



RESEARCH ARTICLE

# Effect of bio-fertilizers inoculation on the growth and biomass productivity on the seedlings of shola tree species under nursery condition

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## Abstract

Under nursery conditions, this study evaluates the impact of biofertilizer inoculation on the biomass productivity and growth of three Shola tree species: *Syzygium montanum*, *Syzygium arnottianum* and *Elaeocarpus oblongus*. Treatments included the individual and combined application of *Azospirillum*, Phosphobacteria and VAM fungi, with an uninoculated control group for comparison. *S. montanum* recorded the highest growth parameters (shoot length: 39.23 cm, root length: 41.98 cm, dry weight: 5.35 g), highlighting that the combined application of *Azospirillum* + Phosphobacteria + VAM significantly increased shoot length, root length, total dry weight and shoot/root ratio across all species. Growth measures were consistently lowest in the uninoculated control. Over the six-month study period, all growth metrics gradually rose, indicating the synergistic effect of biofertilizer combinations in fostering the biomass productivity and growth of Shola tree seedlings. These results demonstrate the potential of biofertilizers in reforestation and sustainable forestry methods.

**Keywords:** *Azospirillum*; *Elaeocarpus oblongus*; phosphobacteria; *Syzygium arnottianum*; *Syzygium cumini*; VAM

## Introduction

*Syzygium cumini*, commonly known as Indian blackberry or Jamun, belongs to the Myrtaceae family and is widely distributed across India, including the Western Ghats. This evergreen tree is valued for its edible fruits, which are rich in antioxidants, vitamins and minerals (1). The tree's dense canopy helps regulate microclimates and helps prevent soil erosion. Research on *Syzygium cumini* has highlighted its high antioxidant content, which contributes to its medicinal properties and potential in managing diabetes and oxidative stress-related diseases. Additionally, studies have emphasized its ecological benefits, including microclimate regulation and soil conservation, making it a valuable species for afforestation efforts.

*Syzygium arnottianum*, a lesser-known species, thrives in the moist, shaded conditions of shola forests and is recognized for its ecological significance in these montane ecosystems. It is characterized by glossy leaves, small white flowers and dark fruits that attract birds, thereby facilitating seed dispersal. Although less studied, *Syzygium arnottianum*, has been the subject of ecological research focusing on its importance in seed dispersal networks. Investigations into its interactions with avian frugivores suggest that it plays a crucial

role in maintaining biodiversity and facilitating forest regeneration in shola ecosystems. Though less studied compared to *S. cumini*, this tree is vital for maintaining biodiversity and supporting wildlife populations in shola habitats (2).

*Elaeocarpus oblongus*, a member of the Elaeocarpaceae family, is another ecologically significant tree species native to shola forests. Known for its evergreen foliage and oblong fruits, this species thrives in cool, moist environments typical of high-altitude forests (3). Studies on *Elaeocarpus oblongus* have examined its adaptation to such conditions, highlighting the role of its evergreen canopy in carbon sequestration and microhabitat stabilization. Research on its reproductive biology and fruiting patterns has further provided insights into the ecological dynamics of shola forests.

VAM fungi establish a symbiotic relationship with plant roots, significantly enhancing nutrient absorption, particularly phosphorus, from the soil. This interaction is particularly beneficial for shola tree species growing in nutrient-deficient soils. Studies on VAM fungi have consistently demonstrated their effectiveness in enhancing phosphorus uptake in nutrient-poor soils, which is particularly beneficial for montane tree species like *Syzygium* and *Elaeocarpus*. In addition to enhancing

nutrient acquisition, VAM fungi also contribute to enhanced resistance against environmental stresses, thereby promoting the health and longevity of these forest ecosystems (4).

*Azospirillum* enhances nitrogen availability by fixing atmospheric nitrogen and producing growth-promoting substances like auxins and cytokinins. In species such as *Syzygium* and *Elaeocarpus*, *Azospirillum* can promote better root development, leading to improved water and nutrient uptake (5). *Azospirillum* has been widely studied for its nitrogen-fixing capabilities, with experiments confirming its role in increasing root biomass and water uptake efficiency in tree seedlings. Investigations into its interactions with *Syzygium* and *Elaeocarpus* suggest potential applications in sustainable forest restoration programs.

The use of Phosphobacteria ensures that shola tree species thrive in phosphorus-deficient soils, which are common in montane regions. By enhancing phosphorus availability, Phosphobacteria promote better growth and biomass production (6). The use of Phosphobacteria in phosphorus-deficient soils has been a subject of agronomic and ecological research, with findings indicating improved tree growth, enhanced biomass production and better stress resistance in shola tree species.

## Materials and Methods

### Location of the study

The experiments were conducted at the Department Nursery, Bhandishola, Coonoor, under the Forest Department. The site is situated at an elevation of 1800 m above MSL. The region receives an average annual rainfall of 1339 mm. The temperature ranges from 11.7 °C to 16.9 °C, with relative humidity varying between 70 to 80 %. Laboratory analyses were carried out at the Forest College and Research Institute, Mettupalayam.

### Materials

The nursery stocks of three shola tree species viz., *Syzygium montanum* Gamble (Poonagay), *S.arnottianum* Walp. (Kattu naval) and *Elaeocarpus oblongus* Gaertner (Bikki) served as the plant material for this study.

### Methods

Six-month-old seedlings of the selected shola tree species were planted on 04-01-1992 in a sufficient number of poly bags, each measuring 30 x 20 cm and filled with 2 kg of shola soil exhibiting the following properties:

#### Shoot length

The measurement was taken from the root collar to the tip of the shoot and expressed in cm.

#### Root length

The seedlings were carefully removed from the containers without damaging the root and the length was measured from the collar region to the tip of the root, expressed in cm.

#### Total dry weight

This was determined by adding the dry weights of the root and shoot and the total was expressed in g.

#### Shoot/root ratio

This was calculated based on the dry weights of the shoot and root of the seedlings.

## Results

### Shoot length

Inoculation with *Azospirillum* Phosphobacteria + VA-mycorrhizal fungi significantly increased shoot length in *Syzygium montanum* (39.23 cm), *S.arnottianum* (34.83 cm) and *Elaeocarpus oblongus* (31.25 cm) compared to other bio-fertilizers treatment. In contrast, the uninoculated control recorded lower shoot lengths of 30.01 cm, 25.17 cm and 19.76 cm, respectively. The influence of VA-mycorrhizal fungi on shoot length was consistently significant and positive, whether applied individually or in combination with *Azospirillum* or Phosphobacteria. Across all species, shoot length increased significantly over the course of the growth period (Table 1).

### Root length

Inoculation with *Azospirillum* Phosphobacteria and VA-mycorrhizal fungi resulted in the maximum root length in *S. montanum* (41.98 cm), *S.arnottianum* (29.20 cm) and *E. oblongus* (29.65 cm) when compared to individual or other combinations of bio-fertilizers. The uninoculated control recorded the minimum root lengths of 21.13cm, 17.61cm and 19.60 cm, respectively. The effect of VA-mycorrhizal fungi on root length was found to be significant both when applied individually and in combination with *Azospirillum* or Phosphobacteria. In all species, root length increased significantly over the duration of the growth period (Table 2).

### Total dry weight

Seedlings inoculated with *Azospirillum* Phosphobacteria and VA-mycorrhizal fungi recorded higher total dry weight in *S. montanum* (5.35 g), *S.arnottianum* (5.16 g) and *E. oblongus* (4.67 g) compared to those treated with other bio-fertilizer combinations and the uninoculated control. The control group exhibited lower dry weights of 3.86 g, 3.67 g and 2.54 g respectively. The response of this parameter to VA-mycorrhizal fungi was significant, whether applied individually or in combination with other bio-fertilizers. Across all species, total dry weight was highest at 6 months after bio-fertilizer application (Table 3).

### Shoot/Root ratio

Among the bio-fertilizers inoculation, Phosphobacteria + VA-mycorrhizal fungi *Azospirillum* inoculations recorded higher shoot/root ratios in *S. montanum* (1.16) and *S.arnottianum* (1.15) compared to other bio-fertilizers treatments. Seedlings of *E. oblongus* inoculated with *Azospirillum* + Phosphobacteria exhibited the highest shoot/root ratio (1.23) when compared to other bio-fertilizers treatments and the uninoculated control. The shoot/root ratio increased significantly with the growth period in all species (Table 4).

## Discussions

The present investigation revealed a significant increase in shoot length of *S. montanum*, *S.arnottianum* and *E. oblongus* due to the combined inoculation of *Azospirillum* Phosphobacteria and VA-mycorrhizal fungi when compared to the uninoculated control.

**Table 1.** Effect of biofertilizers inoculation on the shoot length (cm) in Shola tree species under nursery conditions

Biofertilizers	<i>Syzygium montanum</i>				<i>Syzygium arnottianum</i>				<i>Elaeocarpus oblongus</i>			
	Months after inoculation				Months after inoculation				Months after inoculation			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
Control	25.57	29.69	34.78	30.01	18.64	25.25	31.64	25.17	16.26	19.27	23.76	19.76
Azospirillum	25.94	30.09	35.65	30.56	18.92	26.49	32.92	26.11	16.20	22.84	25.94	21.66
Phosphobacteria	26.01	32.73	37.26	32	18.50	28.65	34.62	27.25	17.26	22.96	25.98	22.06
VAM	26.49	33.65	40.42	33.52	18.94	30.21	35.94	28.36	17.43	25.76	28.64	23.94
Azospirillum + Phosphobacteria	25.78	35.92	42.19	34.63	19.45	33.76	36.66	29.95	18.00	27.43	30.79	25.40
Azospirillum + VAM	26.74	36.56	44.68	36	19.01	33.92	39.00	30.64	16.77	33.64	35.26	28.55
Phosphobacteria + VAM	27	37.73	44.94	36.55	18.32	39.46	39.55	32.43	18.21	33.19	25.41	28.93
Azospirillum + Phosphobacteria + VAM	26.92	42.84	47.95	39.23	19.00	41.82	43.68	34.83	16.94	37.95	39.57	31.25
Mean	26.30	34.90	40.98	34.03	18.85	32.44	36.75	29.34	17.13	27.79	30.67	25.19
	SEd		CD		SEd		CD		SEd		CD	
Bio fertilizer	0.432		0.863		0.316		0.631		0.279		0.557	
Period	0.265		0.529		0.192		0.386		0.171		0.341	
Biofertilizer period	0.749		1.495		0.547		1.093		0.489		0.964	

**Table 2.** Effect of biofertilizers inoculation on the root length (cm) in Shola tree species under nursery conditions

Biofertilizers	<i>Syzygium montanum</i>				<i>Syzygium arnottianum</i>				<i>Elaeocarpus oblongus</i>			
	Months after inoculation				Months after inoculation				Months after inoculation			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
Control	16.69	20.18	26.52	21.13	13.68	17.43	21.72	17.61	12.43	20.14	26.24	19.60
Azospirillum	16.89	26.43	33.68	25.67	13.65	21.71	26.46	20.60	13.00	23.76	28.17	21.64
Phosphobacteria	17.00	24.56	28.72	23.42	12.94	20.29	25.21	19.48	13.81	24.15	28.92	22.29
VAM	18.20	31.29	37.29	28.92	13.08	25.16	29.43	22.55	13.00	29.65	34.16	25.60
Azospirillum + Phosphobacteria	16.91	39.46	45.53	33.96	13.00	22.74	26.23	20.65	12.84	25.14	30.92	22.96
Azospirillum + VAM	18.00	43.69	53.32	38.00	12.89	23.94	26.92	21.25	12.09	27.00	31.00	23.36
Phosphobacteria + VAM	16.78	45.71	54.68	39.05	12.86	30.17	33.71	25.28	12.52	32.42	36.86	27.26
Azospirillum + Phosphobacteria + VAM	18.12	49.19	58.64	41.98	13.97	34.68	38.96	29.20	13.21	34.95	40.81	29.65
Mean	17.32	35.06	42.17	31.15	13.26	24.51	28.58	22.11	12.86	27.15	32.13	24.04
	SEd		CD		SEd		CD		SEd		CD	
Bio fertilizer	0.543		1.085		0.426		0.851		0.355		0.708	
Period	0.333		0.664		0.261		0.521		0.217		0.434	
Biofertilizer period	0.941		1.879		0.738		1.475		0.614		1.227	

**Table 3.** Effect of biofertilizers inoculation on the total dry weight (g) in Shola tree species under nursery conditions

Biofertilizers	<i>Syzygium montanum</i>				<i>Syzygium arnottianum</i>				<i>Elaeocarpus oblongus</i>			
	Months after inoculation				Months after inoculation				Months after inoculation			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
Control	2.43	3.96	5.21	3.86	2.13	3.53	5.36	3.67	1.39	2.23	4.01	2.54
Azospirillum	2.48	4.26	5.73	4.15	2.17	3.69	5.85	3.90	1.41	2.45	4.12	2.66
Phosphobacteria	2.39	4.05	5.36	3.93	2.16	4.05	5.56	3.92	1.38	2.75	4.62	2.91
VAM	2.40	4.97	6.65	4.67	2.20	4.73	6.04	4.32	1.37	3.56	5.03	3.32
Azospirillum + Phosphobacteria	2.41	4.69	5.98	4.36	2.19	4.39	5.97	4.18	1.42	3.25	5.29	3.32
Azospirillum + VAM	2.39	5.86	6.73	5.00	2.18	5.78	6.84	4.93	1.40	4.36	5.96	3.90
Phosphobacteria + VAM	2.45	5.90	6.69	5.01	2.17	5.36	6.41	4.64	1.42	4.27	5.90	3.86
Azospirillum + Phosphobacteria + VAM	2.41	6.31	7.34	5.35	2.15	6.05	7.29	5.16	1.45	5.62	6.93	4.66
Mean	2.42	5.00	6.21	4.54	2.16	4.69	6.16	4.33	1.40	3.56	5.23	3.39
	SEd		CD		SEd		CD		SEd		CD	
Bio fertilizer	0.046		0.093		0.048		0.097		0.039		0.079	
Period	0.028		0.057		0.029		0.059		0.024		0.048	
Biofertilizer period	0.080		0.161		0.084		0.168		0.068		0.137	

**Table 4.** Effect of biofertilizers inoculation on the shoot/ root ratio in Shola tree species under nursery conditions

Biofertilizers	<i>Syzygium montanum</i>				<i>Syzygium arnottianum</i>				<i>Elaeocarpus oblongus</i>			
	Months after inoculation				Months after inoculation				Months after inoculation			
	0	3	6	Mean	0	3	6	Mean	0	3	6	Mean
Control	0.89	0.90	1.07	0.95	0.83	0.93	1.06	0.94	0.85	0.97	1.04	0.95
Azospirillum	0.92	0.97	1.11	1.00	0.85	0.96	1.08	0.96	1.04	1.02	1.07	1.04
Phosphobacteria	0.92	0.95	1.07	0.98	1.07	1.04	1.07	1.06	1.02	1.06	1.13	1.07
VAM	0.93	1.12	1.29	1.11	1.85	1.03	1.09	1.05	1.04	1.15	1.20	1.13
Azospirillum + Phosphobacteria	0.92	1.14	1.22	1.09	1.14	0.99	1.09	1.04	1.05	1.07	1.22	1.11
Azospirillum + VAM	0.93	1.12	1.26	1.10	1.06	1.04	1.10	1.06	1.05	1.30	1.35	1.23
Phosphobacteria + VAM	0.94	1.12	1.21	1.09	1.06	1.03	1.09	1.06	1.06	1.30	1.32	1.22
Azospirillum + Phosphobacteria + VAM	0.95	1.24	1.30	1.16	1.10	1.15	1.22	1.15	1.07	1.23	1.27	1.19
Mean	0.92	1.07	1.19	1.06	1.01	1.02	1.10	1.04	1.02	1.13	1.20	1.11
	Sed		CD		Sed		CD		Sed		CD	
Bio fertilizer	0.015		0.031		0.013		0.026		0.019		0.039	
Period	0.009		0.019		0.007		0.015		0.011		0.023	
Biofertilizer period	0.027		0.054		0.022		0.044		0.033		0.067	

Such an increase in shoot length due to the combined inoculation of these three bio-fertilizers has been previously reported in *R.nilagiricum*, *S.arnottianum* and *S.cumini* (7), as well as in *Thea sinensis* (8).

The present finding of enhanced shoot length due to individual inoculation of VA-mycorrhizal fungi supports the observations of earlier studies on *Magnolia grandiflora* (9), *Casuarina equisetifolia* (10), *Acacia scleroxyla* (11), *Acacia nilotica*, *Leucaena leucocephala*, *E.tereticornis*, *C.equisetifolia* (12), *A.auriculiformis*, *A.mangium*, *Albizia falcatoria* (13) and *A.cyanophylla* (14). The positive effect of VA-mycorrhizal fungi on shoot growth is primarily due to their ability to enhance nutrient uptake, particularly phosphorus, which is often a limiting factor in plant development. VAM fungi extend their hyphal networks into the soil, increasing root surface area and facilitating better absorption of essential nutrients and water. This improved nutrient uptake leads to better overall plant vigor, increased photosynthetic efficiency and greater biomass accumulation.

The combined inoculation of Phosphobacteria, VA-mycorrhizal fungi and *Azospirillum* was found to record the longest root system in all three species. This finding aligns with the results in tea (8). The possible reason for the increased root length in seedlings inoculated with the combination is the synergistic interaction between the three beneficial microorganisms, which likely resulted in enhanced plant growth and nutrient uptake. A similar increase in root length was observed in *Alnus incana* due to the combined inoculation of *G. fasciculatum*, *Paxillux involutus* and *Frankia* (15). The researcher attributed this effect to the ability of mycorrhizal plants to produce more growth regulators, resist root pathogens and survive in adverse soil conditions and higher soil temperature (16).

Phosphorus is a vital macronutrient essential for root and shoot development, yet it often exists in insoluble forms in the soil. Phosphobacteria facilitate the solubilization of phosphate compounds, making phosphorus more available for plant uptake. This increased phosphorus availability supports energy transfer, root expansion and ultimately shoot growth.

When the S/R ratio was calculated based on shoot and root biomass, it was found to be significantly higher in seedlings inoculated with the combined treatment of Azospirillum + Phosphobacteria and VA-mycorrhiza than uninoculated control. According to (11), the S/R ratio was much higher in mycorrhizal seedlings than in non-mycorrhizal seedlings of *A.scleroxyla*. Similar findings were also summarised in *Fraxinus pennsylvanica* and in *Acacias* (17, 18).

The beneficial effects of mycorrhizae on the S/R ratio may partially be interrupted as a response to improved mineral nutrition. VA-mycorrhizal fungi enhance the growth rate of plants and influence the partitioning of phyto mass between the shoot and root systems. This is attributed to the increased nutrient uptake facilitated by fungal activity and its efficient translocation to the aerial parts, which enhances photosynthate utilization in the shoot. Consequently, a relatively smaller portion of photosynthates is allocated to the roots, resulting in a higher S/R ratio in VA-mycorrhizal plants compared to their non-mycorrhizal counterparts (11, 19).

## Conclusion

The study demonstrates the significant positive impact of biofertilizer inoculation on the growth and biomass productivity of Shola tree species, specifically *Syzygium montanum*, *Syzygium arnottianum* and *Elaeocarpus oblongus*. The combined application of *Azospirillum*, Phosphobacteria and VA-mycorrhizal fungi was particularly effective in enhancing shoot and root length, total dry weight and the shoot/root ratio compared to the uninoculated control. These results highlight the synergistic effects of biofertilizer combinations in promoting plant growth by improving nutrient uptake and overall biomass production.

The biofertilizer-treated seedlings exhibited progressive improvements in all growth parameters over the six-month study period. This research underscores the potential of using biofertilizers as a sustainable approach for enhancing the productivity and health of Shola tree species in nursery conditions, which could contribute to successful reforestation and biodiversity conservation efforts in montane ecosystems.

Furthermore, the study emphasizes the benefits of incorporating biofertilizers in forestry practices to enhance the growth performance of tree seedlings, offering a promising tool for sustainable forestry and ecosystem restoration.

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## Authors' contributions

IS and HBR carried out the research. HBR participated in the statistical analysis. MS participated in sequence alignment. All authors read and approved of the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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