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Biotechnological Approach to Enhance the Growth and Biomass of *Tectona grandis* Linn. F. (Teak) Seedlings

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Abstract

Bio-fertilizers play a significant and complex role in plant growth of forestry species, which have been proved in the present study. In the present investigation various bio–fertilizers were applied alone and in combination. The growth and biomass response of T. grandis were enhanced due to inoculation of bio-fertilizers. This was primarily due to amelioration of soil pH, organic matter per cent, phosphorus, nitrogen and other nutrients which were assimilated by plants with higher capacity in the presence of microbial inoculants. In neutral pH range (6.5 to 7.5), the bio-fertilizers were found to be more effective. In the case of organic matter effects of bio-fertilizers were found to be more effective with the increase of organic matter in soil.

The results revealed that soil organic matter and pH plays an important role in determining plant growth and survival. The bio-fertilizers, which are now in use world wide in integrated plant nutrition system to ameliorate soil condition showed better response for teak in this study.

Keywords: *Biotechnology, organic matter, ph, biofertilizers, biomass*

1. Introduction

Decreasing non-renewable reserves- all over the world and increasing cost of chemical fertilizers have necessitated the demand for alternative renewable sources to meet the need of plant nutrients. Bio-fertilizers are an effective, cheap and renewable supplement to chemical fertilizers. Considering the problem of chemical fertilizers it has been globally recognized to incorporate bio-fertilizers in integrated plant nutrition system (IPNS) for meeting the nutritional demand of plants. In this system judicious combination of fertilizers, organic manure and bio-fertilizers are applied to provide ideal nutrition for plants and their optimum utilization along with maintenance of soil productivity.

At present when the country is passing unprecedented crisis in availability of chemical fertilizers, the importance of bio-fertilizer is increasing day by day. More over harmful effects of chemical fertilizers obligate users to think of using bio-fertilizers which are eco-friendly and economically viable.

Bio-fertilizers are group of microorganisms consisting of bacteria, fungi, algae etc. These alone or in combination are known to be increasing plant growth by way of various biochemical activities in the soil. Mainly there are two groups of biofertilizers i.e. symbiotic and non symbiotic. Symbiotic group comprises *Rhizobium, Frankia* (Nitrogen fixing

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organism) and *Mycorrhizae*. (especially for phosphorus) and covers most of the terrestrial and aquatic plant community. While non-symbiotic group includes *Azotobactor*, *Azospiricullum, Pseudomonas* etc. living in free environment. The role of each micro-organism is very specific and plants interact with them to fulfil their requirement for various minerals. Organic matter interaction and VAM fungi was reported first for sustainable agriculture production in tropics. Brechelt (1988) have studied the different levels of organic matter with VAM fungi in Capsicum production. The application of bio-fertilizers in agriculture sector is well recognized but is lacking in the forestry sector. The use of bio-fertilizer practice is scarce in forest nurseries and plantations.

Soil organic matter and pH play an important role in determining plant growth and survival. Usually both the factors vary in forest soil due to climatic and edaphic changes. Higher organic matter is known to enhance better plant growth, while optimum ranges of pH is needed for particular plant species.

The bio-fertilizers, which are now in use world wide in integrated plant nutrition system to ameliorate soil conditions, also show differential response under varying status of soil organic matter and pH. However, no screening of bio-fertilizer under various levels of pH and organic matter has been done so far. Application practices of various bio-fertilizers are being increased in various plantations to improve degraded soil and to increase the bio-mass per unit area. These are showing excellent results in plant growth by better utilization of nutrients. But in some cases the performance of bio-fertilizers was noticed to be unappreciable due to some limitations of microbial species. The present paper deals to search out the scope of different bio-fertilizers inoculation in soil having variation in pH and organic matter.

2. Materials and Methods

The study was conducted in the green net house of State Forest Research Institute, Jabalpur (M.P.) India. *Tectona grandis* (Teak) was selected for the study. Keeping in view, the teak has commercial value and demand. Isolation, authentication and mass multiplication of PSB and Azotobacter was done adopting standard procedure by Subba Rao (1984). VAM was isolated and mass multiplied as per methods described by Seiverding (1991). The detailed procedure is described as under:

1. Preparation of Potting Mixture and Adjustment of pH

Potting mixture consisting of Soil: Sand: FYM in 1:1:0.5 ratios were analyzed for their initial pH. Subsequently adding of HCL and NaOH was made as per initial pH value of potting mixture. Finally the pH levels were adjusted to 5.5, 6.5, 7.5, 8.5 and 9.0. 40 poly-bags were prepared for each pH level (Table 1).

2. Adjustment of Organic Matter

The initial content of organic matter was analyzed from potting mixture. The potting mixture of desired organic matter levels was prepared by activated Charcoal, compost materials and sand etc. On the basis of organic matter value of soil, five levels of organic matter were prepared as 0.67, 2.08, 3.25, 4.03 and 5.17%. Total 400 poly-pots (80 for each levels) were prepared (Table 2) with potting mixture having different levels of organic matter.

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3. Production of Teak seedlings

The seedling production was done in green net house of State Forest Research Institute. Phenotypic superior seeds of Teak were sown in beds and after germination; plants were transplanted in to the polythene bags. One month old poly-bag seedlings were used for the study.

4. Inoculation of bio-fertilizers:

VAM, PSB and Azotobacter were selected for the study to screen on different pH and organic matter levels. A mixture of 20g VAM, l0g PSB and l0g Azotobacter was inoculated separately in different pH level poly-pots. While 20g VAM, l0g PSB and l0g Azotobacter was inoculated separately and in combinations in teak seedlings. For the inoculation 3-4 holes of size 3-5 cm depth were made around the root zone of each seedlings and inoculation of VAM, PSB, Azotobacter in both isolation and in combination was made into these holes. Subsequently, the holes were filled up with soil followed by watering of the plants. Control seedlings were also maintained for the purpose of comparison.

5. Experimental Design:

Randomized block design was adopted.

In case of different pH levels four treatments viz; Control-TO, VAM-T1, PSB- T2 and Azotobacter-T3, (Table 3) and for different levels of organic matter seven treatments viz; Control (T0), VAM (T1), PSB (T2), Azotobacter (T3), VAM+PSB (T4), VAM+Azotobacter (T5), PSB+Azotobacter (T6) and VAM+PSB+Azotobacter (T7) were taken. In each treatment, 10 seedlings were used.

6. Observations

After 1 year of bio-fertilizer inoculations, following observations were taken to assess the response of seedlings inoculated with the bio-fertilizers:

(a) Growth performance (Height and Girth).
(b) Plant dry matter (Bio-mass).
(c) Soil nutrient status

7. Soil Nutrient Analysis

pH of soil was estimated by pH meter in 1:2.5 soil water ratio. Organic matter content in soil was estimated using Wakley and Black (1934) method. Nitrogen estimation was done through auto Kjeltec 2300, while estimation of available phosphorus in soil was made by extraction with $NaHCO_3$ (Olsen *el al*., 1954) and potassium using Flame Photometer.

8. Measurement of growth and biomass

The representative sample of 10 plants from each treatment was selected randomly (3 from each treatment) for growth performance. The height and girth of seedlings were measured by tape. The dry biomass was estimated after keeping plant material in oven at 70°C for 3 days.

The biomass yield improvement percentage was calculated on the basis of total biomass as per the formula given below:

$$\text{Improvement percent} = \frac{\text{Biomass of treated plant}}{\text{Biomass of control (untreated) plant}} \times 100$$

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3. Results and Discussion

1. Growth Performance

The growth performance of *Tectona grandis* in different treatments under different pH and organic matter levels showed variation to considerable extent. The result of study was shown in Table 5 and 6.

The height of *Tectona grandis* was found significantly increased with 7.5 pH, while height in other pH levels (5.5, 6.5, 8.5 and 9.0) remained non-significant at $P<0.05$ after 1 year of inoculation. However, inoculation of VAM, PSB and Azotobacter showed improved plant height in all the pH levels under study in comparison to un-inoculated seedlings. The height of teak seedlings was found maximum (15.90cm in VAM, 15.67 in Azotobacter, 14.10cm in PSB and 12.67cm in control) in neutral (7.5) value of pH. The minimum height of seedlings was observed in 9.0 pH. Among the bio-fertilizers the maximum height was obtained with Azotobacter in 5.5, 8.5 and 9.0 pH value, while VAM gave better results with pH value of 7.5 (Table-5). It was also observed that the height of teak seedlings increased with the increasing of pH values in soil and pH onwards 7.5 showed decreasing trends in the plant height.

Similarly, the inoculation of VAM, PSB and Azotobacter in isolation as well as in combination had improved plant height in all the organic matter levels in comparison to un-inoculated seedlings. The effect of different bio-fertilizers in various organic matter levels was found significant. However, the most beneficial effect of bio-fertilizer pertaining to seedlings height was found in dual inoculation of VAM and Azotobacter (T5) in all the organic matter levels. The seedlings were found healthy with feeder roots in the presence of VAM and Azotobacter. With the increasing of organic matter level up to 3.25% height of seedlings was found increasing significantly, however, it showed a slight decreasing trend thereafter with more increase in organic matter (Table 6). The maximum height of seedling was found in soil with 3.25 per cent organic matter followed by 4.03 percent and 5.17 per cent, while the minimum height was observed in 0.67 per cent organic matter level.

2. Biomass Production

Promising results were obtained with regard to bio-mass (Table 5 & 6) production in relation to bio-fertilizer inoculation. The variation in total bio-mass production was observed among different pH and organic matter levels. The bio-mass production of plant was found to be better with VAM in all the pH levels in comparison to biomass observed in PSB. It was observed that the maximum bio-mass production of plants was found in 7.5 pH value followed by 6.5 and 5.5 pH, while the minimum bio-mass was found in 9.0 pH of soil, followed by 8.5 pH. The benefits of bio-fertilizer in increasing the total bio-mass are calculated in term of improvement percentage (Table 4). The results revealed that VAM improved 70.84, 41.74 and 26.83% bio-mass over control with 7.5, 6.5 and 8.5 pH values, respectively. Similarly, Azotobacter improved biomass production of teak plants up to 38.95, 33.03, 32.475, 11.09 and 2.86% over control with 7.5, 6.5, 5.5, 8.5 and 9.0 pH levels, respectively after 1 year of inoculation. The PSB also improved 30.55%, 23.06%, 16.97%, 15.72% and 4.06% of biomass with 5.5, 8.5, 6.5, 7.5 and 9.0 pH values. However, it was found that with the increase of pH values, the biomass production of plants in all the biofertilizers treatments was found to be increasing up to 7.5 pH value, but subsequently showing decreasing trend thereafter.

Perhaps the greatest general influence of pH on plant growth is its effect on the availability of nutrients. A good overall nutrient availability is found near pH range from 6.5 to 7.5 in

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high base status soil. Soil pH is related to the percentage of base saturation. When the base saturation is less than 100 per cent, an increase in pH is associated with an increase in the availability of calcium and magnesium in the soil solution, resulting to increase in plant growth. At low pH, molybdenum toxicity for plants increases in some soils. Phosphorus and boron also tend to be unavailable in very acidic soils. Potassium availability is usually good in neutral and alkaline soil that reflects the limited leaching of exchangeable potassium (Mohammad, 1993). The availability of some nutrients was found decreasing with an increase in pH. Phosphorus and boron also tend to be unavailable in calcareous soils that results from reaction with calcium, copper and zinc reducing availability in both highly acidic and highly alkaline soils.

In case of different organic matter levels, the variation in total biomass production was also observed. The maximum (8.94g / plant) biomass production was found with 3.25% organic matter level having 82.45% improvement over control, followed by 4.035 and 5.17 % organic matter levels. In teak it was found that with the increase of organic matter levels the biomass production of plants was increased. The improvement per cent of biomass in various treatments of biofertilizers over control was maximum with 5.175% of organic matter followed by 4.03% in combined inoculation of VAM and Azotobacter (T5). It revealed that the biofertilizers performed well in higher levels of organic matter than the lower levels. The biomass and height of plants increased with more availability of organic matter but after the level of 3 .25% of organic matter the growth of plants in terms of height and biomass was found almost stable.

The inoculation of VAM, PSB and Azotobacter both in isolation and in combination resulted in improvement in growth and biomass of *Tectona grandis* in all the pH and organic matter levels. However, higher level of organic matter percentage and neutral value pH gives better height and biomass production of plants. According to previous experiments, the improvement in growth and biomass of inoculated seedlings was due to the uptake of higher nutrients through VAM (Marschner, H and Dell, 1994; Smith *et al*., 1994) and secretion of phospho-enzymes and higher availability of phosphorus through PSB and through fixation of nitrogen and secretion of other growth promoting substances by Azotobacter (Bongale and Nadiger, 1989). In this study, variations were recorded in growth and bio-mass with different pH and organic matter levels as influenced with bio-fertilizers.

In case of the different organic matter, the maximum biomass production was found in 3.25% organic matter level over control followed by 4.03 and 5.17% organic matter level. The plant height and biomass of teak seedlings with 6.5 to 7.5 pH and soil organic matter of 3.5 to 5.17% levels proved statistically significant over control and other treatment (Annexure -1 & 2).

3. Soil Nutrient Status

In both the experiments (pH and organic matter level) soil nutrient status was increased with biofertilizers inoculation. Available nitrogen, phosphorus and potassium contents were found maximum with 6.5 and 7.5 pH levels and with 2.08 and 3.25% organic matter levels. Nitrogen content was found maximum in VAM and VAM + Azotobacter inoculated soil. The availability of phosphorus and potassium status depleted in soil even with biofertilizers inoculation with PSB and VAM + PSB + Azotobacter as compared to control and other combinations of biofertilizers.

Plant growth and biomass mainly depends on soil nutrient reserve which is available to plant. But once the microbial inoculants are inoculated, soil nutrients status is affected due to its fast mobilization and assimilation by the plant. This has resulted in to decreasing of soil N and P status which is compensated as per the efficiency of inoculants. The improvement in

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soil P and N with PSB and Azotobacter is attributed to solubilisation of insoluble phosphate (Raj *et al*., 1981) and fixation of atmospheric nitrogen (Subba Rao, 1984) respectively. Depletion of soil P was found with VAM inoculated soil which might be due to greater harvesting of P by plants with extra radical mycelium beyond root zone (Hatting, 1975). Similarly low nutrients status was also observed in some cases even after microbial inoculations was done which may be due to its higher removal by growing plants (Brady, 1990) than the rate of nutrient mineralization.

Table 1. Various pH Level, Potting Mixture (Soil: Sand: FYM, 1:1:0.5) and HCL / NaOH Quantity

SN	pH levels	No of poly-pots	HCL/NaOH quantity
1	5.5	40	1.2 lt. (0.1 N HCL)
2	6.5	40	0.6 lt. (0.1 N HCL)
3	7.5	40	Nil (Normal)
4	8.5	40	0.6 lt. (10% NaOH)
5	9.0	40	1.8 lt. (10% NaOH)
Total No. of bags		**200**	

Table 2. Treated Organic Matter Level, Potting Mixture Ratio and No. of Poly-bags

SN	Organic matter level (%)	Potting mixture ratio			No. of level (%)
		Soil	Sand	Charcoal/compost material	
1	0.67	1	2	0	80
2	2.08	1	1	0.5	80
3	3.25	1	0.5	0.5	80
4	4.03	1	0.5	1	80
5	5.17	1	0	1	80
		Total No. of polybags			**400**

Table 3. Treatments for Various pH Levels (5.5, 6.5, 7.5, 8.5 and 9.0)

SN	Treatments	Biofertilizers doses	No. of poly-bags
1	Control (T0)	NA	10
2	VAM (TI)	20g / bag	10
3	PSB (T2)	l0g/bag	10
4	*Azotobacter* (T3)	10g/bag	10

Table 4. Treatment for Various Organic Matter Levels (0.67, 2.08, 3.25, 4.03 and 5.17%)

Treatment No.	Treatments	Bio-fertilizer doses	No. of polybags
T0	Control	-	10
T1	VAM	20g / bag	10
T2	PSB	l0g/bag	10

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T3	*Azotobacter*	l0g/bag	10
T4	VAM + PSB	20+10g / bag	10
T5	VAM + *Azotobacter*	20+10g / bag	10
T6	PSB + *Azotobacter*	l0+l0g / bag	10
T7	VAM+PSB+ *Azotobacter*	20+10+10g / bag	10

Table 5. Effect of Bio-fertilizers on Growth, Bio-mass Production of Teak and Nutrient Status of Soil under Varying pH Levels

SN	Treatments	Growth			Available nutrients		
		Height (cm)	Biomass (g/plant)	Improvement (%)	N (%)	P (ppm)	K (%)
			5.5 pH				
1	Control	9.73	4.25	00.00	0.16	2.70	0.01
2	VAM	12.10	6.01	41.41	0.20	8.39	0.02
3	PSB	12.17	5.54	30.55	0.17	9.43	0.01
4	*Azotobacter*	12.43	5.63	32.47	0.22	8.01	0.02
			6.5 pH				
1	Control	10.30	4.36	00.00	0.18	2.61	0.02
2	VAM	13.93	6.18	41.74	0.24	6.79	0.03
3	PSB	13.90	5.10	16.97	0.20	7.08	0.02
4	*Azotobacter*	13.73	5.80	33.03	0.26	6.09	0.06
			7.5 pH				
1	Control	12.67	4.39	00.00	0.17	2.51	0.02
2	VAM	15.90	7.50	70.84	0.22	6.93	0.03
3	PSB	14.10	5.08	15.72	0.21	7.08	0.02
4	*Azotobacter*	15.67	6.10	38.95	0.24	6.94	0.06
			8.5 pH				
1	Control	10.57	4.51	00.00	0.15	2.10	0.01
2	VAM	13.67	5.72	26.83	0.10	6.39	0.03
3	PSB	14.10	5.55	23.06	0.18	6.42	0.02
4	*Azotobacter*	14.73	5.01	11.09	0.20	6.09	0.04
			9.0 pH				
1	Control	8.97	4.19	00.00	0.10	1.90	0.01
2	VAM	10.07	5.13	22.43	0.14	5.90	0.03
3	PSB	10.13	4.36	4.06	0.12	6.12	0.02
4	*Azotobacter*	10.27	4.31	2.86	0.17	5.84	0.03

Table 6. Effect of Bio-fertilizers on Growth, Bio-mass Production of Teak and Nutrient Status with Reference to Soil under Varying Organic Matter (O.M.) Levels

TN	Treatments	Growth			Available nutrients		
		Height (cm)	Biomass (g/plant)	Improvement (%)	N (%)	P (ppm)	K(%)
			0.67% O.M.				
T0	Control	13.2	3.56	0	0.06	2.84	0.03
T1	VAM	18.3	4.54	27.53	0.23	5.74	0.05
T2	PSB	18	4.04	13.48	0.42	5.47	0.03
T3	*Azotobacter*	16.2	4.17	17.13	0.27	5.12	0.02
T4	VAM + PSB	17.6	4.66	30.9	0,21	5.23	0.05
T5	VAM + *Azotobacter*	20.4	4.01	12.64	0.24	5.43	0.05
T6	PSB + *Azotobacter*	12.9	4.04	13.48	0.22	5.5	0.06
T7	VAM+PSB+ *Azotobacter*	18.1	4.62	29.78	0.21	5.94	0.04

TN	Treatments	Growth			Available nutrients		
		Height (cm)	Biomass (g/plant)	Improvement (%)	N (%)	P (ppm)	K(%)
			2.08% O.M.				
T0	Control	15.7	4.19	0	0.08	3.13	0.04
T1	VAM	18.4	4.9	16.95	0.3	11.74	0.05
T2	PSB	18.1	4.4	5.01	0.28	14.36	0.06
T3	*Azotobacter*	17.8	4.5	7.4	0.21	9.34	0.05
T4	VAM + PSB	18.1	5.15	22.91	0.27	10.8	0.05
T5	VAM + *Azotobacter*	21.6	5.3	26.49	0.36	12.81	0.08
T6	PSB + *Azotobacter*	17.8	4.8	14.56	0.24	11.94	0.04
T7	VAM+PSB+ *Azotobacter*	18.3	5.5	31.26	0.32	13.44	0.06
			3.25% O.M.				
T0	Control	16.2	4.9	0	0.08	3.39	0.03
T1	VAM	20.5	6.18	26.12	0.31	4.79	0.03
T2	PSB	15.7	5	19.33	0.27	4.4	0.05
T3	*Azotobacter*	15.9	5.1	18.57	0.24	4.04	0.04
T4	VAM + PSB	21.1	7.67	56.53	0.27	5.14	0.04
T5	VAM + *Azotobacter*	24.5	8.94	82.45	0.29	4.93	0.03
T6	PSB + *Azotobacter*	16.5	5.7	16.33	0.26	5.79	0.05
T7	VAM+PSB+ *Azotobacter*	23.2	8.3	69.39	0.3	6.7	0.06
			4.03% O.M.				
T0	Control	16.2	4.45	0	0.07	2.87	0.02
T1	VAM	21.2	6.14	37.98	0.21	2.93	0.04
T2	PSB	19.9	5.76	29.44	0.24	3.43	0.05
T3	*Azotobacter*	18.4	5.66	27.19	0.32	3.74	0.05
T4	VAM + PSB	17.6	5.32	19.55	0.34	5.97	0.04
T5	VAM + *Azotobacter*	24.2	8.93	100.67	0.37	4.08	0.05
T6	PSB + *Azotobacter*	19.1	6.69	50.34	0.24	6.11	0.03
T7	VAM+PSB+ *Azotobacter*	22.1	7.93	78.2	0.33	6.34	0.04
			5.17% O.M.				
T0	Control	15.7	3.94	0	0.04	2.71	0.02
T1	VAM	18.3	6.11	55.08	0.29	2.34	0.04
T2	PSB	17.2	5.62	42.64	0.3	3.78	0.03
T3	*Azotobacter*	17	5.6	42.13	0.31	3.73	0.04
T4	VAM + PSB	16.9	5.03	27.66	0.29	4.89	0.05
T5	VAM + *Azotobacter*	22.8	8.5	115.74	0.34	3.09	0.06
T6	PSB + *Azotobacter*	19.2	6.61	67.77	0.29	5.38	0.04
T7	VAM+PSB+ *Azotobacter*	20.9	7.07	79.44	0.26	6.24	0.05

Annexure-1. One-way ANOVA-1. Effects of biofertilizers on growth and biomass of teak seedlings under varying pH level in soil

		Sum of Squares	df	Mean Square	F	Sig.
Height	Between Groups	155.066	4	38.767	21.861	0.000
	Within Groups	97.535	55	1.773		
	Total	252.601	59			
Biomass	Between Groups	10.314	4	2.578	4.412	0.004
	Within Groups	32.143	55	0.584		
	Total	42.457	59			

*The mean different is significant at 0.05% level

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Annexure-2. One-way ANOVA-2. Effect of bio-fertilizers on growth of teak seedlings under varying organic matter in soil

		Sum of Squares	df	Mean Square	F	Sig.
Height	Between Groups	122.795	4	30.699	4.884	0.001
	Within Groups	722.890	115	6.286		
	Total	845.684	119			
Biomass	Between Groups	97.790	4	24.448	19.133	0.000
	Within Groups	146.945	115	1.278		
	Total	244.735	119			

*The mean different is significant at 0.05% level

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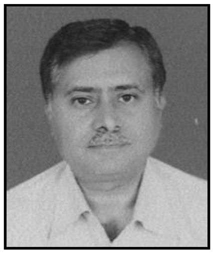

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