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Screening of Mineral Solubilizing Microbes and Rhizobium for Growth Promotion and Development of *Acacia leucocephala* Grown under Nursery Conditions

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ARTICLE INFORMATION	ABSTRACT
Corresponding author: Nibha Gupta E-mail: nguc2003@yahoo.co.in	The application aspect of microbial strains especially mineral solubilization in individual and /or combination under glasshouse and field conditions with respect to enhancement in plant of <i>Acacia leucocephala</i> was the main objective of the present for which 11 fungi, 5 bacteria, and 11 isolates of Rhizobia had been used. Screening of ten fungal species and 5 bacterial isolates
Keywords:	for their effects on the growth of Acacia leucocephala, done under pot culture
Acacia leucocephala Phosphate solubilization Rhizobium Penicillium Aspergillus Growth	in conditions, elucidated the best performance of the combination of PF3 (<i>Penicillum crysogenum</i> Thom. 1) and IF5 (<i>Cunninghmella elegans</i> Lendn.) and <i>Rhizobium</i> isolates R10 and /or R11 in <i>A. leucocephala</i> . The combination of selected microbial inoculants for the individual test tree species was evaluated for their individual performance and /or in combination with the other selected inoculants in a specific ratio of their inoculums. Under the dual inoculation
Received: 28.07.23 Received in revised form: 02.08.23 Accepted: 04.08.2023	experiment, selected mineral solubilizers (fungi/bacteria) were evaluated along with the combination of different isolates of Rhizobium and resulted in the final formulation of <i>Aspergillus</i> sp. + <i>Cunnighmella elegans</i> with <i>Rhizobium</i> isolate no. 11 tree legumes tested. The present study done under controlled greenhouse conditions has provided an experimental approach to adopt pre- inoculation of the symbiotic microbes to seedlings in the nursery for better

plantation results.

INTRODUCTION

The application of chemical fertilizer provides nutrition in high concentration in the soil and plants. However, the entire contents would not be absorbed by the plants and the remaining parts would bind to the soil. Microorganisms are useful for biomineralization of bound soil and make nutrients available to their host and / or its surroundings. Microorganisms facilitate plant mineral nutrition by changing the amounts, concentrations and properties of minerals available to plants. These changes lead to change in growth, development and chemical composition of plant that are common and substantial enough to encourage the exploitation of plant microbe interaction for improvement of crop productivity. There are various groups of organisms that can solubilize and / or leaching of phosphate, iron and other mineral metals. (Kumar et al.2004; Afzal et al.2005; Mehrvarz et al. 2008; Patil et al. 2002; Mehrvarz and Chaichi, 2008; Reis et al. 2008). All



tropical legumes fix the atmospheric nitrogen by Rhizobium which requires optimum level of phosphorus in plant tissue. Their seedlings establish better in presence of mineral solubilizers because more of the tropical soils are phosphate fixing and make it unavailable to the plants (Dabas and Kaushik, 1998; Sahgalet al. 2004; Tilaket al. 2005; Hameeda et al. 2008; Gupta et al. 2007;Huda et al. 2007).

It is known that a large number of seedlings of forest species useful for afforestaion in dry deciduous conditions are required for raising plantations. Microbial application at the nursery stage was also found to be useful in enhancing productivity in some forest trees like Albizzia, Acacia and Dalbergia etc. (Rahangdale and Gupta, 1998; Sahgalet al. 2004; Thatoi et al. 1993). Acacia auriculoformis, Acacia nilotica, and Acacia leucocephala are three legumes suitable for agroforestry because their litter contains more than 2% nitrogen (Puri, 1960; Mishra and Sharma, 2004). It is however equally important to screen some suitable bioinoculants for these species.

Acacia leucocephala or Leucaena leucocephala is the most productive and versatile multi-purpose legume tree in tropical regionsits plantation in degraded land helps in the recovery of soil microbiological properties (Valpassos et al. 2007; Ruiz et al. 2006; Forrester et al. 2006; Line et al.2006). Acacia leucocephala has been the focus of a great deal of research in the past few decades for its nitrogen fixing potential (Hogberg and Kvarnstrom, 1982;Sanginga et al. 1985 a and b). The high nitrogen fixing potential of this tree is related to its abundant nodulation under specific soil conditions (Hogberg and Kvarnstrom, 1982; Lulandala and Hall, 1986; Halliday and Somasegaran, 1982). In view of the above-cited aspects of plant-microbe interaction, their potential towards biomineralization of unavailable sources of minerals and elements and application in transplantation tree legumes useful for the revegetation and reclamation of disturbed land, encourage us to evaluate the microflora for their effect on growth and development of some tree legumes at nursery conditions.

MATERIALS AND METHODS

1. Microbial strains used

Six phosphate solubilizing fungi, PF1 (*Penicillium grisefulvum* Diercks.), PF2 (*Penicillium restrictum* Gilman & Abott.), PF3 (*Penicillium chrysogenum* Thom. 1), PF4 (*Aspergillus species* 1), PF5 (*Aspergillus ornatus*

Raper, Fernele, Tresner.) and PF6 (*Aspergillus wentii*), four iron leaching fungi IF1 (*Penicillum expansum*), IF4 (*Paecilomyces variotii*), IF5 (*Cunnighamella elegans*) and IF6 (*Penicillium chrysogenum* Thom 2), five phosphate solublizing bacteria and 11 *Rhizobium* were used for the inoculation studies (The *Rhizobium* strains 1a, 18, 20, 28a, 6, 23, 5a, 16a, 29, 13 and 7a were selected and re-coded as R1, R2, R3, R4, R5, R6, R7, R8, R9, R10 and R11 respectively).

2. Experimental

1) The experiment was set at the glass house of the Regional Plant Resource Centre at a temperature of $35\pm2^{\circ}C$ & and $80\pm5\%$ relative humidity in Pot size: 8x11'' polybags containing 2.5 kg soil. The soil contains 83.8% of sand, 8.8% of slit, and 7.4% of clay. The textural class of the soil was loamy sand the soil pH was 6.27. The salt content of the soil was 0.504. The nitrogen (N), phosphate (P2O5), and potassium (K2O) of the soil were 168.7Kg/Ha, 237.2kg/Ha, and 645.12kg/Ha respectively.

2) Screening experiment

One factor at a time i. e. individual microbial culture was used to inoculate the poly pot 25ml of 7-day-old culture prepared in Czapekdox medium (4.5 pH) was added to each pot prior to seed sowing. 25 ml of 5day-old culture prepared in potato dextrose broth (7.0 pH) was added to each pot prior to seed sowing. The experiment was set in 20 replications. Pretreated, and healthy seeds (3 no.) per pot. Daily watering was done through a sprinkler mist system. Finally, observations were recorded of 120 days of plants for Shoot height, Number of leaves, leaflets, Branches developed, and fresh and dry biomass of leaves.

3) Dual inoculation experiment

In the second phase of the experiment, the two best microbial strains were selected for each tree legume on the basis of their performances in the previous experiment. The experiment was set up according to the first experiment done on screening except the different microbial inoculums used for specific tree legumes. The experimental sets were taken into consideration. [1] Control: without any inoculation, [2] PF4: 50ml in each pot., [3] IF5: 50ml in each pot, [4] PF4+IF5: 50ml of PF4 + 50ml of IF5 in each pot, [5] PF4 (more)+IF5: 100ml of PF4 + 50ml of IF5 in each pot, [6]



4) Dual Inoculation with Rhizobium:

PF4+IF5 (more): 50ml of PF4 + 100ml of IF5 in each pot.

In second phase of experiment, the two best microbial strains were selected on the basis of their performances in the previous experiment. The experiment was set up according to the first experiment done on screening except for the different combinations of microbial inoculum used for specific tree legumes. [1] controluninoculated, [2] inoculated with PF4, [3] Inoculated with IF5 [4] Individual inoculation of Rhizobium (11 no.), [5] Inoculation with PF4 and Individual Rhizobium, [6] Inoculation with IF 5 with individual Rhizobium.

5. Growth analysis

Growth parameters were recorded for the experimental plants such as shoot height (in cm), root length (in cm), number of leaves, leaflets and branches, total seedling height (in cm), collar diameter (in mm), fresh and dry biomass of leaf, stem, root, shoot and seedling (in gram) (Al-Garni, 2006, Sahet al. 1998 and Tewari et al. 2006). Statistical analysis for one-way ANOVA was done by following Sockal et al.1981).

6. Soil analysis

Basic Soil analysis was done by Solution Analyser (Sandeep Instrument). After the experiment was completed the potting soil was subjected to analysis. For each treatment, the soil was pooled for each replication. 1 gram soil was added to 10 ml of distilled water and stirred. The soil dilution was subjected to analysis for pH, (Mishra et al. 2002; Sangha and Jalota, 2005; Chanderet al. 1998). Soil N, P, K was analyzed by wet oxidation method through commercial laboratories (Greenwood et al. 2001).

RESULTS

Effect of microbial inoculation on Acacia leucocephala

The plants of *A. leucocephala* grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and other plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, fungal strains PF 4 (Aspergillus species 1), IF 1 (PaecilomycesvariotiiBainier.), and IF 5 (Cunnighamella elegancs) showed better effects in enhancing plant height, number and biomass of leaves as compared to other microbial inoculants. Bacterial strains also performed well in improving plant health. Finally, these two fungal strains PF4 and IF5 were selected for further experimentations on dual inoculations (Table1). In Acacia leucocephala different growth parameters were observed. In the number of leaves, the highest value exhibited by PF4 i.e. 69.60 ± 15.66 and then in IF5 i.e. 47.60 ± 14.51. In the case of a number of leaflets 324.60 ± 80.39 was observed in the case of PF4 which is highest than 239.40 ± 68.82 in the case of IF5. The best result in the case of root length was observed in the case of PF3 i.e. 24.68 ± 2.67 and in PF2 i.e. 23.06 ± 2.55. In the case of shoot height highest result was observed in the case of PF4 i.e. 62.72 ± 12.42 then in IF5 i.e. 53.62 ± 21.77. In the case of fresh biomass of leaves best result exhibited by PF4 i.e. 5.52 ± 1.37 and PF6 i.e. 4.15 ± 0.90. In the case of dry biomass of leaves PF6 and PF1 exhibited better results than others i.e. 3.34 ± 1.75 and 2.28 ± 0.90. In the case of collar diameter IF4, IF5, and IF6 were found to be the best among others. By analyzing the growth parameters we have selected PF4 and IF5 from the primary screening to be used for the secondary screening. The result was found in PF3 i.e. 21.40 ± 1.67mm than in IF5 and PF2 i.e. 21.00 ± 0.70mm and 21.00 ± 2.54mm, respectively. From the primary screening fungi PF3 and bacteria PB6 found to be the best among treatments were selected for the secondary screening.

Effect of dual inoculation on Acacia leucocephala

The plants of *A. leucocephala* grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, fungal strains PF 4, and IF 5 in equal quantity showed better



effects in enhancing plant height, number, and biomass of leaves as compared to other treatments. (Table 2). In the case of secondary screening in case of the number of leaves and leaflets best result was by the single inoculation of IF5 i.e. 28.29 ± 6.15 cm root length and 67.86 ± 16.20 cm shoot height, but the combination of PF4 and IF5 in 1: 1 ratio attained a shoot height of 65.42 ± 19.96 cm. In the case of fresh biomass of leaves, the best result was obtained by PF4 (M) + IF5 i.e. 2.42 ± 1.75 . In the case of the

exhibited by the combination of PF4 and IF5 in the combination of 1: 1 i.e. 63.00 ± 32.54 no. of leaves and 269.60 ± 142.89 no. of leaflets. In the case of root length and shoot height, the best result was obtained collar diameter of the plant best result was obtained by a single inoculation of IF5 (28.20 ± 10.28 mm). By observing all the parameters we found that the combination of PF4 and IF5 in the ratio of 1: 1 found to be the best.

Treatments	No. of Leaves	Shoot height (cm)	Fresh biomass	Dry biomass
			leaves (gm)	leaves (gm)
Control	29.80 ±10.77	29.96 ±11.47	1.08 ±0.87	1.63 ±0.57
PF1	45.40 ±15.04	39.90 ±5.10	2.28 ±0.90	3.19 ±1.00
PF2	24.60 ±4.82	20.22 ±9.55	0.99 ±0.69	0.73 ±0.21
PF3	17.00 ±4.35	21.10 ±14.01	3.06 ±0.91	1.45 ±2.29
PF4	69.60 ±15.66	62.72 ±12.42	5.52 ±1.37	1.41 ±0.57
PF5	38.40 ±9.71	38.14 ±9.47	1.90 ±1.14	0.92 ±0.79
PF6	43.20 ±19.48	34.50 ±11.20	4.15 ±0.90	3.34 ±1.75
IF1	47.40 ±8.50	44.56 ±4.50	4.00 ±1.26	2.62 ±1.00
IF4	41.00 ±9.19	48.24±9.00	2.68 ±1.76	0.57 ±0.61
IF5	47.60 ±14.51	53.62±21.77	3.18 ±3.24	3.16 ±1.61
IF6	38.00 ±15.52	42.12±8.32	3.63 ±1.00	2.65 ±1.21
PB2	46.00 ±17.14	42.96±7.46	2.94 ±1.30	1.52 ±0.43
PB3	40.40 ±14.65	47.38±8.54	2.12 ±1.61	0.99 ±1.26
PB4	32.60 ±17.68	40.20±13.93	3.07 ±0.95	2.12 ±1.61
PB5	28.60 ± 4.72	45.38±7.40	2.36 ±0.84	0.73 ±0.62
PB6	39.40 ±10.73	51.88±8.74	3.03 ±0.53	2.62 ±0.60

Table -1: Growth performance of Acacia Teacocephala under screening experiment	Table -1: Growth	performance of Acacia	leucocephala under s	creening experimen
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PF1 (*Penicillium grisefulvum* Diercks.), PF2 (*Penicillium restrictum* Gilman &Abott.), PF3 (*Penicillium rysogenum* Thom. 1), PF4 (*Aspergillus* species 1), PF5 (*Aspergillus ornatusRaper*, Fernele, Tresner.) and PF6 (*Aspergillus wentii*), four iron leaching fungi IF1 (*Penicillum expansum*), IF4 (*Paecilomyces variotii*), IF5 (*Cunnigham ellaelegans*) and IF6 (Penicillium crysogenumThom 2), PB2: Streptomyces sp.1, PB3: Micrococcus luteus, PB 4 : Micrococcus luteus, PB5: Micrococcus varians, PB6 : Streptomyces sp. 2

Table -2 Effect of dual inoculation of selected n	microbes on the growth of Acacia leucocephala
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parameters	1	2	3	4	5	6
Leaf no.	38.60 ± 23.86	41.40 ± 16.16	42.60 ±	63.00 ±	50.80 ±	50.60 ±
			10.74	32.94	9.52	16.83
Leaf lets no.	165.40 ±	167.20 ± 76.32	248.20 ±	269.60 ±	308.00 ±	265.00 ±
	90.15		76.32	142.89	46.92	97.49
Root length (cm)	21.64 ± 8.45	26.14 ± 6.15	28.20 ± 6.15	22.68 ±	24.50 ±	23.26 ±
				5.14	5.66	1.93
Shoot length (cm)	55.52 ± 18.71	49.60 ± 8.83	67.86 ±	65.42 ±	73.18 ±	73.36 ±
			16.20	19.96	21.99	8.46
Biomass leaf (g)	1.64 ± 0.67	1.52 ± 0.63	2.65 ± 0.58	2.61 ± 1.68	6.09 ± 2.10	4.34 ±
					**	2.52*

Dry biomass leaf(g)	0.85 ± 0.43	1.07 ± 0.59	1.80 ± 0.32	2.42 ± 1.75	2.38 ± 0.86	2.40 ± 1.26
± Standard deviation		·	·			
Abbreviations for tre	atments					
1- control, 2- PF4 (Aspergillus sp. 1) 3 - IF5 (Cunnighmella elgans), 4 -both fungal strains equal amount, 5- both						

fungal isolates in 2:1 ratio, 6- both fungal isolates in 1:2 ratio

Effect of microbial inoculants and Rhizobium on *Acacia leucocephala*

The plants of A. leucocephala grown under different treatments (47) along with control untreated plants exhibited good growth in terms of plant height, biomass and plant parts. Besides this, significant variations could be observed among all the treatments in affecting plant growth performances as compared to uninoculated control. However, the combination of PF4, IF5 and R11 showed better effects in enhancing the number and biomass of leaves as compared to other treatments (Table -3). In the dual inoculation or the third experiment, the parameter shoot height showed the best result in single inoculation of IF5 (60.08 ± 9.43cm), second best was single inoculation of PF4 (52.56 ± 6.55cm) and then PF4 + R9 (51.08 ± 7.87cm). In case of root length highest value obtained from PF4 + R9 i.e. 27.46 ± 12.32cm then PF4 + R6 i.e.22.46 ± 2.30cmthen PF4 + R5 i.e. 26.58 ± 2.79. In the case of total seedling height best result was obtained by a single inoculation of IF5 (80.38 ± 11.25cm) then single inoculation of PF4 (43.34 ± 6.56cm) then single inoculation of R2 (73.06 ± 7.74cm). In case of no. of leaves highest value obtained from PF4 + IF5 + R11 (39.80 ± 9.98) then PF4 + IF5 + R10 (39.20 ± 7.56) then PF4 + R6 (37.60 ± 8.32).in the case of no. of primary leaflets highest value obtained from PF4 + IF5 + R11 (210.20 ± 49.97) then IF5 + R5 (192.20 ± 45.36) then PF4 + IF5 + R10 (189.00 ±19.09). In case of collar diameter best result found in PF4 + IF5 + R10 (22.60 ± 1.52mm), then PF4 + IF5 + R11 (22.60 ± 1.10mm) then PF4 + R6 (21.80 \pm 1.80mm). In the case of fresh biomass of leaves best result was found in the case of IF5 + R6 (7.47 ± 1.26g) then PF4 + IF5 + R11 (7.28 ± 0.87g) then PF4 + R11 (7.26 ± 1.24g). In the case of dry biomass of leaves best result was obtained from PF4 + IF5 + R11 (4.71 ± 1.19g) then PF4 + R11 (4.55 ± 1.39g) then PF4 + IF5 + R8 (4.41 ± 1.27g). In the case of fresh biomass of root best result obtained by PF4 + $IF5 + R3 (2.85 \pm 1.53g)$ then in PF4 + IF5 + R5 (3.81 ± 0.57g) then PF4 + IF5 + R11 (2.57 ± 0.49g). In the case of dry biomass of root highest value was obtained by

PF4 + IF5 + R5 (1.63 \pm 0.96g), then PF4 + IF5 + R3 (1.60 \pm 1.11g) then PF4 + IF5 + R11 (1.43 \pm 0.27g). By analyzing the result of the growth parameter of *Acacia leucocephala* we found out that the combination of PF4 (*Aspergillus* species 1), IF5 (*Cunnighamella elegans*) and R10 (*Rhizobium* species) and the combination of PF3, IF5 and R11 (*Rhizobium* species) found to be the best inoculums for the plant. Though the test plants were found to be infected with Rhizobium a very poor performance was observed as far as root nodule formation is concerned. Hence total number of nodules, size and structure could not be recorded.

DISCUSSION

The present study revealed the suitability of some inoculantsfor enhancing growth and biomass and indirect P uptake in test plants when compared with the uninoculated control. Among them, fungal inoculants exhibited better performance in providing benefits of mineral uptake to these plants in general. The findings on better growth performance of test plants showed the impact of inoculation and affirmed their potential in afforestation of problematic soil. The plants of Acacia leucocephala grown under different treatments along with control untreated plants exhibited good growth in terms of plant height, biomass, and plant parts with significant variations. Though all microbial strains perform better in laboratory conditions may vary in field conditions, and screening and selection of microbial inoculants for the development of biofertiliser are needed. Their affectivity depends upon the type of host they associate with. It is clearly evident that the uninoculated plants of A. leucocephala exhibited better growth as compared to the few fungal inoculants used in this study. Inoculations with bacterial strains did not show better performance in seedling growth compared to control.

Test plants of *A. leucocephala* were inoculated by the most effective mineral solubilizers. Such plants have



double symbiotic benefits in terms of nitrogen and phosphorus that allow plants to grow well under experimental conditions. Inoculation results have been quite promising in the tree species taken. Microbial inoculation of tree legume species with mineral solubilizes and nitrogen-fixing organisms not only enhanced the nutrient content in the aboveground plant material but also provided a wellbalanced and regulated nutrient supply due to an enlarged and symbiotically associated root system developed in such plants. The amelioration of microflora to developing seedlings is useful for certain plantation programs.

Table 3-	Effect of	selected	inoculants	in combination	with	Rhizobium	isolates
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	SHOOT HEIGHT (IN CM)	ROOT LENGTH (IN CM)	TOTAL SEEDLING HEIGHT (IN CM)	NUMBER OF LEAVES	NUMBER OF PRIMARY LEAFLETS
CONTROL	43.66 ± 8.16	15.16 ± 4.43	58.82 ± 5.46	21.20 ± 4.02	136.80 ± 75.29
PF4	52.56 ± 6.55	20.78 ± 1.53	73.34 ± 6.56	28.20 ± 6.10	144.20 ± 28.71
IF5	60.08 ± 9.34**	20.30 ± 2.72	80.38 ± 11.25**	28.00 ± 4.74	146.00 ± 14.78
R1	47.06 ± 7.79	19.88 ± 2.13	66.94 ± 8.27	16.40 ± 8.38	140.80 ± 53.22
R2	51.38 ± 5.56	21.68 ± 4.70*	73.06 ± 7.74	24.00 ± 1.58	156.80 ± 35.52
R3	45.32 ± 9.51	19.68 ± 2.29	65.00 ± 9.35	23.80 ± 3.63	124.20 ± 19.20
R4	28.12 ± 2.93	17.92 ± 5.30	46.04 ± 6.57	26.60 ± 5.77	145.20 ± 24.63
R5	37.52 ± 6.95	18.14 ± 2.23	55.66 ± 6.86	32.40 ± 7.13*	161.80 ± 36.24
R6	38.38 ± 3.19	26.16 ± 2.26**	64.54 ± 4.53	34.80 ± 5.31**	178.40 ± 28.04
R7	35.12 ± 4.87	16.70 ± 3.47	51.82 ± 6.22	23.40 ± 5.46	125.60 ± 33.09
R8	34.70 ± 4.50	17.42 ± 4.39	52.12 ± 5.87	24.60 ± 1.67	125.20 ± 15.58
R9	49.08 ± 6.43	19.64 ± 3.10	68.72 ± 7.74	35.00 ± 11.22**	182.40 ± 54.59
R10	39.94 ± 2.44	20.76 ± 2.83	60.70 ± 1.29	29.00 ± 2.65	179.20 ± 41.97
R11	46.06 ± 5.02	18.04 ± 3.25	64.10 ± 6.05	24.40 ± 7.89	130.00 ± 38.63
PF4 + R1	48.16 ± 7.75	21.72 ± 2.26*	69.88 ± 5.66	30.00 ± 4.30	152.40 ± 16.41
PF4 + R2	43.08 ± 12.25	13.58 ± 2.31	56.66 ± 13.78	26.80 ± 3.70	146.60 ± 17.81
PF4 + R3	39.64 ± 9.32	19.64 ± 1.29	59.28 ± 9.04	26.40 ± 3.13	146.60 ± 26.49
PF4 + R4	40.06 ± 7.42	22.08 ± 2.10*	61.14 ± 7.70	31.80 ± 7.22*	163.40 ± 34.83
PF4 + R5	44.24 ± 6.99	26.58 ± 2.79**	70.82 ± 4.63	33.00 ± 9.62*	172.60 ± 52.69
PF4 + R6	40.08 ± 8.41	22.46 ± 2.30*	62.54 ± 7.17	27.40 ± 7.30	144.00 ± 38.65
PF4 + R7	41.50 ± 8.17	19.62 ± 6.35	61.12 ± 8.20	31.00 ± 5.15	169.20 ± 11.34
PF4 + R8	49.42 ± 5.97	20.38 ± 3.09	69.80 ± 7.00	27.40 ± 2.19	147.00 ± 12.53
PF4 + R9	51.08 ± 7.87	27.46 ± 12.32**	79.26 ± 15.13**	26.00 ± 4.74	132.00 ± 27.99
PF4 + R10	48.82 ± 9.65	19.12 ± 5.82	67.94 ± 14.20	19.80 ± 3.11	102.60 ± 16.40
PF4 + R11	47.80 ± 9.70	21.60 ± 1.10	69.40 ± 10.14	28.80 ± 8.53	143.40 ± 48.67



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IF5 + R1	50.66 ± 4.91	20.04 ± 2.41	70.70 ± 7.23	30.60 ± 6.15	153.00 ± 33.48
IF5 + R2	42.96 ± 4.81	20.30 ± 2.11	63.28 ± 4.10	30.80 ± 5.12	162.60 ± 17.77
IF5 + R3	40.64 ± 5.72	19.76 ± 4.91	60.40 ± 9.28	28.00 ± 2.00	141.00 ± 11.96
IF5 + R4	44.20 ± 3.85	22.00 ± 2.90*	66.20 ± 5.75	26.00 ± 3.81	132.00 ± 23.10
IF5 + R5	44.30 ± 12.55	21.50 ± 3.74	65.80 ± 12.83	37.60 ± 8.32**	192.00 ± 45.76
IF5 + R6	48.58 ± 13.34	22.44 ± 3.77*	71.02 ± 14.72	34.80 ± 7.26**	180.20 ± 33.12
IF5 + R7	52.08 ± 6.83	20.24 ± 2.48	72.32 ± 5.22	27.00 ± 8.46	167.40 ± 53.95
IF5 + R8	40.40 ± 5.21	21.04 ± 3.37	61.44 ± 2.00	24.80 ± 3.42	154.80 ± 50.53
IF5 + R9	41.48 ± 5.47	18.68 ± 2.36	60.26 ± 6.63	23.00 ± 2.45	119.60 ± 11.35
IF5 + R10	50.56 ± 7.43	18.58 ± 2.79	69.10 ± 9.53	24.60 ± 5.03	137.40 ± 35.62
IF5 + R11	45.82 ± 6.26	18.38 ± 2.62	64.20 ± 5.47	26.00 ± 2.92	145.60 ± 16.01
PF4 + IF5 + R1	44.08 ± 7.89	18.98 ± 2.36	63.06 ± 9.54	26.40 ± 2.61	156.00 ± 20.05
PF4 + IF5 + R2	38.24 ± 6.51	13.82 ± 4.73	52.06 ± 4.46	21.40 ± 3.05	138.40 ± 62.14
PF4 + IF5 + R3	49.22 ± 4.59	15.38 ± 4.58	64.60 ± 8.31	28.80 ± 5.36	151.00 ± 29.78
PF4 + IF5 + R4	48.86 ± 6.97	19.90 ± 4.45	68.76 ± 6.41	32.80 ± 11.69*	173.60 ± 45.42
PF4 + IF5 + R5	44.54 ± 6.69	16.22 ± 3.08	60.76 ± 6.05	29.80 ± 4.44	155.20 ± 23.97
PF4 + IF5 + R6	43.58 ± 6.07	18.26 ± 2.24	61.84 ± 5.83	31.40 ± 6.91	163.20 ± 25.59
PF4 + IF5 + R7	40.46 ± 7.90	20.40 ± 0.84	60.86 ± 8.20	34.80 ± 2.17**	166.60 ± 16.56
PF4 + IF5 + R8	37.00 ± 8.33	20.26 ± 3.08	57.26 ± 10.11	33.40 ± 6.69*	173.20 ± 34.69
PF4 + IF5 + R9	39.30 ± 6.53	18.14 ± 1.31	57.44 ± 7.23	30.80 ± 3.56	158.60 ± 7.60
PF4 + IF5 + R10	46.68 ± 7.80	19.48 ± 3.62	66.16 ± 6.54	39.20 ± 7.56**	189.00 ± 19.05
PF4 + IF5 + R11	45.10 ± 12.59	22.34 ± 3.32*	67.44 ± 14.28	39.80 ± 9.98**	210.20 ± 49.97*

	COLLAR DIAMETER (IN MM)	FRESH BIOMASS OF LEAF (IN GRAM)	DRY BIOMASS OF LEAF (IN GRAM)	FRESH BIOMASS OF ROOT (IN GRAM)	DRY BIOMASS OF ROOT (IN GRAM)
CONTROL	19.80 ± 0.84	3.00 ± 0.81	1.58 ± 0.41	1.56 ± 0.17	0.70 ± 0.16
PF4	20.20 ± 3.63	4.36 ± 2.07	2.50 ± 1.19	2.05 ± 0.81	0.88 ± 0.24
IF5	19.00 ± 2.00	$4.96 \pm 0.99^*$	2.70 ± 0.51	1.93 ± 0.49	1.24 ± 0.61
R1	20.80 ± 0.84	4.97 ± 0.89*	$2.99 \pm 0.60^*$	1.40 ± 0.25	0.67 ± 0.20
R2	21.20 ± 5.81	$5.03 \pm 0.99^*$	$3.05 \pm 0.92^*$	2.32 ± 1.14	1.22 ± 0.88
R3	20.00 ± 1.41	5.13 ± 1.81*	3.14 ± 1.36**	2.23 ± 0.68	1.25 ± 0.45
R4	18.80 ± 2.17	4.62 ± 0.70	2.77 ± 0.50	1.73 ± 0.39	1.00 ± 0.14
R5	18.60 ± 2.68	4.92 ± 0.99	2.86 ± 1.08*	1.93 ± 0.62	1.02 ± 0.36
R6	20.00 ± 0.71	$6.58 \pm 0.75^{**}$	4.14 ± 0.56**	2.03 ± 0.82	1.04 ± 0.42

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R7	21.00 ± 1.41	4.60 ± 2.03	2 81 + 1 1/	1.84 ± 0.02	0.91 + 0.45
R8	20.00 + 1.22	$5.12 + 1.60^*$	2.01 ± 1.14 2.67 ± 0.75	1.04 ± 0.32	1.03 ± 0.51
R9	19.40 + 2.41	3.94 + 0.63	2.23 ± 0.46	1.95 + 0.50	1.02 ± 0.45
R10	19.80 + 1.30	6.04 + 1.60**	3.68 + 1.50**	1.42 + 0.41	0.75 + 0.33
R11	19.80 + 2.39	5.43 + 0.82**	3.25 + 0.60**	1.46 + 0.34	0.79 ± 0.28
PF4 + R1	20.00 ± 0.71	$5.56 \pm 0.66^*$	$3.19 \pm 0.47^{**}$	1.72 ± 0.94	0.97 ± 0.54
PF4 + R2	20.80 ± 0.84	$5.32 \pm 1.16^*$	2.81 ± 0.74	1.58 ± 0.44	1.05 ± 0.47
PF4 + R3	20.60 ± 1.14	$5.03 \pm 0.45^*$	2.49 ± 0.28	1.77 ± 0.62	0.89 ± 0.34
PF4 + R4	21.80 ± 1.30	$5.29 \pm 0.47^*$	2.79 ± 0.32	2.02 ± 0.44	1.03 ± 0.29
PF4 + R5	21.00 ± 1.00	4.67 ± 1.36	2.78 ± 0.75	1.85 ± 0.53	0.91 ± 0.34
PF4 + R6	21.80 ± 2.68	5.31 ± 1.81*	3.00 ± 1.18*	1.49 ± 0.66	0.85 ± 0.34
PF4 + R7	20.00 ± 1.22	5.36 ± 0.74**	3.00 ± 0.96*	1.76 ± 0.48	0.97 ± 0.32
PF4 + R8	20.00 ± 3.00	5.34 ± 0.81**	3.07 ± 0.45*	1.81 ± 0.65	0.91 ± 0.39
PF4 + R9	19.20 ± 2.68	4.64 ± 1.04	2.85 ± 1.14*	1.80 ± 0.35	0.97 ± 0.36
PF4 + R10	20.80 ± 3.11	4.58 ± 0.34	2.68 ± 0.24	1.65 ± 0.80	0.83 ± 0.42
PF4 + R11	20.60 ± 1.14	7.06 ± 1.24	4.55 ± 1.39**	1.28 ± 0.47	0.78 ± 0.33
IF5 + R1	18.40 ± 2.61	4.20 ± 0.72	2.51 ± 0.65**	1.50 ± 0.42	0.70 ± 0.38
IF5 + R2	20.60 ± 1.67	5.92 ± 0.84**	3.26 ± 0.65**	1.32 ± 0.63	0.64 ± 0.31
IF5 + R3	19.40 ± 3.85	6.66 ± 0.80**	3.78 ± 1.01**	1.63 ± 0.46	0.93 ± 0.23
IF5 + R4	19.60 ± 2.97	5.92 ± 0.71**	3.53 ± 0.84**	1.67 ± 0.53	1.00 ± 0.19
IF5 + R5	18.30 ± 2.28	5.92 ± 0.43**	3.45 ± 0.37**	1.92 ± 0.40	1.13 ± 0.23
IF5 + R6	20.00 ± 2.00	7.47 ± 1.25**	5.04 ± 1.02**	2.10 ± 0.07	0.99 ± 0.47
IF5 + R7	20.00 ± 1.22	$5.04 \pm 0.20^*$	3.34 ± 0.63**	1.79 ± 0.32	1.02 ± 0.33
IF5 + R8	20.80 ± 1.30	5.07 ± 2.48*	3.18 ± 1.54**	1.54 ± 0.18	1.00 ± 0.38
IF5 + R9	19.80 ± 2.39	5.15 ± 0.78*	3.10 ± 0.66**	1.53 ± 0.31	0.94 ± 0.36
IF5 + R10	19.80 ± 1.30	6.08 ± 1.07**	3.87 ± 0.53**	1.78 ± 0.56	0.99 ± 0.44
IF5 + R11	21.20 ± 1.10	$5.00 \pm 0.96^*$	3.17 ± 0.59**	1.96 ± 0.48	1.03 ± 0.25
PF4 + IF5 +	18.40 ± 1.82	4.99 ± 1.03*	$3.20 \pm 0.92^{**}$	2.09 ± 0.34	1.33 ± 0.30
PF4 + IF5 + R2	19.80 ± 1.48	6.58 ± 0.78**	4.36 ± 0.81**	2.15 ± 0.38	1.36 ± 0.38
PF4 + IF5 +	20.80 ± 1.64	5.78 ± 0.90**	3.47 ± 0.88**	2.85 ± 1.53*	1.60 ± 1.11*
R3 PF4 + IF5 + R4	21.20 ± 1.48	4.72 ± 0.76	2.77 ± 0.27	1.84 ± 0.25	0.94 ± 0.32
PF4 + IF5 + R5	21.00 ± 1.00	6.41 ± 1.07**	4.01 ± 0.97**	2.82 ± 0.58*	1.65 ± 0.96**
PF4 + IF5 + R6	19.80 ± 2.28	4.61 ± 0.86	2.61 ± 0.63	1.83 ± 0.44	0.98 ± 0.43
PF4 + IF5 + R7	20.40 ± 2.19	4.55 ± 1.22	2.64 ± 0.80	1.88 ± 0.29	1.08 ± 0.33
PF4 + IF5 + R8	21.60 ± 0.89	6.37 ± 0.69**	4.41 ± 1.27**	1.98 ± 0.47	1.34 ± 0.68
PF4 + IF5 + R9	21.40 ± 0.89	5.85 ± 0.49**	3.68 ± 0.73**	2.03 ± 0.21	1.27 ± 0.15
PF4 + IF5 + R10	22.60 ± 1.52	6.07 ± 1.50**	3.58 ± 1.41**	2.15 ± 0.70	1.18 ± 0.33
PF4 + IF5 + R11	22.20 ± 1.10	7.28 ± 0.87**	4.71 ± 1.19**	2.57 ± 0.49	1.43 ± 0.27

Screening of ten fungal species and 5 bacterial isolates for their effects on the growth of Acacia

leucocephala done under pot culture in greenhouse conditions, elucidated the best performance of the

combination of PF3 (Penicillumcrysogenum Thom. 1) and IF5 (CunninghmellaelegansLendn.) and Rhizobium isolates R10 and /or R11 in A. leucocephala.

Penicilliumchrysogenum Thom. (1) showed to be the suitablefor A. leucocephalain increasing the plant dry biomass of leaves and total shoot dry biomass, as the biomass increment (P<0.001). Variation among different inoculants used in this study is well reflected in the growth and development of host species (Rahangdale and Gupta, 1998).

Microbial inoculations may also increase the dualroot shoot ratio, leaf numbers and length of the branches of host plants (Al-Garni, 2006). The findings of increment in the number of branches, and leaf area of inoculated A. leucocephala plants (12 no. per plant) over control (3.0 per plant). Are corroborated with studies of Weih and Nordh (2005) on Salix sp.. Who stated that the total leaf area of the pot-grown plants is a better predictor of shoot biomass and branches in the field than the pot-grown plants. The result showed that the selected inoculants contributed to a higher rate of shoot growth amongst 15 no. of microbial strains tested, Pencilliumchrysogenum Thom. 1 was found to be the most effective in increasing plant height, biomass and morphological quality of seedlings among all the inoculants used.

Nodulated legumes generally have а high requirement for phosphorus to generate ATP which is required for nitrogenase function. It was observed that growth and nodulation increase by use of phosphate fertilizers (Huda et al.2007). Most tropical soils are phosphate fixing, use of mineral solubilizers from microbial origin may make them available to the host plants. Microbial inoculants are also found to be useful in enhancing the growth of forest tree seedlings grown under stress conditions (Dabas and Kaushik, 1998; Dash et al. 2013). The plants of Acacia leucocephala inoculated with different phosphate solubilizersand iron ore leaching fungi also exhibited good growth in terms of plant height, biomass and plant parts compared to the uninoculated control. Besides this, significant variations could be observed among all the treatments in affectingplant growth performances. Bacterial strains performed poorly in improving plant health grown under this experiment. Increase in plant height of tree seedlings over uninoculated control is indicative of the potential effect of these inoculants. The suitability of Penicilliumchrysogenum and Cunninghmellaelegans.

(1) are observed to be an effective inoculants for this tree species influencingthe plant dry biomass of leaves and total shoot dry biomass (P<0.05 & P<0.001).

It is clearly evident that microbial inoculations respond differentially towards the growth and development of host species. It became evident that fungal inoculation is likely to increase the number of branches in seedlings. Enhancement in the number of branches may lead to the development of tree crowns. Jankiewicz and Stecki (1976) reported that branching patternsindicate the type and form of tree crown.

The leguminous species possess physiological specialization relative to rhizobial affinities and also exhibit symbiotic promiscuity. Allen and Allen (1958) have considered that plant is the dominant partner in symbiosis and that nodule formation merits recognition. In the present study, the nodulation pattern in A. leucocephala was very poor. Since the study was carried out for only four months; the role of inoculated Rhizobium and their effects on the performance of host plants is difficult to be interpreted. However, during screening experiments evaluation of bioinoculants of mineral for solubilizersespecially phosphate solubilizing microbes and Rhizobium isolates, the seedlings of Acacia leucocephala was found to be manifested with root nodule though it was very poor and could not be recorded for its number, size and structure. Hence, the role of Rhizobium inoculants in the performance of test plants can not be interpreted well.

Many of the tropical leguminous trees are reported to be fixing atmospheric nitrogen through nodule development, endowed with VA mycorrhizaland other microbial associations. Plantation of such species enriches poor soils, seedlings fortified with microbial inoculations may lead to the successful in establishment of plants in poor sites (Sahet al. 1998 and Sahgalet al. 2004). The effects of mineral solubilizersneed to be evaluated under field conditions, on a long-term basis, before the application of these inoculations to plantation seedlings is considered a viable proposition.

CONCLUSION

The present study done under controlled greenhouse conditions has provided an experimental approach to



adopt pre-inoculation of the symbiotic microbes to seedlings in the nursery for better plantation results. This aspect may become very practicable and costeffective proposition in plantation programs in tropicalconditions. The present study was restricted with the time schedule and the working environment, further studies may be taken for the development of the package of practice for the tree legumes useful for of reclamation of wastelands and revegetation of barren and/or overburdened mine lands. However, the outcome of the present study is very important due to the requirement of microbial manifestation in the tree improvement programin a stressful environment.

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