Influence of Biofertilizers on the Biomass Production of *Casuarina equisetifolia* in Farm Forestry

K. Rajendran*, V. Sugavanam and P. Devaraj
Institute of Forest Genetics and Tree Breeding
P. O. Box No. 1061, Coimbatore-641 002
Tamil Nadu, India

Abstract

Casuarina equisetifolia seedlings inoculated with different biofertilizers such as *Azospirillum*, Phosphobacterium, VAM fungi and *Frankia* and their combinations were planted in farmland. Growth parameters were collected at bimonthly intervals up to 30 months. Biomass was estimated 24 months after planting. Maximum height, girth at breast height (gbh) and total biomass were obtained in the combined application of *Azospirillum*, Phosphobacterium, VAM and *Frankia*. The combination of VAM + *Frankia* among double inoculation, and the combination of *Azospirillum*, VAM and *Frankia* in triple inoculation also proved to be the best treatment in promoting the significant total height, gbh and total biomass production.

সারসংক্ষেপ

বিভিন্ন জীব-সার, যথা *Azospirillum*, Phosphobacterium, VAM ছত্রাক ও *Frankia* এবং তাদের মিলিত যৌগ *Casuarina equisetifolia* চারাতে ইনোকুলেশন করা হয় এবং পরে চারাগুলো মাঠে লাগানো হয়। দু'মাস পর পর ৩০ মাস পর্যন্ত ঐসব চারার বৃদ্ধি পরিমাপ করা হয়। চারা লাগানোর ২৪ মাস পর তাদের বায়োমাস নির্ণয় করা হয়। *Azospirillum*, Phosphobacterium, VAM ছত্রাক এবং *Frankia* -এর মিলিত যৌগ প্রয়োগকৃত চারাগুলোতে উচ্চতা, বুক উচ্চতায় বেড় এবং মোট বায়োমাস বেশি পাওয়া যায়। উল্লেখযোগ্য সর্বাধিক উচ্চতা, বুক উচ্চতায় বেড় এবং মোট বায়োমাস উৎপাদনে সহায়তাকারী হিসেবে VAM + *Frankia* -এর মিশ্রণের দ্বৈত ইনোকুলেশন এবং *Azospirillum*, VAM এবং *Frankia* -এর তৈরী মিশ্রণের ত্রয়ী ইনোকুলেশন সবচেয়ে ভাল হিসেবে প্রমাণিত হয়েছে।

Key words : Biofertilizers, biomass production, *Casuarina equisetifolia*, farm forestry

Introduction

The huge demand-supply gap and rising prices of industrial wood are making farm forestry and agroforestry plantations viable and attractive alternative land use option. Farm forestry will play a very important role in meeting the country's future wood requirement (Lal 1993). With increased pressure on available land resource, maximum harvestable quality biomass production per unit area is the prime concern.

*Corresponding author : Lecturer in Botany, Thiagarajar College, Post Box No. 107, 139-140 Kamarajar Salai, Madurai-625 009, Tamil Nadu, India. Email : kuppurajendran@usa.net

In the context of increasing importance of intensive silviculture and multiple land use approach, a large number of fast growing exotics are grown in India. Among them *Casuarina equisetifolia* is commonly used for wasteland development due to its ability to form symbiotic nitrogen fixing microorganism of *Frankia*. Fertilizers play an important role in increasing biomass production of plantations and forestry crops.

In modern days, intensive crop cultivation requires chemical fertilizers, but chemical fertilizer is not only in short supplies but also very expensive in developing countries like India. Alternatively, biofertilizers are ecofriendly and economically viable to increase the biomass production. VAM fungi help in protecting the plants from harmful pests, especially soil borne microorganisms (Myers 1993).

Evidences are available on the beneficial effect of biofertilizers in increasing the growth and biomas of *Casuarina equisetifolia* in laboratory and nursery conditions. The effect of *Casuarina equisetifolia* seedlings inoculated with *Frankia* (Reddell 1990), *Azospirillum* (Rodriguez-Barrueco *et al.* 1991) and VAM (Vasanthakrishna *et al.* 1995) have been well documented. The dual inoculation of *Azospirillum* and *Frankia* (Rajendran 1993) and VAM and *Frankia* (Ramirez *et al.* 1992) was also reported in the seedlings of *C. equisetifolia.* However, the effect of combined inoculation of *Azospirillum*, Phosphobacteria, VAM fungi and *Frankia* in field conditions needs to be studied. Hence, the present investigation was carried out to assess the compatibility of microorganisms and their influence on the growth and biomass production of *C. equisetifolia* in farm forestry.

Materials and methods

Biofertilizer inoculum

The VAM fungi, *Gloms fasciculatum*, was isolated and recorded as dominant species in the rhizosphere soil of four years old *C. equisetifolia* plantation at Pudukkottai, Tamil Nadu, India. It was multiplied in pot culture in the sterilised mixture of said : vermiculite (1 : 1 v/v) and maintained in the roots of *Sorghum vulgare* as the host plant. The inoculum contained extra-matrical hyphae, chlamydospores and infected root segments. Inoculum potentials were determined by the most probable number (Porter 1979) and approximately 12,500 infective propagules. Ten grams of the VAM fungi was added in the root zones of each seedlings of respective treatments.

Peat based culture of *Azospirillum* and Phosphobacterium with a population load of 10^{-9} and 10^{-8} colony forming unit/gram of peat soil respectively were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Frankia nodules were collected from the plus tree of *C. equisetifolia.* Nodules were washed and surface sterilised with 30% hydrogen peroxide for 20 minutes followed by 70% alcohol for 1 minute and then rinsed three times in distilled water, ground with a pestle and mortar and diluted with 2% sucrose solution (Reddell *et al.* 1988).

Seeds

Seeds were collected from the plus tree of five years old *C. equisetifolia* located at Vaithikovil of Pudukkottai district, Tamil Nadu, India. Matured cones were collected and the seeds were separated, graded and only bigger seeds were used for raising seedlings.

Potting media, transplanting of seedling and application of biofertilizers

Seeds were germinated in sand medium. Healthy and uniform size (about 5 cm height) seedlings were transplanted to 13 x 26 cm size polythene bags with a potting mixture of unsterilised sand : red soil : farm yard manure (2 : 3 : 1). Ten days after transplanting, peat based culture of 10 grams of *Azospirillum*, Phosphobacterium, vermiculite based VAM fungi and 10 ml of *Frankia* nodule suspension were applied to polythene bag at 5 cm depth near the root zone by making holes. There were 16 treatment combinations :

T1 - *Azospirillum*

T2 - Phosphobacterium

T3 - Vesicular arbuscular mycorrhizae (VAM)

T4 - *Frankia*

T5 - *Azospirillum* + Phosphobacterium

T6 - *Azospirillum* + VAM

T7 - *Azospirillum* + *Frankia*

T8 - Phosphobacterium + VAM

T9 - Phosphobacterium + *Frankia*

T10 - VAM + *Frankia*

T11 - *Azospirillum* + Phosphobacterium + VAM

T12 - *Azospirillum* + Phosphobacterium + *Frankia*

T13 - *Azospirillum* + VAM + *Frankia*

T14 - Phosphobacterium + VAM + *Frankia*

T15 - *Azospirillum* + Phosphobacterium + VAM + *Frankia*

T16 - Control (uninoculated)

Biofertilizer inoculated seedlings were maintained in the nursery for six months, then the seedlings were planted in farmland.

Experimental design and planting

Six months old *C. equisetifolia* seedlings inoculated with biofertilizers were planted in 30 cm^3 of pits at a spacing of 1 m x 1.5 m. The experiment was set up in a Randomised Block Design (RBD) with three replications. Each treatment blocks contained 36 plants. Totally 1728 seedlings were planted.

Study area

The study was conducted in a farm land at the Bank of Noiyal river in Coimbatore, Tamil Nadu, Inda at latitude $11^0$02'N and longitude 76^0 58'E, at an elevation of 409 m above mean sea level with an annual precipitation averages of 450-650 mm. Soil type is sandy clay loam (sand 63.6% : silt 26.8% : clay 9.6%) with a pH of 7.1. Organic content of the soil is 1.75%. The nitrogen, phosphorus, potassium, calcium and magnesium contents are 0.55%, 0.11%, 0.47%, 0.71% and 0.36% respectively.

Soil pH was determined in 1:2.5 ratio of soil water suspension using a pH meter (Jackson 1973). Nitrogen was determined by Kjeldahl method using Kjeltech Auto analyser 1030 (Piper 1966). Phosphorus was estimated by Olsens method using Venado Molybdate yellow colour (Jackson 1973). Potassium was estimated by using Flame photometer with neutral normal ammonium acetate solution (Standford and English 1949). Calcium and magnesium were determined by Versenate method (Jackson 1973). Organic carbon was estimated by Walkley and Black wet digestion method (Piper 1966).

Biometric observations

Total tree height and girth at breast height (gbh) were measured 6, 12, 18, 24 and 30 months after planting. Biomass (oven dry) components such as foliage, twig and stem were estimated 24 months after planting.

Estimation of VAM root infection and microbial population

Soil and plant root samples were collected 24 months after planting for the estimation of VAM infection, VAM spore density, *Azospirillum* and Phosphobacterium population in the rhizosphere soil. VAM spore density in each soil sample was estimated by a modified wet sieving and decanting technique as described by Gerdemann and Nicolson (1963). Mycorrhizal root infection was assessed following the procedure of Phillips and Hayman (1970). Dilution plate counting method was employed for the enumeration of microbial population in the soil samples. N-free semi-solid malate medium was used for *Azospirillum* (Dobereiner *et al.* 1976), Pikovskaya's medium was used for Phosphobacteria (Sundara Rao and Sinha 1963).

Frankia nodultion

Percentage of *Frankia* nodulated trees was assessed through excavating the soil portion of root system at depth of 0-30 cm. The number of nodule bearing trees were counted and percentage of *Frankia* nodulated trees were calculated using the following formula :

$$\text{Percentage of nodulated trees} = \frac{\text{Number of nodulated trees}}{\text{Total number of trees examined}} \times 100$$

Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA), and treatment means were separated using Duncan's Multiple Range Test ($P<0.05$) (Duncan 1955).

Results

Height

The result shows that combined inoculation of *Azospirillum* + Phosphobacterium + VAM + *Frankia* (T15) recorded maximum height in all the stages, that was registered 90.4%, 23.6%, 28.1%, 20.56% and 30.2% increased over control at 6, 12, 18, 24 and 30 months respectively. Statistically there is no height difference between the triple inoculations except *Azospirillum* + Phosphobacterium + VAM (T11) in 6 months after planting. *Azospirillum* + Phosphobacterium + *Frankia* (T12) in 12 months, *Azospirillum* + VAM + *Frankia* (T13) in 18, 24 and 30 months after planting registered better height over control (Table 1). Among double inoculations *Azospirillum* + VAM (T6) in six months, VAM + *Frankia* (T10) in 12, 18, 24 and 30 months after planting showed better height over control.

GBH

In general, combined inoculation of *Azospirillum* + Phosphobacterium + VAM + *Frankia* (T15) recorded maximum gbh at 6, 12, 18, 24 and 30 months after planting when compared with all other treatments. Among the triple inoculations, Phosphobacterium + VAM + *Frankia* (T14) and *Azospirillum* + VAM + *Frankia* (T13) in 6 months, *Azospirillum* + VAM + *Frankia* (T13) inoculated trees in 12, 18, 24 and 30 months after planting had better gbh (Table 1).

Among double inoculation, there was no significant variation between the treatments except *Azospirillum* + Phosphobacterium (T5) at 6 months after planting. But VAM + *Frankia* (T10) had better gbh among the double inoculations at 12, 18, 24 and 30 months after planting compared to control. In single inoculations, *Frankia* (T4) and VAM fungi (T3) recorded better gbh than other single inoculation in all the stages of growth.

Total above ground biomass

The results revealed that biofertilizers inoculated trees had significantly enhanced above ground biomass at 24 months after planting compared with uninoculated control trees (Table 2). *Azospirillum* + Phosphobacterium + VAM + *Frankia* (T15) inoculated trees were found to have higher above ground biomas than other treatments. Among triple inoculations, *Azospirillum* + VAM + *Frankia* (T13) registered next highest biomass than that of the control. Among double inoculations, VAM + *Frankia* (T10) registered more biomass than other treatments, but it was at par with *Azospirillum* + Phosphobacterium + VAM (T11) and *Azospirillum* + Phosphobacterium + *Frankia* (T12). In single inoculations, VAM fungi (T3) inoculated trees registered higher biomass when compared to other single inoculations.

Total below ground biomass

Combined inoculation with *Azospirillum* + Phosphobacterium + VAM + *Frankia* (T15) showed the highest root biomass (Table 2). However, it

Table 1. Growth of *Casuarina equisetifolia* inoculated with biofertilizers in the farmland.

Treatment	6 months		12 months		18 months		24 months		30 months	
	GBH (cm)	Height (m)	GBH (cm)	Height (m)	GBH (cm)	Height (m)	GBH (cm)	Height (m)	GBH (cm)	Height (m)
T1	2.3def	2.68ef	9.4b	5.4	13.67a	6.73g	17.07a	10.73c	23.70bc	12.35cd
T2	2.5ef	2.18cd	9.5bc	5.13b	14.87c	6.43cde	17.97b	9.93a	23.67bc	11.25ab
T3	2.2cde	1.79ab	10.1cde	5.24b	15.23de	6.67fg	18.70bcd	10.87cde	24.80cd	12.30bc
T4	2.9fg	1.56a	10.2de	5.60cd	15.43efg	6.50def	18.77cd	10.97e	24.47bcd	12.72cd
T5	2.0bcd	1.87abc	9.4b	4.64a	15.73g	6.30bcd	18.23bc	10.77cd	23.07ab	12.73cd
T6	2.6ef	2.61ef	9.7bcd	5.15b	15.0cd	6.18ab	19.13de	10.90de	23.63bc	12.48cd
T7	2.3def	2.05bc	9.7bcd	4.82a	14.47b	6.57efg	18.80cd	10.50b	24.33bcd	12.17bcd
T8	2.4ef	2.48de	9.2b	5.61cd	14.77bc	6.37b-e	19.67ef	10.87cde	24.30bcd	13.04cde
T9	2.4ef	1.80abc	10.2de	5.29b	15.77g	6.28bc	20.30f	10.37b	24.20bcd	12.37cd
T10	2.2c-f	1.75ab	10.9fg	5.84de	16.63h	7.00h	22.03hi	11.47f	26.43e	13.22de
T11	1.8ab	1.73ab	10.5ef	5.36bc	15.33ef	6.55efg	21.27g	11.00e	24.27bcd	12.79cd
T12	2.6fg	2.62ef	10.6ef	5.91e	15.67fg	6.57efg	21.33gh	11.53f	24.47bcd	12.86cd
T13	3.0gh	2.92f	11.2g	5.39bc	16.93h	7.45i	21.83ghi	11.03f	26.70e	12.86cd
T14	3.2h	2.87ef	10.2de	4.80a	15.63fg	7.12h	22.13i	11.60g	25.57be	13.04cde
T15	3.3h	2.99f	11.9h	5.66de	17.40i	7.73j	23.20j	11.90g	28.73f	13.96e
T16	1.5a	1.57a	8.5a	4.58a	13.43a	6.03a	16.80a	9.87a	21.70a	10.72a

Means followed by a common letter are not significantly different at the 5% level by DMRT

Treatments : T1 *Azospirillum*; T2 Phosphobacterium; T3 Vesicular arbuscular mycorrhizae (VAM); T4 *Frankia*; T5 *Azospirillum* + Phosphobacterium; T6 *Azospirillum* + VAM; T7 *Azospirillum* + *Frankia*; T8 Phosphobcterium + VAM; T9 Phosphobacterium + *Frankia*; T10 VAM + *Frankia*; T11 *Azospirillum* + Phosphobacterium + VAM; T12 *Azospirillum* + Phosphobacterium + *Frankia*; T13 *Azospirillum* + VAM + *Frankia*; T14 Phosphobacterium + VAM + *Frankia*; T15 *Azospirillum* + Phosphobacterium + VAM + *Frankia* and T16 Control.

Table 2. Biomass (kg/tree) of two years old *Casuarina equisetifolia* inoculated with biofertilizers in the farmland.

Treatment	Stem weight	Bark weight	Branch & twig weight	Needle weight	Total agb weight	Root weight	Root nodule	Total bgd	Total biomass
T1	7.675b	1.142bcd	1.797bc	3.749bcd	14.363c	2.486cd	0.041a	2.527cd	16.89c
T2	7.603c	0.660a	1.625b	2.639a	12.527b	1.553a	0.016a	1.569a	14.096b
T3	8.833cd	1.442def	1.740bc	3.567bc	15.582d	2.517cd	0.038a	2.555cd	18.137d
T4	9.133d	0.764ab	1.824bc	2.962a	14.683c	1.742ab	0.108b	1.840ab	16.533c
T5	8.127bc	1.347cde	1.877c	3.962cde	15.313cd	1.761ab	0.018a	1.780ab	17.093cd
T6	8.167bc	0.965abc	1.808bc	4.097def	15.037cd	2.226bc	0.030a	2.256bc	17.293cd
T7	8.533cd	0.627a	1.162a	4.215efg	14.537c	2.520cd	0.117a	2.367cd	16.904cd
T8	8.250bc	1.452def	3.348h	3.525b	16.576e	2.592cd	0.023a	2.615cd	19.191e
T9	11.142e	0.999abc	2.364d	4.338e-h	18.834f	2.520cd	0.098b	2.618cd	21.461f
T10	14.43g	1.290cde	2.571def	4.759hi	23.050g	3.275ef	0.129b	3.404ef	26.454h
T11	13.450f	1.345cde	2.653ef	4.534ghi	21.985g	3.312ef	0.035a	3.347e	25.204g
T12	13.667g	1.477def	2.554de	4.461fgh	22.159g	2.926de	0.119b	3.045de	25.204g
T13	15.750h	1.50def	2.719fg	4.524ghi	24.573h	3.876fg	0.140b	4.015fg	28.588i
T14	15.373h	1.776f	2.593ef	4.699 hi	24.441h	3.388ef	0.111b	3.499ef	27.490i
T15	16.600i	1.571ef	2.879g	4.895i	25.955i	4.282g	0.135b	4.417g	30.362j
T16	6.460a	0.988abc	1.076a	2.734a	11.258a	1.219a	0.012a	1.303a	12.561a

Means followed by a common letter are not significantly different at the 5% level by DMRT; agb = Above ground biomass; bgd = Below ground biomass.

Treatments : T1 *Azospirillum*; T2 Phosphobacterium; T3 Vesicular arbuscular mycorrhizae (VAM); T4 *Frankia*; T5 *Azospirillum* + Phosphobacterium; T6 *Azospirillum* + VAM; T7 *Azospirillum* + *Frankia*; T8 Phosphobcterium + VAM; T9 Phosphobacterium + *Frankia*; T10 VAM + *Frankia*; T11 *Azospirillum* + Phosphobacterium + VAM; T12 *Azospirillum* + Phosphobacterium + *Frankia*; T13 *Azospirillum* + VAM + *Frankia*; T14 Phosphobacterium + VAM + *Frankia*; T15 *Azospirillum* + Phosphobacterium + VAM + *Frankia* and T16 Control.

Table 3. Microbial population, VAM and *Frankia* infection in the rhizosphere of *Casuarina equisetifolia* inoculated with biofertilizer in the farmland (two years after planting).

Treatment	*Azospirillum*	Phosphobacterium	VAM colonization %	*Frankia* nodulation %
T1	3.8×10^6f	1.3×10^4b	34.63a	24.77ab
T2	0.1×10^6ab	4.2×10^4def	42.33c	27.57bc
T3	0.8×10^6b	1.5×10^4b	93.63fg	35.33de
T4	0.4×10^6ab	1.3×10^4b	44.97c	82.60g
T5	2.2×10^6cd	2.6×10^4c	36.90ab	32.33cd
T6	3.2×10^6e	0.5×10^4a	73.60e	24.67ab
T7	2.8×10^6ce	1.6×10^4b	41.40bc	76.00f
T8	0.5×10^6ab	2.6×10^4c	69.00e	32.43cd
T9	0.4×10^6ab	4.5×10^4ef	44.33c	79.90fg
T10	0.4×10^6ab	2.2×10^4c	92.67fg	92.23 hi
T11	0.1×10^6a	3.8×10^4d	73.00e	41.13e
T12	2.6×10^6cde	4.6×10^4f	56.83d	86.17gh
T13	2.7×10^6be	1.6×10^4b	90.53f	93.90 hi
T14	2.0×10^6c	4.0×10^4de	96.63g	85.00 gh
T15	2.8×10^6de	3.9×10^4de	93.83fg	98.63i
T16	1.0×10^6a	1.35×10^4b	30.00a	20.53a

Means followed by a common letter are not significantly different at the 5% level by DMRT

Treatments : T1 *Azospirillum*; T2 Phosphobacterium; T3 Vesicular arbuscular mycorrhizae (VAM); T4 *Frankia*; T5 *Azospirillum* + Phosphobacterium; T6 *Azospirillum* + VAM; T7 *Azospirillum* + *Frankia*; T8 Phosphobcterium + VAM; T9 Phosphobacterium + *Frankia*; T10 VAM + *Frankia*; T11 *Azospirillum* + Phosphobacterium + VAM; T12 *Azospirillum* + Phosphobacterium + *Frankia*; T13 *Azospirillum* + VAM + *Frankia*; T14 Phosphobacterium + VAM + *Frankia*; T15 *Azospirillum* + Phosphobacterium + VAM + *Frankia* and T16 Control.

was statistically at par with *Azospirillum* + VAM + *Frankia* (T13) and Phosphobacterium + VAM + *Frankia* (T14) treated trees. Among single inoculations, VAM (T3) inoculated trees showed higher root biomass when compared with other single treatments. In double inoculations, VAM + *Frankia* (T10) showed better root biomass but it was at par with Phosphobacterium + VAM (T9), *Azospirillum* + Phosphobacterium + VAM (T11) and Phosphobacterium + VAM + *Frankia* (T14).

Total biomass

The highest total biomass was obtained in the combined application of *Azospirillum* + Phospobacterium + VAM + *Frankia* (T15) followed by *Azospirillum* + VAM + *Frankia* (T13) and Phosphobacterium + VAM + *Frankia* (T14). Among the double and single inoculations, VAM + *Frankia* (T10) and VAM (T3) treated trees registered better biomass compared to control trees (Table 2).

Microbial population

Maximum *Azospirillum* population was enumerated in rhizosphere soil of *Azospirillum* (T1) treated trees followed by the *Azospirillum* combined application with other microorganisms. Similarly, maxmium Phosphobacterium population was enumerated in rhizosphere soil of Phosphobacterium (T2) treated trees, and it was statistically at par with Phospobacterium coinoculated with other beneficial microorganisms (Table 3).

Percentage of VAM infection

VAM infection percentage was heighest in (T14) followed by (T15), and it was statistically at par with *Azospirillum* + VAM + *Frankia* (T13), VAM + *Frankia* (T10) and VAM (T3). Lowest VAM infection was calculated in control plants, and it was statistically at par with (T1) and (T5).

Percentage of *Frankia* nodulation

Seedlings inoculated with *Frankia* and in combination with VAM had maximum nodulated trees. Percentage of nodulated trees was highest in *Azospirillum* + VAM + *Frankia* (T13), and it was statistically at par with Phosphobacterium + VAM + *Frankia* (T14) and VAM + *Frankia* (T10). Among individual inoculation *Frankia* (T4) inoculated trees had better nodulation than other individual inoculation. Lowest percentage of nodulated trees was control (T16), and it was statistically at par with *Azospirillum* inoculated (T1) trees (Table 3).

Discussions

In India intensive management of land has brought in to the prominence the need for a comprehensive knowledge of mineral requirements of forest tree species for suggesting suitable nutrient amendments in the nurseries and in the field. Application of suitable biofertilizers to tree crops is the most convenient method to satisfy the nutritional requirement of plants. *Casuarina equisetifolia* is one of the most important tree species grown widely in nutrient deficient, high alkaline and sandy soils. It has the ability to fix the atmospheric nitrogen and mobilize phosphorus due to the tripartite association of actinomycetes and mycorrhizal fungi.

C. equisetifolia attained 204.8 cm height and 3.6 cm diameter at the age of two years under arid conditions in Egypt (El - Lakany 1983). Halos (1983) reported that the height and diameter growth of *Casuarina* 1, 2 and 3 years old shows 1.6, 2.2 and 2.5 m respectively. In the present study the excellent growth of *C. equisetifolia* were observed in height ranging from 9.87-11.90 m and GBH ranging from 16.8-23.2 cm. The growth might be due to application of biofertilizers, regular irrigation, and intensive care.

In the present study, *C. equisetifolia* plants inoculated with biofertilizers had better growth. Combined inoculation of *Azospirillum* + Phospobacterium + VAM + *Frankia* (T15) recorded excellent growth, biomass and nodulation. The increase in growth and biomass due to co-inoculation of all the four biofertilizers might be strongly correlated with improved accumulation of both nitrogen due to *Azospirillum* and *Frankia* (Wong and Stenberg 1979, Reddell *et al.* 1988) and phosphorus due to VAM, and Phosphobacteria inoculation (Habte and Manjunath 1987).

The percentage of VA mycorrhizal colonization in the present study was high due to conjoint inoculation of *Azospirillum* + Phosphobacterium + VAM + *Frankia* in *C. equisetifolia* trees. VAM fungi application either individually or in combination with other microorganism had also increased the colonization in *C. equisetifolia* (Gunjal and Patil 1992). The increase in the VAM mycorrhizal infection in the root may be enhanced in the presence of Phosphate solubilizing bacteria (Azcon *et al.* 1976) or due to higher uptake of N and P (Manjunath *et al.* 1984, Dela Cruz *et al.* 1988).

In the uninoculated control, the colonization was 30%. This indicates the presence of native mycorrhizal fungi in the soils. Inspite of the presence of the native VAM fungi in untreated control trees, their growth response was much less than in specific VAM treated trees. It is concluded that the natural VAM fungi in the soil may be insufficient as expressed by Powell and Daniel (1978) and there is need to apply most suitable VAM fungi to increase the growth and biomass production. In the present study also artificial inoculation improved the plant growth more than the indigenous VAM fungi.

Plants inoculated with *Frankia* had more nodulation and higher nodular biomass than uninoculated control trees. Combined inoculation of *Frankia* + Phosphobacteria + VAM had excellent nodule development than single inoculation of *Frankia*. Growth of all plants inoculated with *Frankia* alone was poor and comparable to that of uninoculated trees. Nodulation was significantly low even after inoculation with effective *Frankia*. Poor nodulation may be attributed to the P stress in this P deficient soil brought about by the absence of VAM fungi which otherwise could provide the Prequired for nodulation and N_2 fixation. Nodulation was maximum in trees inoculated with *Frankia* + VAM fungi + Phosphobacteria. This implies that effective VAM fungi such as the species used played a major role in nodulation as reported also by Manjunath *et al.* (1984).

Dual inoculation with effective *Frankia* and mycorrhizal fungi has potential to significantly increase *Casuarina* productivity whilst minimizing the need for fertilizer application in P deficient soils (Diem and Gautheir 1982). The present study clearly demonstrates the importance of VAM and Phosphobacteria for good nodulation in association with *Frankia*. This is also the first report to show that in unsterilized soils in the field inoculation with combined inoculation of *Azospirillum*, VAM, *Frankia* and Phosphobacteria had enhanced growth and biomass of *C. equisetifolia*.

It is suggested that under appropriate management, the use of efficient biofertilizrs lead to increased growth and biomass of *C. equisetifolia* in field conditions. The present study had clearly shown that the combined application of *Azospirillum* + Phosphobacterium + VAM + *Frankia* plays a signification role in improving the growth and biomass of *C. equisetifolia*.

References

Azcon, R.; Barea, J. M . and Hayman, D. S. 1976. Utilisation of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate solubilizing bacteria. *Soil Biology and Biochemistry* 8 : 135-138.

Dela Cruz, R. Z.; Manalo, M. Q.; Aggannan, N. S. and Tambalo, J. D. 1988. Growth of three legume trees inoculated with VAM fungi and *Rhizobium*. *Plant and Soil* 108 : 111-115.

Diem, H. G. and Gauthier, D. 1982. Effect de lingection endomycorhizienne (*Glomus mosseae*) sur la nadulation *et al.* crossance de *Casuarina equisetifolia C. R. Academy of Science, Paris* 294 : 215-218.

Dobereiner, J.; Marriel, J. E. and Nery, J. 1976. Ecological distribution of *Spirillum lipoferum* Beijerinick. *Canadian Journal of Microbiology* 22 : 1461-1473.

Duncan, D. B. 1955. Multiple Range and Multiple f-tests. *Biometrics* 11 : 1-42.

El-Lakany, M. H. 1983. A review of breeding drought resistant *Casuarina* for shelterbelt establishment in arid regions with special reference to Egypt. *Forest Ecology and Management* 6 : 129-137.

Gerdemann, J. M. and Nicolson, T. H. 1963. Spores of mycorrihzal Endgone species extracted from soil by wet sieving and decanting. *Transaction of the British Mycological Society* 46 : 235-244.

Gunjal, S. S. and Patil, P. L. 1992. Mycorrhizal control of wilt in *Casuarina. Agroforestry Today* 4 : 14-15.

Habte, M. and Manjunath, A. 1987. Soil solution phosphorus status and mycorrhizal dependency in *Leucaena leucocephala. Applied Environmental Microbiology* 53 : 797-801.

Halos, S. C. 1983. *Casuarina* in Philippine forest development. *In* : Midgley, S. J.; Turnbull, J. W. and Johnston, R. D. (eds.). Casuarina *Ecology, Management and Utilization.* Proceedings of the First International *Casuarina* Workshop, CSIRO, Melbourne, Australia. pp. 89-98.

Jackson, M. K. 1973. *Soil Chemical Analysis*. Printice Hall of India (Pvt.) Ltd., New Delhi.

Lal, P. 1993. Farm forestry in India-problems and potential. National Workshop on "Farm Forestry Management" organized by Indian Institute of Foresty Management, Bhopal, June 23-26.

Manujunath, A.; Bagyaraj, D. J. and Gopala Gowda, H. S. 1984. Dual inoculation with VAM and *Rhizobium* is beneficial to *Leucaena. Plant and Soil* 78 : 445-448.

Myers, M. 1993. Mycorrhizae : their use as biofertilizers. *Horticulturist* 2 : 8-12.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transaction of British Mycological Society* 55 : 158-161.

Piper, C. S. 1966. *Soil and Plant Analysis.* Hans Publications, Bombay.

Porter, W. M. 1979. The most probale number method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research* 17 : 515-518.

Powell, C. L. and Daniel, J. 1978. Growth of white clover in undisturbed soils after inoculation with efficient mycorrhizal fungi. *New Zealand Journal of Agricultural Research* 21 : 675-681.

Rajendran, K. 1993. Effect of *Frankia* and *Azospirillum* on the growth and biomass production of *Casuarina equisetifolia* Forst. Proceedings of the National Seminar on Biodiversity Strategies for Conservation and Future Challenges. Bharathiar University, Coimbatore, India. pp. 187-190.

Ramirez, S. H.; Valdes Ramires, M. and Cruz-Cisneros, R. 1992. Inoculation and irrigation with waste water of *Casuarina* in nurseries. *Turrialba* 42 : 476-481.

Reddell, P. 1990. Increasing productivity in plantations of *Casuarina* by inoculation with *Frankia*. *In* : El-Lakany, M.; Turnbull, J. W. and Brewbaker, J. L. (eds.). *Advances in* Casuarina *Research*. Desert Development Centre, AUC, Cairo. pp. 133-140.

Reddell, P.; Rosbrook, P. A.; Bowen, G. D. and Gwale, D. 1988. Growth response in *Casuarina cunninghamiana* plantings inoculation with *Frankia*. *Plant and Soil* 108 : 76-86.

Rodriguez-Barrueco, C.; Cervantes, E.; Subba Rao, N. S. and Rodriguez-Caceres, E. 1991. Growth promoting effect of *Azospirillum brasilense* on *Casuarina cunninghamiana* Mig. *Plant and Soil* 135 : 121-124.

Stanford, S. and English, L. 1949. Use of flame photometer in rapid soil tests of K and Ca. *Agronomy Journal* 41 : 446-447.

Sundara Rao, W. V. B. and Sinha, M. K. 1963. Phosphate dissolving organisms in the soil and rhizosphere. *Indian Journal of Agricultural Science* 33 : 272-278.

Vasanthakrishna, M.; Bagyaraj, D. J. and Nirmalnath, P. J. 1995. Selection of efficient VAM fungi for *Casuarina equisetifolia* second screening. *New Forests* 121 : 157-162.

Wong, P. P. and Sternberg, N. E. 1979. Characterization of *Azospirillum* isolates from nitrogen fixing roots of harvested sorghum plants. *Applied and Environmental Microbiology* 38 : 1189-1191.