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Nursery Production of *Cedrus deodara* G. don (Deodar) Seedlings of Kashmir Himalayas: Influence of microbial inoculation

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ABSTRACT

The present study demonstrated the use of microorganisms as bio-inoculants in forest species. The experiment was conducted to study the influence of microbial inoculation on the growth of Deodar (*Cedrus deodara* G. don) seedlings. Ten different treatments of microbial formulations namely *Azotobacter chroococcum*, Phosphorous Solubilising Bacteria (PSB), Potassium Solubilising Bacteria (KSB), Zinc Solubilising Bacteria (ZnSB), Vesicular Arbuscular Mycorrhiza (VAM) and combinations of VAM + PSB, VAM + KSB, VAM+ ZnSB, VAM + *Azotobacter chroococcum* including control were applied to few weeks old seedlings. After one growing season, growth characteristics viz; plant height, collar diameter, seedling survival per cent, root length, root-shoot ratio and total fresh and dry biomass production significantly increased in all the treatments. The results suggested that the dual inoculation of VAM + *Azotobacter chroococcum* showed maximum increase in all the growth parameters. However, survival of 95% was observed in seedlings treated with Zinc solubilising bacteria. The present study reflects the potential approach of different microbial consortia to be considered for nursery production of conifer seedlings to procure their rapid regeneration in general and reduce the pressure on natural forests for meeting the demand of wood derived fuel and timber in particular.

Key words: *Azotobacter chroococcum, Deodar, Potassium Solubilising Bacteria (KSB), Vesicular Arbuscular Mycorrhiza (VAM) and Zinc Solubilising Bacteria (ZnSB)*

Introduction

Cedrus deodara, belonging to family Pinaceae is a large evergreen coniferous tree with a trunk up to 3 m (10 ft) in diameter and a height of 40–50 m (131–164 ft). With level branches and drooping branchlets, it has a conic crown (Muthoo and Wali, 1965). The needle-like leaves are 2.5–5 cm (0.98–1.97 in) long, occasionally up to 7 cm (2.8 in), slender (1 mm (0.039 in) thick, borne singly on long shoots and in dense clusters of 20–30 on short branches, and range in colour from bright green to glaucous blue-green. Male cones measure 4–6 cm (1.6–2.4 in) in length, and female cones measure 4–6 cm (1.6–2.4 in) in length. It start appearing in temperate areas of the UT i.e., from Kudh onward and grows chiefly in the north-western Himalayas of Kashmir. Deodar wood is one of India's most durable woods. The

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heartwood is aromatic, fine and close-grained, rot and termite resistant, and long-lasting. It is utilized in the construction of boat homes, doors, furniture, and wooden carvings.

According to the Jammu and Kashmir State Forest Policy-2010" past few decades have witnessed exponential increase in human and livestock population, rapid industrialisation, and a spurt in developmental activities." These developmental processes, says the document, have resulted in loss of forest area accompanied by an overall degradation of forest vegetation and forest soils. Moreover, natural regeneration is nearly impossible in woods with a crown density less than 40%. Natural succession takes thousands of years for forest species to reach the climax stage, especially in temperate regions where species such as *Cupressus torulosa* D. Don (Himalayan Cypress), *Abies pindrow* Spach (Silver Fir), *Cedrus deodara* (Roxb.) G. Don (Deodar), *Pice asmithiana Wall*. (Spruce), and *Pinus wallichiana Jackson* (Kail) predominate. Because the indiscriminate use of inorganic fertilisers and pesticides is both environmentally and economically unsustainable, there is a high demand for the use of microbial inoculants in the nursery and in the creation of plantations to boost productivity. As a result, in order to meet the enormous demand for timber, fuel wood, and firewood, raising such forest species on degraded forest patches, supplemented with a variety of microbial inoculants, could be an excellent and viable option for sustaining current resources and ensuring a better and greener future.

Materials and Methods

Experimental site

A field experiment was conducted to study the "Effect of Microbial Inoculation on "Growth of Deodar seedlings of Kashmir Himalayas under Nursery Conditions" at Faculty of Agriculture and Regional Research Station, SKUAST-K Wadura, Sopore to evaluate the effect of various microbial inoculants on the growth of seedlings. The experiment was laid in Completely Randomized Design (CRD) with four replications comprising of ten treatments.

Experimental detail

The materials used in the present investigation consisted of conifer seeds of Deodar were raised in polybags and different microbial inoculants were obtained from Bio-fertilizer Laboratory (Basic Sciences and Humanities), FoA, Wadura Sopore. The experimental medium was prepared by mixing soil, sand and vermi-compost in the ratio of 2:1:1 respectively. The prepared mix was transferred to the trays in which seeds were later sown. Polybags of size 6" × 9" filled with soil, sand and vermi-compost in the ratio of 2:1:1 were prepared. The seedlings were transplanted in each polybags. For the purpose of microbial inoculation, seedlings were inoculated by applying 25 ml of liquid inoculant per germinant in the rhizosphere soil after transplanting in polybags. As for VAM application, it was applied as top dressing to the required polybags.The experimental plot was kept weed free by hand weeding whenever necessary. Additionally, the water requirements were fulfilled by manually applying water separately to each treatment as and when required.

Growth analysis

Growth parameters viz., plant height, collar diameter at various intervals; root length, total fresh and dry biomass (root and shoot), root-shoot ratio and seedling survival at the end of one growing season were evaluated. The performance of microbial inoculants was evaluated individually and in combination with one other in contrast to control for screening the best microbial inoculants.

Statistical Analysis

Data generated during the study on each experiment was analyzed applying suitable methods of analysis (Gomez and Gomez, 1984 and OP STAT). Appropriate transformations were made and significant results were compared on the basis of critical difference.

Results and Discussion

The results depicted that among all the microbial inoculants applied to the coniferous species, VAM + *Azotobacter chroococcum* proved superior in terms of various growth parameters viz; maximum plant growth of 6.87 cm and diameter of 1.11 mm was recorded with the application of VAM + *Azotobacter chroococcum*. Other parameters viz., root length, root-shoot ratio, fresh and dry biomass production was also recorded maximum with the dual application of VAM + *Azotobacter chroococcum*. Seedling survival of 95% was recorded by the application of Zinc Solubilising Bacteria.

Plant growth (in height and diameter)

In case of plant growth (in height and diameter), there was a significant variation in plant height as well as collar diameter due to different microbial inoculations. The plant growth (in height and diameter) increased continuously in all the treatments of both with and without biofertilizer application. In the study, the inoculations of Vesicular *Arbuscular mycorrhiza* (VAM) + *Azotobacter chroococcum* followed by VAM + Zinc Solubilsing Bacteria (ZnSB), VAM + PSB and VAM alone significantly increased the height of one year old seedlings. Maximum growth (height) of 6.87 cm by the application of VAM + *Azotobacter chroococcum* was recorded followed by 6.60 cm the application of VAM + ZnSB while control recorded height of 2.84 cm only (Table 1). Similar results were obtained in Neem when inoculated with the G. *mosseae* + A. *chroococcum* + B. *coagulans* (Sumana *et al*., 2003).

The growth in diameter significantly varied in all the treatments for the species under study. After one complete growing season, maximum growth in diameter as 1.11 mm in VAM + *Azotobacter chroococcum* was recorded followed by 1.02 mm by the application of VAM + ZnSB while control recorded 0.49 mm of growth in diameter (Table 1). The findings are in line with that of Seema *et al.* (2000) who reported that better growth and biomass was observed by the inoculation of AM fungi and *Azotobacter chroococcum* on *Gmelina arborea, Bambusa arundinacea, Tectona grandis* and *Dilbergia sissoo.*

Root Length

By the application of various microbial inoculants, like shoot height root length also varied significantly in different treatments after one growing season. Maximum root length was measured as 12.95 cm by the application of VAM + *Azotobacter chroococcum* followed by 12.64 cm in VAM + ZnSB treated seedlings while root length of only 6.40 cm was recorded in control plot after one growing season (Table 2). VAM fungi interact with other soil microbes like free-living nitrogen fixers to improve their efficiency for the biochemical cycling of elements to the host plants (Singh and Jamaluddin, 2010; Shamshiri *et al*., 2012 and Ghazi, 2013). The results in the present are in conformity with Bahrani *et al*. (2010) who suggested a synergistic or additive interaction between VAM and *Azotobacter,* therefore the absorption of more nutrients by plants because *Azotobacter* in association with AMF provided access to more soil volume as extra matrical hyphae of AM fungi enlarge the effective surface outside of the roots in promoting plant growth.

Seedling survival % age

The percentage survival of seedlings under study increased after microbial inoculation. Generally, bacterial inoculants proved to give high percentage of survived seedlings after one complete growing season however seedlings inoculated with VAM singly or in combination also increased seedling survival significantly over control. After one complete growing season 95% of seedlings survived in treat-

Table 1. Effect of microbial inoculation on plant growth (in height and diameter)

Treatments	Plant growth (in height)			Plant growth (in diameter)		
	Height at the time of transplanting (cm)	Height after one growing season (cm)	Growth in one season (cm)	Diameter at the time of transplanting (mm)	Diameter after one growing season (mm)	Growth in one season (mm)
Control	2.33	5.22	2.84	0.36	0.85	0.49
Azotobacter chroococcum	2.46	8.77	6.28	0.31	1.23	0.92
Phosphorous Solubilising Bacteria (PSB)	3.04	8.40	5.34	0.38	1.12	0.74
Potassium Solubilising Bacteria (KSB)	2.71	8.17	5.47	0.41	1.04	0.62
Zinc Solubilising Bacteria ZnSB	2.26	8.50	6.23	0.41	1.14	0.64
VAM	2.61	8.90	6.30	0.47	1.31	0.84
VAM + PSB	2.81	9.05	6.16	0.32	1.33	0.98
VAM + KSB	2.30	8.57	6.28	0.45	1.19	0.74
VAM + ZnSB	2.96	9.12	6.60	0.39	1.37	1.02
VAM + Azotobacter chroococcum	2.89	9.50	6.87	0.49	1.52	1.11
C.D (pd”0.05)	0.039	0.081	0.034	0.017	0.036	0.011
SE(m)	0.013	0.028	0.12	0.006	0.12	0.04

ment ZnSB followed by 94.50% and 94.10% in KSB and PSB respectively, 94.40% in VAM + PSB, 93.40% in VAM + ZnSB, 93.25% in VAM + KSB, 89.52% in VAM + *Azotobacter chroococcum*, 88.80% in VAM which is significantly at par with survived seedlings of 88.20% in *Azotobacter chroococcum* and only 78.50% survival in control (Table 2).

According to Hafeez *et al*. (2001) investigations that used Zn-mobilizing PGPR inoculants as biofertilizers accelerated the regeneration of damaged land and improved soil fertility. Plant survival and growth rates were improved, grain output was increased, malnutrition rates were reduced, and chemical fertilizer dependence was reduced.

Total fresh and dry (root and shoot) Biomass

Fresh as well as dry biomass increased after one complete growing season by the application of microbial inoculants. The maximum total fresh biomass of 1.53 g in VAM + *Azotobacter chroococcum* was recorded followed by 1.41 g in VAM + ZnSB treated seedlings and control plot recorded 0.31g of fresh total biomass. Total dry biomass was recorded 0.79g in VAM + *Azotobacter chroococcum* treated seedlings followed by 0.73 g in VAM + ZnSB treatment while only 0.17 g of total dry biomass was recorded in control after one growing season (Table 2).

The positive influence of AM fungi however, might be due to growth promotory effect of AM fungi that had increased phosphorus availability and thereby causing higher protein synthesis resulted in more morphological growth (Singh and Singh, 2004). The rhizotrophic microorganism viz. AMF, PSB and *Azotobacter* inoculated with *Azadiracta indica* made congenial environment, protected from diseases (Lax *et al*., 2011), increase absorptive capacity by AMF mycelium network (Hassan *et al*., 2012), compensated stress and drought resulted into enhanced uptake of nutrients which converted into higher biomass and improved the quality parameter of the seedlings. Non- inoculated control plants exhibited very weak shoot growth and produced little biomass compared to the inoculated plants. The absence of the AMF in soil and root and other microorganisms is a factor that could limit seriously the growth and the development of control plants.

Root-shoot ratio

The root and shoot ratio varied significantly among all the treatments. Maximum ratio of 1.40 was recorded in VAM + *Azotobacter chroococcum*, followed by 1.38 in VAM + ZnSB and 1.33 in VAM treated seedlings. 1.19 root and shoot ratio was recorded in control (Table 2). The non-inoculated seedlings had relatively less root-shoot ratio than the inoculated ones. The "sturdiness quotient," which contrasts root collar diameter (in mm) with height (in cm), is a less vigorous but still non-destructive assessment. If the quotient is low, the plant is robust and has a better chance of surviving, especially in windy or dry conditions. A sturdiness quotient higher than 6 is an indicative of being undesirable (Gregorio *et al*., 2007). From the study of Sreedhar and Mohan (2016) with two observational years, *Neolamarckia cadamba* seedlings had appropriate sturdiness quotient val-

Table 2. Effect of microbial inoculation on various plant growth parame

Treatments	Root length (cm)	Total fresh (root and shoot) biomass (g)	Total dry (root and shoot) biomass (g)	Seedling survival (%)	Root-shoot ratio
Control	6.40	0.31	0.17	78.50	1.19
Azotobacter chroococcum	11.35	0.97	0.49	88.20	1.29
Phosphorus Solubilising Bacteria (PSB)	10.65	0.74	0.42	94.10	1.26
Potassium Solubilising Bacteria (KSB)	9.95	0.71	0.35	94.50	1.24
Zinc Solubilising Bacteria (ZnSB)	10.80	0.92	0.48	95.00	1.27
Vesicular Arbuscular Mycorrhiza (VAM)	11.87	1.13	0.58	88.80	1.33
VAM + PSB	12.53	1.36	0.67	94.40	1.36
VAM + KSB	11.26	1.08	0.52	93.25	1.31
VAM +ZnSB	12.64	1.41	0.73	93.40	1.38
VAM +Azotobacter chroococcum	12.95	1.53	0.79	89.52	1.40
C.D (pd"0.05)	0.128	0.027	0.019	0.236	0.005
SE(m)	0.044	0.009	0.007	0.081	0.002

ues (6.0). However, in both situations, seedlings were inoculated with AM fungus and PGPR, which improved the sturdiness quotient values towards < 6.

Conclusion

Natural forests are facing extensive pressure worldwide as they have been exploited for valuable products used in timber, fuel and medicinal purposes. Due to slow regeneration of conifers in temperate conditions, nursery raising becomes cumbersome. The utilization of variety of beneficial microbial inoculants potentially enhances conifer seedlings growth, therefore, can be introduced for commercial nursery raising that confers protection to the natural forest species. Interestingly, this study can aid in the conversion of wastelands to forest lands or afforestation of new areas with aim of providing natural habitats to various species. Among all the microbial treatments, the dual inoculation of VAM and *Azotobacter chroococcum* proved to be best for all growth parameters over control. However, seedling survival was recorded maximum with application of Zinc Solubilising Bacteria.

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