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Effect of Arbuscular Mycorrhizal (AM) Fungi and Plant Growth Promoting Rhizobacteria (PGPR) as Bio-fertilizers on Growth Enhancement of Economically Important Native Tree Species, *Neolamarckia cadamba* Seedlings

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ABSTRACT

The demand for large scale development of plantations of economically important indigenous as well as fast growing exotic tree species through ToF (Trees Outside Forests) programme has been progressively increasing in the recent past in Tamil Nadu and other parts of the country. Successful establishment of plantations is based on the survivability of the planted healthy and quality saplings in the field. Hence, an efficient production of quality seedlings of economically important tree species is of paramount importance. In this study, an attempt was made to determine the effect of commercially available beneficial microbes as bio-inoculants on growth enhancement of economically important fast growing native tree species, *Neolamarckia cadamba* in nursery. It was observed that dual application of bio-inoculants was found better than single inoculation indicating the synergistic effect. Further, dual combination involving both nitrogen fixers and phosphorus solubilizer/ mobilizer were found highly effective.

Keywords: AM fungi, *Azospirillum*, *Azotobacter*, *Neolamarckia cadamba*, phosphobacterium

INTRODUCTION

India has achieved self sufficiency in food production, after the Green Revolution. One of the main factors, which contributed for this achievement was the utilization of chemical fertilizers but indiscriminate use of these fertilizers in most of the areas, had its toll on soil health. The natural forest areas have fortunately been kept out of the influence of chemical fertilizers, while plantation forestry and tree nurseries found use of inorganic fertilizers. Of late, eco-friendly organic manures and bio-fertilizers are being encouraged in a big way to ensure sustainable productivity from plantation forests and agro-forestry.

Bio-fertilizers are beneficial microorganisms such as Arbuscular Mycorrhizal (AM) fungi, Ectomycorrhizal (ECM) fungi, Plant Growth Promoting Rhizobcteria (PGPR), *Frankia*, *Rhizobium*, etc. involved in breakdown of organic matter, N_2 fixation, secretion of plant growth hormones and increase of available mineral nutrients in soil. They are also helpful to build up other beneficial microorganisms and in turn improve soil health. The bio-fertilizers also control soil or root borne diseases, parasitic nematodes and maintain soil structure. They are ready-to-use as carrier based formulations. They can be applied to seed, root or soil in nurseries. The bio-fertilizers can make a significant contribution towards the development of strategies for production of high quality seedlings, which will help for better greenery in wastelands and unproductive lands of the country.

Neolamarckia cadamba (Roxb.) Bosser (Syn. *Anthocephalus cadamba)* is a large deciduous tree (family: *Rubiaceae*) having wide distribution from the sub-Himalayan tract, Nepal eastwards, Bengal, Assam, Bihar, Chattisgarh, Andhra Pradesh and the west coast from North Kanara southwards. It also occurs in Sri Lanka and Southeast Asian countries like Malaysia, Indonesia, etc. (Krisnawati *et al.*, 2011). It is a tree of moist warm regions frequenting in moist deciduous and evergreen forests on alluvial grounds. The wood is highly preferred for pencil making. The wood is also used for making ceiling boards, packing cases, light furniture, plywood, carving and turnery articles. It is also used for light constructional work such as beams and rafters. Bark and leaves are used for medicinal purposes.

The rhizosphere is a dynamic soil environment formed by living plant roots and their associated microorganisms and fauna. Among different microbes, plant growth promoting microbes like AM fungi, *Azospirillum*, *Azotobacter*, phosphobacteria are present in rhizosphere soil and they are able to exert a beneficial effect upon plant growth. These microbes have multiple functions like nitrogen fixation, phosphorus solubilization and mobilization (Sen and Paul, 1957) and stimulating root development by producing metabolites like IAA and other growth hormones (Lynch, 1990). They are eco-friendly, renewable, cost effective and pollution free. Therefore, use of these beneficial microorganisms as bio-fertilizers provides an effective alternative to the chemical fertilizers. The effect of various beneficial micro-organisms as bio-fertilizers in agriculture have been well documented (Subba Rao, 1993; Dash and Gupta, 2011; Brahmaprakash and Sahu, 2012).

The bio-fertilizers can also serve as important tools in forestry programme including reclamation of degraded lands, mined overburdens and other problematic areas so as to make these programme more effective (Grove and Le Tacon, 1993). The success or failure of a plantation can be forecasted by the quality of the nursery that has been maintained. Hence, to establish a good plantation, raising of high quality elite seedlings is necessary (Durvey and Landis, 1984). The apparent result of the beneficial microorganisms may not be evident under all natural conditions because of insufficient population naturally occurring in the soil (Powell and Daniel, 1978). Therefore, application of most suitable beneficial microorganisms becomes imminent. Bio-fertilizers are capable of making afforestation programme successful by improving planting stock quality and ultimately increasing productivity. The use of bio-fertilizers in forestry appears to be more important than in agriculture because, firstly, in countries like India no large scale provisions exist to irrigate, fertilize and protect the plantations and secondly, the chemical fertilizers are costly and are in short-supply. Therefore, inoculating nursery seedlings with selective bio-fertilizers holds promise for improving seedling quality, out planting performance and increased resistance to root diseases and climatic stresses in the field. Also the use of these bio-fertilizers would reduce the cost of chemical fertilizers involved in plantation programme. The effective utilization of bio-fertilizers for trees will not only provide economic benefits but also improve and maintain the soil fertility and sustainability in natural soil ecosystem. Since these bio-resources represent a great diversity in chemical, physical and biological characteristics, their efficient use depend on, among others, identification of suitable type of bio-fertilizers. Since very limited reports are available on use of AM fungi, *Azospirillum, Azotobacter* and phosphobacteria on forest tree species, especially the economically important fast growing native tree species, the study with an aim of elucidating the information on the efficacy of these bio-fertilizers on the growth improvement of *Neolamarckia cadamba* in nursery.

MATERIALS AND METHODS

Seed source and potting medium: Healthy seeds of *N. cadamba* were obtained from Seed Division, Kerala Forest Research Institute (KFRI), Peechi, Kerala. The seeds were not given any pretreatment. Potting medium used in the present study was a mixture of solar sterilized sand: soil: farmyard manure in the ratio 1:2:1. Potting medium was analyzed for its physico-chemical parameters such as pH, Electrical Conductivity (EC), available Nitrogen (N) (Jackson, 1973), available Phosphorus (P) (Jackson, 1973), available Potassium (K) (Sankaram, 1966) and micronutrients such as copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) (Lindsay and Norvell, 1978) following standard procedures. The physico-chemical properties of the potting medium are presented in **Table 1**.

Bio-fertilizers: The plant growth promoting microbes as bio-fertilizers used in the present study were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. The soil based inoculum of Arbuscular Mycorrhizal (AM) fungi consortium consisted of *Glomus fasciculatum* and *G. mosseae* at the rate of 12-15 spores per gram of soil. Peat soil based inocula of *Azospirillum, Azotobacter* and phosphobacterium consisted of *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megaterium,* respectively, each with a population count of 10^8 bacterial cells per gram of peat soil. The commercial chemical fertilizer, Diammonium Phosphate (DAP) also used in the study was procured from the market.

Raising and transplantation of seedlings in poly bags: Seeds of *Neolamarckia cadamba* were sown in nursery bed containing solar sterilized sand for raising seedlings. The bio-fertilizers/ DAP were applied to polythene bags (10 x 20 cm size) already filled with the potting medium up to 5cm below the top surface as per treatment schedule in completely randomized design by layering inoculation technique. On the bio-fertilizers/DAP layer, the potting medium was again filled up to the surface. Seedlings of uniform size from mother bed were immediately transplanted into the bags. The seedlings were maintained under nursery conditions and watered twice daily for further studies.

Table 1. Physico-chemical properties of potting medium used in the nursery experiments

Texture	pH	E.C. (dS m^{-1})	Macro Nutrients ($kg\ ha^{-1}$)			Micro Nutrients (ppm)			
			N	P	K	Cu	Zn	Fe	Mn
Sandy loam	7.3	0.14	180	19	225	1.8	1.54	10.8	11.08

Nursery experiment

Species and Treatment structure

Number of treatments : 13
Number of replicates/treatment : 3
Number of plants per replicate : 50
Design : CRD

Bio-fertilizers and DAP dosage

AM fungi - *Glomus fasciculatum + G. mosseae* : 10g per poly bag
Azospirillum - Azospirillum brasilense : 5g per poly bag
Azotobacter - Azotobacter chroococcum : 5g per poly bag
Phosphate solubilizing bacterium - *(Bacillus megaterium)* : 5g per poly bag
Diammonium Phosphate (DAP) : 5g per poly bag

Experimental design

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum*
T4 *Azotobacter*
T5 Phosphobacterium
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum + Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum + Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

OBSERVATIONS

Observations were taken at regular intervals of 90 and 180 days after inoculation (DAI). The growth data such as shoot height, collar diameter, total dry weight (shoot + root) were recorded after harvesting the seedlings. Growth indices like absolute growth rate (AGR), relative growth rate (RGR), leaf area ratio (LAR) and net assimilation rate (NAR) were subsequently calculated. Further, quality indices like root : shoot ratio, sturdiness index, volume index and Dickson's quality index were also calculated. Microbial Inoculation Effect and Benefit Cost Ratio were worked out.

Statistical Analysis: All data were subjected to analysis of variance and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) at P=0.05 level using SPSS (Version 10.0, SPSS Inc.) statistical software to determine the effects due to treatments.

RESULTS

Data on the growth parameters of *N. cadamba* seedlings inoculated with the selected bio-fertilizers *viz.*, AM fungi *Glomus fasciculatum + G. mosseae*), *Azospirillum brasilense* (Azo), *Azotobacter chroococcum* (Azoto) and Phosphate solubilizing bacterium (PSB - *Bacillus megaterium*) individually and in combinations as well as chemical fertilizer *viz.*, Di-ammonium phosphate (DAP) alone is presented in **Tables 2-4** and **Figs. 1** and **2**.

Table 2. Effect of bio-fertilizers on the shoot height, collar diameter and total dry weight of *Neolamarckia cadamba* seedlings

Treatment	Shoot Length (cm)		Collar diameter (mm)		Total Dry weight (g)	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T1	8.677 a	19.337 a	2.47 a	4.53 a	1.157 a	4.243 a
T2	11.597 c	25.617 bc	3.13 b	6.80 bc	1.787 bc	6.530 bc
T3	10.833 b	25.370 bc	3.07 b	6.63 b	1.733 b	6.380 bc
T4	12.057 c	25.007 b	3.30 bc	7.23 cd	1.710 b	6.307 bc
T5	12.240 c	25.470 bc	3.33 bc	7.27 cd	1.780 bc	6.707 c
T6	13.120 d	26.870 de	3.47 bc	7.50 d	1.883 cd	7.197 d
T7	14.057 e	26.300 cd	3.67 c	7.57 de	1.893 cd	7.453 e
T8	13.130 d	25.567 bc	3.40 bc	7.47 d	1.807 bc	6.737 bc
T9	12.180 c	26.167 bcd	3.43 bc	7.43 d	1.787 bc	6.733 b
T10	15.807 f	27.927 e	3.53 bc	7.57 de	2.030 e	7.527 e
T11	15.937 f	27.717 e	3.70 c	7.63 de	1.980 de	7.567 e
T12	17.073 g	29.217 f	4.23 d	8.10 ef	2.187 f	8.107 f
T13	17.040 g	29.987 f	4.40 d	8.33 f	2.170 f	7.917 f

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI - Days after inoculation.

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum* (Azo)
T4 *Azotobacter* (Azoto)
T5 Phosphobacterium (PSB)
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* +Phosphobacterium
T13 Diammonium phosphate (DAP)

Shoot height

Data on shoot height of *Neolamarckia cadamba* seedlings inoculated with selected bio-fertilizers is presented in **Table 2**. It was observed that the seedlings inoculated with combined application of all the bio-fertilizers (AMF + Azo + Azoto + PSB) and DAP (alone) showed significantly greater shoot height at both ages over uninoculated control and other treatments, but between them they were at par. These were followed by the seedlings treated with dual applications of Azoto + PSB and Azo + PSB, but between them they were at par. In general, all the seedlings treated with bio-fertilizers/DAP had significantly greater shoot height when compared to the uninoculated control seedlings during both ages of the observation.

Collar diameter

Data on the collar diameter of *N. cadamba* seedlings inoculated with different bio-fertilizes individually and in combinations and DAP (alone) is presented in **Table 2**. There was a gradual increase in collar diameter with increase in the age of the seedlings. The collar diameter was more in all the bio-fertilizers and DAP treated plants when compared to the uninoculated control. There was a significant variation among the treatments. The collar diameter was found greater and highly significant in seedlings treated with DAP (alone) and combined application of all four bio-fertilizers (AMF + Azo + Azoto + PSB) at both the ages, but was found at par between them. Among the other treatments, seedlings inoculated with Azoto + PSB and Azo + PSB showed more collar diameter followed by AMF + Azoto and AMF + Azo inoculated seedlings. At both age levels, the seedlings with individual application of bio-fertilizers showed the lesser collar diameter values but they had significantly greater values than the uninoculated control seedlings.

Total dry weight

Data on total dry weight (shoot + root) of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 2**. There was significant variation in total dry weight of seedlings among the treatments. It was observed that the seedlings inoculated with combination of all the four bio-fertilizers (AMF + Azo + Azoto + PSB) as well as DAP (alone) resulted in highly significant total dry weights at both ages. These were followed by the seedlings treated with dual inoculations of Azoto + PSB; Azo + PSB and AMF + Azoto. All the seedlings treated with bio-fertilizers as well as DAP (alone) showed significantly higher total dry weight values than uninoculated control seedlings at both the ages of observation.

Growth indices

Absolute Growth Rate (AGR): Data on the absolute growth rate (AGR) of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 3**. It was found that seedlings treated with combined inoculation of all the four bio-fertilizers (AMF + Azo + Azoto + PSB) showed highest AGR values, followed by individual application of DAP (alone), but were at par between them. These were followed by seedlings treated with dual inoculations of Azoto + PSB, Azo + PSB and AMF + Azoto, but were at par among them. In general, the seedlings treated with dual inoculations showed better AGR values than with single inoculations. It was interesting to note that all the treatments with bio-inoculants and DAP showed significantly higher AGR than the uninoculated control seedlings.

Relative Growth Rate (RGR): Data on the relative growth rate (RGR) of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP

Table 3. Effect of bio-fertilizers on the AGR, RGR, LAR and NAR of *Neolamarckia cadamba* seedlings.

Treatment	AGR (mg/g/day)	RGR (mg/g/day)	LAR (cm^2/g)	NAR (mg/cm^2/day)
T1	34.296 a	14.442 a	4.759 d	3.034 a
T2	53.334 bc	14.497 a	3.697 bc	3.921 a
T3	51.629 bc	14.479 a	3.105 ab	4.663 abc
T4	51.073 b	14.501 a	3.506 abc	4.136 ab
T5	54.741 c	14.739 a	2.546 abc	5.789 abc
T6	59.037 d	14.895 a	3.306 abc	4.506 ab
T7	61.778 de	15.226 a	2.239 ab	6.800 bc
T8	54.777 c	14.623 a	3.043 ab	4.806 abc
T9	54.962 c	14.741 a	3.386 abc	4.353 ab
T10	61.074 de	14.560 a	2.118 a	6.874 bc
T11	62.074 cde	14.896 a	2.231 ab	6.675 bc
T12	65.778 f	14.559 a	2.110 a	6.901 c
T13	63.852 ef	14.380 a	2.078 a	6.919 c

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI - Days after inoculation.

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum* (Azo)
T4 *Azotobacter* (Azoto)
T5 Phosphobacterium (PSB)
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

Table 4. Effect of bio-fertilizers on the volume index and quality index of *Neolmarckia cadamba* seedlings

Treatment	Root Shoot ratio		Sturdiness Quotient		Volume Index		Quality Index	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T1	0.592 a	0.869 a	3.517 a	4.266 c	1.147 a	7.319 a	0.124 a	0.471 a
T2	0.763 d	0.926 ab	3.701 a	3.767 ab	2.367 b	20.637 bc	0.198 b	0.862 bc
T3	0.757 cd	0.926 ab	3.532 a	3.825 b	2.208 b	19.523 b	0.193 b	0.821 b
T4	0.763 d	0.994 cd	3.654 a	3.457 a	2.671 bc	22.885 bcd	0.196 b	0.894 cd
T5	0.751 bcd	0.848 a	3.672 a	3.505 ab	2.779 bc	23.523 cde	0.201 b	0.918 cd
T6	0.749 bcd	0.990 cd	3.784 ab	3.583 ab	3.275 cd	26.175 def	0.205 b	0.998 ef
T7	0.732 bcd	0.986 cd	3.833 ab	3.476 a	3.807 de	26.429 def	0.208 b	1.047 fg
T8	0.689 b	0.945 cd	3.862 ab	3.424 a	3.034 bcd	24.995 def	0.197 b	0.954 de
T9	0.691 bc	1.020 d	3.548 a	3.520 ab	2.977 bcd	25.177 def	0.203 b	0.897 d
T10	0.716 bcd	0.998 cd	4.474 c	3.691 ab	3.771 de	27.334 ef	0.204 b	1.028 f
T11	0.757 d	1.002 d	4.307 ab	3.631 ab	4.185 e	27.651 f	0.207 b	1.048 fg
T12	0.713 bcd	0.984 cd	4.033 abc	3.607 ab	6.006 f	32.728 g	0.235 c	1.130 h
T13	0.709 bcd	0.963 bcd	3.873 ab	3.599 ab	6.494 f	35.462 g	0.240 c	1.104 gh

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

DAI -Days after inoculation

T1 Control
T2 AM fungi (AMF)
T3 *Azospirillum* (Azo)
T4 *Azotobacter* (Azoto)
T5 Phosphobacterium (PSB)
T6 AMF + *Azospirillum*
T7 AMF + *Azotobacter*
T8 AMF + Phosphobacterium
T9 *Azospirillum* + *Azotobacter*
T10 *Azospirillum* + Phosphobacterium
T11 *Azotobacter* + Phosphobacterium
T12 AMF + *Azospirillum* + *Azotobacter* + Phosphobacterium
T13 Diammonium phosphate (DAP)

(alone) is presented in **Table 3**. It was observed that seedlings treated with dual inoculation of AMF + Azoto showed the highest RGR value whereas seedlings treated with DAP (alone) showed the lowest RGR. It was observed that there was no significant difference among the treatments including uninoculated control seedlings.

Leaf Area Ratio (LAR): Data on the leaf area ratio (LAR) of *N. cadamba* seedlings inoculated with different bio-fertilizers individually and in combinations) and DAP (alone) is presented in **Table 3**. It was found that seedlings treated with DAP (alone), combined inoculation of all the four bio-fertilizers (AMF + Azo + Azoto + PSB) and dual inoculation of Azo + PSB showed the best LAR values and were at par among them. These were followed by seedlings treated with dual inoculation of Azoto + PSB and AMF + Azoto. In general, all the treatments showed significantly better LAR values than uninoculated control seedlings.

Net Assimilation Rate (NAR): Data on the net assimilation rate (NAR) of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 3**. It was observed that seedlings treated with DAP (alone), combined inoculation of all the four bio-fertilizers (AMF + Azo + Azoto + PSB) showed maximum and highly significant NAR. These were followed by seedlings treated with and dual inoculations of Azo + PSB, AMF + Azoto and Azoto + PSB. In general, all the treatments showed higher NAR values than uninoculated control seedlings.

Quality indices

Root:shoot ratio: Data on the root - shoot ratio of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 4**. There was a significant variation between uninoculated control seedlings and all other treatments and at 90 DAI. Whereas, there was no significant difference between uninoculated control seedlings and single inoculations at 180 DAI, but the dual inoculations, combination of all four bio-fertilizers and DAP (alone) differed significantly. It was found that seedlings treated with single application of AMF and dual application of Azoto + PSB had maximum root shoot ratio values at 90 DAI and 180 DAI, respectively. In general, the bio-fertilizers treated seedlings showed higher root shoot ratio values than uninoculated control seedlings at both the ages. It was also found that the seedlings showed higher root shoot ratio value at 180 DAI than at 90 DAI.

Sturdiness quotient: Data on the sturdiness quotient of *Neolamarckia cadamba* seedlings inoculated with bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 4**. It was observed that there was no significant difference between the control and other treatments except dual inoculations of Azo + PSB and Azoto + PSB at 90 DAI. However, all the treatments involving bio-fertilizers and DAP differed significantly from uninoculated control at 180 DAI. In general, the inoculated seedlings showed better sturdiness quotient values when compared with uninoculated control seedlings at 180 DAI.

Volume index: Data on the volume index of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Table 4**. When the volume index of the seedlings was calculated, it was observed that a significantly high value was obtained in DAP (alone) treated seedlings, followed by the seedlings treated with combined application of all four bio-fertilizers, but between them they were at par. It was also found that dual inoculated seedlings showed better values than single inoculated seedlings at both ages. In general, all the treatments showed significantly higher values than the untreated control seedlings at both the ages of observation.

Dickson's Quality Index: Data on the quality index of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (individual) is presented in **Table 4**. It was found that seedlings treated with DAP (alone) showed highest quality index values followed by combined inoculation of all the four bio-fertilizers (AMF + Azo + Azoto + PSB) at 90 DAI and vice versa at 180 DAI. They showed highly significant values than all other treatments including uninoculated control seedlings, but between them they were at par at both the ages. These were followed by seedlings treated with dual inoculations of Azoto + PSB and AMF + Azoto, which again were at par between them. It was interesting to note that all the treatments with bio-fertilizers and DAP (alone) showed better performance than the uninoculated control seedlings at both the ages.

Microbial Inoculation Effect (MIE) : Data on microbial inoculation effect (MIE) of *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Fig. 1**. It was observed from the study that the MIE varied with the treatment. Seedlings inoculated with combined application of AMF + Azo + Azoto + PSB showed the highest value of MIE, followed by Azoto + PSB and Azo + PSB inoculated seedlings when compared to all other treatments. In general, the seedlings treated with dual application of bio-fertilizers showed higher values when compared to single inoculation at both the ages of observation.

Benefit cost ratio: Data on the benefit cost ratio for *N. cadamba* seedlings inoculated with different bio-fertilizers (individually and in combinations) and DAP (alone) is presented in **Fig. 2**. It was observed from the study that the BC ratio varied with the treatment. Seedlings inoculated with DAP showed the highest BC ratio followed by single inoculations of Azo, Azoto and PSB. These were followed by dual inoculations of Azo + Azoto, Azo + PSB and Azoto + PSB. In general, the seedlings treated with bio-fertilizers showed higher BC ratios when compared to un-inoculated control seedlings.

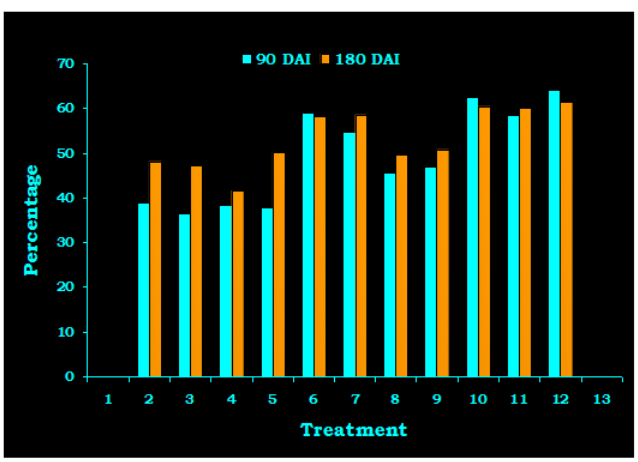

Fig. 1: Microbial inoculation effect (MIE) of bio-inoculants on *Neolamarckia cadamba* seedlings

DISCUSSION

Plant growth response: In the present study, the shoot height, collar diameter and total dry weight were higher in *N. cadamba* seedlings inoculated with AM fungi and PGPRs than uninoculated control (**Table 2**). The present findings were quite in agreement with the results reported by earlier researchers on the native tree species. Kandasamy *et al.* (1987) while working with *Ailanthus excelsa* and two other species reported that inoculation with AM fungus, *Glomus fasciculatum* increased the shoot and root dry weights (62.08% and 43.18%, respectively). Balasubramanian and Srinivasan (1995) have also reported that inoculation with four AM fungi significantly increased the total biomass, leaf area and total chlorophyll of *Ailanthus excelsa, Tectona grandis* and *Dalbergia sissoo* seedlings. Madan *et al*. (1995) reported that AM fungal inoculated *Ailanthus excelsa, Pongamia glabra* and *Cassia siamea* seedlings showed better shoot height, shoot and root dry weight in highly alkaline-saline soils. In the subsequent years, Rahangdale and Gupta (1998) reported that all the twelve AM fungi have contributed to significant increase in shoot height, root and shoot biomass in all the six tree species tested including *Gmelina arborea.*

Talukdar and Thakuria (2000) also reported that AM fungi inoculated *Gmelina arborea* and *Tectona grandis* with organic amendments gave better yield in terms of shoot and root biomass in degraded soils. Chandra and Ujjaini (2002) reported that when *Gmelina arborea* and four other species were inoculated with AM fungi + organic matter, the plant biomass increased up to 36.85%. Recently, Barua *et al*. (2010) have found that length of shoot and root, collar diameter, fresh and dry weight of shoot and root were improved in AM fungi inoculated *Gmelina arborea* plants. They also observed that the mycorrhizal seedlings had as much as 40% higher increment in total growth and 2.4 times higher increment in biomass compared to non-mycorrhizal seedlings. The

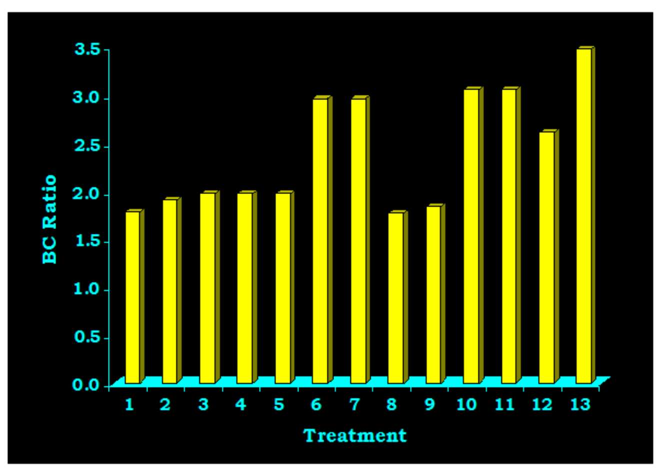

Fig. 2: Benefit cost ratio of bio-inoculants on *Neolamarckia cadamba* seedlings

significant effect of AM fungi and PGPR on different growth parameters of *A. excelsa* seedlings in the current study may be due to the increased production of auxins and gibberellins by the selected plant growth promoting microbes of PGPR and AM fungi. The findings are also in conformity with the studies conducted by De La Cruz *et al.* (1988) and Dubey *et al.* (1997). The increase in seedling biomass production may be strongly correlated with improved accumulation of N due to *Azospirillum* and *Azotobacter* and P due to AM fungi and PSB inoculation (Ratha Krishnan *et al.*, 2004).

In general, seedlings inoculated with combination of all plant growth promoting microbes (AM fungi and PGPR) showed maximum values for the growth parameters at both 90 DAI and 180 DAI in all the four tree species. Further, among the treatments involving bio-inoculants, dual combinations, especially the ones involving a Nitrogen fixer and a Phosphorus solubilizer/ mobilizer were found to be better than single inoculations. These findings are in accordance with the findings of Seema *et al.* (2000) who reported that AM fungi and *Azotobacter chroococcum* inoculation on *Gmelina arborea*, *Bambusa arundinacea*, *Tectona grandis* and *Dalbergia sissoo* resulted in improved growth and biomass. Zambrano and Diaz (2008) have also reported a positive correlation between mycorrhization and plant height and a synergic effect between AM fungi, *Glomus* spp. and *Azospirillum brasilense* on *Gmelina arborea* with two potting media.

The effect of plant growth promoting microbes on the growth performance (shoot height, collar diameter and total dry weight) varied with the treatment and the host plant species. Balasubramanian and Srinivasan (1995) also reported that different AM fungi responded differently to different tree species with *Gigaspora margarita* on *Tectona*, *Glomus mosseae* on *Dalbergia* and *G. monosporum* on *Ailanthus* showing maximum beneficial effect. Rahangdale and Gupta (1998) reported that twelve different AM fungi produced different growth response on six tree species tested. Seema *et al.* (2000) also reported variations among treatments and species while working with AM fungi and *Azotobacter chroococcum* and three tree species. The greater increase in shoot height, collar diameter and total dry weight in treatment with combination of all the plant growth promoting microbes and dual inoculations involving AM fungi/PSB and Azo/Azoto, in *A. excelsa* plants may be due to improved accumulation of N both by *Azospirillum* and *Azotobacter* (Wong and Sternber, 1979) and P by AM fungi and PSB inoculations (Habte and Manjunath, 1987; Saravanan, 1991).

Growth indices: In the present study, Aggregate Growth Rate (AGR) and Net Assimilation Rate (NAR) were higher in *N. cadamba* seedlings inoculated with plant growth promoting microbes than uninoculated control. Relative Growth Rate (RGR) in general, was found to be higher in inoculated *N. cadamba* seedlings. However, Leaf Area Ratio (LAR) was found to be lower in inoculated seedlings of *N. cadamba*, which indicates that lesser leaf area in inoculated seedlings is able to produce more dry matter. These findings are in accordance with the findings of earlier researchers on other tree species. Huante *et al*. (1993) reported that AM fungal inoculation resulted in increased biomass production and RGR in *Caesalpinia eriostachys*, *Cordia alliodora* and *Pithecellobium mangense*. Lovelock *et al.* (1996) reported that inoculation with AM fungi increased RGR of the tropical tree, *Beilschmiedia pendula* seedlings. Shishido and Chanway (2000) reported that hybrid spruce (*Picea glauca* × *Picea engelmannii*) seedlings pre-inoculated with PGPR (*Pseudomonas* sp. and *Bacillus* sp.) exhibited significantly higher shoot and root RGRs (increased by 10-234%) four months after outplanting, at all the four sites tested. Ratha Krishnan *et al.* (2004) reported that inoculation of *Azospirillum, Azotobacter,* PSB and AM fungi on *Simarouba glauca* seedlings resulted in increased RGR. Recently, Zapata *et al.* (2009) reported that AM fungi increased RGR of *Desmoncus orthacanthos* (*Arecaceae*) seedlings at low P level.

Increase in growth rates of different bio-inoculants applied plants may be due to alteration of morphology (more leaf area) and physiology (improved nutrient accumulation and photosynthesis) of the seedlings. NAR and LAR are correlated with RGR of plants. The higher the NAR the more efficient the species, which usually translates into higher growth rates (Larcher, 2003). Inoculation with AM fungi and PGPR increased both total plant growth, as measured by increased AGR, and the efficiency of growth, as measured by increased NAR. This increased growth efficiency allowed the plant to have a smaller shoot system (decreased LAR), which is the source, while still enhancing the size of the root system which is a sink (Leopold and Kriedemann, 1975).

Quality indices: The root: shoot ratio is an important measure for seedling survival. It relates the water absorbing area (roots) to the transpiring area (shoot). A good ratio- one which indicates a healthy plant is 1:1 to 2:1 root:shoot mass (Jaenicke, 1999). Significant difference was found between treated and untreated seedlings of *N. cadamba.* However, the root:shoot ratio was higher in all the treated seedlings than the control and many treatments showed desired values of >1.0. This indicates that the bio-inoculants tend to improve the root shoot ratio of inoculated seedlings by increasing the root biomass. The seedlings of uninoculated control had root:shoot ratio of less than one, which means that shoot biomass was too high compared to root biomass indicating that these seedlings were of sub-optimal quality and unlikely to withstand the adverse conditions in most planting sites.

A less rigorous, but non-destructive, index is the 'sturdiness quotient' which compares height (in cm) over root collar diameter (in mm). A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. A sturdiness quotient higher than 6 is undesirable (Jaenicke, 1999; Gregorio *et al*., 2007). Seedlings of *N. cadamba* had desirable sturdiness quotient values (<6.0) at both the ages of observation. However, in both the cases, the inoculation of seedlings with AM fungi and PGPR has resulted in improvement of sturdiness quotient values towards <6.0.

The volume index and the quality index were higher in *N. cadamda* seedlings inoculated with AM fungi and PGPRs than uninoculated control. These findings are in accordance with the findings of earlier researchers on native and related tree species like *Azadirachta indica* (Sumana and Bagyaraj, 2002), *Tectona grandis* (Ayswarya, 2008; Rajeshkumar *et al*., 2009) *Melia azedirach (*Rajeshkumar *et al*., 2009) as well as exotic ones like *Eucalyptus hybrid* (Sastry *et al.,* 2000), *Simarouba glauca* (Ratha Krishnan *et al.,* 2004) and *Acacia auriculiformis* and *A. mangium* (Tamilselvi, 2005). Sumana and Bagyaraj (2002) also reported that dual inoculation of *Glomus mosseae* and *Azotobacter chroococcum* resulted in maximum volume index and quality index of neem seedlings while Rajeshkumar *et al.* (2009) reported that triple inoculation of *Glomus geosporum, Azotobacter chroococcum,* and *Bacillus coagulans* resulted in maximum volume index and quality index of *Melia azedarach* seedlings.

Microbial Inoculation Effect (MIE): It was observed from the study that the Microbial Inoculation Effect (MIE) varied with the treatment and the seedlings inoculated with combined application of all four bio-fertilizers (AMF + Azo + Azoto + PSB) showed the highest value of MIE, followed by dual inoculations when compared to all other treatments in all the four tree species tested (**Fig. 1**). This indicates that these organisms act synergistically when inoculated in combinations. Similar findings were reported by Muthukumar *et al*. (2001) in Neem.

BC ratio: In the present study, it was observed that the bio-inoculants treated plants especially, combined application of all bio-fertilizers and dual inoculations involving N fixer and P solubilizer/ mobilizer having good quality index and attaining good height and collar diameter gave better benefit cost ratio than other. In terms of benefit cost analyses of the bio-inoculants, it is well known that the beneficial effect of the bio-inoculants is multifaceted and does not result solely from improved nutrient uptake. Thus it may not be appropriate to compare the cost of inoculation directly with the cost of chemical fertilizer additives that achieve equivalent growth response in target plants. Indeed, the benefits of chemical soil additives are usually short lived, unless slow release formulations are used, and hence are not sustainable in low input or natural ecosystems. If the bare economics of the relative costs of chemical fertilizers versus bio-inoculants are compared, the latter will be little more costly in the short term. However, in terms of overall plant health and sustainability, the benefits of establishing an effective AM symbiosis and PGPR are much wider and more long lasting.

CONCLUSION

It is evident from the data that the bio-fertilizers have greatly improved seedling growth and biomass of *N. cadamba* in nursery on par with DAP application. Dual application of bio-inoculants was found to be better than single inoculations indicating the synergistic effect. Further, dual combinations involving a N fixer and a P solubilizer/ mobilizer were found to be highly effective. Application of plant growth promoting microbes as bio-fertilizers will not only give desired benefits in terms of good seedling quality and out planting performance in the field, but also ensure the maintenance of soil health which is essential for sustainable and eco-friendly forestry. Use of efficient AM fungi and PGPR bio-fertilizers in seedling and transplanting stage may be an excellent solution to strengthen seedlings to withstand dry land conditions and support the Trees outside Forest (ToF) efforts, thereby promoting farmers, tree growers and wood based entrepreneurs of the country.

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