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FIELD PERFORMANCE OF BLUE PINE (*PINUS WALLICHIANA*) SEEDLINGS INOCULATED WITH SELECTED SPECIES OF BIO-INOCULANTS UNDER NURSERY CONDITIONS

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ABSTRACT

A pot experiment was carried out during 2009 and 2010 to study the field performance of blue pine seedlings inoculated with selected species of bio-inoculants under nursery conditions .The experiment was laid in Completely Randomized Design with three replications which comprised of seven inoculants (*Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens, Bacillus subtilis, Pisolithus tinctorius*, *Laccaria laccata* and control). Various growth characters viz., shoot height, collar diameter, root length, and seedling survival at various intervals responded significantly to all the microbial inoculants. Among microbial inoculants the two mycorrhizae viz., *Pisolithus tinctorius* and *Laccaria laccata* proved beneficial for all growth parameters than rest of the inoculants. It was followed by *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens* and *Bacillus subtilis*. However for root length *Pseudomonas fluorescens* and *Bacillus subtilis* gave best results than *Azotobacter* sp. and *Azospirillum* sp. Microbial inoculation of *Pisolithus tinctorius* and *Laccaria laccata* gave best results with respect to per cent decrease in seedling mortality rate of the species. Thus the two treatments viz; *Pisolithus tinctorius* and *Laccaria laccata* proved superior for all the studied growth parameters. Our findings show that the application of bio-inoculants improve the growth attributes of blue pine seedlings under natural field conditions.

KEYWORDS: **Blue pine, inoculation, seedlings, nursery**

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INTRODUCTION

Pinus wallichiana Jackson, commonly known as kail, blue pine or Bhutan pine, is an evergreen large conifer tree which has bluish feathery foliage. At young age, it is one of the most beautiful pines in the world. In the Himalayan region, kail is frequently found between 1500-3000 m. However, sometimes it may grow upto 3600 m in the upper reaches. They are largely found in areas where rainfall is 1000-2000 mm annually. From Afghanistan in the west, the kail region extends upto Bhutan and Arunachal Pradesh in the east, although it is absent in considerable portions of Kumaon and Sikkim. Other important places where kail grows abundantly in the sub-continent are from Garhwal through Jaunsar, the Shimla hills, Kulu, Chamba and Muree hills. Kail requires well-drained moist, fresh and deep soils; preferably derived from mica-schist which decomposes in moist fresh soil. In certain cases, the species also grows on deep limestone soils. It sometimes grows up in great abundance on bolder and gravel deposits in the beds of streams owing to its preference for porous soil with a fair amount of sub-soil moisture (Troup, 1921). Kail generally attains large heights, with horizontally spreading branches. The young shoots are glaucous green. Each dwarf shoot has five needles (12.5-20.0 cm long). The female cones are 15-30 cm long with rounded ovuliferous scales. The seeds are winged; wings membranous, about thrice as long as the seed. The kail produces seeds profusely atleast every year, which are very fertile and viable also. It is also a pioneer and strong light demanding species. It can resist moderate shade for sometime but makes little progress and ultimately fails to recover. Adequate light ensures a vigorous growth for the kail. It can resist the wind by its massive root system but suffers from snow which damages its crown and stem. It is sensitive to fire because of absence of thick and protective bark.

The timber of kail finds many uses. It is used for internal fittings of residential houses such as planking, door and window frames, panels, joinery and furniture, for these purposes it is preferred to deodar as it has less pronounced odour and does not pick up dust like the oily deodar wood. The wood after treatment is commonly used for making packing cases, camp furniture, drawing boards, fermentation vats and lorry bodies and shingles and railway sleepers. It is also utilized for making pencils, battery separators, violins and match boxes. It is a good fuel wood and yields excellent charcoal with high calorific value. Kail bark contains a fair amount of colouring matter and is sometimes used for dyeing silk and wool; it gives a fine yellow colour on corah silk and deep orange on wool. It is also employed for roofing huts. The indiscriminate use of inorganic fertilizers and pesticides is neither environmentally safe nor economically feasible. There is pressing demand for microbial inoculants for quality seedling production in nursery and also the establishment of plantation to increase the forest productivity. Bio-inoculants are cost effective, ecofriendly, cheaper and renewable sources of plant nutrients and play a vital role in maintaining long-term soil fertility and sustainability. Thus, to meet the challenges like poor regeneration, deforestation and spread of wastelands, introduction of microbial inoculants at the nursery stage of forest trees has become inevitable. Although various aspects of mycorrhizal impact of the forest trees have been studied, no work has been done on the impact of other microbial inoculants on the regeneration of forest trees viz, blue pine .Therefore, the present study was undertaken to evaluate the field performance of blue pine inoculated with selected bio-inoculants under nursery conditions.

MATERIALS AND METHODS

The present investigations were undertaken at the Forest Nursery of Department of Forestry, Faculty of Agriculture and Regional Research Station, SKUAST-Kashmir, Wadura, Sopore during 2009 and

2010.Microbial inoculants isolated from rhizosphere of blue pine forest stands were used in the studies.

Mass production of microbial inoculants

The two free living aerobic nitrogen fixing bacteria viz., *Azotobacter* sp. and *Azospirillum* sp. were mass cultured using nutrient medium enriched with glucose and peptone. Plant growth promoting rhizobacteria (PGPR) viz., *Pseudomonas fluorescens* and *Bacillus subtilis* were mass propagated in King's B nutrient broth. The two ectomycorrhizae viz., *Pisolithus tinctorius* and *Laccaria laccata* were mass multiplied in Melin Norkran's nutrient broth and Potato Dextrose Agar, respectively.

Field operations

For the microbial inoculation, one year old seedlings of blue pine of uniform heights and collar diameter growing in polyethylene bags (9″ x 7″) containing 1 kg potting material of soil and sand mixture in the ratio of 1:1 were selected.

Microbial inoculation

For inoculation, the different broth cultures of N-fixers, P-solubilizers and ectomycorrhizal inoculants isolated from local forest stands were applied to the potting material (25 ml/seedling) in the month of March, 2009, without disturbing the root system of the seedlings.

Nursery operations

The seedlings were irrigated with rose-cans as and when needed and maintained virtually weed free by manual weeding.

Plant growth measurement

All the growth parameters viz., plant height (cm), collar diameter (mm), seedling survival (%) and root length (cm) were measured at an interval of 2 months up to 12 months. All the growth parameters of the seedlings at the initial stage of the experiment were recorded.

Statistical analysis

The data was statistically analysed by using O.P Stat software developed by Haryana Agriculture University, Hisar.

RESULTS AND DISCUSSION

Plant height

The data on impact of various microbial inoculants on plant height of Blue pine seedlings indicates that mean plant height was significantly more in response to various treatments as compared to control (Table-1; Plate-1). *Azotobacter* and *Azospirillum* inoculation exhibited 37.17 and 36.83 per cent more plant height over control. Similarly *Pseudomonas flourescens* and *Bacillus subtilis* inoculation resulted in 35.56 and 33.91 per cent more plant height while as the inoculation with two ectomycorrhizal fungi viz., *Pisolithus tinctorius* and *Laccaria laccata* resulted in 42.97 and 40.77 per cent more plant height as compared to control respectively. However, the application of *Pisolithus tinctorius* showed maximum increase (42.97%) in plant height over control, thus proved superior over all the individual inoculants. Moreover, there was an increasing trend in plant height from April to October and from December onwards till February there was a slight increase. The interactions between inocula and months were significant till October and from December to February it was non-significant.The increase in shoot height by *P. tinctorius* and *L. laccata* could be attributed to the production of growth promoting substances like auxins (Dehn, 1982) and enhancement of water absorption and nutrient mobilization (Dar *et al*., 1997) by vastly increased surface area network of the fungal mycelia (Myer, 1992). In case of *Azotobacter* and *Azospirillum* sp. inoculation the increase in shoot height could be ascribed to nitrogen fixing ability, synthesis of growth promoting substances like cytokinens, gibberellins, auxins (Reynders and Vlassak, 1979; Hartmann *et al*., 1983; Jain and Patriquin, 1985) and production of antifungal antibiotics (Chahal and Chahal, 1988). However, increase in shoot height by inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* could be through iron chelating siderophores (Schippers, 1988) by releasing phytohormones, solubilizing P and reduction in population of deleterious

microorganisms (Weller, 1988). Further our findings are in close conformity with the results of Oh and Park (1989), Jeffries and Dodd (1991), Natarajan *et al*. (1995) who reported that *P. tinctorius* and *L. laccata* inoculation resulted in enhancement of plant height of *Acaccia nilotica, Quercus serrata, Eucalyptus camaldulensis* and *E. deglupta* seedlings respectively. Similarly, the enhancement in plant height with respect to *Azotobacter* and *Azospirillum* sp. has also been reported in *Quercus serrata* (Pandey *et al*., 1986) in peach (Awasthi *et al*., 1996). Moreover, the inoculation of clover plants with *Pseudomonas putida* has also been reported to enhance the plant height (Meyer and Linderman, 1986). Moreover, the gradual decline in plant height of the species in the later half of study period could be due to below freezing soil temperatures and short growing season of conifers.

Collar diameter

The data presented in Table-2; Plate-2 shows that the microbial inoculants increased the collar diameter of blue pine seedlings significantly. The inoculation with *P. tinctorius* showed 16.87 per cent increment over control. This was followed by *Laccaria laccata* (15.84%), *Azotobacter* (13.70%), *Azospirillum* (12.82%), *Pseudomonas fluorescens* (9.81%) and *Bacillus subtilis* (8.84%), respectively. The application of ectomycorrhizal fungi viz. *P. tinctorius* resulted in maximum collar diameter which was 16.87 per cent more than control. Moreover, plant collar diameter revealed a significant increase from April to October and thereafter till February there was a slight non-significant increase. The interactions between inocula and months were also significant till October and from October onwards it was non-significant. . Enhancement in collar diameter could be due to the release of plant growth substances and increase in the nutrient availability in the root zone (Jackobsen *et al*., 1994). Further, the gradual decline in collar diameter of seedlings in the winter months may be due to low fluctuating soil temperatures which might have stopped the growth of microbial inoculants. Similar observations have been recorded by other workers in various plants (Lee and Koo, 1985; Kumar and Lakhanpal, 1990; Tam and Griffiths, 1994).

Seedling survival

The results on impact of microbial inoculants on seedlings survival of Blue pine at nursery stage are presented in Table-3. The data depict that the seedling survival of Blue pine got significantly improved by the application of various microbial inoculants. The best results with respect to seedling survival percentage were obtained with the inoculation of *Pisolithus tinctorius* which was 21.06 per cent more as compared to control. Similarly it was followed by *Laccaria laccata* (19.07%), *Pseudomonas fluorescens* (15.51%), *Bacillus subtilis* (14.15%), *Azotobacter* (11.26%) and *Azospirillum* (9.73%), respectively. However, seedling survival percentage of Blue pine revealed a declining trend in the last months of winter. Enhancement in seedling survival could be attributed to the ability of microbial inoculants to secrete antifungal antibiotics, uptake of nutrients by converting them into available forms and greater access to water (Stribley, 1987) and production of growth promoting substances (Jackobsen *et al*., 1994). The decrease in survival percentage of the species in winter months could be attributed to low and below freezing soil temperatures which might have stopped the growth of inoculants and other soil microflora present there.

Root length

The data contained in Table-4; Plate-2 reveals a significant increase in root length of Blue pine seedlings due to application of microbial inoculants. Inoculation of kail seedlings with *Azotobacter* and *Azospirillum* resulted in 12.11 and 10.98 per cent more root length, respectively over control. Similarly *Pseudomonas fluorescens*, *Bacillus subtilis* and *L. laccata* inoculations resulted in 15.88, 14.24 and 18.21 per cent more root length over control. However, the application of *Pisolithus tinctorius* resulted in maximum root length which was 19.06 per cent more than control and thus proved superior over all other microbial inoculants. Moreover, there

was an increasing trend in root length between April and October and from October onwards there was a slight increase. Significant interactions were observed between inocula and months upto October and from there onwards it showed non-significant interactions. The increase in root length due to microbial inoculants could be attributed to their capability to synthesize biologically active substances (Jackson, 1964) and increased uptake of essential macronutrients (Bowen, 1973). Further the increase in root length with these microbial inoculants has also been reported by several workers (Tien *et al*., 1979; Navratil and Rochon, 1981; Ljungquist and Stenstrom, 1983; Pandey *et al*., 1986; Gaudin *et al*., 1994). Further the slight decrease in root length could be ascribed to low fluctuating soil temperatures in the winter months which might have lowered the efficiency of microbial inoculants and the already present soil microflora.

Table-1
Impact of microbial inoculation on plant height (cm) of Blue pine (Pinus wallichiana A.B. Jackson) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	11.30	12.70	14.82	15.31	15.33	15.33	14.13
Azotobacter sp.	14.95	18.97	22.98	26.90	22.94	22.94	22.49
Azospirillum sp.	14.20	18.35	22.42	26.40	26.43	26.43	22.37
Pseudomonas fluorescens	13.90	17.95	21.97	25.92	25.94	25.94	21.93
Bacillus subtilis	13.30	17.42	21.50	25.35	25.37	25.37	21.38
Pisolithus tinctorius	16.40	20.82	24.92	28.85	28.87	28.87	24.78
Laccaria laccata	15.87	19.90	23.94	27.82	27.84	27.84	23.86
Mean	14.27 (0.98)	18.01 (1.51)	21.79 (1.90)	25.22 (2.25)	25.24 (2.29)	25.24 (2.29)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.013	0.012	0.032
SEm	0.047	0.043	0.011

Initial plant height = 9.70 cm
Figures in parenthesis indicate CD of individual months

Tabble-2
Impact of microbial inoculation on collar diameter (mm) of Blue pine (Pinus wallichiana A.B. Jackson) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	2.92	3.08	3.38	3.67	3.69	3.69	3.40
Azotobacter sp.	3.15	3.56	3.88	4.35	4.37	4.37	3.94
Azospirillum sp.	3.12	3.51	3.83	4.31	4.33	4.33	3.90
Pseudomonas fluorescens	3.10	3.48	3.79	4.09	4.10	4.10	3.77
Bacillus subtillis	3.07	3.43	3.75	4.05	4.06	4.06	3.73
Pisolithustinctorius	3.28	3.68	4.15	4.48	4.50	4.50	4.09
Laccaria laccata	3.23	3.63	4.10	4.43	4.45	4.45	4.04
Mean	3.12 (0.06)	3.48 (0.11)	3.84 (0.14)	4.19 (0.16)	4.21 (0.21)	4.21 (0.21)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.013	0.012	0.032
SEm	0.047	0.044	0.011

Initial collar diameter = 1.96 mm
Figures in parenthesis indicate CD of individual months

Table-3
Impact of microbial inoculation on seedling survival (%) of Blue pine (Pinus wallichiana A.B. Jackson) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	76.18	73.28	73.28	72.18	70.12	68.14	74.14
Azotobacter sp.	85.52	85.52	85.52	84.31	82.12	78.31	83.55
Azospirillum sp.	84.25	84.25	84.25	83.37	80.23	76.52	82.14
Pseudomonas fluorescens	89.35	89.35	89.35	88.30	87.13	83.13	87.76
Bacillus subtilis	88.42	88.42	88.42	87.40	85.31	80.23	86.36
Pisolithus tinctorius	95.83	95.83	95.83	94.61	93.25	88.21	93.92
Laccaria laccata	93.25	93.25	93.25	92.27	91.21	86.52	91.62
Mean	87.54 (4.25)	87.12 (4.24)	87.12 (4.24)	86.06 (4.23)	84.19 (4.21)	80.15 (4.17)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.019	0.018	0.049
SEm	0.007	0.006	0.017

Initial seedling survival = 100 %
Figures in parenthesis indicate CD of individual months

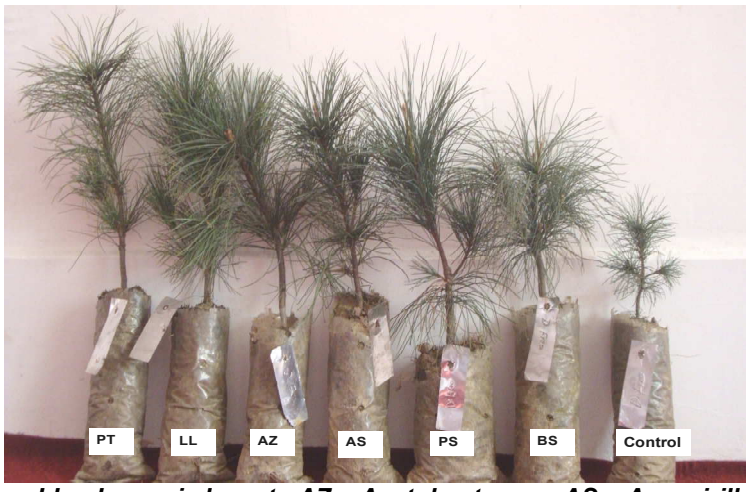
Tab1e-4
Impact of microbial inoculation on root length (cm) of Blue pine (Pinus wallichiana A.B. Jackson) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	14.20	16.30	19.20	22.31	22.32	22.32	19.44
Azotobacter sp.	16.70	19.62	22.50	24.64	24.65	24.65	22.12
Azospirillum sp.	16.35	19.32	22.10	24.42	24.43	24.43	21.84
Pseudomonas fluorescens	17.60	20.42	23.55	25.70	25.71	25.71	23.11
Bacillus subtilis	17.15	20.12	23.10	25.22	25.23	25.23	22.67
Pisolithus tinctorius	18.50	21.30	24.25	26.70	26.71	26.71	24.02
Laccaria laccata	18.10	21.12	24.14	26.42	26.43	26.43	23.77
Mean	16.94 (0.82)	19.74 (0.97)	22.69 (1.00)	25.05 (1.04)	25.06 (1.08)	25.06 (1.08)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.031	0.029	0.078
SEm	0.011	0.010	0.027

Initial root length = 12 cm
Figures in parenthesis indicate CD of individual months

Plate-1
Growth of Pinus wallichiana seedlings in response to microbial inoculations at nursery stage



PT = Pisolithus tinctorius;LL = Laccaria laccata;AZ = Azotobacter sp.; AS = Azospirillum sp.; PS = Pseudomonas fluorescens; BS = Bacillus subtilis

Plate - 2
Collar diameter and root length measurement of blue pine seedlings

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