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Research Article

Field Enactment of *Taxus wallichiana Zucc.* (Himalayan yew) Stem Cuttings Inoculated with Selected and Beneficial Bio-inoculants under Nursery Conditions

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ABSTRACT

The unrelenting demand for the production of trees in forests with a substantial decline in the consumption of man-made chemical fertilizers and pesticides is an immense task at the moment. Plant Growth Promoting Rhizobacteria (PGPR) and fungi being the useful microbes which are extensively considered by microbiologists and agronomists as of their impending in growing crop production and offer innumerable methods to replace man-made chemical fertilizers, pesticides, etc., and therefore has significantly managed to their augmented demand. Throughout the present investigation, the microbial inoculants (*Bacillus subtilis*, *Bacillus safensis*, *Penicillium griseoroseum* and *Trichoderma harzianum*) were inoculated in various treatments to determine the impact on vegetative growth of *Taxus wallichiana* Zucc. (Himalayan yew) stem cuttings under nursery conditions. The pot experiment with 10 treatments including control and 3 replications with plot size comprising of 30 pots was arranged in Completely Randomized Design (CRD). Several growth characteristics viz., plant height, collar diameter, root length, fresh and dry biomass (shoot, root and total plant biomass) after the interlude of two months responded significantly to all the different treatments of microbial inoculants as compared to control. The combined treatment of the microbial inoculants showed the best results for all the growth characteristics as compared to isolated treatments and an increasing trend in all the growth characteristics was noticed up to December of the study period and in February it remains same as no growth was observed. Thus, our outcomes revealed that the application of microbial inoculants enhanced the growth traits of Himalayan yew stem cuttings under nursery conditions.

Keywords: Microbial inoculants; Nursery conditions; PGPR; *Taxus wallichiana*; Vegetative growth

INTRODUCTION

The decrease in forest area is countered usually by afforestation programs involving planting of the trees in deforested areas. But there are some major difficulties in successful afforestation programs because of less percentage of adaptation and acclimatization of planted trees. And as far as natural regeneration is concerned it does not practically take place in forests where crown density is less than 40%. Relying on natural succession, it will take us hundreds of years to regenerate the degraded forests to climax stage with species like *Taxus wallichiana* Zucc. (Himalayan Yew), *P. wallichiana* A.B. Jackson (Kail), *C. deodara* (Roxb.) G. Don (Deodar), *A. pindrow* Spach (silver fir) and *P. smithiana* Wall. (spruce) which dominate the vegetation of our forests. It has been evaluated that more than 100 million tons of inorganic fertilizers are used annually in order to enhance crop yield [1]. However, indiscriminate use of chemical fertilizers regardless of climatic, soil and other factors has affected the environmental quality and soil ecosystem. The potential negative effect of synthetic fertilizers on the global environment and the cost associated with production has led to scrutiny with the purpose of replacing synthetic fertilizers with microbial inoculants. Therefore, inception of befitting microbial inoculants is imperative to ameliorate the survival and quality of planting stock so as to undertake national developmental programs of afforestation, reforestation, wasteland- reclamation and social forestry favorably.

Taxus wallichiana Zucc. is an evergreen small to medium-sized conifer, with red berries, is native to the Himalaya from Afghanistan to China. It grows up to 10–20 m tall at an elevation of about 1800-3300 m above the mean sea level. Its leaves are dark green, flat, arranged spirally on the stem [2]. It grows in various soil types from acidic to neutral soils. As the species are highly similar, they are often easier to separate geographically than morphologically [3]. Typically, ten species are recognized: *T.*

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baccata (European or English yew), *T. canadensis* (Canadian yew), *T. brevifolia* (Pacific yew or Western yew), *T. chinensis* (Chinese yew), *T. floridana* (Florida yew), *T. cuspidata* (Japanese yew), *T. globosa* (Mexican yew), *T. sumatrana* (Sumatran yew), *T. celebica* (Celebes yew) and *T. wallichiana* (Himalayan yew) [4]. It is found in temperate, moderate temperate, and tropical submontane to high montane forests and is extensively distributed in the areas of Afghanistan, China, Bhutan, India, Malaysia, Indonesia, Pakistan, Nepal, Philippines and Vietnam [5]. In Kashmir valley it is distributed in Lolab, Gulmarg and Tangmarg regions.

A significant attention has been generated by the genus *Taxus*, due to the presence of taxol (a diterpene alkaloid content) in shoots and leaves has an exhilarating prospective as an anti-cancer remedy for different cancer treatments viz ovarian cancer and breast cancer, kaposi's sarcoma etc. [6]. Taxene (alkaloid) is used as either monotherapy or in combination with other anti-cancer agents [7,8]. The worldwide demand of the taxol is 800 -1000 kg per annum. Approximately 3 to 4 million kg of taxol is harvested yearly while the expected harvesting rate is to be 0.7 million kg per annum [3]. *Taxus wallichiana* is medicinally used for the treatment of high fever and painful inflammatory conditions and many other diseases are treated including headaches, eruptions, cystitis, Kidney and heart problems, rheumatism, bronchitis, asthma, indigestion and to treat viper bites, heart ailments and as an abortifascient [9]. Although its wood is durable and strong, used for bow making and many other purposes, likewise it is burnt as incense in Nepal and parts of Tibet or used as fuel wood by the local communities [10]. However, due to overexploitation of its bark and slow growth it has been placed in an endangered category of IUCN in 2015 [11].

MATERIAL AND METHODS

The present investigation was conducted at the Kashmir University Nursery, Srinagar during the year (2015-2017). The microbial inoculants isolated from the rhizosphere of Himalayan yew forest stands were used in the study.

Inoculum preparation

The efficient bacterial (*B. subtilis, B. safensis*) and fungal (*P. griseoroseum, T. harzianum*) strains isolated from *T. wallichiana* were scraped from agar slants maintained at -20°C for long term storage with sterile inoculating loop, transferred each into 1 litre broth, using nutrient broth for bacterial strains and potato dextrose broth for fungal strains and were kept on incubator shaker at 2000 rpm for a week.

Experimental details

Design: The pot experiment with 10 treatments including control and 3 replications with plot size comprising of 30 pots was arranged out in Completely Randomized Design (CRD).

Treatment details: The microbial inoculants were inoculated separately and in combination comprising of 10 treatments including control (Table 1).

Field operations: Before microbial inoculation, the rooted stem cuttings of *T. wallichiana* of uniform heights and collar diameter were transferred in pots (9″ × 7) containing 1 kg autoclaved potting material of soil and sand mixture in the ratio of 1:1.

Microbial inoculation: For inoculation, the broth cultures of bacterial and fungal inoculants isolated from rhizospheric soil of *T. wallichiana* were applied to the already rooted stem cuttings in pots (25 ml/ cutting) in the month of March, 2016, without disturbing the roots of the stem cuttings.

Nursery operations: The irrigation to the stem cuttings was done using rose can as per the need and maintenance, weeds were removed manually.

Plant growth measurements: All the parameters related to the plant growth like the plant height, the diameter of the collar, length of the root, the fresh root biomass, fresh shoot biomass, dry root biomass, dry shoot biomass, total fresh plant biomass and total dry plant biomass. All these parameters were measured in an interval of 2 months for about a year and all the growth parameters at an initial stage means before the application of microbial inoculants of the experiment were recorded.

Statistical analysis

The data was analyzed by ANOVA using Duncan's multiple range test (SPSS 17.0) with a significance level of $p < 0.005$.

RESULTS

The current study revealed that the microbial inoculants (*B. subtilis*, *B. safensis*, *P. griseoroseum* and *T. harzianum*) significantly enhanced plant growth viz plant height, collar diameter, root length, fresh and dry shoot biomass, fresh and dry root biomass and total fresh and dry plant biomass as compared to uninoculated control.

Plant height

The microbial inoculants exerted a significant influence on plant height in response to different treatments as compared

Table 1: Treatment details.

Treatments	
Control	**Control**
B1	*Bacillus subtilis*
B2	*Bacillus safensis*
F1	*Penicillium griseoroseum*
F2	*Trichoderma harzianum*
B1+B2	*Bacillus subtilis* + *Bacillus safensis*
F1+F2	*Penicillium griseoroseum*+ *Trichoderma harzianum*
B1+F1+F2	*Bacillus subtilis* + *Penicillium griseoroseum*+ *Trichoderma harzianum*
B2+F1+F2	*Bacillus safensis* + *Penicillium griseoroseum*+ *Trichoderma harzianum*
B1+B2+F1+F2	*Bacillus subtilis* + *Bacillus safensis* + *Penicillium griseoroseum*+ *Trichoderma harzianum*

to uninoculated control (Table 2, Figure 1 and Figure 2A). The maximum increase in plant height of Himalayan yew stem cuttings (44.70 ± 0.20 cm) was observed due to the treatment B1+B2+F1+F2 followed by B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; F1; B1 and B2 treatments. Moreover, the plant height showed an increasing trend up to December and in February plant height remains same because no growth was detected.

Collar diameter

The data presented in Table 3 shows the response of microbial inoculants on increased collar diameter of Himalayan yew stem cuttings as compared to control (Figure 2B). The application of the treatment B1+B2+F1+F2 resulted in maximum (2.38 ± 0.06 mm) collar diameter of Himalayan yew stem cuttings which was followed by treatments B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; F1; B1and B2. Moreover, collar diameter revealed an increasing trend from April to December and remains same in February.

Root length

Perusal of the data on root length of Himalayan yew stem cuttings clearly indicates an increase in root length by the application of microbial inoculants in various treatments as compared to control (Table 4, Figure 2C). The best results (25.00 ± 0.06 cm) with respect to root length was obtained with the inoculation of treatment B1+B2+F1+F2 followed by B1+F1+F2; B2+F1+F2; F1+F2; B1 +B2; F2; F1; B1 and B2 treatments. Further an increasing trend in root length was observed along the months.

Fresh shoot biomass

It was observed in the present study that microbial inoculants improved the fresh shoot biomass of the stem cuttings over control (Table 5). The maximum (25.70 ± 0.05 g) increase in fresh shoot biomass was recorded by the inoculation of the treatment B1+B2+F1+F2 followed by B1+F1+F2; B2+F1+F2; F1+F2; B1 +B2;

Table 2: Impact of microbial inoculants on plant height (cm) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Plant Height (cm)	*Treatments	April	June	August	October	December	February
25.00	Control	^{a}25.06 ± 0.11	^{a}25.07 ± 0.05	^{a}25.07 ± 0.05	^{a}25.08 ± 0.05	^{a}25.09 ± 0.05	^{a}25.09 ± 0.05
	B1	^{abc}26.13 ± 0.57	^{b}27.10 ± 0.10	^{c}27.56 ± 0.32	^{c}27.66 ± 0.20	^{c}27.83 ± 0.11	^{c}27.83 ± 0.11
	B2	^{ab}25.50 ± 0.50	^{a}25.60 ± 0.36	^{b}25.76 ± 0.15	^{b}25.79 ± 0.01	^{b}25.93 ± 0.05	^{b}25.93 ± 0.05
	F1	^{bc}26.36 ± 0.51	^{b}27.30 ± 0.30	^{c}27.83 ± 0.05	^{c}28.06 ± 0.15	^{c}28.13 ± 0.20	^{c}28.13 ± 0.20
	F2	^{c}26.80 ± 0.34	^{c}28.70 ± 0.20	^{d}29.93 ± 0.05	^{d}30.46 ± 0.45	^{d}30.73 ± 0.20	^{d}30.73 ± 0.20
	B1+B2	^{c}27.13 ± 0.30	^{c}28.56 ± 0.49	^{d}29.70 ± 0.26	^{d}30.56 ± 0.49	^{d}31.60 ± 0.20	^{d}31.60 ± 0.20
	F1+F2	^{d}28.56 ± 0.57	^{d}31.40 ± 0.36	^{e}34.36 ± 0.32	^{e}35.33 ± 0.35	^{e}35.66 ± 0.32	^{e}35.66 ± 0.32
	B2+F1+F2	^{d}29.03 ± 0.96	^{e}32.56 ± 0.49	^{f}35.36 ± 0.30	^{f}36.76 ± 0.25	^{f}37.16 ± 0.15	^{f}37.16 ± 0.15
	B1+F1+F2	^{d}29.66 ± 0.57	^{f}34.26 ± 0.30	^{g}38.30 ± 0.26	^{g}40.20 ± 0.26	^{g}40.66 ± 0.20	^{g}40.66 ± 0.20
	B1+B2+F1+F2	^{e}30.83 ± 0.63	^{g}36.10 ± 0.10	^{h}41.20 ± 0.26	^{h}44.26 ± 0.30	^{h}44.70 ± 0.20	^{h}44.70 ± 0.20

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005.

*B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Figure 1: Growth of *Taxus wallichiana* stem cuttings in response to different treatments of microbial inoculants under nursery conditions.

(A) (B) (C)

Figure 2 (A-C): Measurement of plant height, collar diameter and root length.

Table 3: Impact of microbial inoculants on collar diameter (mm) of Himalayan yew (*Taxus wallichiana*Zucc.) at nursery stage during year (2016-2017).

Initial Collar Diameter (mm)	*Treatments	April	June	August	October	December	February
0.8	Control	^{a}0.86 ± 0.06	^{a}0.88 ± 0.05	^{a}0.89 ± 0.05	^{a}0.90 ± 0.05	^{a}0.91 ± 0.05	^{a}0.91 ± 0.05
	B1	^{a}0.90 ± 0.06	^{a}0.94 ± 0.04	^{a}0.97 ± 0.03	^{a}0.99 ± 0.04	^{a}1.00 ± 0.04	^{a}1.00 ± 0.04
	B2	^{a}0.88 ± 0.05	^{a}0.91 ± 0.03	^{a}0.92 ± 0.03	^{a}0.93 ± 0.03	^{a}0.94 ± 0.03	^{a}0.94 ± 0.03
	F1	^{b}1.03 ± 0.05	^{b}1.08 ± 0.05	^{b}1.12 ± 0.03	^{b}1.13 ± 0.03	^{b}1.14 ± 0.05	^{b}1.14 ± 0.05
	F2	^{b}1.12 ± 0.04	^{c}1.26 ± 0.04	^{c}1.29 ± 0.04	^{c}1.31 ± 0.03	^{c}1.33 ± 0.03	^{c}1.33 ± 0.03
	B1+B2	^{c}1.33 ± 0.06	^{d}1.42 ± 0.07	^{d}1.45 ± 0.04	^{d}1.48 ± 0.05	^{d}1.49 ± 0.05	^{d}1.49 ± 0.05
	F1+F2	^{cd}1.41 ± 0.03	^{d}1.48 ± 0.06	^{e}1.54 ± 0.05	^{e}1.58 ± 0.06	^{e}1.60 ± 0.04	^{e}1.60 ± 0.04
	B2+F1+F2	^{e}1.60 ± 0.05	^{f}1.70 ± 0.08	^{g}1.78 ± 0.07	^{g}1.84 ± 0.05	^{g}1.88 ± 0.06	^{g}1.88 ± 0.06
	B1+F1+F2	^{de}1.50 ± 0.07	^{e}1.59 ± 0.09	^{f}1.66 ± 0.06	^{f}1.71 ± 0.06	^{f}1.74 ± 0.06	^{f}1.74 ± 0.06
	B1+B2+F1+F2	^{f}1.80 ± 0.08	^{g}2.10 ± 0.06	^{h}2.21 ± 0.06	^{h}2.30 ± 0.06	^{h}2.38 ± 0.06	^{h}2.38 ± 0.06

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Table 4: Impact of microbial inoculants on root length (cm) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Root Length (cm)	*Treatments	April	June	August	October	December	February
5.00	Control	^{a}5.06 ± 0.04	^{a}5.10 ± 0.04	^{a}5.13 ± 0.04	^{a}5.15 ± 0.04	^{a}5.16 ± 0.04	^{a}5.16 ± 0.04
	B1	^{c}6.11 ± 0.04	^{c}7.01 ± 0.05	^{c}7.61 ± 0.05	^{c}7.71 ± 0.05	^{c}7.79 ± 0.05	^{c}7.79 ± 0.05
	B2	^{b}5.50 ± 0.05	^{b}5.80 ± 0.05	^{b}6.00 ± 0.05	^{b}6.09 ± 0.06	^{b}6.15 ± 0.05	^{b}6.15 ± 0.05
	F1	^{d}6.30 ± 0.03	^{d}7.30 ± 0.02	^{d}7.90 ± 0.04	^{d}8.10 ± 0.04	^{d}8.12 ± 0.05	^{d}8.12 ± 0.05
	F2	^{e}7.00 ± 0.05	^{e}8.80 ± 0.07	^{e}10.01 ± 0.05	^{e}10.61 ± 0.05	^{e}10.64 ± 0.05	^{e}10.64 ± 0.05
	B1+B2	^{f}7.10 ± 0.05	^{f}9.11 ± 0.06	^{f}10.61 ± 0.05	^{f}11.41 ± 0.07	^{f}11.45 ± 0.07	^{f}11.45 ± 0.07
	F1+F2	^{g}8.20 ± 0.06	^{g}11.20 ± 0.08	^{g}13.80 ± 0.07	^{g}14.80 ± 0.06	^{g}14.85 ± 0.05	^{g}14.85 ± 0.05
	B2+F1+F2	^{i}10.00 ± 0.06	^{i}14.60 ± 0.07	^{i}18.60 ± 0.06	^{i}20.40 ± 0.08	^{i}20.80 ± 0.06	^{i}20.80 ± 0.06
	B1+F1+F2	^{h}9.02 ± 0.06	^{h}12.80 ± 0.07	^{h}16.00 ± 0.07	^{h}17.50 ± 0.07	^{h}17.80 ± 0.05	^{h}17.80 ± 0.05
	B1+B2+F1+F2	^{j}11.00 ± 0.10	^{j}16.50 ± 0.06	^{j}21.50 ± 0.05	^{j}24.50 ± 0.07	^{j}25.00 ± 0.06	^{j}25.00 ± 0.06

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

F2; F1; B1 and B2 treatments. It has been observed that the fresh shoot biomass revealed an increasing trend from April to December.

Dry shoot biomass

Perusal of the data presented in Table 6, depicts an increase in dry shoot biomass of the Himalayan yew stem cuttings at nursery stage by the application of different microbial inoculants. Maximum increase (22.51 ± 0.06g) in dry shoot biomass as compared to control was observed due to the application of B1+B2+F1+F2 treatment followed by B1+F1+F2; B2+F1+F2; F1+F2; B1 +B2; F2;

F1; B1 and B2 treatments. Further, dry shoot biomass also revealed an increasing trend with the advancement of seasons.

Fresh root biomass

All the microbial inoculants of various treatments increased the fresh root biomass of Himalayan yew stem cuttings over control (Table 7). Among the various treatments the application of B1+B2+F1+F2 treatment resulted in maximum fresh root biomass (22.71 ± 0.06 g), followed by the treatments B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; B1; F1 and B2. Moreover, an increasing trend was observed in fresh root biomass from April to December and remains same in February.

Dry root biomass

The results presented in Table 8 evidently shows that all the microbial inoculants had a significant impact on the dry root biomass of Himalayan yew stem cuttings at nursery stage as compared to control. Application of B1+B2+F1+F2 treatment gave maximum dry root biomass (21.02 ± 0.05 g) followed by B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; B1; F1 and B2 treatments. Moreover, an increase in dry root biomass was also noticed from April to December.

Total fresh plant biomass

During the present investigation, all the microbial inoculants of various treatments had improved the total fresh plant biomass of Himalayan yew cuttings over control (Table 9). Amongst various treatments B1+B2+F1+F2 was found superior in terms of total fresh plant biomass (28.41 ± 0.04 g) followed by B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; F1; B1 and B2. Moreover, an increasing trend in total fresh plant biomass up to December was observed and remains same in February.

Total dry plant biomass

The application of microbial inoculants of different treatments shown in Table 10 clearly depicted an increase in total dry plant biomass as compared to an uninoculated control. Inoculation by B1+B2+F1+F2 treatment gave the maximum (23.52 ± 0.07 g) of total dry plant biomass as compared to control which was followed by B1+F1+F2; B2+F1+F2; F1+F2; B1+B2; F2; F1; B1 and B2 treatments. Further, an increasing trend was noticed in total dry plant biomass up to December and in February it remains same.

DISCUSSION

Plant height is an important growth attribute as it determines the

Table 5: Impact of microbial inoculants on fresh shoot biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Fresh Soot Biomass (g)	*Treatments	April	June	August	October	December	February
5.70	Control	^{a}5.76 ± 0.05	^{a}5.80 ± 0.06	^{a}5.83 ± 0.06	^{a}5.85 ± 0.05	^{a}5.86 ± 0.05	^{a}5.86 ± 0.05
	B1	^{c}6.80 ± 0.06	^{c}7.70 ± 0.05	^{c}8.30 ± 0.08	^{c}8.41 ± 0.04	^{c}8.48 ± 0.06	^{c}8.48 ± 0.06
	B2	^{b}6.20 ± 0.06	^{b}6.50 ± 0.07	^{b}6.70 ± 0.06	^{b}6.79 ± 0.07	^{b}6.85 ± 0.05	^{b}6.85 ± 0.05
	F1	^{d}7.01 ± 0.07	^{d}8.02 ± 0.05	^{d}8.63 ± 0.06	^{d}8.83 ± 0.08	^{d}8.85 ± 0.07	^{d}8.85 ± 0.07
	F2	^{e}7.71 ± 0.06	^{e}9.50 ± 0.05	^{e}10.70 ± 0.07	^{e}11.30 ± 0.06	^{e}11.33 ± 0.06	^{e}11.33 ± 0.06
	B1+B2	^{e}7.80 ± 0.07	^{f}9.80 ± 0.07	^{f}11.30 ± 0.04	^{f}12.10 ± 0.05	^{f}12.14 ± 0.06	^{f}12.14 ± 0.06
	F1+F2	^{f}8.90 ± 0.07	^{g}11.90 ± 0.08	^{g}14.50 ± 0.07	^{g}15.50 ± 0.05	^{g}15.55 ± 0.05	^{g}15.55 ± 0.05
	B2+F1+F2	^{h}10.70 ± 0.07	^{i}15.30 ± 0.07	^{i}19.31 ± 0.06	^{i}21.11 ± 0.05	^{i}21.50 ± 0.07	^{i}21.50 ± 0.07
	B1+F1+F2	^{g}9.70 ± 0.04	^{h}13.70 ± 0.06	^{h}16.70 ± 0.08	^{h}18.20 ± 0.06	^{h}18.50 ± 0.09	^{h}18.50 ± 0.09
	B1+B2+F1+F2	^{i}11.70 ± 0.07	^{j}17.20 ± 0.06	^{j}22.20 ± 0.07	^{j}25.20 ± 0.08	^{j}25.70 ± 0.05	^{j}25.70 ± 0.05

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Table 6: Impact of microbial inoculants on dry shoot biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Dry Soot Biomass (g)	*Treatments	April	June	August	October	December	February
2.50	Control	^{a}2.56 ± 0.06	^{a}2.60 ± 0.05	^{a}2.63 ± 0.05	^{a}2.65 ± 0.05	^{a}2.66 ± 0.05	^{a}2.66 ± 0.05
	B1	^{c}3.60 ± 0.06	^{c}4.50 ± 0.06	^{c}5.10 ± 0.06	^{c}5.20 ± 0.06	^{c}5.28 ± 0.07	^{c}5.28 ± 0.07
	B2	^{b}3.01 ± 0.07	^{b}3.30 ± 0.08	^{b}3.50 ± 0.07	^{b}3.59 ± 0.06	^{b}3.65 ± 0.05	^{b}3.65 ± 0.05
	F1	^{d}3.80 ± 0.07	^{d}4.80 ± 0.07	^{d}5.40 ± 0.06	^{d}5.60 ± 0.06	^{d}5.62 ± 0.05	^{d}5.62 ± 0.05
	F2	^{e}4.50 ± 0.06	^{e}6.30 ± 0.06	^{e}7.50 ± 0.07	^{e}8.10 ± 0.05	^{e}8.13 ± 0.05	^{e}8.13 ± 0.05
	B1+B2	^{e}4.60 ± 0.07	^{f}6.60 ± 0.04	^{f}8.10 ± 0.06	^{f}8.91 ± 0.06	^{f}8.94 ± 0.05	^{f}8.94 ± 0.05
	F1+F2	^{f}5.71 ± 0.06	^{g}8.71 ± 0.05	^{g}11.30 ± 0.07	^{g}12.30 ± 0.06	^{g}12.35 ± 0.07	^{g}12.35 ± 0.07
	B2+F1+F2	^{h}7.51 ± 0.06	^{i}12.10 ± 0.04	^{i}16.10 ± 0.07	^{i}17.90 ± 0.07	^{i}18.30 ± 0.05	^{i}18.30 ± 0.05
	B1+F1+F2	^{g}6.50 ± 0.07	^{h}10.30 ± 0.07	^{h}13.50 ± 0.05	^{h}15.00 ± 0.07	^{h}15.30 ± 0.06	^{h}15.30 ± 0.06
	B1+B2+F1+F2	^{i}8.50 ± 0.07	^{j}14.01 ± 0.07	^{j}19.01 ± 0.05	^{j}22.01 ± 0.08	^{j}22.51 ± 0.06	^{j}22.51 ± 0.06

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Table 7: Impact of microbial inoculants on fresh root biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Fresh Root Biomass (g)	*Treatments	April	June	August	October	December	February
2.71	Control	^{a}2.77 ± 0.07	^{a}2.81 ± 0.07	^{a}2.84 ± 0.06	^{a}2.86 ± 0.06	^{a}2.86 ± 0.07	^{a}2.86 ± 0.07
	B1	^{c}3.81 ± 0.05	^{c}4.71 ± 0.08	^{c}5.31 ± 0.06	^{c}5.41 ± 0.06	^{c}5.49 ± 0.07	^{c}5.49 ± 0.07
	B2	^{b}3.21 ± 0.06	^{b}3.51 ± 0.05	^{b}3.71 ± 0.06	^{b}3.80 ± 0.07	^{b}3.86 ± 0.06	^{b}3.86 ± 0.06
	F1	^{d}4.01 ± 0.05	^{d}5.01 ± 0.04	^{d}5.61 ± 0.08	^{d}5.81 ± 0.07	^{d}5.83 ± 0.06	^{d}5.83 ± 0.06
	F2	^{e}4.71 ± 0.07	^{e}6.51 ± 0.07	^{e}7.71 ± 0.06	^{e}8.31 ± 0.08	^{e}8.34 ± 0.05	^{e}8.34 ± 0.05
	B1+B2	^{e}4.81 ± 0.06	^{f}6.81 ± 0.06	^{f}8.31 ± 0.06	^{f}9.10 ± 0.05	^{f}9.15 ± 0.05	^{f}9.15 ± 0.05
	F1+F2	^{f}5.91 ± 0.07	^{g}8.91 ± 0.06	^{g}11.51 ± 0.05	^{g}12.51 ± 0.05	^{g}12.56 ± 0.07	^{g}12.56 ± 0.07
	B2+F1+F2	^{h}7.71 ± 0.08	^{i}12.31 ± 0.07	^{i}16.31 ± 0.08	^{i}18.11 ± 0.06	^{i}18.51 ± 0.05	^{i}18.51 ± 0.05
	B1+F1+F2	^{g}6.71 ± 0.06	^{h}10.51 ± 0.08	^{h}13.71 ± 0.05	^{h}15.21 ± 0.07	^{h}15.51 ± 0.08	^{h}15.51 ± 0.08
	B1+B2+F1+F2	^{i}8.71 ± 0.06	^{j}14.21 ± 0.07	^{j}19.21 ± 0.06	^{j}22.21 ± 0.04	^{j}22.71 ± 0.06	^{j}22.71 ± 0.06

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Table 8: Impact of microbial inoculants on dry root biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Dry Root Biomass (g)	*Treatments	April	June	August	October	December	February
1.02	Control	^{a}1.08 ± 0.07	^{a}1.12 ± 0.04	^{a}1.15 ± 0.05	^{a}1.17 ± 0.05	^{a}1.18 ± 0.06	^{a}1.18 ± 0.06
	B1	^{c}2.12 ± 0.06	^{c}3.02 ± 0.07	^{c}3.62 ± 0.06	^{c}3.72 ± 0.07	^{c}3.80 ± 0.07	^{c}3.80 ± 0.07
	B2	^{b}1.52 ± 0.07	^{b}1.82 ± 0.07	^{b}2.02 ± 0.06	^{b}2.11 ± 0.05	^{b}2.17 ± 0.06	^{b}2.17 ± 0.06
	F1	^{d}2.32 ± 0.07	^{d}3.32 ± 0.07	^{d}3.92 ± 0.07	^{d}4.12 ± 0.08	^{d}4.14 ± 0.05	^{d}4.14 ± 0.05
	F2	^{e}3.02 ± 0.05	^{e}4.82 ± 0.06	^{e}6.02 ± 0.06	^{e}6.62 ± 0.07	^{e}6.65 ± 0.06	^{e}6.65 ± 0.06
	B1+B2	^{e}3.12 ± 0.06	^{f}5.12 ± 0.05	^{f}6.62 ± 0.07	^{f}7.42 ± 0.05	^{f}7.46 ± 0.07	^{f}7.46 ± 0.07
	F1+F2	^{f}4.22 ± 0.05	^{g}7.22 ± 0.04	^{g}9.82 ± 0.06	^{g}10.82 ± 0.06	^{g}10.87 ± 0.07	^{g}10.87 ± 0.07
	B2+F1+F2	^{h}6.02 ± 0.05	^{i}10.62 ± 0.06	^{i}14.62 ± 0.07	^{i}16.42 ± 0.05	^{i}16.82 ± 0.08	^{i}16.82 ± 0.08
	B1+F1+F2	^{g}5.02 ± 0.06	^{h}8.82 ± 0.05	^{h}12.02 ± 0.06	^{h}13.52 ± 0.07	^{h}13.82 ± 0.07	^{h}13.82 ± 0.07
	B1+B2+F1+F2	^{i}7.02 ± 0.06	^{j}12.52 ± 0.07	^{j}17.52 ± 0.07	^{j}20.52 ± 0.06	^{j}21.02 ± 0.05	^{j}21.02 ± 0.05

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

Table 9: Impact of microbial inoculants on total fresh plant biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Total Fresh Plant Biomass (g)	*Treatments	April	June	August	October	December	February
8.41	Control	^{a}8.47 ± 0.05	^{a}8.51 ± 0.07	^{a}8.54 ± 0.05	^{a}8.56 ± 0.07	^{a}8.57 ± 0.04	^{a}8.57 ± 0.04
	B1	^{c}9.51 ± 0.06	^{c}10.41 ± 0.05	^{c}11.01 ± 0.06	^{c}11.11 ± 0.05	^{c}11.19 ± 0.06	^{c}11.19 ± 0.06
	B2	^{b}8.91 ± 0.06	^{b}9.21 ± 0.05	^{b}9.41 ± 0.05	^{b}9.50 ± 0.07	^{b}9.56 ± 0.04	^{b}9.56 ± 0.04
	F1	^{d}9.71 ± 0.05	^{d}10.71 ± 0.07	^{d}11.31 ± 0.05	^{d}11.51 ± 0.06	^{d}11.53 ± 0.06	^{d}11.53 ± 0.06
	F2	^{e}10.41 ± 0.06	^{e}12.21 ± 0.04	^{e}13.41 ± 0.06	^{e}14.01 ± 0.07	^{e}14.04 ± 0.04	^{e}14.04 ± 0.04
	B1+B2	^{f}10.51 ± 0.05	^{f}12.51 ± 0.06	^{f}14.01 ± 0.06	^{f}14.81 ± 0.05	^{f}14.85 ± 0.05	^{f}14.85 ± 0.05
	F1+F2	^{g}11.61 ± 0.06	^{g}14.61 ± 0.05	^{g}17.21 ± 0.07	^{g}18.21 ± 0.04	^{g}18.26 ± 0.05	^{g}18.26 ± 0.05
	B2+F1+F2	^{i}13.41 ± 0.05	^{i}18.01 ± 0.07	^{i}22.01 ± 0.05	^{i}23.81 ± 0.05	^{i}24.21 ± 0.07	^{i}24.21 ± 0.07
	B1+F1+F2	^{h}12.41 ± 0.07	^{h}16.21 ± 0.04	^{h}19.41 ± 0.04	^{h}20.91 ± 0.06	^{h}21.21 ± 0.04	^{h}21.21 ± 0.04
	B1+B2+F1+F2	^{j}14.41 ± 0.06	^{j}19.91 ± 0.04	^{j}24.91 ± 0.06	^{j}27.91 ± 0.06	^{j}28.41 ± 0.04	^{j}28.41 ± 0.04

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. *B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

vigor of the yield. These findings have been supported by various workers who reported that the inoculation with beneficial microbial inoculants have positively affected the plant height at seedling stage [12-14]. Yang et al. [15], who revealed a significant increase in plant height by the application of *Bacillus* and *Pseudomonas* species inoculated on *P. massoniana* plants. In combined inoculation of all the four microbial inoculants (*B. subtilis* + *B. safensis* + *P. griseoroseum* + *T. harzianum*) the plant height showed an abrupt increase which may be attributed to the synergistic effect on growth promoting action of microbial inoculants. Moreover, the plant height during the last months of the study period of the plant could be due to short growing season of conifers and below freezing soil temperatures.

The release of plant growth substances and enhancement in

Table 10: Impact of microbial inoculants on total dry plant biomass (g) of Himalayan yew (*Taxus wallichiana* Zucc.) at nursery stage during year (2016-2017).

Initial Total Dry Plant Biomass (g)	*Treatments	April	June	August	October	December	February
3.52	Control	^{a}3.58 ± 0.06	^{a}3.62 ± 0.04	^{a}3.65 ± 0.04	^{a}3.67 ± 0.06	^{a}3.68 ± 0.05	^{a}3.68 ± 0.05
	B1	^{c}4.62 ± 0.05	^{c}5.52 ± 0.05	^{c}6.12 ± 0.06	^{c}6.22 ± 0.05	^{c}6.30 ± 0.06	^{c}6.30 ± 0.06
	B2	^{b}4.02 ± 0.06	^{b}4.32 ± 0.04	^{b}4.53 ± 0.05	^{b}4.61 ± 0.05	^{b}4.67 ± 0.05	^{b}4.67 ± 0.05
	F1	^{d}4.82 ± 0.05	^{d}5.82 ± 0.06	^{d}6.42 ± 0.06	^{d}6.62 ± 0.05	^{d}6.64 ± 0.08	^{d}6.64 ± 0.08
	F2	^{e}5.52 ± 0.07	^{e}7.32 ± 0.06	^{e}8.51 ± 0.07	^{e}9.12 ± 0.04	^{e}9.15 ± 0.05	^{e}9.15 ± 0.05
	B1+B2	^{e}5.62 ± 0.07	^{f}7.62 ± 0.07	^{f}9.12 ± 0.05	^{f}9.92 ± 0.06	^{f}9.96 ± 0.06	^{f}9.96 ± 0.06
	F1+F2	^{f}6.72 ± 0.06	^{g}9.72 ± 0.06	^{g}12.33 ± 0.05	^{g}13.32 ± 0.07	^{g}13.37 ± 0.06	^{g}13.37 ± 0.06
	B2+F1+F2	^{h}8.52 ± 0.06	^{i}13.12 ± 0.04	^{i}17.12 ± 0.05	^{i}18.92 ± 0.06	^{i}19.32 ± 0.05	^{i}19.32 ± 0.05
	B1+F1+F2	^{g}7.53 ± 0.06	^{h}11.32 ± 0.04	^{h}14.52 ± 0.07	^{h}16.02 ± 0.07	^{h}16.32 ± 0.06	^{h}16.32 ± 0.06
	B1+B2+F1+F2	^{i}9.52 ± 0.06	^{j}15.02 ± 0.07	^{j}20.02 ± 0.05	^{j}23.02 ± 0.06	^{j}23.52 ± 0.07	^{j}23.52 ± 0.07

Values are represented as mean ± SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. B1=*Bacillus subtilis*; B2= *Bacillus safensis*; F1=*Penicillium griseoroseum*; F2=*Trichoderma harzianum*

availability of nutrients in the root zone of plant by the application of microbial inoculants could be ascribed for the increase in size of collar diameter [16]. Likewise the highest increase in collar diameter of Himalayan yew stem cuttings could be attributed to the fact that the cuttings have got an efficient nutrient transport by the application of microbial inoculants. Further, no increase in collar diameter of Himalayan yew stem cuttings in the winter months is due to low soil temperatures which might have stopped the growth of microbial inoculants. Similar observations have been recorded by other workers on various plants Lee and Koo [17], Kumar and Lakhanpal [18], Arshad and Frenkenberger [19], Tam and Griffiths [20]. The results obtained during the current study uphold the results observed by Daiho and Upadhyay [21] and also by other workers [22-24] on various forest trees.

Since our findings are in line with Refs. [25,26] on *Calotropis procera* and *Dalbergia sisso* connected to the increase of root length by the application of microbial inoculants and has been observed a remarkable increase in the root length of *Pinus sylvestris* and *Robinia pseudoacacia* seedlings following inoculation with mixed rhizobacteria and mycorhizosphere fungus [27]. The capability of microbial inoculants to synthesize biologically active substances and essential micronutrients could be ascribed to increase the root length [28,29]. Further no increase in root length of the plant in later months of the study could be ascribed due to low soil temperatures in the winter months which might have lowered the efficiency of microbial inoculants as well as the already present soil microflora.

The enhancement in the fresh and dry shoot biomass corroborate with the findings of several other workers [30-33]. Increased fresh and dry shoot biomass of Himalayan yew stem cuttings could be due to better nutrient absorption and water uptake by its efficient root system by these microbes [34-36]. Further, no response in fresh and dry shoot biomass in the winter months of the study period could be ascribed to the heavy precipitation and sub-zero temperature of soil which can lessen and stopped the productivity and growth of microbial inoculants along with the natural soil micro flora present there.

The consequence of intensive colonization in rhizosphere by application of microbial inoculants resulted in fresh and dry root biomass improvement which may have promoted the availability of more nutrients in the rhizosphere and the synthesis of growth promoting compounds [37] and secondly, these microbes attributed to the ability to produce growth substances and helps in root proliferation [38], mineral uptake by plants and indirect plant growth stimulation [39]. Moreover, no response in fresh and dry root biomass in winter months of the study period is because of decreased efficiency of microbial inoculants due to low soil temperature.

The enhancement in the total fresh and dry plant biomass corroborates with the results of several other workers [40]. The improvement in total fresh and dry plant biomass is a result of intensive colonization of roots by microbial inoculants which stimulates the synthesis of plant growth promoting substances and more nutrient accessibility in rhizosphere [41]. The increasing trend in total fresh and dry plant biomass in the early months may be ascribed to favorable environmental conditions which might have triggered the growth of microbial inoculants in the rhizosphere and no response in total fresh and dry plant biomass towards winter may be due to adverse climatic conditions.

CONCLUSION

Microbial inoculants isolated and selected from rhizosphere soil of *Taxus wallichiana* Zucc. stands improved the plant growth under nursery conditions. Plant growth characteristics of *T. wallichiana* stem cuttings on the subject of plant height, collar diameter, root length and biomass were highest under the combined treatment B1+B2+F1+F2 followed by B1+F1+F2; B2+F1+F2; F1+F2; B1 +B2; F2; F1; B1 and B2 treatments. Moreover, all the growth characteristics showed an increasing trend up to December and in the month of February no growth was detected so all the growth characteristics remain same. Himalayan yew, being a very slow growing conifer, significantly retaliated to the microbial inoculation and showed maximum growth. With this approach Himalayan yew proved a very promising tree species for afforestation of poor soils, reforestation and wastelands. Altogether, the use of microbial inoculants viz. *B. subtilis*, *B. safensis*, *P. griseoroseum* and *T. harzianum* at nursery stage of Himalayan yew stem cuttings for plant growth characteristics, proved the most efficacious, potent and productive.

DECLARATION OF COMPETING INTERESTS

All authors declare no conflict of interest.

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