

Effect of fluorescent pseudomonas and *Glomus* sp. on the bamboo root morphology

Abstract

Dendrocalamus strictus (male bamboo) is one of the most important bamboo species in India. The bamboo plays an important role in various areas, such as socio-economy of the country as well as the environment. As it also the fastest growing giant grass in tropical region, especially during the monsoon season, gathering of biomass at such quick time is also remains a mystery. Bamboo has a very shallow root system (upto 3–4 feet) with fibrous in nature, the dependency of roots on surrounding microbes for nutrient uptake needs an attention. In the current study, researchers attempted to explore the role of rhizospheric microbes isolated from the roots of such bamboos from the forest on the root architecture of bamboo roots. It was found that fluorescent pseudomonads and arbuscular mycorrhizae played a definite role in shaping the root morphology, which indirectly or directly influenced in gathering biomass in the plants more quickly, especially in case of bamboo.

Keywords: rhizosphere, PGPR, mycorrhizae, bamboo, P solubilisation

Volume 2 Issue 5 - 2018

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Received: August 02, 2018 | **Published:** September 28, 2018

Introduction

India is one of the 12 mega diversity countries of the world which has 7% of the world's biodiversity and total forest cover of 21.05% (692,027 km²) of the geographical area of the country.¹ India is the second richest country in the world after China in terms of bamboo genetic resources. Indian bamboo forests contain 23 genera including 19 indigenous; and 136 species with 125 indigenous and 11 exotic.¹ The bamboo is also called 'green gold' in India; the country that has the second-largest reserve of bamboo in the world.² Bamboo in many ways is the mainstay of the rural Indian economy, sparking considerable social and ecological spin-offs. Among the bamboo species, *Dendrocalamus strictus* (Roxb.) Nees (English name= male bamboo) is an important bamboo species in India.³

The root system of *D. strictus* consist of a dense mass of short rhizome in which nodes are close to each other and provided with the clusters of small rootlets. Every node may have one or more eyes, which are capable of developing into new culm, even though, they, generally, remain dormant. The new rhizome grows horizontally for a short distance and, then, turns upwards, generally producing new culms in the same year. If the old rhizome die and rot, the new rhizomes grow towards the center. The root system of *D. strictus* is superficial and does not grow to more than a meter deep. The culms are prone to uprooting by strong winds. Due to the superficial root system, changes in soil moisture have marked effect on the growth, and young plantation of bamboo is likely to be adversely affected during spells of prolonged drought.⁴

Bhattacharya et al.⁵ studied the relevance of mycorrhiza for bamboo cultivation in laterite wastelands along with mycorrhizal dependency and phosphorous utilisation efficiency. The solubilisation of phosphate is reported to be the common mode of action for PGPR and studies by Singh and Kapoor⁶. They showed that PSB such as *B. circulans* together with arbuscular mycorrhizal (AM) fungi increased plant yield and P uptake of wheat. Phosphate solubilising bacteria

(PSB) have great prospects to improve plant growth under phosphate deficient soils when used in conjunction with AM fungi.⁷ They are known to mobilise phosphate ions from sparingly soluble organic and inorganic phosphate sources. However, the released phosphate does not reach the root surface as a result of inadequate diffusion.^{8,9} It was proposed that AM fungi could improve the uptake of the solubilised phosphate, hence, this combined interaction should improve phosphate nutrition and supply to plants.¹⁰ Muthukumar and Udaiyan³ studied the effect of inoculation of arbuscular mycorrhizal fungi and PGPR on the growth of bamboo (*D. strictus*) in different tropical soils. They observed that combined inoculation of AM fungi, phosphate solubilising bacteria (PSB) and *A. brasilense* resulted in maximum growth response under both fertilised and unfertilised conditions and soil types (alfisol & vertisol).

In the current study, researchers attempted to find the effect of AM fungi and selected fluorescent pseudomonads on the root architecture of *D. strictus*. The role of rhizospheric microbes around root system of *D. strictus* on the root architecture, which influence the nutrient uptake is studied in the present research.

Materials and methods

Isolation of fluorescent pseudomonads and arbuscular mycorrhizae from the rhizospheres of *D. strictus*

The study area included three locations: first, reserve forest of the FRI, Dehradun, which lies between the latitudes of 30° 20'N and longitudes of 78° 00'E; second, Shivpuri near Byasi (Rishikesh), which lies between latitudes of 30° 06'N and longitude of 78° 25'E; and third, Chirapur range (Haridwar district), which lies between the latitude of 29° 52'N and longitude of 78° 11'E. These locations were selected based on the presence of different growth forms of *D. strictus*.¹¹ Fluorescent pseudomonads and arbuscular mycorrhizae were isolated from the roots of these different growth forms and were selected in-vitro based on their growth and biocontrol efficiency.

In-vivo Selection of fluorescent pseudomonads based on root colonisation, growth and proliferation of *D. strictus*

The host

As a host, *D. strictus* seedlings were used for the study. These seedlings were raised from seeds collected from Nahan (Paonta Sahib, H.P.) forest range. The seeds were surface sterilised using 0.5% NaOCl and rinsed 5–6 times by sterilised distilled water. Once washed, these seeds were soaked overnight in sterilised distilled water. Next day, *D. strictus* seeds were sowed in sterile soil (pots) and kept in humid and warm place for germination. After 15–20 days, 3–5 leaves stage seedlings were used for the experiment. The seedlings were raised in two types of soils. First, of sterilised soil and other sterilised soil inoculated with culture of mycorrhizal fungus, *Glomus etunicatum*.

The soil

Mixture of sand: soil: farm yard manure (FYM) was used in the 2:1:2 % ratio. Potting mixture was used having the physico-chemical characteristics of the experimental soil (Table1).

Table 1 Physico-chemical characteristics of experimental soil

pH	Organic matter (%)	Organic carbon (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
7.9	1.99	1.16	301.5	19.5	345

Container

The seedlings were raised in mud pots of about 5 kg capacity. Roughly 150 seedlings were maintained in each pot. These seedlings once raised were transferred to root trainers of 25 cups of 150 ml capacity. After these seedlings achieved the height of 4–5 cm, they were transferred to root trainers of 12 cups of 300 ml capacity.

Preparation of microbial inoculum

Thirty bacterial isolates (10 for each location) were screened out from total 90 biochemically characterised fluorescent pseudomonads. This selection was again based on the functional (growth & biocontrol) attributes of bacterial populations. The quantity estimation of *in-vitro* P solubilisation was conducted using NBRIP media based on method given by Nautiyal¹².

Bacteria were grown on King's B broth for 3 d at 27°C. The bacterial suspension was centrifuged at 2,000 g for 15 min, the supernatant was discarded, and the pellet was re-suspended in 0.1 M hydrated magnesium sulphate (MgSO₄·7H₂O). The suspension was centrifuged and re-suspended two more times. The optical density of the bacterial suspension was measured at 640 nm on a spectrophotometer, and was adjusted to 0.6 absorbance units, which correspond to approximately 1×10⁹ cells/ml. Root dip method was used. *D. strictus* seedlings roots were surface sterilised in 0.05% NaClO solution for 30 seconds and rinsed six times in sterilised water. After, these seedlings were dipped into bacterial suspension (10⁹/ml) for 20min and planted.

Treatment

The experiment was carried out in sterilised as well as unsterilised soil condition with uninoculated control (T1).

Plants were inoculated with seven fluorescent pseudomonads isolates. Each bacterial isolate was taken as a single treatment, such as, PFF1, PFF3, PFF13, PFC31, PFC71, PFC72 and PFC 125. As per experiment conducted in the nursery, the most efficient *P. fluorescens* (PFC 125) was used for inoculation in the study.

Replication

In all, 8 replicates were maintained in each treatment.

Duration and evaluation of the experiment

The root colonisation and proliferation of *D. strictus* were evaluated at the end of 42 days after the transplantation of the seedlings into the root trainers. The 42 days were decided due to the average time taken for arbuscular mycorrhizae to establish in the roots of bamboo.

Observation

Observations on different plant parameters were taken weekly for six weeks. Plant height was taken in centimeters (cm) by measuring scale. The shoots were included in counting the tiller number. Collar diameter was taken in mm from shoot's initiating point at soil level with the help of electronic digital Vernier Calipers (NSK MAX-Series, Micrometer Mfg. Co. Ltd., Japan). The roots were washed with tap water to remove adhering soil particles and excess water was removed with the help of blotter paper. The root and rhizome volume was determined by the water displacement method in a graduated cylinder.¹³ Excluding old ones, all other rhizomes were numbered. After uprooting plants from root trainers, their shoot and root portions were washed, cut and dried by placing them between the folds of blotter paper. Then, plants were kept in an oven at 60°C till their weight (g) measured constantly.

Evaluation of bacterial population on the roots of bamboo seedlings

Roughly 1 cm long freshly uprooted roots were carefully, randomly cut from each plant. Some of the rhizospheric soil was also collected in plastic bags. It was made sure that the roots were not washed for this step. The roots and soil were kept in 5° to 10°C to maintain their moisture and microbial activity until the study was completed. Combining roots and rhizospheric soil, 1g of sample was serially diluted up to 10⁻⁵. Now from dilutions 10⁻³ to 10⁻⁵, 100µl sample was spread on a plate with King's B media and incubated at 28°C for 2 days. Under the colony counter or UV lamp, the fluorescent colonies were counted. Once the colonies are counted, the log CFU (colony forming unit) was estimated using following formula:

$$\frac{\text{Number of CFU}}{\text{Volume plated (ml)} \times \text{total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{ml}}$$

For example, for the 1×10⁻⁸ dilution plate, 0.1 ml of the diluted cell suspension was plated that counted 200 CFU, then the calculation would be:

$$200 / 0.1 \text{ ml} \times 10^{-8} \text{ or } 200 / 10^{-9} \text{ or } 2.0 \times 10^{11} \text{ bacteria / ml.}$$

The endophyte

The mycorrhizal culture of *G. etunicatum* Backer & Gerdemann was borrowed from G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand. The culture was mass multiplied

in pot (sterilised sand) having maize plants. The seedlings were raised in a potting mixture containing one part of inoculum (having root bits, mycelium and spores) mixed with 9 parts of a sterilised potting mixture. The root colonisation and spore number were estimated before the inoculation. After 40 days in earthen pots, the seedlings are transferred into root trainers.

Results

Root architecture

The effect of different treatments on the root architecture is registered (Figures 1–4).

Average root volume

Irrespective of soil conditions, the controls registered minimum and significantly lower root volume than treated plants (1.04 cc; Table 2). The seedlings treated with combination of *G. etunicatum*, PFC125 and standard dose of NPK (T11: 1.93 cc) registered maximum volume,

which was at par with plants treated in combination of *G. etunicatum* and standard dose of NPK (T7: 1.79 cc), PFC125 and standard dose of NPK (T9: 1.78 cc) and PFC125 and half dose of NPK (T10: 1.75 cc). Irrespective of treatments, no significant difference in root volume was registered in seedlings raised in unsterilised and sterilised soils.

Interactions between treatment and soil condition (TxS) revealed that untreated plants raised in sterilised soil registered minimum and significantly low root volume (0.91 cc; Table 2) that was at par with controls (1.17 cc) and plants treated with *G. etunicatum* raised in unsterilised soil (T2: 1.13cc). Seedlings of unsterilised soil treated with T11 (2.0 cc) attained maximum and significantly high root volume which was at par with four treatments of unsterilised (T5: 1.73 cc; T7: 1.84 cc; T9: 1.88 cc; T10: 1.85 cc) and six treatments of sterilised soil (T3: 1.74 cc; T5: 1.72 cc; T7: 1.74 cc; T9: 1.68 cc; T11: 1.87 cc; T12: 1.74 cc). Plants treated with *G. etunicatum* and raised in sterilised soil (T2) had significantly more root volume (1.56 cc) than raised in unsterilised soil (1.13 cc).

Table 2 Average root volume of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/ Av. root vol. (cc)		Mean
	Unsterilised	Sterilised	
Control (T1)	1.17(20.5)*	0.91(10.3)	1.04
<i>Glomus etunicatum</i> (G.E.; T2)	1.13(17.6)	1.56(40.3)	1.35
<i>Pseudomonas fluorescens</i> (PFC125; T3)	1.55(37.2)	1.74(65.1)	1.64
Std. NPK (T4)	1.47(35.7)	1.61(49.3)	1.54
½ NPK (T5)	1.73(67.6)	1.72(54.3)	1.72
G.E + PFC125 (T6)	1.65(52.9)	1.45(29.7)	1.55
G.E + Std. NPK (T7)	1.84(119.8)	1.74(59.1)	1.79
G.E + ½ NPK (T8)	1.52(37.0)	1.65(47.1)	1.59
PFC125 + Std. NPK (T9)	1.88(77.7)	1.68(49.7)	1.78
PFC125 + ½ NPK (T10)	1.85(70.5)	1.65(46.9)	1.75
G.E + PFC125 + Std. NPK (T11)	2.00(104.6)	1.87(84.2)	1.93
G.E + PFC125 + ½ NPK (T12)	1.61(41.6)	1.74(56.3)	1.67
Mean	1.62	1.61	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.08	0.03	0.12
CD (5%)	0.23	0.09	0.32

*Original values in Parenthesis

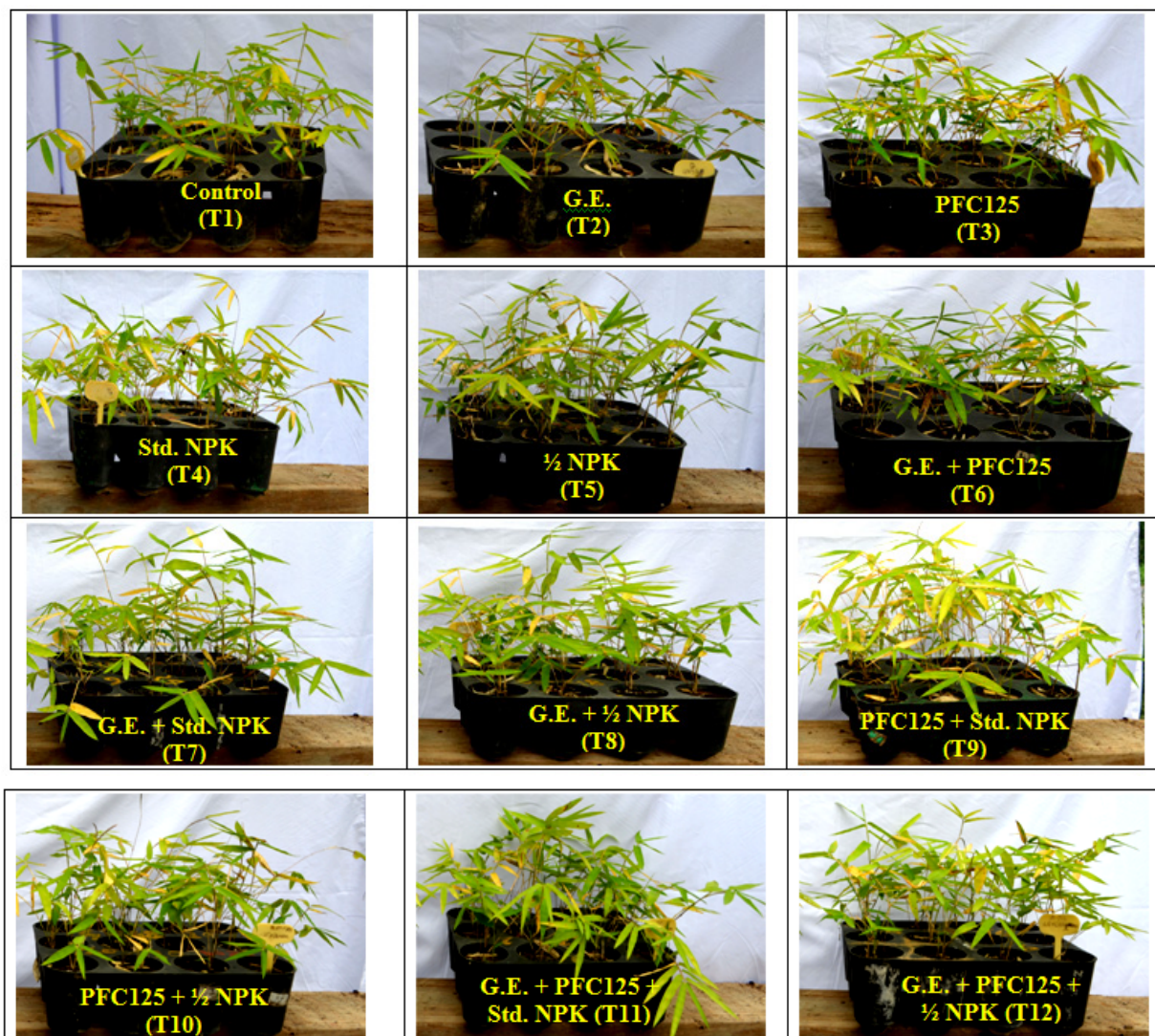


Figure 1 Effect of different treatments on growth and proliferation of *D. strictus* seedlings raised in unsterilised soil.

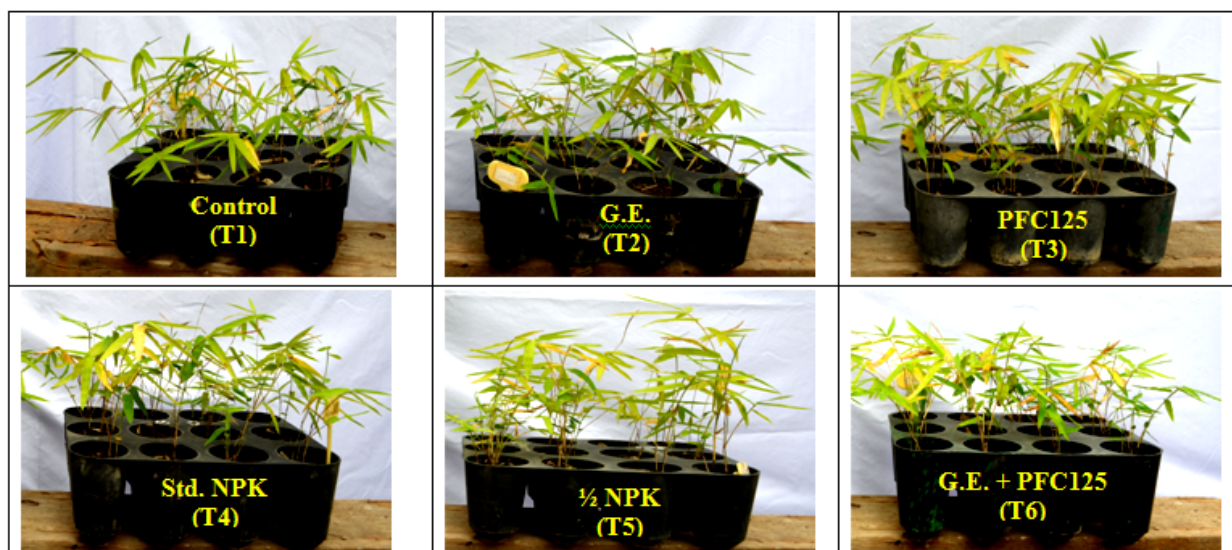
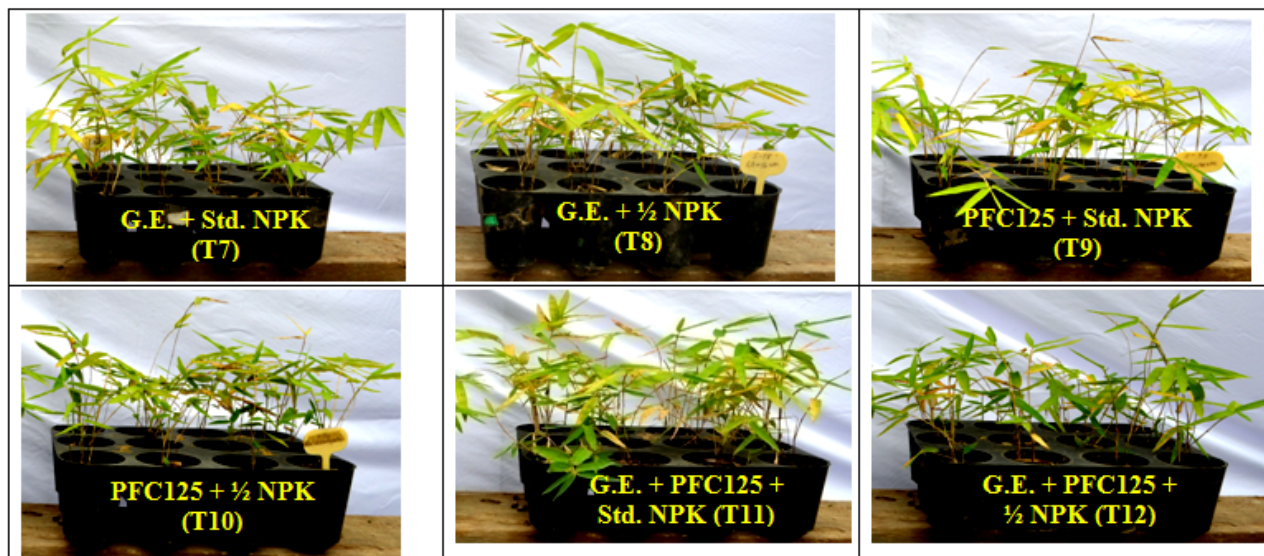
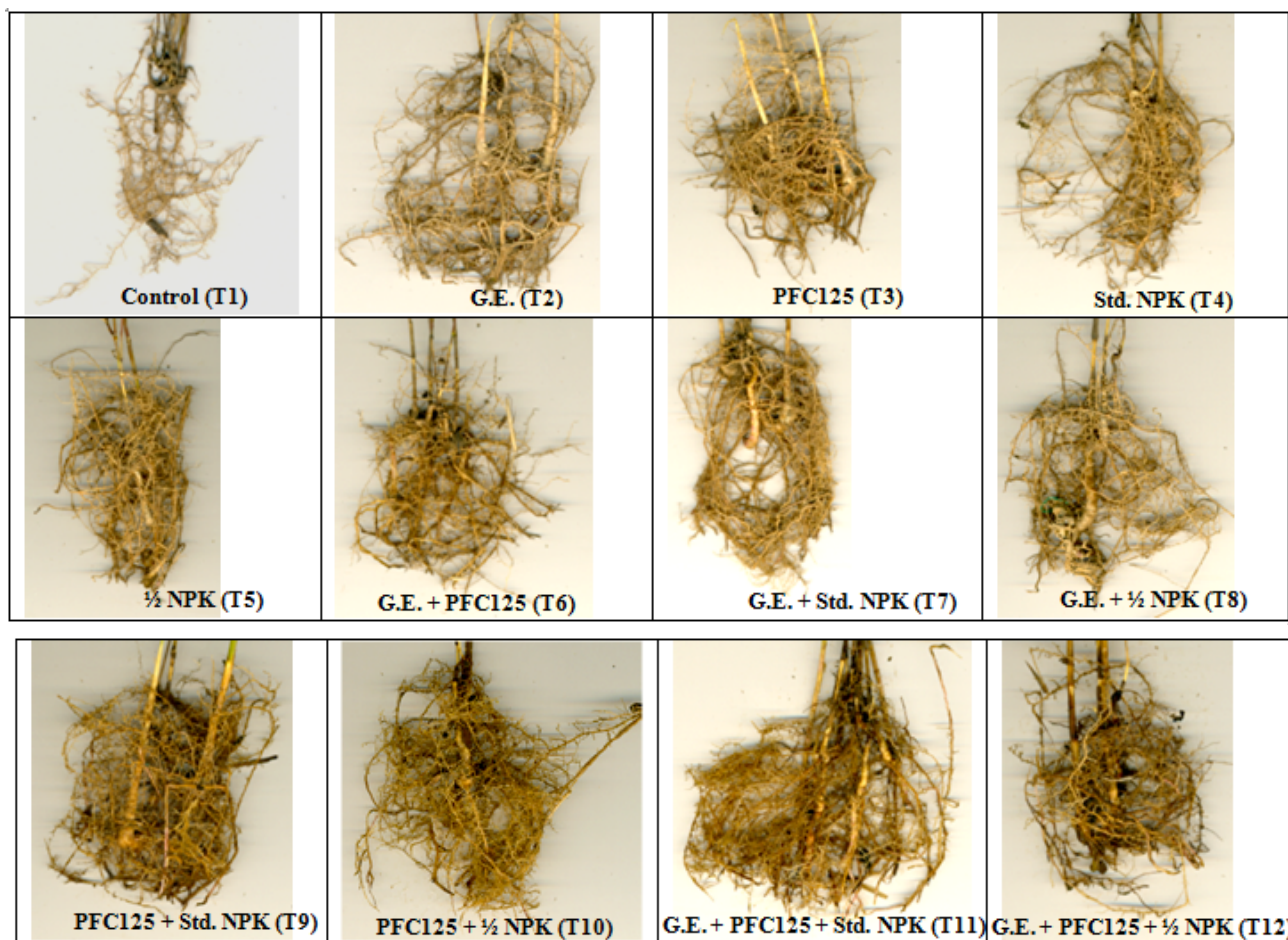


Figure Continued...

**Figure 2** Effect of different treatments on growth and proliferation of *D. strictus* seedlings raised in sterilised soil.**Figure 3** Effect of different treatments on root of *D. strictus* seedlings raised in unsterilised soil.

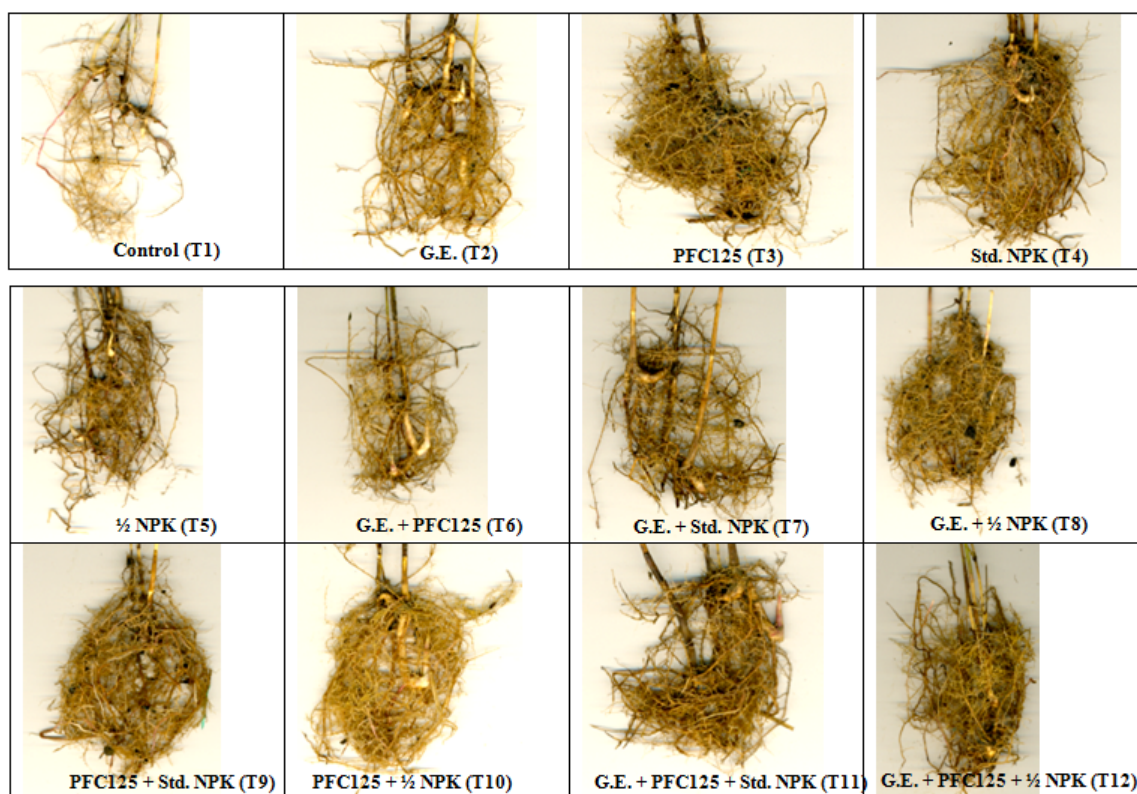


Figure 4 Effect of different treatments on root of *D. strictus* seedlings raised in sterilised soil.

Average root diameter

Irrespective of soil conditions, controls (T1) registered minimum and significantly low root diameter than treated plants (0.45mm; Table 3). Seedlings treated with combination of *G. etunicatum*, PFC125 and standard dose of NPK (T11: 1.03mm) registered maximum and significantly more diameter, which was at par with plants dually treated with *G. etunicatum* and standard dose of NPK (T7: 0.88mm), PFC125 and standard dose of NPK (T9: 0.95mm) and PFC125 and half dose of NPK (T10: 0.93mm). Irrespective of treatments, plants raised in sterilised soil (0.82mm) recorded significantly more average root diameter than seedlings of unsterilised soil (0.79mm).

Considering interactions between treatment and soil condition (TxS), untreated plants (T1: 0.41mm; Table 4) raised in sterilised soil recorded minimum and significantly low root diameter which was at with two treatments of unsterilised soil (T1: 0.50mm & T2: 0.49mm). The plants raised in unsterilised soil treated with combination of *G. etunicatum*, PFC125 and standard dose of NPK (T11: 1.08mm) registered maximum and significantly high root diameter which was at par with four treatments raised in unsterilised (T5: 0.86mm; T7: 0.89mm; T9: 1.03; T10: 0.98mm) and eight treatments of sterilised soil (T3: 0.91mm; T5: 0.86mm; T7: 0.90mm; T8: 0.89mm; T9: 0.86mm; T10: 0.88 mm; T11: 0.99 mm; T12: 0.88mm). Plants raised in sterilised soil and treated with *G. etunicatum* had significantly more root diameter (T2: 0.75mm) than their unsterilised counterparts (0.49 mm).

Average root length

The untreated plants measured minimum and significantly lower

root length (26.8 cm; Table 4), irrespective of soil conditions. The combination of *G. etunicatum*, PFC125 and standard dose of NPK (T11) treated seedlings achieved significantly higher root length (69.9 cm) that was at par with plants treated with standard dose of NPK (T4: 62.8 cm), *G. etunicatum* and standard dose of NPK (T7: 62.7 cm) and PFC125 and standard dose of NPK (T9: 61.0 cm). Plants raised in unsterilised soil registered 59.5 cm root length which was significant than roots of sterilised soil (55.5 cm), irrespective of treatments.

Interaction between treatment and soil condition (TxS) reported that control plants raised in unsterilised (26.6 cm; Table 4) and sterilised (27.0 cm) soils registered minimum and significantly low root length. Among the treated plants, T11 (74.3 cm) treated seedlings raised in unsterilised soil registered maximum and significantly high root length which was at par with five treatments of unsterilised (T4: 66.5 cm; T5: 67.8 cm; T6: 67.0 cm; T7: 67.4 cm; T9: 63.8 cm) and two treatments of sterilised soil (T5: 62.9 cm; T11: 65.5 cm). Seedlings raised in unsterilised soil and dually treated with *G. etunicatum* and PFC125 (T6: 67.0 cm) recorded significantly more root length than plants raised in sterilised soil (49.9 cm).

Degree of root branching

The controls registered minimum and significantly lower root branching (0.80; Table 5), irrespective of soil conditions. The seedlings treated with *G. etunicatum* (T2: 1.52) recorded maximum and significantly more branching which was at par with plants treated dually with *G. etunicatum* and half dose of NPK (T8: 1.35). No significant effect of soil was observed in the experiment as plants raised in both types of soils had similar values, irrespective of treatments.

Table 3 Average root diameter of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/Av. root dia. (cc)		Mean
	Unsterilised	Sterilised	
Control (T1)	0.50	0.41	0.45
<i>Glomus etunicatum</i> (G.E.;T2)	0.49	0.75	0.62
<i>Pseudomonas fluorescens</i> (PFC125;T3)	0.73	0.91	0.82
Std. NPK (T4)	0.65	0.80	0.72
½ NPK (T5)	0.86	0.86	0.86
G.E + PFC125 (T6)	0.76	0.72	0.74
G.E + Std. NPK (T7)	0.89	0.90	0.88
G.E + ½ NPK (T8)	0.73	0.89	0.81
PFC125 + Std. NPK (T9)	1.03	0.86	0.95
PFC125 + ½ NPK (T10)	0.98	0.88	0.93
G.E + PFC125 + Std. NPK (T11)	1.08	0.99	1.03
G.E + PFC125 + ½ NPK (T12)	0.76	0.88	0.82
Mean	0.79	0.82	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.06	0.02	0.08
CD (5%)	0.16	0.06	0.22

Table 4 Average root length of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/Av. root length (cm)		Mean
	Unsterilised	Sterilised	
Control (T1)	26.6	27.0	26.8
<i>Glomus etunicatum</i> (G.E.;T2)	55.3	58.2	56.8
<i>Pseudomonas fluorescens</i> (PFC125;T3)	55.3	59.9	57.6
Std. NPK (T4)	66.5	59.0	62.8
½ NPK (T5)	67.8	62.9	65.4
G.E + PFC125 (T6)	67.0	49.9	58.5
G.E + Std. NPK (T7)	67.4	57.9	62.7
G.E + ½ NPK (T8)	54.8	53.1	54.0
PFC125 + Std. NPK (T9)	63.8	58.1	61.0
PFC125 + ½ NPK (T10)	60.7	52.6	56.6
G.E + PFC125 + Std. NPK (T11)	74.3	65.5	69.9
G.E + PFC125 + ½ NPK (T12)	54.8	61.4	58.1
Mean	59.5	55.5	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	3.2	1.3	4.5
CD (5%)	8.9	3.6	12.6

Table 5 Degree of root branching of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/ Deg. of branch.		Mean
	Unsterilised	Sterilised	
Control (T1)	0.78	0.83	0.80
<i>Glomus etunicatum</i> (G.E.;T2)	1.67	1.36	1.52
<i>Pseudomonas fluorescens</i> (PFC125;T3)	1.48	1.17	1.32
Std. NPK (T4)	1.35	1.32	1.34
½ NPK (T5)	1.12	1.25	1.19
G.E + PFC125 (T6)	1.27	1.30	1.29
G.E + Std. NPK (T7)	1.15	1.26	1.21
G.E + ½ NPK (T8)	1.42	1.27	1.35
PFC125 + Std. NPK (T9)	1.15	1.31	1.23
PFC125 + ½ NPK (T10)	1.35	1.21	1.28
G.E + PFC125 + Std. NPK (T11)	1.20	1.11	1.15
G.E + PFC125 + ½ NPK (T12)	1.29	1.22	1.26
Mean	1.27	1.22	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.06	0.02	0.09
CD (5%)	0.17	0.07	0.24

Considering interactions between treatment and soil condition (TxS), controls raised in unsterilised (0.78; Table 6) and sterilised (0.83) soil registered minimum and significantly low root branching. Among the treated plants, seedlings raised in unsterilised soil treated with T2 (1.67) registered maximum and significantly highest root branching that was at par with plants treated with PFC125 (T3: 1.48) of same soil condition. Also T2 and T3 treated plants raised in unsterilised soil (1.67 & 1.48) had significantly more root branching than their counterparts in sterilised soil (1.36 & 1.17).

Dry root weight

The controls recorded minimum and significantly lower root weight (T1: 0.59 g; Table 7), irrespective of soil conditions. The seedlings treated dually with PFC125 and standard dose of NPK (T9: 1.69 g) registered maximum and significantly higher weight which was at par with plants treated with half dose of NPK (T5: 1.42 g), *G. etunicatum*

and standard dose of NPK (T7: 1.62 g), and *G. etunicatum*, PFC125 and standard dose of NPK (T11: 1.60 g). Irrespective of treatments, plants raised in unsterilised soil (1.40 g) recorded significantly more weight than sterilised soil (1.18 g).

The interactions between treatment and soil condition (TxS) recorded that untreated plants of unsterilised (0.62 g; Table 5) and sterilised soils (0.56 g) had minimum and comparable dry root weight that was at par with seedlings raised in unsterilised (0.97 g) and sterilised (0.92 g) soil treated with *G. etunicatum* (T2). Among the treated plants, treatment T7 (1.81 g) raised in unsterilised soil registered highest and significantly higher weight which was at par with T5 (1.52 g) treated seedlings raised in unsterilised soil and with T11 treated plants raised in unsterilised (1.61 g) and sterilised (1.58 g) soils. Three treatments (T4: 1.42 vs. 1.04 g; T7: 1.81 vs. 1.42 g; T9: 2.24 vs. 1.15 g) raised in unsterilised soil gained significantly more root weight their counterparts in sterilised soil.

Table 6 Dry root weight of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/Dry root wt. (g)		Mean
	Unsterilised	Sterilised	
Control (T1)	0.62	0.56	0.59
<i>Glomus etunicatum</i> (G.E.;T2)	0.97	0.92	0.95
<i>Pseudomonas fluorescens</i> (PFC125;T3)	1.38	1.24	1.31
Std. NPK (T4)	1.42	1.04	1.23
½ NPK (T5)	1.52	1.31	1.42
G.E + PFC125 (T6)	1.19	0.94	1.06
G.E + Std. NPK (T7)	1.81	1.42	1.62
G.E + ½ NPK (T8)	1.30	1.38	1.34
PFC125 + Std. NPK (T9)	2.24	1.15	1.69
PFC125 + ½ NPK (T10)	1.39	1.17	1.28
G.E + PFC125 + Std. NPK (T11)	1.61	1.58	1.60
G.E + PFC125 + ½ NPK (T12)	1.32	1.41	1.37
Mean	1.40	1.18	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.10	0.04	0.14
CD (5%)	0.28	0.11	0.37

Table 7 Fluorescent pseudomonads count of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/Bac. count (cfu/ml)		Mean
	Unsterilised	Sterilised	
Control (T1)	5.04	3.31	4.17
<i>Glomus etunicatum</i> (G.E.;T2)	4.02	2.13	3.07
<i>Pseudomonas fluorescens</i> (PFC125;T3)	5.28	4.80	5.04
Std. NPK (T4)	5.13	1.72	3.42
½ NPK (T5)	4.52	4.06	4.29
G.E + PFC125 (T6)	4.97	4.75	4.86
G.E + Std. NPK (T7)	5.80	4.88	5.34
G.E + ½ NPK (T8)	5.02	3.44	4.23
PFC125 + Std. NPK (T9)	3.68	4.04	3.86
PFC125 + ½ NPK (T10)	4.23	2.89	3.56
G.E + PFC125 + Std. NPK (T11)	4.00	4.99	4.49
G.E + PFC125 + ½ NPK (T12)	4.64	3.72	4.18
Mean	4.69	3.73	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.39	0.16	0.55
CD (5%)	1.11	0.45	1.57

Root colonization by fluorescent pseudomonads

Plants treated with *G. etunicatum* (T2) supported minimum and significantly lower fluorescent pseudomonads (3.07 log cfu/ml; Table 6) which was at par with control seedlings (T1; 4.17 log cfu/ml), plants treated with standard dose of NPK (T4; 3.42 log cfu/ml), dually with PFC125 and standard dose of NPK (T9; 3.86 log cfu/ml), PFC125 and half dose of NPK (T10; 3.56 log cfu/ml) and combination of *G. etunicatum*, PFC125 and half dose of NPK (T12; 4.18 log cfu/ml), irrespective of soil conditions. The maximum bacterial counts were recorded in seedlings treated with *G. etunicatum* and standard dose of NPK (T7 : 5.34 log cfu/ml) which was at par with plants treated with PFC123 (T3: (5.04 log cfu/ml), half dose of NPK (T5: 4.29 log cfu/ml), *G. etunicatum* and PFC125 (T6: 4.86 log cfu/ml), *G. etunicatum* and half dose of NPK (T8: 4.23 log cfu/ml) and *G. etunicatum*, PFC125 and standard dose of NPK (T11: 4.49 log cfu/ml). Irrespective of treatments, plants raised in unsterilised soil supported more number of fluorescent pseudomonads (4.69 log cfu/ml) than seedlings raised in sterilised soil (3.73 log cfu/ml).

Considering interactions between treatment and soil condition (TxS), plants treated with T4 (1.72 log cfu/ml; Table 6) registered minimum and significantly low fluorescent pseudomonads which was

at par with T2 (2.13 log cfu/ml) and T10 (2.89 log cfu/ml) treated seedlings raised in sterilised soil. Plants raised in unsterilised soil and treated with T7 (5.8 log cfu/ml) registered maximum and significantly higher bacterial count that was at par with eight treatments of unsterilised (T1: 5.04; T3: 5.28; T4: 5.13; T5: 4.52; T6: 4.97; T8: 5.02; T10: 4.23; T12: 4.64 log cfu/ml) and four treatments of sterilised soil (T3: 4.80; T6: 4.75; T7: 4.88; T11: 4.99). Plants of four treatments and raised in unsterilised soil (T1: 5.04 vs 3.31; T2: 4.02 vs. 2.13; T4: 5.13 vs. 1.72; T8: 5.02 vs. 3.44 log cfu/ml) registered significantly more fluorescent pseudomonas than seedlings raised in sterilised soil.

Root colonisation by *G. etunicatum*

Irrespective of soil condition, maximum root colonization was registered in seedlings treated dually with *G. etunicatum* and standard dose of NPK (T6: 72.4%; Table 8; Figure 5) which was at par with plants treated with *G. etunicatum* (T2: 72.2%). Minimum percent root colonization was registered in plants inoculated with PFC125 (T3: 19.9%) which was at par with control seedlings (T1: 25.9%) along with plants treated with standard dose of NPK (T4: 25.1%) and half dose of NPK (T5: 21.4%). Plants raised in unsterilised soil supported significantly more root colonization (49.8%) than sterilised raised seedlings (40.9%), irrespective of treatments.

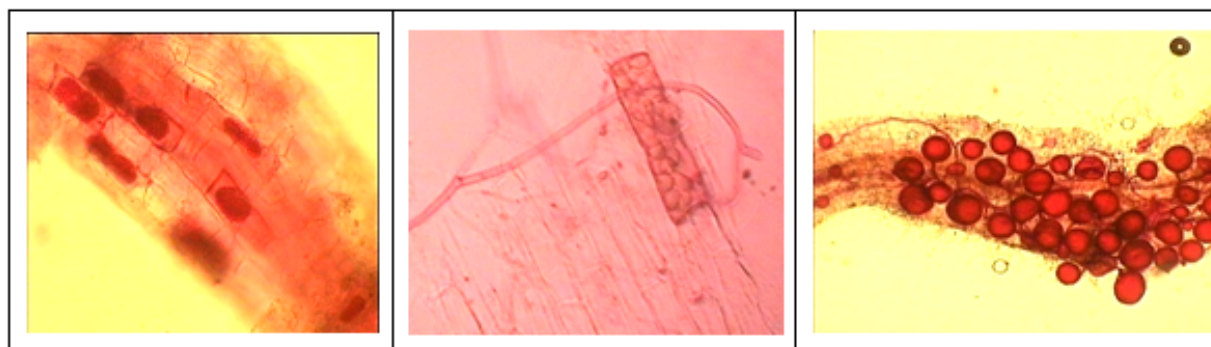
**Figure 5** Root colonisation by *G. etunicatum* in roots of *D. strictus* seedlings.

Table Continued.....

Table 8 Root colonisation by *G. etunicatum* of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/root colonization (%)		Mean
	Unsterilised	Sterilised	
Control (T1)	37.8	13.9	25.9
<i>Glomus etunicatum</i> (G.E.;T2)	62.7	81.7	72.2
<i>Pseudomonas fluorescens</i> (PFC125;T3)	30.6	9.2	19.9
Std. NPK (T4)	41.6	8.6	25.1
½ NPK (T5)	30.9	11.9	21.4
G.E + PFC125 (T6)	55.6	70.5	63.1
G.E + Std. NPK (T7)	72.9	71.9	72.4
Treatment	Soil condition/root colonization (%)		Mean
G.E + ½ NPK (T8)	62.1	64.6	63.4
PFC125 + Std. NPK (T9)	34.8	17.2	26.0
PFC125 + ½ NPK (T10)	45.9	17.8	31.8
G.E + PFC125 + Std. NPK (T11)	53.5	70.6	62.0
G.E + PFC125 + ½ NPK (T12)	68.8	52.6	60.7
Mean	49.8	40.9	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	2.1	0.9	3.0
CD (5%)	6.1	2.5	8.6

Interactions between treatment and soil condition (TxS) revealed that plants raised in sterilised soil treated with standard dose of NPK (T4: 8.6%) registered minimum and significantly low root colonisation which was at par with seedlings raised in same soil condition treated with four treatments (T1: 13.0%; T3: 9.2%; T5: 11.9%; T9: 17.2%). Plants raised in sterilised soil treated with *G. etunicatum* (T2: 81.7%) recorded maximum and significantly high root colonisation followed by seedlings treated with T7 raised in unsterilised (72.9%) and sterilised soil (71.9%) which was at par with T6 (70.5%), T8 (64.6%) and T11 (70.6%) of sterilised soil and T12 (68.8%) treated plants raised in unsterilised soil. In majority of the cases where *G. etunicatum* is part of treatment, the plants raised in sterilised soil had more root colonisation than their counterparts.

Spore population

Irrespective of soil conditions, plants treated with standard dose of NPK (T4) and half dose of NPK (T5) recorded minimum spore population of mycorrhizae (1.02 each; Table 9; Figure 6) which was at

par with controls (T1: 1.03), PFC125 (T3: 1.04), dually treated PFC125 and standard dose of NPK (T9: 1.05) as well as dually supplied with PFC125 and half dose of NPK (T10: 1.04). Seedlings treated with both *G. etunicatum* and standard dose of NPK supported maximum and significantly higher spore population (T7: 2.24). Irrespective of treatments, plants raised in unsterilised soil (1.54) had significantly more spores than sterilised ones (1.21).

Interactions between treatment and soil condition (TxS) revealed that untreated seedlings raised in sterilised soil registered minimum and significantly low spore population (0.55; Table 9) which was at par with plants of five treatments of same soil condition (T3: 0.56; T4: 0.57; T5: 0.57; T9: 0.61; T10: 0.59). Among the treated plants, T7 seedlings raised in unsterilised (2.16) and sterilised (2.32) soils registered maximum and significantly high spore population which was at par with three treatments of sterilised soil (T8: 2.09; T11: 2.09; T12: 2.18). Plants treated with T6 (1.64 vs. 1.42) and T7 (2.16 vs. 2.32) registered no significant change in spore population between unsterilised and sterilised soils.

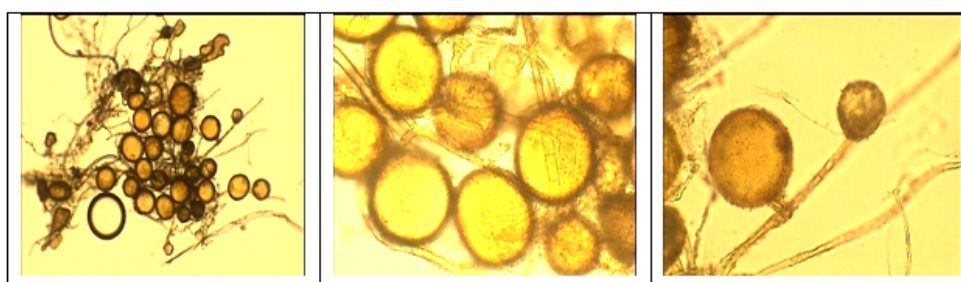
**Figure 6** Spores of *G. etunicatum* from the rhizospheric soil of inoculated *D. strictus* seedlings.

Table 9 Spore population of *G. etunicatum* of *D. strictus* seedlings raised in unsterilised and sterilised soils after 105 days of transplanting

Treatment	Soil condition/Spore no. (/ml)		Mean
	Unsterilised	Sterilised	
Control (T1)	1.51(32.7)*	0.55(3.6)	1.03
<i>Glomus etunicatum</i> (G.E.;T2)	1.54(38.0)	0.97(9.8)	1.26
<i>Pseudomonas fluorescens</i> (PFC125;T3)	1.51(33.2)	0.56(3.7)	1.04
Std. NPK (T4)	1.47(30.1)	0.57(3.8)	1.02
½ NPK (T5)	1.47(30.2)	0.57(3.8)	1.02
G.E + PFC125 (T6)	1.64(44.2)	1.42(27.2)	1.53
G.E + Std. NPK (T7)	2.16(153.0)	2.32(219.7)	2.24
G.E + ½ NPK (T8)	1.60(40.6)	2.09(132.0)	1.85
PFC125 + Std. NPK (T9)	1.49(30.6)	0.61(4.1)	1.05
PFC125 + ½ NPK (T10)	1.50(31.7)	0.59(3.9)	1.04
G.E + PFC125 + Std. NPK (T11)	1.77(61.4)	2.09(144.4)	1.93
G.E + PFC125 + ½ NPK (T12)	1.47(30.1)	2.18(153.6)	1.82
Mean	1.59	1.21	
	Treatment (T)	Soil condition (S)	Interaction (TxS)
SEM	0.06	0.02	0.08
CD (5%)	0.16	0.07	0.23

*Original values are in parentheses.

Discussion

The effect of microbial inoculants on the root architecture of bamboo seedlings was also investigated. The study revealed that the combination of *G. etunicatum*, PFC125 and standard dose of NPK (T11) increased the root volume, average root diameter and average root length. The degree of root branching was increased by the application of *G. etunicatum* (T2). These results are in agreement with the observation reported by Gamalero et al.¹⁴ They too found that co-inoculation of *P. fluorescens* and *G. mosseae* strongly affected the root architecture of tomato plants and helped in improving plant mineral nutrition by increasing leaf P content. Influence of *P. fluorescens* on root volume and length in *D. strictus* seedlings is in coherence with the above findings. Berta et al.¹⁵ stated that longer root systems are more adapted and concentrated to soil exploration and exploitation.¹⁵ Enhancement of total root volume and length of shallow root system of bamboo by the bacterium and fungus can be of extra advantage of this grass where reaching out for nutrients and water becomes more easy due to boost to root growth. Berta et al.¹⁶ and Gamalero et al.¹⁵ reported that mycorrhizae (*G. mosseae*) can affect root architecture, especially the degree of root branching.¹⁵ They stated that increase in root branching improves the ability of plants to absorb and transport nutrients. The synergistic effect of microbial inoculants (*G. etunicatum* and *P. fluorescens* (T11)) on root volume, length and branching in bamboo seedlings might be promoting efficient exploration of surrounding soil, nutrient transport and uptake that could express in increased tillering, plant height, collar diameter, dry shoot weight and total shoot P content.

Acknowledgements

I acknowledge the Forest Research Institute for their support in providing facilities for the research.

Conflict of interest

Authors declare that there is no conflict of interest.

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