Swietenia macrophylla* King*, Albizzia lebbeck* (L.) Benth.) and *Dalbergia sissoo* Roxb grown in calcareous soil. Results showed significant increases in all seedling growth parameters (seedling height, stem diameter, root length and fresh weight of shoots and roots) compared with un-inoculated seedlings. Also, growth parameters showed superiority for co-inoculation with VAM+B.M or with B.P compared to other treatments. Inoculation increased total chlorophyll content in leaves and carbohydrate percentages in stems compared to un-inoculated seedlings of all studied tree species. Also, results revealed increments in N, P and K % in leaves of inoculated seedlings with superiority to VAM + B.M and VAM+M.P. On the other hand, the results showed that mycorrhiza and phosphoate dissolving bacteria treatments improved soil properties as it decreased pH and EC and increased organic matter (O.M.), beside increasing N, P and K availability forms especially P, which changed from insoluble to soluble form.

Key words: Mycorrhiza · Bacillus megatherium · Bacillus polymexa · Swietenia macrophylla · Albizzia lebbeck · Dalbergia sissoo · Calcareous soil

INTRODUCTION

During the last five decades, the reclamation and improvement of new lands in Egypt is an absolute must to face the ever-increasing demand of growing population. One of the most important problems of Egyptian desert soils is high pH level, leading to fix phosphorus in insoluble form such as tri-calcium phosphate. Microorganisms can stimulate, or inhibit root growth, depending on the type of microorganisms, plant species, and environmental conditions [1]. From this point of view Bowen and Rovira [2] classified the soil microorganisms into categories in relation to their effects on plant growth, negatives (detrimental): such as root pathogens, sub clinical pathogens; neutr al rhizobacteria, cyanide producers and positives (beneficial): such as rhizobia, mycorrhizae; antagonists (biocontrol) hormone producers, plant growth promoting bacteria. It's well known that the phosphate dissolving bacterium *Bacillus megatherium* is classified as beneficial, and it is widely used as biofertilizer.

Phosphorus availability in soils is one of the main factors that limiting vegetative growth. Under conditions of limited P, microbes aid in mitigating Puses and increasing its availability and vegetation clearly affects the microbial community and P cycling [3].

Mycorrhizae are obligate fungi that predominate in the roots and soil of higher plants. They form association with plant roots in a host-nonspecific manner. Seven types of mycorrhizae have been known i.e. arbuscular, ecto, arbutoid, ectendo, ericoid, monotropoid and orchidaceous mycorrhizae. Out of these, arbuscular and ectomycorrhizae are the most abundant and wide spread. They promote plant growth by enhancing nutrient acquisition and promoting growth hormones. They also increase the resistance in plants against plant pathogens

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and surface area of root system for better absorption of nutrients from soil. Therefore, they can be used as biofertilizer and as biocontrol agent [4, 5].

The average soil phosphorus content is 0.05% (w/w) of which only 0.1% is available to plants due to soil pH [6]. Nearly 80%of applied phosphorus may be unavailable to plants [7]. Global P fertilizer consumption for 2010 was approximately 37.6 Mt with an annual 3%increase in demand thereafter to be over 45 Mt [8, 9]. Reserves of mineable rock phosphate (RP), provides the base raw material for inorganic fertilizer production, [10]. The release of P adsorbed on the solid phase of soil solution is very slow, and consequently, P fertilization is compulsory [11]. The availability of Pto crops for uptake and utilization is declining in alkaline and calcareous soils due to the decreases of solubility of calcium phosphate minerals [12, 13].

Under Egyptian soils condition the conversion of applied, inorganic P-fertilizer to precipitated form of CO_3 $(PO_4)_2$, is a major problem, which is unavailable to the growing plants [14, 15].VAM symbiosis can promote and increase the uptake of mineral nutrients such as P, Zn, and Cu by plant for growth [16].

On the other side *Bacillus megatherium* produces large amounts of organic acids, which increase the soil acidity and converts the insoluble forms of phosphorus into soluble ones [17, 18]. Consequently, the use of these bacteria as biofertilizers in the alkaline soils is very important and essential to increase the availability of soil phosphorus. Furthermore, the obligate symbiotic microorganisms vesicular- arbuscular mycorrhizal (VAM) fungi are associated with plant roots in a host monospecific manner [19].

Forest trees are renewable resources available to the mankind are not only necessary for ecology and aesthetics but also as a source for obtaining basic necessities for people. Though timber production as of now does not suffice the needs of the ever-growing human population, there is definitely a scope to increase the production of timber. Keeping these needs in view, increasing the production of timber seedlings at nursery level is most essential. This could be possible, if the production can be increased with the application of biofertilizers like phosphate solubilizing bacteria (PSB) and vesicular- arbuscular mycorrhiza (VAM), the mycobont undergoes pronounced alterations of root system besides ensuring ecological sustainability [20]. VAM fungi offer a great potential for sustainable plant growth [21]. Recently, the potential of PSB and VAM fungal association on different plants has been well documented [22, 23].

Swietenia macrophylla, belongs to family Meliaceae otherwise, known as Big Leaf Mahogany, is a slow-growing, tall, tropical tree reaching a height of about 40-60 m with a diameter of 1.5 to 2 m [24]. Various medicinal uses of this plant have been reported. The bark is used to treat diarrhea and fever. The tree is not cultivated for food for no plant part is edible. Else, it is used in reforestation projects or as a shade tree in plantation crops. Crushed fruit shells are used as a potting medium. The bark produces gums, and used for dyeing and tanning leather. Seed kernels yield oil which is very bitter and purgative. The wood is valued for high quality woodwork and furniture, musical instruments, veneer, etc. [24, 25].

Albizzia lebbeck (L.) Benth belongs to family Fabaceae, it is a multipurpose tree for semiarid regions. *A. lebbeck* has been widely distributed around the tropics and mainly planted as a shade tree [26]. It is used in medicine in different purposes. Its wood is dense and used for making cabinet timber and also some types of furniture.

Dalbergia sissoo, belongs to family Fabaceae, and is one of the most useful timber species. It is used for high-quality furniture, cabinets, decorative, veneers, marine and aircraft grade plywood, ornamental turnery, carving, engraving, tool handle and sporting goods. Its root wood is used for tobacco pipes. In village industry, *D. sissoo* is popular for doors and windows. Oil obtained from the seeds is used to cure skin diseases. It is used as a wind break in mango, coffee and tea plantation. The tree nodulates, it therefore improves soil fertility [27].

Using mineral P along with slow release P fertilizer (rock phosphate) plus P bacterial transformats namely *Bacillius megatherium*var. Phosphaticum (phosphorene and mycorrhizal) was very effective in stimulating growth aspects and vine nutritional status as well as improving yield and quality of grapevine crops [28-31]. The solubility of P in triple calcium super- phosphate and slow release P fertilizer added to the soil can be governed by soil type, pH, organic matter and biofertilization. Using different organic manures enriched with B.M. var. phosphaticum as well as the application of mycorrhiza substantially was associated with enhancing the availability of P to plants [32, 33].

This study focused on the response of *Swietenia macrophylla* King , *Albizzia lebbeck* (L.) Benth.) and *Dalbergia sissoo* Roxb seedlings, grown in calcareous soil, to mycorrhiza and phosphate dissolving bacteria inoculation.

MATERIALS AND METHODS

The present investigation was carried out at the experimental farm of Horticultural Research Station of El-Kanater El-Khayria, Qaluobeia Governorate, Egypt, along two successive years (30months starting from March 2018 to August 2020) to study the effects of Mycorrhiza (VAM) and the phosphate dissolving bacteria *Bacillus megatherium* (B.M.) and *Bacillu spolymexa* (B.P) inoculation on seedlings of *Swietenia macrophylla* King*, Albizzia lebbeck* (L.) Benth .and *Dalbergia sissoo* Roxb grown in calcareous soil.

Plant Materials: One-year-old seedlings of *Swietenia macrophylla* king., *Albizzia lebbeck* (L.) Benth. and *Dalbergia sissoo* Roxb*.,* were chosen. The seedlings used were in average of 15, 20 and 18 cm., in height and 0.7, 0.5 and 0.4 cm., in diameter respectively. The seedlings were transplanted on the first week of March 2018 in polyethylene bags of 20 cm. diameter, and 35 cm. depth, filled with 8 kg of calcareous soil transported from Bilpies desert, Sharkia Governorate, Egypt.

Physical and Chemical properties of the used soil were determined according to Black *et al.* [34] as shown in Table (1).

The seedlings were placed in shaded area and common cultural practices including irrigation were followed (twice weekly in winter and four times in summer).Every transplant received starter nutrition consisted of NPK 0.5 gm from Kristalon 19: 19: 19 for bacterial activity.

Microbial Inoculum: Mycorrhiza inoculum of *Glomus marrocarpum* fungi was supplied by the Microbiology Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. While, *Bacillus megatherium* and *Bacillus polymexa* were supplied by the Microbiology Department, Soil, Water and Environment Research Institute, Agricultural Research Center, Giza. Bacillus strains were grown in KB medium (King's B Medium) and incubated at 25°C for three days until early log phase was developed and cell density reached about 10^9 ml^{-1} container medium.

Inoculation of Seedlings: VMA was inoculated according to Menge *et al*. [35]. The gridlines intersect method was used to calculate VAM infection% according to Giovannetti and Mosse [36] which reached 90 %. While, the seedlings received suspensions of bacteria *Bacillus megatherium* (B.M.) and *Bacillus polymexa* (B.P.) three times on April 2018, 2019 and 2020 from planting as a soil drench at the rate of 10 ml per bag. The control plants were left without any biofertilization.

Data Concerned: At the end of the experiment) August 2020), the vegetative growth parameters were recorded including seedling height, stem diameter, root length (cm), root and shoot fresh weights (g), as well as shoot/root ratio on fresh weight basis.

Chemical Composition:

- Total chlorophylls, was determined according to the method described by A.O.A.C [37].
- Carbohydrates (% D.W) in stem was estimated using the method recommended by Dubois *et al.* [38].
- Nitrogen content was determined using the modified micro Kjeldahl method as described by Pregl [39].
- Phosphorus content was estimated using the method recommended by King [40].
- Potassium content was estimated using the flame spectrophotometer method of Piper [41].

Layout of Experiment: A number of 150 seedlings for each tree species was selected for this study then divided to 6 treatments, each treatment included 25 seedlings in 5 replicates (5 seedlings/replicate), which were arranged in a complete randomized design. All the obtained data were subjected to analysis of variance according to Snedecor and Cochran [42]. The new (LSD) was used to compare the average of the determined parameters.

Table 1: Soil physical and chemical analyses

pH	EC (dsm^{-1})	Calcium carbonate%	Coarse sand%	Fine sand%	Clay%	Silt%	Textural grade
8.19	6.45	1.7	19.88	56.70	4.12	19.30	Calcareous

Water soluble cation (m mole L^{-1})				Water soluble anion (m mole L^{-1})				Available nutrients			
Ca^{++}	Mg^{+}	Na^{+}	K^{+}	Co_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}	Available N (ppm)	Available P (ppm)	Available K (ppm)	S.P.
22.31	9.11	30.48	2.00	0.0	2.34	42.0	19.56	35.85	6.45	185.15	17.00%

RESULTS AND DISCUSSION

Vegetative Growth: Data in Tables (2) showed that inoculation with Vesicular- arbuscular mycorrhiza (VAM) or phosphate dissolving bacteria *Bacillus megatherium* (B.M.) or *Bacillus polymexa* (B.P.)to growing media had generally favorable effects on the vegetative growth parameters, compared to noninoculated seedlings (control). Also, results showed that the combined treatments of VAM with phosphate dissolving bacteria had significant effect on growth parameters, for all plant species.

Concerning the effect on seedling heights, the tallest plants were obtained with VAM + B.P. giving (104.00 and 95.00 cm.) for *Swietenia macrophylla* and *Albizzia lebbeck* seedlings with percentages of increases by 48.6 and 50.8% over control, respectively, while *Dalbergia sissoo* seedlings recorded the highest value when seedlings were inoculated by VAM+B.M (117.0 cm) achieving increase by 51.95% over control.

For stem diameter, inoculation with VAM + phosphate dissolving bacteria gave the thickest stems compared to control. The mixture of VAM + B.M. gave the highest value with *Swietenia macrophylla,* (2.60 cm) recording increase by 63% over control, while the mixture of VAM + B.P. recorded the highest values of stem diameter (2.40 and 1.70cm) for *Albizzia lebbeck* and *Dalbergia sissoo* seedlings with 118.18 and 112.5% increases over control respectively.

Regarding root length, data showed that inoculation of VAM or phosphate dissolving bacteria positively affected the root length for seedlings of all species comparing with control, but the highly significant values were obtained with VAM+B.P. recording (36.00, 33.00 and 33.00 cm) with 63.64, 83.33 and 94.12 % increases over control for *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo* seedling respectively.

From the previous results it's worthy to notice that applying the mixture of VAM+ phosphate dissolving bacteria as soil drench boifertilizers produced healthy seedlings.

Data in Table (3) indicated highly significant increases in fresh weights of shoots and roots of seedlings inoculated by VAM and bacterial strains either alone or as a mixture. The highest increments of fresh weights of roots and shoots were observed with using the mixture of VAM + B.M., (28.00, 19.00, 16.00, 42.00, 36.00 and 41.00 g.) for *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo* seedlings, respectively. It is observed that the increments in fresh weights of roots represented 133.3,116.7 and 75 % over control in the plants treated with VAM+B.M, VAM+B.P and VAM alone, respectively in *Swietenia macrophylla* during extended growing season (30 months), the same line was observed with *Albizzia lebbeck* and *Dalbergia sissoo* seedlings.

Also, data in Table (3) showed that the highest significant values in fresh weight of shoot of *Swietenia macrophylla ,Albizzia lebbeck* and *Dalbergia sissoo* seedlings were recorded by adding both VAM+B.M or VAM+ B.P compared with all used treatments. On the other hand, the lowest ones were obtained by control treatments.

Concerning shoot/roots fresh weight ratio of studied seedlings, data in Table (3) showed that this ratio in *Swietenia macriphylla* seedlings significantly reduced with inoculation by microorganism's strains, (VAM + B.M or VAM + B.P) compared with control consequently, the reduction in shoot/roots ratio F.W. was 9.87% than control, which mean that the roots increased in inoculated seedlings compared to non- inoculated ones. On the other hand, shoot/roots ratios of *Albizzia lebbeck* and *Dalbergia sissoo* seedlings exhibited that control gave the lowest values of 1.51 and 1.22. So the results of *Swietenia macrophylla* and *Albizzia lebbeck* more affected by inoculation with microorganism's strains as a mixture or alone, while *Dalbergia sissoo* seedlings were more affected by the inoculation with mycorrhiza fungi (VAM).

Chemical Analyses:.

Total Chlorophylls (mg/g F.W.) and Total Carbohydrates (%D.W.): Regarding total chlorophylls, data in Tables (4) indicate that there were significant increases in leaves content of chlorophyll in seedlings inoculated with VAM+B.P .giving (4.91, 5. 68 and 5.83 mg/ g F.W.), representing 22.44, 41.65, 41.85% increases over controls for *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo*, respectively. Statistically, all other treatments showed insignificant differences in the chlorophyll contents.

Concerning the effects of micro-organism inoculation on the content of total carbohydrates (%) of *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo* seedlings, the obtained values ranged from 22.11, 10. 79 and 9.42% in controls recording increases by (24.07% with B.P., 12.14% with VAM and 9.61% with B.M.) for the studied seedlings respectively.

Table 2: Effect of mycorrhiza and phosphate dissolving bacteria treatments on increment of seedling height, stem diameter and root length at the end of the experiment

	Swietenia macrophylla			*Albizzia lebbeck*			*Dalbergia sissoo*		
Treatments	Seedling height (cm)	Stem diameter (mm)	Root length (cm)	Seedling height (cm)	Stem diameter (mm)	Root length (cm)	Seedling height (cm)	Stem diameter (mm)	Root length (cm)
Control	70.00	1.60	22.00	63.00	1.10	18.00	77.00	0.80	17.00
VAM	83.00	1.90	33.00	72.00	1.40	23.00	85.00	1.00	25.00
B.M	80.00	2.30	26.00	70.00	1.70	27.00	90.00	1.01	22.00
B.P	81.00	2.02	27.00	74.00	1.90	29.00	93.00	1.06	27.00
VAM+B.M.	102.00	2.60	34.00	93.00	2.20	31.00	117.00	1.06	31.00
VAM + B.P.	104.00	2.50	36.00	95.00	2.40	33.00	110.00	1.70	33.00
LSD at 5 %	0.56	0.08	0.65	0.58	0.09	0.67	0.59	0.09	0.66

VAM = Vesicular Arbuscular Mycorrhiza B.M. = *Bacillus megatherium* B.P.=*Bacillus polymexa*

Table 3: Effect of mycorrhiza and phosphate dissolving bacteria treatments on, fresh weights of roots and shoots and shoot / root ratios at the end of the experiment

	Swietenia macrophylla			*Albizzia lebbeck*			*Dalbergia sissoo*		
Treatments	Root fresh weight (g)	Shoots fresh weight (g)	Shoot/roots ratio fresh weight (g)	Root fresh weight (g)	Shoots fresh weight (g)	Shoot/roots ratio fresh weight (g)	Root fresh Weight (g)	Shoots fresh weight (g)	Shoot/roots ratio fresh weight (g)
Control	12.00	21.00	1.75	10.00	15.00	1.51	9.00	11.00	1.22
VAM	21.00	34.00	1.62	12.00	24.00	2.02	12.00	19.00	1.59
B.M	15.00	28.00	1.89	11.00	29.00	2.64	10.00	26.00	2.64
B.P	16.00	29.00	1.82	16.00	31.00	1.95	14.00	27.00	1.96
VAM+B.M.	28.00	42.00	1.58	19.00	36.00	1.59	16.00	41.00	2.06
VAM+B.P.	26.00	41.00	1.58	19.00	34.00	1.79	15.00	33.00	2.76
LSD at 5 %	1.07	0.72	0.08	1.01	0.77	0.10	0.99	0.71	0.11

VAM = Vesicular Arbuscular Mycorrhiza B.M. = *Bacillus megatherium* B.P.=*Bacillus polymexa*

Table 4: Effect of mycorrhiza and phosphate dissolving bacteria treatments on total chlorophyll in leaves (mg/g F.W.) and total carbohydrates contents in stem at the end of the experiment

	Swietenia macrophylla		*Albizzia lebbeck*		*Dalbergia sissoo*	
Treatment	Total chlorophyll (mg/g.FW.)	Total carbohydrates (%D.W.) in stem	Total chlorophyll (mg/g.F.W.)	Total carbohydrates (%D.W) in stem	Total chlorophyll (mg/g.f.w.)	Total carbohydrates (%D.W.) in stem
Control	4.01	22.11	4. 01	10.79	4.11	9.42
VAM	4.81	24.01	4.37	12.14	4.22	9.33
B.M	4.62	23.91	4.81	11.75	4.43	9.61
B.P	4.50	24.07	4.87	12.03	4.48	9.58
VAM+B.M.	4.73	20.76	5.30	11.60	5.40	8.50
VAM+B.P.	4.91	21,40	5.68	11.77	5.83	8.40
LSD at 5 %	0.13	0.16	0.14	0.14	0.12	0.15

VAM = Vesicular Arbuscular Mycorrhiza B.M. = *Bacillus megatherium* B.P.=*Bacillus polymexa*

Table 5: Effect of mycorrhiza and phosphate dissolving bacteria treatments on N, P and K percentages in leaves at the end of the experiment

	Swietenia macrophylla			*Albizzia lebbeck*			*Dalbergia sissoo*		
Treatments	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	2.01	0.22	0.91	2.12	0.18	1.21	1.27	0.26	0.66
VAM	2.55	0.35	0.96	2.30	0.26	1.23	1.38	0.52	0.66
B.M	2.11	0.33	1.01	2,19	0.20	1.30	1.40	0.50	0.68
B.P	2.42	0.35	1.02	2.22	0.23	1.32	1.40	0.44	0.67
VAM+B.M.	2.50	0.36	1.04	2.73	0.25	1.36	1.41	0.60	0.69
VAM+B.P.	2.47	0.37	1.08	2.55	0.27	1.40	1.41	0.61	0.69
LSD at 5 %	0.11	0.02	0.02	0.11	0.02	0.03	0.10	0.02	0.02

VAM = Vesicular Arbuscular Mycorrhiza *B.M. = Bacillus megatherium B.P.=Bacillus polymexa*

Nitrogen, Phosphorus and Potassium (%D.W.): Data in Table (5) revealed that the application of mycorrhiza and phosphate dissolving bacteria treatments caused significant elevation of N% compared to control in all studied species, and the highest N % values occurred in the seedlings treated with VAM for *Swietenia macrophylla* 2.55% in leaves whilst, the highest values of N% for *Albizzia lebbeck and Dalbergia sissoo* seedlings were 2.73 and 1.41% in leaves, for the treatment of VAM+B.M compared to their control.

Data presented in Table (5) showed that P% in leaves of *Swietenia macrophylla*, *Albizzia lebbeck and Dalbergia sissoo* significantly increased by different inoculation treatments compared to control. The best results were obtained with the treatments of VAM + B.P. which gave 0.37,0.27 and 0.61 P%, followed by those treated with VAM+B.M 0.36, 0.25 and 0.60 P% of seedling of *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo* respectively.

Results in Table (5) showed that K% in leaves of *Swietenia macrophylla*, *Albizzia lebbeck and Dalbergia sissoo* seedlings increased under all inoculation treatments compared to controls of these species which recorded the lowest values of 0.91,1.21 and 0.66 K% respectively. This increase was significant only with the treatment of VAM+B.P which reached to maximum values 1.08, 1.40 and 0.69 K% followed by those treated with VAM+B.M. giving 1.04,1.36 and 0.69 K% respectively.

Effect of Mycorrhiza and Phosphate Dissolving Bacteria Treatments on Soil Properties: Results obtained in Table (6) revealed that, the inoculation by mycorrhiza and phosphate dissolving bacteria had beneficial influences on soil properties.

As regard soil pH, inoculation decreased it with all treatments and the lowest pH was obtained with VAM+ B.P recording decrease percentages of 5.11, 0.85, 0.97% compared to control.

For EC values, also the VAM+B.P inoculation recorded the lowest percentages (1.80, 1.66, 1.65%) over the untreated soil for *Swietenia macrophylla, Albizzia lebbeck* and *Dalbergia sissoo,* respectively.

Concerning soil organic matter (O.M), the data in Table (6) indicated positive effects in increasing the percentage of (O.M) to reach 15.38,15.09, 11.76% for VAM+ B.P inoculation over the control for *Swietenia macrophylla*, *Albizzia lebbeck* and *Dalbergia sissoo*, respectively.

Generally, VAM, B.M and B.P. play the main role in facilitating the presence of phosphorus in the soil in dissolving form through the material it produces, that reduce the acidity of the soil, which brings the triphophosphate to a unique phosphate that can be absorbed by plant roots.

Concerning the effect of mycorrhiza and phosphate dissolving bacteria inoculation on N.P and K contents of the soil, results showed increments with all treatments and the high superiority of the values were obtained with VAM+B.P recording 7.69, 8.26 and 8.34 % for N, 31.21,30,07 and 39.52% for P and 22.73, 23.12 and 20.87% for K over control for *Swieteniamacrophylla, Albizzia lebbeck* and *Dalbergia sissoo,* respectively. These results agree with Mahfouz and Sharaf- Eldin [43]; Hasaneen *et al.* [44]; Runyan and Odorico [3]; Sushanta *et al.* [11] and Ghafoor [13].

It is worthy to mention that the results of the present study indicated that the inoculation of the studied seedlings with VAM and Phosphate dissolving bacteria (PDB) exhibited positive effects on growth parameters of the inoculated plant seedlings. This was previously reported by Mahfouz and Sharaf-Eldin [43] on (*Foeniculum vulgare* Mill.); Hasaneen *et al.* [44] on *Lactuca sativa*; Ahmed and Abada [29]; Shaheen *et al.* [30] and Shaaban [31] on superior grapevine.

Also, it's evident that microorganism's strains, mixture or alone was beneficial for the growth of both roots and shoots also, biofertlization promoted and formed more secondary hairs roots. These results agreed with Motosugi *et al.* [45] on some annual plants as they reported that the highest ratio of roots / shoots and leaves was given by adding biofertlizers and its evident that resulted in more formation of hair roots.

Concerning the effect of biofertilizer on enhancing the content of total chlorophylls and total carbohydrates, the obtained results were in harmony with Shanan and Higazy [46] who found that algalization enhanced the biochemical characters such as carbohydrates and chlorophyll in leaves. Also, Grzesik *et al.* [47] on (*Salix viminalis* L.) reported that the biofertlizers treatments increased the stability of chlorophyll content, intensity and of net photosynthesis.

Prasanna *et al*. [48] on wheat (*Triticum vulgar* L.) and Anand *et al.* [49] on maize crop showed significant increase in chlorophyll a, and Mohsen *et al.* [50] on lettuce plants. The authors found that all different biofertilizer treatments considerably increased chlorophyll content and total carbohydrate.

Table 6: Effect of mycorrhiza and phosphate dissolving bacteria treatments on soil properties at the end of the experiment

	Swietenia macrophylla					
Treatment	pH	EC(dsm^{-1})	O.M. (%)	N ppm	P ppm	K ppm
Control	8.60	6.65	0.52	35.75	7.05	179.9
VAM	8.18	6.55	0.59	36.93	9.11	216.0
B.M.	8.20	6.56	0.59	36.77	8.33	212.2
B.P.	8.19	6.56	0.59	36.80	8.35	212.2
VAM+B.M.	8.23	6.53	0.60	38.46	9.26	220.2
VAM+B.P.	8.16	6.53	0.60	38.50	9.25	220.8
Albizzia lebbeck						
Control	8,22	6.64	0.53	35.84	7.15	179.5
VAM	8.17	6.58	0.54	38.11	9.58	215.0
B.M.	8.19	6.59	0.54	37.70	8.46	216.3
B.P.	8.19	6,59	0.58	37.20	8.48	216.0
VAM+B.M.	8.16	6.53	0.58	38.80	9.26	220.6
VAM+B.P.	8.15	6.53	0.61	38.80	9.30	221.0
Dalbergia sissoo						
Control	8.23	6.66	0.51	35.87	7.06	179.2
VAM	8.17	6.59	0.54	34.40	9.80	215.1
B.M.	8.20	6.60	0.54	36.80	8.41	214.0
B.P.	8.19	6.60	0.54	36.78	8.35	214.60
VAM+B.M.	8.17	6.58	0.57	38.80	9.99	216.0
VAN+B.P.	8.15	6.55	0.57	38.86	9.85	216.6

VAM = Vesicular Arbuscular Mycorrhiza B.M. = *Bacillus megatherium* B.P.=*Bacillus polymixa*

These results are in agreement with Hammad *et al*. [18] on *Taxodium disticum*; Nelson and Achar [19] on *Brassica oleracea*, Al Harbi *et al.* [12] and Ghafoor [13] on wheat, who explained that improving effects arising from microbial inoculation are due to dissolving phosphate to be available form by plants, and producing growth promoting substance such as auxins.

Many results are inconclusive, but encouraging enough to improve selection procedures and the production of quality inocula for practical application.

Despite only a small proportion of angiospermic species having been examined, mycorrhiza form a ritualistic of relationship with the roots of nearly eighty percent of such plant species [5]. Arbuscular mycorrhiza (AM) symbiosis can promote host plant growth by increasing the uptake of mineral nutrition such as P, Zn, and Cu [16]. AM Fungi and plant roots, improve water and nutrient uptake like phosphorus, nitrogen and micronutrients and thus enhance plant growth [51].

As mycorrizal inoculation mediated processes involved the availability of phosphate and other nutrients in the soil. Arbuscula rmycorrhizal (AM) fungi are found among the soil flora and interact with approximately 85%of the plants on the ground [52].

Mycorrhizae are obligate fungi that predominate in the roots and soil of higher plants. They form association with plant roots in a host-nonspecific manner. They also increase the resistance in plants against plant pathogens and surface area of root system for better absorption of nutrients from soil. Therefore, they can be used as bio-fertilizer and as biocontrol agents. The primary establishment effect of AM is the improvement of phosphate uptake by plants due to the ability of the external mycelium of AM fungi to act as a bridge between roots and the surrounding soil microhabitats. This gives access to the phosphate ions from the soil solution beyond the phosphate- depletion zone surrounding the roots [53]. The AM fungi can contribute to P capture and supply, by linking the biotic and geochemical portions of the soil ecosystem, therefore affecting P cycling rates and patterns in both agricultural and natural ecosystems [54].Phosphate dissolving bacteria (PDB) solubilize insoluble P by producing chelating substances and various organic acids and hence this available P is taken up by plants [55-57]. The hyphae length density of plants inoculated with PDB and AMF was longer than the other plants inoculated with AMF alone and there was significant difference between them. That is could be attributed to the role of PDB which increased the total percentage of root colonization by native AMF by producing phytohormones which apparently stimulate mycorrhizal infection, the phosphate made available by PSB (phosphate solubilizing bacteria) acting on sparingly soluble P sources may not reach the root surface due to limited diffusion, it was proposed that if the solubilized phosphate was taken up by an AM mycelium. This synergistic microbial interaction should improve P supply to the plant [58].

In the present investigation, results revealed that the combined inoculation of VAM+ B.P. or B.M. significantly increased seedling height, over the treatments of PSB or VAM. These results are consistent with earlier reports of Kalavathi *et al.* [59]. It is relevant to mention that the possible synergistic effect would be the uptake by AM fungal hyphae and translocation into the plant of P released by PSB in soil [21, 23].

Certain plant hormones like IAA and GA produced by the bacterial culture might induce the growth of other associated organisms like VAM [58].

Thus, using of these biofertilizers treatments VAM +B.P. or B.M. produced vigour growth of *Swietenia macrophylla*, *Albizzia lebbeck* and *Dalbergia sissoo* seedlings as well as healthy timber seedling stock at nursery level, for forest plantation projects.

CONCLUSION

It is necessary to mention that the use of biofertilizers is important for cheap production and, also to provide hard currency for the import of mineral fertilizers, in addition to improve the soil and reducing air pollution resulting from the oxidation of mineral nutrients, in addition to the utilization of non-absorbable phosphorus. In order to get the most out of it, you must combine various microorganisms that are useful for feeding the plant. From the results of our study it's worthy to be recommended that applying VAM+ phosphate dissolving bacteria to the plants as bio fertilizers for the production of healthy seedlings of *Swietenia macrophylla Albizzia lebbeck* and *Dalbergia sissoo* seedlings especially when grown in calcareous soil.

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