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Influence of different strains of bio-inoculants on relative growth rate of blue pine and Himalayan cypress under temperate nursery conditions

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Abstract
A pot experiment was carried out during 2009-2010 to study the impact of bio-inoculants on relative growth rate of Blue pine (*Pinus wallichiana* A.B. Jackson) and Himalayan cypress (*Cupressus torulosa* Don) under temperate nursery conditions. The experiment was laid in Completely Randomized Design with three replications which comprised forty-two treatment combinations of seven inoculants (*Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pisolithus tinctorius*, *Laccaria laccata* and control).The growth character viz., relative growth rate at various intervals responded significantly to all the bio- inoculants. Among bio- inoculants the two mycorrhizae viz., *Pisolithus tinctorius* and *Laccaria laccata* proved best for the relative growth rate parameter than rest of the inoculants. It was followed by *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens* and *Bacillus subtilis*. Moreover, there was a significant increase in relative growth rate of Himalayan cypress than Blue pine by the application of different strains of bio- inoculants.

Keywords: blue pine, himalayan cypress, bio-inoculation, nursery, relative growth rate

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) [12].

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 utilised 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 increasing pressure of human and livestock population, indiscriminate extraction of forest produce, regular forest fires and mining activities have resulted in soil erosion, loss of fertility and moisture content and decreasing productivity of forests which pose manifold problems to restore the ecosystem. Thus, microbial inoculants present in the soil form a strong and important component of our soils, mainly owing to their role to promote plant growth by providing access to the nutrients, nitrogen fixation, mobilization of some unavailable nutrients and production of antifungal antibiotics.

Similarly Himalayan cypress belonging to the family Coniferae is a large evergreen tree with a

pyramidal crown and drooping branch lets. Trees upto 47 m height and 7.15 m in girth have been measured in Tehsil Garhwal (Troup, 1921) [12]. Bark greyish brown, peeling off in long thin strips; leaves small, scale like seeds compressed with an orbicular wing, light reddish brown. The tree has a local distribution in the western Himalayas from Chamba to Nepal between 1800-2750 m elevations. The tree is naturally found on limestone. In its natural habitat the absolute maximum shade temperature is probably about 90ºF, the absolute minimum about 15ºF and the normal rainfall varies from 1000 to 2400 mm per annum. The heartwood is light brown with dark streaks, moderately hard, suitable for making furniture and building materials. It is an excellent timber for making railway sleepers. The timber of cypress shapes smoothly; as compared to teak. Its working quality index is 116 (Pant *et al.* 1989) [8]. Due to limited availability, its uses are not explored fully.

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. Moreover, they form an important component of organic farming practices. 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. Therefore, the present study will give us an idea about the impact of inoculants on relative growth rate of two important conifers of temperate belt viz, Blue pine and Himalayan cypress 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 2009-2010. Microbial inoculants isolated from rhizosphere of blue pine and Himalayan cypress forest stands were used in the studies

Isolation of bio- inoculants

A field survey of two districts of Kashmir valley viz, Kupwara and Bandipora was carried out during 2008-09 for the collection of rhizosphere soil samples of Blue pine and Himalayan cypress stands. The collected rhizosphere soil samples of both the species were brought directly to the laboratory for isolation of bacterial and fungal inoculants. The fungal inoculants were isolated by dilution plate method (Johnson *et al.*, 1957) [4]. on potato dextrose agar medium. The soil samples were thoroughly homogenized. Ten grams of soil was placed in 90 ml distilled sterile water and different dilutions made. One ml of each 10^4 and 10^5 dilution was pipetted out and poured into sterile Petri-dishes. Later, 15 ml molten PDA medium was poured in petriplates which were gently rotated and incubated at 26±2ºC for 36 hours. The cultures obtained were purified by single spore/hyphal tip method and maintained for further studies. The identification of isolated fungal inoculants was done on the basis of cultural and morphological characteristics viz. growth, colour and shape of the colonies, colour, shape and size of hyphae, basidiospores, cap, mycelia spines, gleba, conidiosphores and conidia (Arx von, 1981) [1]. The bacterial inoculants were isolated from the rhizosphere soil samples of blue pine and Himalayan cypress stands by serial dilution technique. One gram of rhizosphere soil sample was transferred to 250 ml conical flask containing 100 ml sterile water. After thorough shaking for 15 minutes in a shaker, serial dilutions upto 10^{-7} were prepared. One ml of each 10^{-6} and 10^{-7} dilution was pipetted out and poured into the sterile petri-dishes. Fifteen ml molten King's B medium (KB) (King *et al.*, 1954) [5] was poured in plates which were rotated gently and incubated at 28±2ºC for 24 hours. The bacterial growth developed was purified by the dilution plate technique. The bacterial cultures were maintained on King's B medium in culture tubes at 4ºC.

Field operations

For the microbial inoculation, one year old seedlings of blue pine and Himalayan cypress 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

Plant growth parameter viz., relative growth rate (mg/month) was measured at an interval of 2 months up to 12 months.

Findings and Discussions.

It was observed in the present study that Bio- inoculants enhanced the relative growth rate of kail and Himalayan cypress seedlings over control in a non-significant manner. *P. tinctorius* among inoculants resulted in maximum plant relative growth rate and was superior over rest of the inoculants. Similarly it was followed by *L. laccata* and then *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas fluorescens* and *Bacillus subtilis*, respectively. Moreover relative growth rate demonstrated a non-significant increase upto October and from October onwards it showed a decreasing trend (Table-1, 2). Further there was a higher relative growth rate in case of Himalayan cypress as compared to blue pine. Increase in relative growth rate could be attributed to better nutrient absorption and uptake by these inoculants (Balakrishnan, 1988) [3] and their ability to produce growth promoting substances like auxins, gibberellins, indole acetic acid etc. and antifungal antibiotics like HCN, in addition to fixing molecular nitrogen (Brown and Carr, 1984) [2] and greater access to water and nutrients in the soil (Stribley, 1987) [10]. Further our findings are in conformity with the findings of several workers like O'Neill *et al*. (1987), Marx and Cordell (1989), Klopper *et al*. (1989) and Skvarna *et al*. (2002) [8, 7, 6, 11]. The increase in relative growth rate in the early months may be attributed to favourable environmental conditions which might have triggered the growth of inoculants in the rhizosphere soil and similarly the decline in relative growth rate of seedlings in winter months may be due to adverse climatic conditions. Further the maximum relative growth rate in Himalayan

cypress seedlings could be due to better nutrient and water uptake by its root system as compared to Blue pine which has got a poor root system and is otherwise a slow growing species among conifers.

Table 1: Impact of bio- inoculation on relative growth rate (mg/month) of Blue pine (*Pinus wallichiana* A.B. Jackson) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	0.07	0.09	0.11	0.13	0.03	0.01	0.07
Azotobacter sp.	0.12	0.14	0.17	0.19	0.09	0.07	0.13
Azospirillum sp.	0.10	0.12	0.15	0.17	0.08	0.06	0.11
Pseudomonas fluorescens	0.09	0.11	0.14	0.16	0.07	0.05	0.10
Bacillus subtilis	0.08	0.10	0.13	0.15	0.07	0.05	0.09
Pisolithus tinctorius	0.15	0.18	0.21	0.23	0.11	0.09	0.16
Laccaria laccata	0.14	0.16	0.19	0.21	0.10	0.08	0.14
Mean	0.10 (0.020)	0.12 (0.021)	0.15 (0.025)	0.17 (0.027)	0.07 (0.017)	0.05 (0.015)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.013	0.012	NS
SEm	0.004	0.003	0.011

Figures in parenthesis indicate CD of individual months

Table 2: Impact of bio- inoculation on relative growth rate (mg/month) of Himalayan cypress (*Cupressus torulosa* Don) at nursery stage

Treatment	2009					2010	Mean
	April	June	August	October	December	February	
Control	0.10	0.18	0.21	0.23	0.04	0.02	0.13
Azotobacter sp.	0.19	0.26	0.29	0.31	0.13	0.09	0.21
Azospirillum sp.	0.17	0.25	0.28	0.30	0.11	0.08	0.19
Pseudomonas fluorescens	0.16	0.23	0.27	0.29	0.09	0.07	0.18
Bacillus subtilis	0.15	0.22	0.26	0.27	0.08	0.06	0.17
Pisolithus tinctorius	0.23	0.29	0.32	0.34	0.16	0.11	0.24
Laccaria laccata	0.21	0.28	0.31	0.32	0.14	0.10	0.22
Mean	0.17 (0.021)	0.24 (0.028)	0.27 (0.036)	0.29 (0.038)	0.10 (0.017)	0.07 (0.014)	

	Treatment (T)	Month (M)	T x M
CD ($p \leq 0.05$)	0.013	0.012	NS
SEm	0.006	0.004	0.011

Figures in parenthesis indicate CD of individual months

Conclusion

In light of the results of the present investigations it could be concluded that bio-inoculants isolated from rhizosphere soil of blue pine and Himalayan cypress stands improved plant growth viz; relative growth rate under nursery conditions. Himalayan cypress, being a fast growing species responded significantly to the bio- inoculation and showed maximum growth than the extremely slow growing species viz. kail. This makes Himalayan cypress a very promising species for afforestation of poor soils, marginal lands and wastelands.

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