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Research Article

Diversity of Beneficial Rhizosphere Microbes Associated with *Neolamarckia cadamba* Clones in Nursery

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Abstract

Neolamarckia cadamba, popularly known as 'Kadamba' in India is a tropical evergreen tree species of Indo-Malayan Origin. In India, it is one of the promising candidate agro-forestry tree species having varied timber utility. Root colonizing microbes associated with the tree species assumes greater importance owing to their unique properties in supplementing the rhizosphere soil with essential growth requirement for the plants. Studies on such beneficial microorganism in nursery reared seedlings are scanty. In view of the importance of the species, the diversity status of different beneficial microbes such as soil spore population and percent root colonization of Arbuscular Mycorrhizal (AM) fungi, Actinomycetes, Plant Growth Promoting Rhizobacteria (PGPR) comprising of *Azospirillum*, *Azotobacter* and Phosphobacteria as well as soil fungi associated with the roots and rhizosphere soil of six different clonal plants of *N. cadamba* collected from experimental nursery of IFGTB, Coimbatore was studied. The results revealed that all the beneficial microorganisms varied in abundance among different clonal plants screened. Among PGPR, population density of *Azatobacter* and *Azospirillum* was recorded more in all the clonal plants compared to that of phosphate solubilising bacteria. Population density of Actinomycetes was seen well in all the clones, while that of *Pseudomonas* and PSB was very less.

Keywords: Actinomycetes, *Azospirillum, Azotobacter, Neolamarckia cadamba,* Phosphobacteria.

Introduction

Neolamarckia cadamba is a tropical evergreen tree species of Indo-Malayan origin. The tree is commonly known by the name 'Kadamba' in India and is distributed in almost every part of the subcontinent except few pockets at the NW region. It is one of the potential candidate agro-forestry species and is very commonly cultivated in gardens. Characteristically, the tree attains nearly 45 m height, 100-160 cm stem diameter and sometimes up to 2 m small buttress and is having broad umbrella-shaped crown, straight cylindrical bole and branches arranged in tiers (Chaturvedi *et al*., 2017). The tree is being planted for wood having multi-utility, such as plywood, construction materials, packaging materials (boxes and crates), and pencils and even in paper industries (Sreedhar and Mohan, 2016). The species is proved to be highly promising for Tamil Nadu as reported by Vijayaraghavan (2014) that clonal plantation and site clone-matching can increase the yield by 10-15% than that realized of the seed origin by about 70-100 tonnes/ha at a rotation of 6-7 years. In order to achieve good results from clonal origin planting stock including consistent performance in terms of yield and productivity, good coppicing behaviour, good rooting capacity and resistance to insects pests and diseases, the use of microbial bio-fertilizers which are known for various plant growth promoting abilities has become a regular practice for the production of quality planting stock in forest nurseries. The rhizosphere region of a growing tree is a highly conducive habitat for the subsistence some of the very important microorganisms, which are regarded as beneficial to plants as they promote plant's growth in multitude of ways besides enhancing their defense against insects and pathogens. Among the beneficial microbes, the Arbuscular Mycorrhizal (AM) fungi also known as Vesicular Arbuscular Mycorrhizal (VAM) fungi are obligate symbionts that form beneficial symbiosis with roots of angiosperms and other plants. The symbiotic association between AM fungi and host roots provides a significant contribution to the plant's nutrition and growth. It enhances the uptake of essential mineral ions like P, K, Ca, Zn, Cu, Mn and Fe by the plants. Plant Growth Promoting Rhizobacteria (PGPR) are heterogeneous group of bacteria mainly belong to *Pseudomonas, Azospirillum, Azotobacter,* Phosphate Solubilising Bacteria, *Rhizobium, Actinomycetes* found in the rhizosphere soil and are extensively used as microbial bio-inoculants or bio-fertilizes for growth improvement of many plants.

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Effective utilization of bio-inoculants for specific crop not only provides economic benefits, but also improves and maintains the soil fertility and sustainability in natural ecosystem. Moreover, these bio-resources represent a great diversity in chemical, physical and biological characteristics. The uses of bio-fertilizers have been proved much helpful in afforestation programme and reclamation of wastelands and other adverse sites. The tree nurseries primarily aim for production of quality planting stock for the benefit of a larger group of stakeholders including farming communities besides SFDs. Hence, production of high quality tree seedlings is important for increasing the area under tree cultivation to a greater extent thereby contributing to achieve the carbon sequestration, which otherwise reported to go uncontrollably due to degradation of natural forests and woodlands.

Thus, it is of paramount importance to ascertain the status of beneficial microbes associated with the seedlings raised and maintained in various tree nurseries for better selection of beneficial microbial consortia as bio-fertilizers for application at the time of seed sowing and raising seedlings or clones in nursery conditions. Mohan *et al.* (2007) studied the status of Arbuscular Mycorrhizal (AM) fungal associations in seedlings of important forest tree species in tree nurseries in Tamil Nadu and observed that the root colonization and soil spore population of AM fungi were minimum and the application of different beneficial microbes as bio-fertilizer extremely necessary during seedling production in tree nurseries. Many earlier studies have revealed the efficacy of different beneficial microbes on enhanced plant growth and biomass production in nursery. The literature on status of different beneficial microbes associated with the commercially important forestry tree species in nurseries is very scanty. Hence, the present study was taken up to investigate the diversity status of different beneficial microorganisms such as Arbuscular Mycorrhizal (AM) fungi and Plant Growth Promoting Rhizobacteria (PGPR) in the roots and rhizosphere soils of six different clonal plants of *N. cadamba* plants raised and maintained by the Experimental Nursery at IFGTB, Coimbatore.

Materials and methods

Collection of rhizosphere soil and root samples: The rhizosphere soil and root samples were collected from six different clonal seedlings of *Neolamarckia cadamba* (NC-1, NC-2, NC-3, NC-4, NC-5 and NC-6) which were raised and maintained in experimental nursery of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu. All the samples were collected during the month of January, 2019 and stored in freezer till processing.

Isolation and enumeration of rhizosphere microbes: The soil samples were analyzed for isolation of different rhizosphere microbes such as PGPR and Actinomycetes by adopting standard techniques (Parkinson *et al.*, 1971; Subba Rao, 1993; Oskay *et al.*, 2004). One gram soil was dispensed in 100 ml distilled water, the 10 fold dilution was prepared and 0.1 ml was spread plated on to Nutrient Agar for isolation of PGPR and Starch Casein Agar for Actinomycetes. Plates were kept for incubation at appropriate temperature for 2-7 days and then the colonies were selected based on morphology and selected ones were pure cultured and stored in 4°C for further study.

Isolation and identification of Arbuscular Mycorrhizal (AM) fungi: Arbuscular Mycorrhizal (AM) fungal spores from soil samples were isolated and estimated by using wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and sucrose density gradient technique (Daniels and Skipper, 1982). Root colonization of AM fungi was done by using root clearing and staining techniques (Phillips and Hayman, 1970) and data on percent root colonization of AM fungi was also estimated by using gridline intersect method (McGonigle *et al.*, 1990), The intact and the crushed spores were examined under a compound microscope and genus & species level identification was done by using standard keys (Trappe, 1982; Schenck and Perez, 1987). Spores were identified based on spore morphology and sub cellular characters and compared with original descriptions (Schenck and Perez, 1987).

Results and discussion

The present study analyzed the diversity status and population density of PGPR *viz.*, *Azospirillum, Azotobacter,* Phosphate Solubilizing Bacteria (*Pseudomonas*); Actinomycetes and AM fungi from the rhizosphere soil samples of six different clonal plants of *N. cadamba* collected from experimental nursery of IFGTB, Coimbatore. The results of the analysis in detail are summarized as follows:

Azospirillum: The population density of *Azospirillum* colonies isolated from the rhizosphere soil samples of six different clonal plants of *N. cadamba* is presented in Table 1 and Fig. 1. *Azospirillum* population was high in the rhizosphere of NC-2 with mean value of 41.50×10^{4}cfu/g followed by NC-4 with 34.75×10^{-4}cfu/g, NC-1 with 28.0×10^{-4} cfu/g, NC-5 with 14.50×10^{-4}cfu/g and the same was very low in the rhizosphere of NC-6 clone with 9.50×10^{-4}cfu/g and no *Azospirillum* colonies were recorded in the rhizosphere of NC-3 clone.

Azotobacter: The population density of *Azotobacter* colonies isolated from the rhizosphere soil samples of six different clonal plants of *N. cadamba* is presented in Table 2 and Fig. 2.

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Table 1. Total population density of *Azospirillum* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

PGPR (cfu/g) x 10^{-4}	Clone No.	Min.	Max.	Mean ± SD
Azospirillum	NC-1	15.0	44.0	28.00 ± 12.78
	NC-2	26.0	66.0	41.50 ± 17.45
	NC-3	0.0	0.0	0.00 ± 0.00
	NC-4	14.0	66.0	34.75 ± 23.14
	NC-5	3.0	36.0	14.50 ± 14.80
	NC-6	2.0	21.0	9.50 ± 8.35

Table 2. Total population density of *Azotobacter* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

PGPR (cfu/g) x 10^{-4}	Clone No.	Min.	Max.	Mean ± SD
Azotobacter	NC-1	3.0	9.0	5.75 ± 2.50
	NC-2	9.0	34.0	19.50 ± 11.39
	NC-3	2.0	11.0	6.00 ± 3.74
	NC-4	2.0	15.0	7.50 ± 5.57
	NC-5	0.0	4.0	1.75 ± 1.71
	NC-6	0.0	2.0	0.75 ± 0.96

Table 3. Total population density of *Pseudomonas* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

PGPR (cfu/g) x 10^{-4}	Clone No.	Min.	Max.	Mean ± SD
Pseudomonas	NC-1	0.0	0.0	0.00 ± 0.00
	NC-2	0.0	1.0	0.50 ± 0.58
	NC-3	1.0	2.0	1.50 ± 0.58
	NC-4	0.0	0.0	0.00 ± 0.00
	NC-5	1.0	6.0	2.75 ± 2.36
	NC-6	2.0	14.0	7.00 ± 5.29

Table 4. Total population density of *Actinomycetes* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

PGPR (cfu/g) x 10^{-4}	Clone No.	Min.	Max.	Mean ± SD
Actinomycetes	NC-1	0.0	0.0	0.00 ± 0.00
	NC-2	2.0	5.0	3.00 ± 1.41
	NC-3	0.0	2.0	1.00 ± 0.82
	NC-4	1.0	4.0	2.25 ± 1.26
	NC-5	0.0	0.0	0.00 ± 0.00
	NC-6	0.0	2.0	0.75 ± 0.96

Table 5. Total spore population of AM fungi recorded from the rhizosphere of six different clones of *Neolamarckia cadamba.*

AM spores/100 g soil	Clone No.	Min.	Max.	Mean ± SD
AM spore population	NC-1	102.0	191.0	144.00 ± 36.72
	NC-2	102.0	141.0	119.25 ± 16.64
	NC-3	115.0	122.0	119.00 ± 2.94
	NC-4	124.0	132.0	128.00 ± 3.65
	NC-5	142.0	164.0	151.75 ± 9.32
	NC-6	105.0	123.0	113.00 ± 7.62

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Fig. 1. Mean population density of *Azospirillum* colonies organisms isolated from the rhizosphere region of six different clonal plants of *Neolamarckia cadamba.*

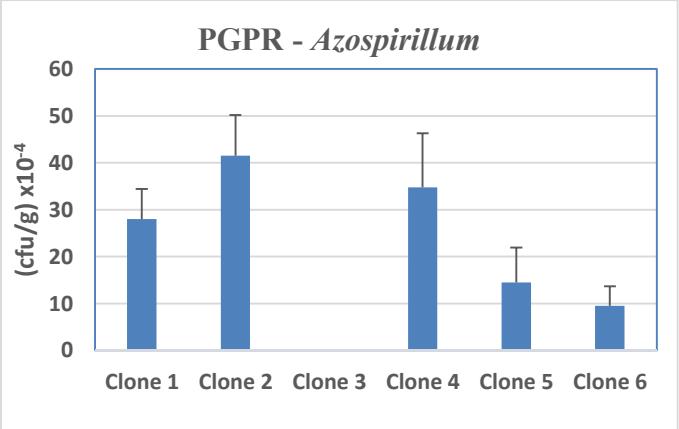


Fig. 2. Mean population density of *Azotobacter* colonies organisms isolated from the rhizosphere region of six different clonal plants of *Neolamarckia cadamba.*

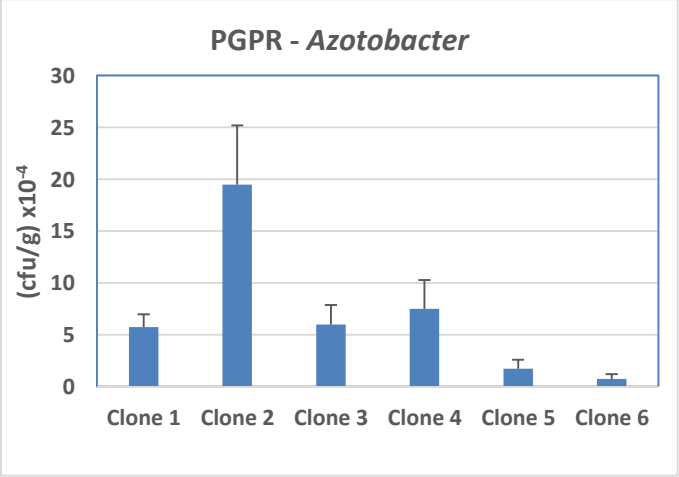


Fig. 3. Total population density of *Pseudomonas* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

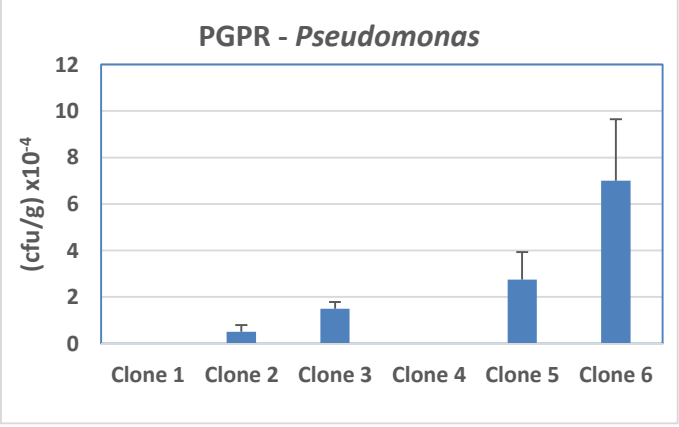


Fig. 4. Total population density of *Actinomycetes* colonies isolated from the rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba.*

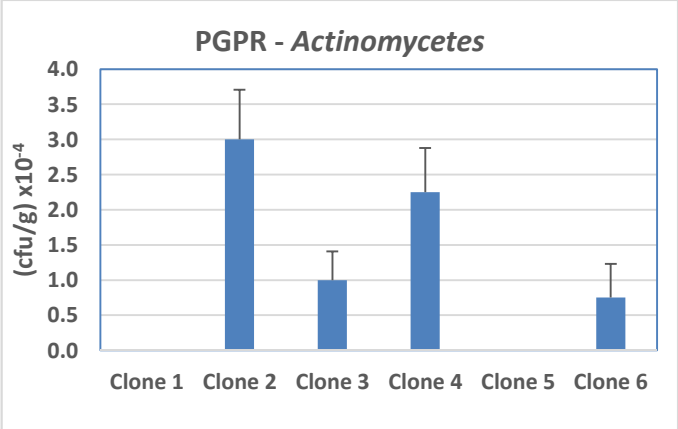


Fig. 5. Mean spore population of AM fungi recorded from the rhizosphere of six different clones of *Neolamarckia cadamba.*

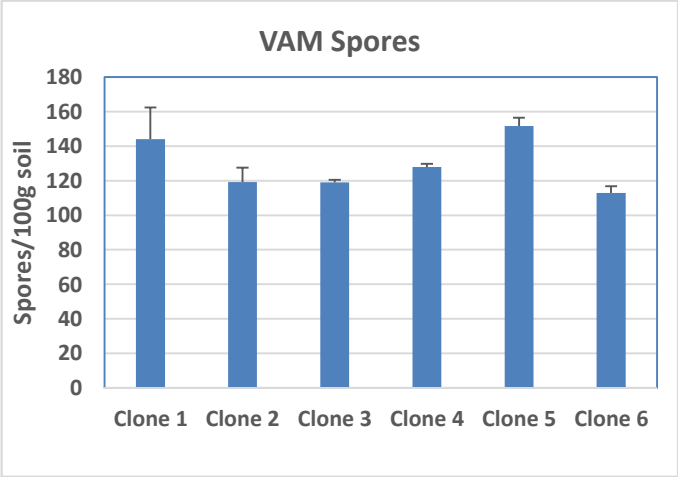
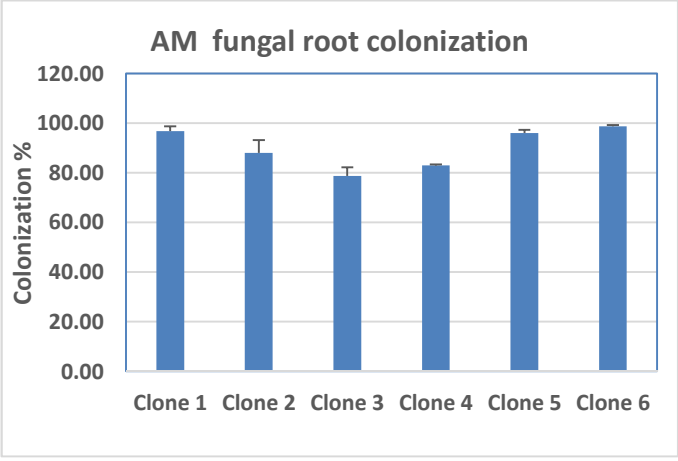


Fig. 6. Mean percent root colonization of AM fungi in six different clones of *N. cadamba.*



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Azatobacter population was high in the rhizosphere of NC-2 with mean value of 19.50x10^{-4}cfu/g and relatively less population density was recorded from the rhizosphere soil of all the other clones and minimum among them was recorded from NC-6 with 0.75x10^{-4}cfu/g.

Pseudomonas: The population density of *Pseudomonas* colonies isolated from the rhizosphere soil samples of six different clonal plants of *N. cadamba* is presented in Table 3 and Figure 3. *Pseudomonas* population was recorded only in four out of six colonies analyzed. It was high in the rhizosphere of NC-6 with mean value of 7.00x10^{-4} cfu/g and low in NC-2 with 0.50x10^{-4} cfu/g.

Actinomycetes: The population density of *Actinomycetes* colonies isolated from the rhizosphere soil samples of six different clonal plants of *N. cadamba* is presented in Table 4 and Figure 4. *Actinomycetes* population was recorded from the rhizosphere region of only four out of six clones studied and the mean value of its density varied from 0.75x10^{-4} cfu/g in NC-6 to 3.00x10^{-4} cfu/g in NC-2.

Population of AM fungal spores isolated from rhizosphere region of six different clonal plants of Neolamarckia cadamba: The rhizosphere soil samples of six different clonal plants of *Neolamarckia cadamba* were processed for estimating the spore population and the results of the analysis are given in Table 5 and Figure 5. The mean AM fungal spores population varied from 151.75 spores/100 g of soil in NC-5 clone to 113 spores/100 g of soil in NC-6. In general, all the clones analyzed had their rhizosphere soil with AM fungal spores.

AM fungal root colonization (%) in six different clones of Neolamarckia cadamba: Root colonization of AM fungi was estimated for six different clonal plants of *Neolamarckia cadamba* and the results of the analysis are provided in Table 6 and Figure 6. It was observed that all the samples had AM fungal colonization, but in varying percentage. The mean percentage colonization of AM fungi varied from 78.75% in NC-3 clone to 96.75% in NC-6 clone.

Occurrence and distribution of AM fungal spores in the rhizosphere of six different clonal plants of Neolamarckia cadamba: A total of twelve different AM fungi taxa belonging to three genera such as *Acaulospora, Gigaspora* and *Glomus* were recorded from the rhizosphere of six different clonal plants of *Neolamarckia cadamba* (Table 7). The genus *Glomus* was found to be the dominant one with ten species. *Glomus geosporum* was observed in the rhizosphere of all the six clonal plants of *N. cadamba* followed by *Glomus fasciculatum* with 5 different clonal plants and *Glomus etunicatum* with 4 different clonal plants. *Acaulospora scrobiculata* was recorded from the rhizosphere of two clones (NC-1 and NC-5), while *Gigaspora gigantea* from the rhizosphere of only one clonal plant (NC-3). The total number of AM fungal diversity in the clones varied from 3 in NC-4 to 8 each in NC-1 and NC-6. Diversity of microbial community in a specific rhizosphere environment was dependent on the plant species (Germida *et al.*, 1998). Many of the environmental factors such as temperature, light and atmospheric CO_2 influence the diversity and population of different microbes (Rovira, 1959). Koeberl *et al.* (2013) suggested that the plant species were vital drivers in structural and functional diversity of microorganisms in soil. The effective utilization of potential beneficial microbes as bio-fertilizers will not only provide economic benefits, but also improve and maintain the soil fertility and sustainability in natural soil ecosystem. This will in turn help in afforestation programme and reclamation of wastelands and other adverse sites. Tropical soils are either poor in phosphorus (P) or other essential nutrients or have an immobile form of P. In such condition, beneficial microorganisms can play an important role in improving the plant growth by increasing the supply of mineral nutrients to plant roots.

The present study analyzed the presence of different beneficial microorganisms in roots and rhizosphere soil of six different clonal plants of *N. cadamba*. It was observed that almost all the samples recorded the occurrence of PGPR and Actinomycetes. The findings of the present study are in accordance with the observations made by many earlier researchers on other plant species in India and elsewhere. Karthikeyan *et al.* (2008) reported that the microbial population was more in the rhizosphere soils as compared to non-rhizosphere soils of the medicinal plants such as *Ocimum sanctum, Coleus forskohlii, Catharanthus roseus* and *Aloe vera*. Sangeetha Menon and Mohan (2012) isolated potential phosphate solubilizing bacterial isolates from the rhizosphere of *Alianthus excelsa* in different locations of Tamil Nadu. Mohan and Menon (2015) isolated and identified a total of 51 PGPR isolates comprising of 18 PSB isolates, 16 isolates of *Azotobacter* and 17 isolates of *Azospirillum* from soils collected from different salt affected areas in Tamil Nadu and Pudhucherry, South India. Recently, Elham *et al.* (2016) studied the status of Plant Growth-Promoting Rhizobacteria (PGPR) from the rhizosphere of sugarcane in saline and non-saline soil and also determined some growth-promoting properties of the isolated PGPR organisms. Four strains were selected as having growth-promoting potential, which based on biochemical and phylogenetic analysis were identified as *Enterobacter cloacae* R13, *Enterobacter cloacae* R33, *Paenibacillus lactis* and *Pseudomonas* sp.

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Table 6. Total percent root colonization of AM fungi in six different clones of *N. cadamba.*

Percent root colonization	Clone No.	Min.	Max.	Mean ± SD
AM fungal root colonization	NC-1	92.0	100.0	96.75 ± 3.95
	NC-2	75.0	100.0	88.00 ± 10.30
	NC-3	75.0	89.0	78.75 ± 6.85
	NC-4	82.0	84.0	83.00 ± 0.82
	NC-5	92.0	98.0	96.00 ± 2.71
	NC-6	98.0	100.0	98.75 ± 0.96

Table 7. Distribution of AM fungal spores in the rhizosphere of six different clonal plants of *Neolamarckia cadamba.*

S. No.	AM fungal spores	Clone Number						Total
		NC-1	NC-2	NC-3	NC-4	NC-5	NC-6	
1	*Acaulospora scrobiculata*	*				*		2
2	*Gigaspora gigantea*			*				1
3	*Glomus etunicatum*	*		*		*	*	4
4	*Glomus fasciculatum*	*	*	*		*	*	5
5	*Glomus fulvus*	*		*			*	3
6	*Glomus geosporum*	*	*	*	*	*	*	6
7	*Glomus intraradices*		*				*	2
8	*Glomus microcarpum*	*				*	*	3
9	*Glomus monosporum*			*	*		*	3
10	*Glomus occultum*		*		*	*		3
11	*Glomus pubescens*	*					*	2
12	*Glomus* sp.	*		*		*		3
	Total	8	4	7	3	7	8	37

The status of percent root colonization and soil spore population of AM fungi were analysed in roots and rhizosphere soil samples of six different clonal plants of *N. cadamba* and the results of the study indicated that all the root samples had AM fungal colonization. It was also found that the vesicular, hyphal and arbuscular structures were found in the root segments of different clonal plants analyzed. Among these structures, vesicular and hyphal structures were larger in number and found in almost all the medicinal plant roots, while Arbuscular structures were found in few clonal plant root samples. The AM fungal spore number varied among different clonal plants analyzed, which may be attributed to various factors. The findings of the study are in accordance with that of by many earlier researchers in India and other parts of the world (Anderson *et al.*, 1983; Howeler *et al.*, 1987; Thapar and Khan, 1985; Muthukumar *et al.*, 1994; Mohan *et al.*, 1995; Mohan and Singh, 1996; 1997; Mohan and Babbar, 1997; Muthukumar and Udaiyan, 2000; 2001; Muthukumar *et al.*, 2006). Presence of AM fungal spores in the rhizosphere soil is a good sign of plant and soil health and it also indicates the involvement of AM fungi in symbiosis of these plants. Life cycle of AM fungi is mainly dependent on the plant roots, as they directly influence the spore germination, germination rate and direction of germ tubes, hyphal branching and recognition of host, root penetration, growth of hyphae in soil and in the sporulation of AM fungi.

Many investigators studied the AM colonization in medicinal plants and reported that most of the medicinal plants showed mycorrhizal colonization (Radhika and Rodrigues, 2010; Gogoi and Singh, 2011; Tanvir Burni and Farrukh Hussain, 2011; Jayaa *et al.*, 2012; 2013).

Conclusion

The present study revealed that the occurrence of PGPR, Actinomycetes and AM fungi from the roots and rhizosphere samples of six different clonal plants of *N. cadamba*. Although reports on the diversity status of AM fungi and PGPR are available on many tropical and temperate tree species, no such reports are available on the clonal plants of *N. cadamba* and thus the findings of this study are reported for the first time. Potential isolates of these beneficial microbes can be utilized for quality seedling production of many clonal plants for their better survival, establishment and productivity in field conditions.

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