Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions

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Submitted to
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2020

To

my beloved Grandparents and
parents for their endless love,
persistent support, constant hope and
encouragement.

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Agriculture, Division of Basic Sciences and Humanities

Certificate - I

This is to certify that the thesis entitled **"Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Microbiology),** to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Ms. Asifa Yousuf Wani (Regd. No. 2017-A-1165-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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We, the members of the Advisory committee of **Ms. Asifa Yousuf Wani (Regd. No. 2017-A-1165-M)**, a candidate for the degree of **Master of Science in Agriculture (Microbiology)**, have gone through the manuscript of the thesis entitled, **"Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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This is to certify that the thesis, **"Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions"** submitted by **Ms. Asifa Yousuf Wani (Regd. No. 2017-A-1165-M)** to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Microbiology)** was examined and approved by the Advisory Committee and external examiner on ………………..

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ABSTRACT

An experiment was carried out during Spring 2018 to study the influence of microbial inoculants on the growth of two important temperate forest coniferous species Himalayan Cypress (*Cupressus torulosa* G. Don) and Deodar (*Cedrus deodara* D. Don) under nursery conditions and to ascertain the impact of microbial inoculants on the plant growth and nutrient concentration (N, P and K) of plants. In addition to this, the experimental trial studied the nutrient status and microbial population of the experimental medium. The experiment was laid in Completely Randomized Design with four replications comprising of ten inoculants viz., *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* and control. Growth characteristics viz., plant height, collar diameter, seedling survival percentage, root length, root-shoot ratio and total fresh and dry biomass production was significantly better in all the microbial inoculants. Maximum plant growth of 19.48 cm and diameter of 4.57 mm in Himalayan Cypress was recorded with the application of VAM + *Azotobacter chroococcum.* In Deodar maximum growth in plant height of 6.87 cm and diameter of 1.11 mm was also 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 100% and 95% in Himalayan Cypress and Deodar respectively was recorded by the application of Zinc Solubilising Bacteria.

Microbial inoculants significantly increased N, P and K concentration in both Himalayan Cypress and Deodar. Maximum NPK of 1.42%, 0.09% and 0.53% in Himalayan Cypress and 1.41%, 0.24% and 0.92% in Deodar was recorded with dual application of VAM + *Azotobacter chroococcum*, VAM + PSB and VAM+ KSB respectively. The nutrient status and microbial population in the growing medium also increased significantly by the application of microbial inoculants. The available N, P, K and Zn of 522.4 kg ha^{-1}, 23.0 kg ha^{-1}, 194.6 kg ha^{-1} and 20.4 kg ha^{-1} was recorded maximum in VAM + *Azotobacter chroococcum,* VAM + PSB, VAM + KSB and VAM + ZnSB respectively in Himalayan Cypress. In Deodar also, maximum N, P, K and Zn of 521.8 kg ha^{-1}, 24.1 kg ha^{-1}, 186.8 kg ha^{-1} and 22.3 kg ha^{-1} was recorded by the application of VAM + *Azotobacter chroococcum,* VAM + PSB, VAM + KSB and VAM + ZnSB respectively. The highest population of total viable bacteria with 158×10^6 and 115.5×10^6 $CFUg^{-1}$ of soil was recorded by the application of VAM + *Azotobacter chroococcum* in Himalayan Cypress and Deodar respectively. The highest population of total viable fungi with 11.0×10^5 $CFUg^{-1}$ of soil was recorded by the application of VAM + Phosphorous Solubilising Bacteria in both the species. With respect to soil pH, the pH of experimental medium significantly decreased by the application of microbial inoculants. VAM + *Azotobacter chroococcum* recorded lowest soil pH of 7.19 and 7.18 in Himalayan Cypress and Deodar respectively.

Key words: *Cedrus deodara*, *Cupressus torulosa*, Microbial inoculation, Total viable bacteria and Total viable fungi.

Signature of Student	Signature of Major Advisor
Dated : ___________	Dated: ________________

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Asifa Yousuf Wani

Place : Wadura, Sopore

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Chapter 1

INTRODUCTION

Living gymnosperms are one of the great antiquity and include about 700-800 species in 70-75 genera. Among gymnosperms, conifers with about 550-600 species in 50 genera form the most dominant group. Conifer forests are typical of Himalaya and provides cool and soothing environment for recreation and health. Forests, verily the green gold of the state of Jammu and Kashmir, are dominated by conifers. These cover considerable proportion of the area of our State and contribute to its most important industries, hence mainstay of economy. Most of the timber used in buildings is derived from conifers because of their straight-grain and ease of manipulation. Deodar is the strongest of the Himalayan coniferous woods. Almost all conifers are trees, so they create forests that provide habitat for wildlife and a wide variety of insects, fungi and smaller plants. Some conifer forests support extremely complex ecosystems with very high levels of biodiversity (Dallimore *et al.*, 1967).

The geographical area of Jammu and Kashmir is 42241 km^2 (16309 sq miles) excluding Ladakh and Kargil. The forest area of Jammu and Kashmir (including Ladakh and Kargil) upto August 2019 was 20230 km^2 accounting for 19.95% of total geographical area (101387 km^2) of these four regions on the side of line of control. According to Forest Survey of India and Released by Ministry of Environment Forestry and Climate Change, Total Forest cover and Tree cover of India is 24.39% of total geographic area. The total forest cover is 21.54% of total geographic area. Due to timber mining, more than 60 per cent of our demarcated forests have been declared as uncommercial/degraded (Anonymous, 2005). Natural regeneration does not practically take place in forests where crown density is less than 40 per cent. Natural succession takes thousands of years in the regeneration of forest species to reach the climax stage especially in temperate regions where species like *Cupressus torulosa* D. Don (Himalayan Cypress), *Abies pindrow* Spach (Silver Fir), *Cedrus deodara* (Roxb.) G. Don (Deodar), *Picea smithiana* Wall. (Spruce) and *Pinus wallichiana* Jackson (Kail) dominates the forest cover. Indiscriminate use of inorganic fertilizers and pesticides being environmentally unsafe and economically unfeasible has pressed a huge demand for the application of microbial inoculants for quality seedling production in nursery also in the establishment of plantation to increase the productivity. Therefore, in order to meet the huge demand and supply of timber, fuel wood and firewood, raising of such forest species supplemented with variety of microbial inoculants on degraded forest patches can be an excellent and viable opportunity for sustenance of the present resources towards better and green future.

Deodar (*Cedrus deodara G.Don*):

Himalayan cedar or Deodar is a species native to the Western Himalayas in Eastern Afghanistan, Northern Pakistan and India. In the country, it is found in the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. It is a large evergreen coniferous tree reaching 40-50m (131-164 ft) tall, exceptionally 60 m (197 ft) with a trunk upto 3 m (10 ft) in diameter. It has a conical crown with level branches and drooping branchlets. *Cedrus deodara* occurs on moderate to precipitous rocky slopes, generally dominating cool Northern slopes where it forms pure crops of considerable extent. It is typically a gregarious tree, a light demander, frequently found in the form of pure stands. It tolerates drought at advanced age, but not at the seedling stage. The trees are also resistant to atmospheric pollution (Farjon, 1990).

Deodar is in great demand as building material because of its durability and rot-resistant character and fine, close grain which is capable of a high polish. Its historical use to construct religious temples and in landscaping around temples is well recorded. Its rot-resistant character also makes it an ideal wood for constructing the well-known houseboats of Srinagar, Jammu and Kashmir (McGowan, 2008).

Himalayan Cypress (*Cupressus torulosa* D. Don)

The Himalayan cypress belonging to family Coniferae is a large evergreen tree with a pyramidal crown and drooping branchlets. Trees upto 47m height and 7.15 m in girth have been measured in a Tehsil Garhwal (Troup, 1921). Bark grayish 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 1, 800-2, 750 m elevations. The tree is naturally found on limestone. In its natural habitat the absolute maximum shade temperature is probably about 32.2^0 C, the absolute minimum about-9.4^0 C and the normal rainfall vary from 1, 000-2, 400 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). Due to limited availability its uses are not explored fully.

The coniferous forests of Kashmir Himalayas serve as an excellent habitat for macro-fungi emanating in different seasons due to wide variability in climate, altitude, slope and type of forests etc. but the study on Kashmir Himalayas regarding diversity of macro-fungi and their ectomycorrhizae have only been started in the recent years and is still in an exploratory or pioneering stage (Watling and Abraham, 1992). A few preliminary studies done in the different forest sites of the region more specifically deal with the symbiosis rather than the function of ectomycorrhizae in the forest ecosystem. Up to late 2009 hardly 250 macrofungal species were reported from the whole of Jammu and Kashmir. The most dominant ectomycorrhizae include Amanita, Russula, Boletus, Lactarius, Suillus and Cortinarius etc. (Dar *et al*., 2009). Endomycorrhizae are developed in 80% of plants mostly deciduous and herbaceous (Agarwal and Sah, 2009 and Moore, 2011).

Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake and support the health of plants (Barea *et al*., 1998; Dobbelaere *et al.,* 2001; Hodge *et al*., 2001; Bonfante, 2003; Vessey, 2003; Kloepper *et al.,* 2004; Han and Lee, 2005; Weller, 2007; Adesemoye *et al.,* 2008). Benefits to plants from plant-PGPR interactions have been shown to include increases in seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root weights, bio-control and delayed senescence (Mahaffee and Kloepper 1994; Raaijmakers *et al.,* 1997; Bashan *et al.,* 2004; Mantelin and Touraine, 2004; Bakker *et al.,* 2007; Yang *et al.,* 2009). Other beneficial effects of PGPR strains include enhancing phosphorus availability (Rodriguez and Fraga, 1999); fixing atmospheric nitrogen (Bashan *et al.,* 2004); sequestering iron for plants by production of siderophores (Raaijmakers *et al*., 1997; Bakker *et al.,* 2007); enhancing biosynthesis of furanone flavor compounds in strawberry (*Fragaria × ananassa*); (Zabetakis, 1997); producing plant hormones (Gutierrez-Manero *et al.,* 2001) such as gibberellins, cytokinins and auxins; and synthesizing the enzyme 1-amino cyclopropane-1-carboxylate (ACC) deaminase, which lowers plant levels of ethylene, thereby reducing environmental stress on plants (Glick *et al.,* 2007). The Arbuscular Mycorrhiza Fungi (AMF) have a high-affinity phosphorous-uptake mechanism that enhances phosphorous nutrition in plants. The Arbuscular Mycorrhiza Fungi (AMF) are able to scavenge the available Phosphorous through their hyphae that have large surface areas on which the extra-radical hyphae act as a bridge between the soil and plant roots (Liu *et al.,* 2000; Bianciotto and Bonfante 2002). Considering the capacity of both PGPR and AMF to help plants in uptake of nutrients, a tripartite interaction of PGPR-plant-AMF is highly promising, especially with the proposition that AMF may act as a vehicle to spread PGPR throughout the rhizosphere (Kim *et al.,* 1998; Bianciotto and Bonfante 2002; Morrissey *et al*., 2004). Many PGPR and AMF have been used separately and as combinations to investigate the impacts on the uptake of individual or multiple elements. Although the applications of the tools to sustainable agriculture are yet to be well understood, advances in genomic technology have provided substantial information in plant-PGPR and/or plant-AMF interactions.

Azotobacter is heterotrophic free living nitrogen-fixing bacteria. They are Gram negative, large ovoid pleomorphic cells of 1.5-2.0 µm or more in diameter ranging from rods to coccoid cells. They occur singly, in paired or irregular clumps and sometime in chains of varying length. They do not produce endospores but form cysts. They are motile by peritrichous flagella or non-motile. *Azotobacter* spp. is most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (Indole-Acetic-Acid and other auxins, such as gibberllins and cytokinins), vitamins and siderophores. Azotobacter is capable of converting nitrogen to ammonia, which in turn is taken up by the plants (Kamil *et al.*, 2008). *Azotobacter* can also produce antifungal compounds to fight against many plant pathogens (Jen-Hshuan, 2006).

Phosphate Solubilizing Bacteria (PSB) are used as bio-fertilizer since 1950s. These microorganisms secrete different types of organic acids e.g., carboxylic acid thus lowering the pH in the rhizosphere and consequently dissociate the bound forms of phosphate like Ca_3 $(PO_4)^2$ in calcareous soils. Efficiency of Phosphorous fertilizer throughout the world is around 10-25% and concentration of bio-available Phosphorous in soil is very low reaching the level of 1.0 mg kg^{-1} soil. Among the whole microbial population in soil, PSB constitute 10-50%, while Phosphorus Solubilizing Fungi (PSF) are only 0.1-0.5% in Phosphorous solubilization potential (Aftab and Asghari, 2008). This group covers bacteria, fungi and some actinomycetes. These organisms solubilize the unavailable forms of inorganic Phosphorous like tricalcium, iron, aluminum and rock phosphates into soluble forms by release of a variety of organic acids like succinic, citric, malic, fumaric, glyoxalic and gluconic acids (Venkateswarlu *et* *al.*, 2007). Phosphorous Solubilising Microorganisms include different groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but they also cause a large portion of soluble phosphates to be released in quantities in excess of their requirements. Species of *Aspergillus* and *Penicillium* are among fungal isolates identified to have phosphate solubilizing capabilities. Among the bacterial genera with this capability are *Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Serratia, Enterobacter, Acinetobacter, Flavo-bacterium* and *Erwinia* (Richa, 2003).

Potassium Solubilizing Bacteria (KSB) such as *Bacillus mucilagenosus* and *Bacillus edaphicus* are example of microorganisms that are used as bio-fertilizer. They are able to solubilize potassium rock through production and secretion of organic acids. KSB is a heterotrophic bacterium which is obtaining all their energy and cellular carbon from preexisting organic material. Besides, KSB are aerobic bacteria which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates. In addition, this gram positive bacterium can produce substance that stimulate plant growth or inhibit root pathogens (Bin-Zakaria, 2009).

The majority of plants growing under natural conditions are associated with mycorrhizae. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Wua *et al.,* 2004). Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots (Contra, 2003). Different types of mycorrhizae occur, distinguished by their morphology and to a certain extent, in their physiology. These include ectomycorrhizae (EM) and endomycorrhizae (AM) (Turk *et al.,* 2006). Ectomycorrhizal fungi like *Pisolithus, Laccaria, Amanita, Scleroderma, Russula, Tricholoma,* etc., form ectomycorrhizal associations with the tree species belonging to the family Pinaceae, Betulaceae and Fagaceae. These fungi increase the surface area of absorption of the roots and thus, help in absorption of nutrients, especially those less mobile in the soil solution like phosphorous. They also help in the uptake of water and protect roots from root pathogens. These fungi are culturable, hence mass produced and used as carrier-based inoculants for inoculating forest nurseries (Bagyaraj and Padmavati, 1993). Vesicular Arbuscular Mycorrhiza (VAM) fungi colonize roots of several crop plants, important in agriculture, horticulture and tropical forestry. They are Zygomycetous fungi belonging to the genera *Glomus, Gigaspora, Acaulospora, Sclerocystis*, etc. These are obligate symbionts and cannot be cultured on laboratory media. They help plant growth through improved phosphorous nutrition and protect the roots against pathogens. They are multiplied as pot cultures, using a suitable host and the root pieces along with the substrate as inoculum. They are currently recommended for use in transplanted and nursery raised crops because of the difficulty in inoculum production. Several studies have shown that they improve seedling growth and vigour of many plants important in Agriculture like tobacco, finger millet, chillies, etc., horticulture like tomato, citrus, cardamom, mango, etc. and forestry like *Leucaena, Dalbergia, Acacia, Casuarina, Tectona* etc. (Bagyaraj, 1992).

Mycorrhizal inoculants significantly improve the quality of seedling production in nursery and also the establishment of plantation to increase the forest productivity. Biofertilizers are cost effective, eco-friendly, cheaper and renewable sources of plant nutrients and play a vital role in maintaining long term soil fertility and sustainability. Mycorrhizae reportedly aid young seedlings in their early survival and establishment through intricate and complex system of hyphal networks thereby not only ensures the sustainable supply but also provides protection against invading pathogens. 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 have become inevitable. Although various aspects of biofertilizers impact on different vegetable crops and forest crops in different ecosystems have been studied, but only a little work has been done on the impact of other microbial inoculants on growth attributes of Deodar and Himalayan cypress under nursery conditions.

Importance of biofertilizers

Biofertilizers are widely accepted as low cost supplement to chemical fertilizers and have no deleterious effect either on soil health or environment. Biofertilizers, which can supply nitrogen and phosphorous, two major plant nutrients, have been developed. Nitrogen abundantly present in the atmosphere is fixed by rhizobia living in symbiosis with legumes. Free-living nitrogen fixing bacteria like *Azotobacter* not only fixes nitrogen but produces growth promoting substances. Blue green algae (Cyanobacteria) fixing atmospheric nitrogen, is used in rice cultivation. Phosphate solubilizing bacteria, fungi solubilize unavailable forms of phosphorous in soil through the production of organic acids and make it available for plant growth. Mycorrhizal fungi living in symbiotic association with roots of crop plants and forest tree species improve uptake of phosphorous. Biofertilizers obtained after systematic screening are now mass multiplied and used for inoculating plants to improve biomass, yield and soil fertility.

Thus, it can be concluded that biofertilizers are available for supplying the two major plant nutrients, nitrogen and phosphorous. Because of high cost of raw materials required for the production of chemical fertilizers, their demand in higher quantities to meet the requirement of growing world population and counter the hazards on human and soil health, biofertilizers may serve as the key to sustain agricultural productivity. Biofertilizers on application remain in soil, multiply and benefit crops. They do not get depleted as chemical fertilizers. They are cost effective, environment friendly and improve soil health. Their effect on plant growth may not be spectacular as that of chemical fertilizers, being biological material. In developing countries, they are a boon to farmers (Bagyaraj and Indira, 1994).

The present study entitled “Influence of Microbial Inoculation on Growth of Deodar and Himalayan Cypress under Nursery Conditions” was endeavored to meet the following objectives:

- To study the effects of microbial inoculants on growth of Deodar and Himalayan cypress.
- To assess the concentration of nutrients in Deodar and Himalayan cypress seedlings as influenced by microbial inoculants.
- To ascertain the nutrient status and microbial population of the experimental medium as influenced by the application of microbial inoculants.

Chapter 2

REVIEW OF LITERATURE

In this chapter all the available and relevant literature pertaining to the "Influence of Microbial Inoculations on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions" has been reviewed. The effect of microbial inoculants as bio-fertilizers (*Azotobacter*, Phosphorus Solubilising Bacteria (PSB), Potassium Solubilising Bacteria (KSB), Zinc Solubilising Bacteria (ZnSB) and Vesicular Arbuscular Mycorrhiza (VAM) on different parameters of Deodar and Himalayan Cypress species as well as other related coniferous trees have been discussed under following headings:

2.1 Effect of microbial inoculants on growth characteristics as influenced by the application of microbial inoculants.

2.2 Assessment of the nutrient concentrations as influenced by application of microbial inoculants.

2.3 Nutrient status and microbial population of experimental medium as influenced by the application of microbial inoculants.

2.1 Effect of microbial inoculants on growth characteristics as influenced by the application of microbial inoculants

Arshad *et al.* (2015) experimented using various strains of Zinc solubilising bacteria for improving growth of maize. Several bacteria were isolated from rhizosphere of maize through dilution plate technique. The selected bacterial isolates, capable of solubilizing ZnO, were further screened for their plant growth promoting activity under axenic conditions. Out of ten bacterial isolates, AZ6 was found best strain on the basis of maximum zinc solubilization potential and growth promotion of maize. The selected bacterium was identified as *Bacillus* sp. AZ6 (Accession # KT221633). *Bacillus* sp. AZ6 had different growth promoting attributes and also ability to produce organic acids.

Goteti *et al.* *(*2013) through their experiment using ten strains of Zinc solubilising bacteria five each of *Pseudomonas* spp. designated as P17, P21, P29, P33 and P74 and *Bacillus* spp. as B40, B61, B114, B116 and B118 studied that seed bacterization with zinc solubilizing plant growth promoting bacteria resulted in increased plant height (root volume and shoot height), leaf area and dry mass.

Malik *et al.* (2012) conducted an experiment which comprised forty-two treatment combinations 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, relative growth rate, fresh and dry biomass production 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 and root biomass, *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 both the species.

Gajbhiye *et al.* *(*2011) found that among the two *Azotobacter* species, Natrin application on bell pepper cv. “California Wonder” shows better result. He observed better results in case of plant height, days to first picking and nitrogen uptake in shoots at flowering stage. Maximum plant height (57.44 cm) and minimum days to first picking (87.27) was statistically significant over control (no biofertilizers).

Dar *et al.* (2009) studied the response of deodar seedlings to inoculations with ecto-mycorrhizae (*Pisolithus tinctorius, Laccaria laccata* and *Suillus granulatus*), forest litter and different levels of phosphorous and reported that various plant growth characteristics (plant height, root length, collar diameter and plant biomass) and ectomycorrhizal root colonization responded significantly to ectomycorrhizal inoculation, forest litter and phosphorous. Among the microbial inoculants *Pisolithus tinctorius* proved beneficial for all the studied parameters, followed by *Laccaria laccata* and *Suillus granulatus.* The treatment combination of *Pisolithus tinctorius* along with phosphorous dose of 75 mg per plant proved to be the best treatment for all the studied characters.

Mahato *et al.* (2009) evaluated the response of bio-fertilizer and inorganic fertilizer on germination and growth of tomato plant. They revealed that *Azotobacter* as bio-fertilizer reported better than inorganic fertilizer in relation to seed germination and all plant growth parameters. In the study application of bio-fertilizer resulted in the increase of shoot length and more number of leaves per plant.

Archana *et al.* (2008) conducted an experiment to study the effect of Potassium Solubilising Bacteria (KSB) on growth and yield of maize. Efficient Potassium Solubilizing Bacteria *Bacillus* spp. was used and the result showed that there was a further increase in growth and yield of maize under treatment than the untreated maize plants.

Menkis *et al. (*2007) studied the inoculation of conifer seedling with three ectomycorrhizal fungi. They investigated whether inoculation of *Pinus sylvestris* L. and *Picea abies* Karst. seedlings with mycorrhizas of *Cenococcum geophilum* Fr., *Piceirhiza bicolorata* and *Hebeloma crustuliniforme* has any impact on; 1) Survival and growth of many out planted seedlings on abandoned agricultural land and 2) subsequent mycorrhizal community development. The results showed limited ability to increase tree survival and growth and to manipulate the mycorrhizal community even by extensive pre-inoculants, indicating that fungal community formation in root systems is governed mainly by environmental factors.

Sharma and Thakur (2001) conducted an experiment with three levels of *Azotobacter* (no *Azotobacter, Azotobacter* M4 strain and *Azotobacter* Natrin a commercial form of *Azotobacter*) on tomato cv. “Yashwant”. They reported that individual application of Natrin (*Azotobacter*) resulted in improvement in plant height (213.5 cm) and number of branches (7.6 and 9.2) during the respective year of study i.e. 1995 and 1997.

Mukherjee and Rai (2000) conducted a field experiment to study the effect of Vesicular Arbuscular Mycorrhizas (VAM) and Phosphate-Solubilizing Bacteria (PSB) on the wheat (*Triticum aestivum* cv. Kundan) and chickpea (*Cicer arietinum* cv. Pusa 372) growth, yield and phosphorus uptake. Main plot treatments comprised: 0 and 60 kg P/ha; and subplot treatments comprised: inoculation with *Glomus fasciculatum*, *Glomus macrocarpum*, *Pseudomonas striata* or no inoculation (control). Application of phosphorus exhibited perceptible influence on yield of the crops. Biofertilizer and phosphorus nutrition interaction showed significant influence on growth and phosphorus uptake by wheat and chickpea compared with either of the components applied separately. Among the biofertilizers, *Pseudomonas* s*triata* registered the highest grain yield in both crops at both levels of phosphorus, closely followed by *Glomus fasciculatum.* Among the VAM fungi, *Glomus fasciculatum* had positive effects on yield and phosphorus uptake, unlike *Glomus macrocarpum.*

Paroha *et al*. (2000) experimented that when one month old seedlings of *Bambusa arundinacea*, *Gmelina arborea, Tectona grandis and Dalbergia sissoo* in the nursery were inoculated with 20 g VAM (200 chlamydospores of *Glomus intraradices, Glomus mosseae* and *Acaulospora scrobiculata*, along with 10 g infected roots of the trap species *Panicum maximum*) and/or with 10 g of culture of *Azotobacter chroococcum* (10 cells/g culture), the inoculated seedlings exhibited improved growth and biomass. In all four species root length development was maximum either with VAM or with VAM + *Azotobacter, Tectona grandis* and *Gmelina arborea* doubled their biomass yield just by mycorrhization.

Dixon *et al*. (1997) conducted an experiment to study Vesicular Arbuscular mycorrhiza fungal inoculation of *Prosopis juliflora* seedlings using *Glomus macrocarpum* in the nursery that significantly increased the juvenile growth response over three months. The results showed that the three month old nursery raised mycorrhizal seedlings gave enhanced establishment (survival and growth) 3 months after outplanting (i.e., at 6 months old) in 30 cubic cm pits in nutrient deficient, alkaline soil in Asola Wildlife Sanctuary, a semi-arid area.

2.2 Assessment of the nutrient concentrations in plants as influenced by the application of microbial inoculants

Girmay (2019) reported that application of Phosphate Solubilising Microorganisms (PSMs) by inoculating in soil appears to be an efficient way to convert the in soluble Phosphorous compounds to plant-available Phosphorous form, resulting in better plant growth, crop yield and quality. *Bacillus, Pseudomonas, Rhizobium, Aspergillus, Penicillium* and Arbuscular Mycorrhizal Fungi are the most efficient Phosphorous solubilizers for increasing bioavailability of Phosphorous in soil. Phosphorous Solubilising Microorganism provokes immediate plant growth by providing easily absorbable Phosphorous form and production of plant growth hormones such as Indole Acetic Acid and Giberrilic Acid.

Zeb *et al. (*2018) conducted a series of experiments to study the efficacy of Zn-enriched composts (Zn-EC's) with Zinc Solubilising Bacteria (ZnSB). The results concluded that ZnSB improves the grain quality and Zn-bio-accumulation in rice.

Goteti *et al. (*2013) through their experiment using 10 strains of Zinc solubilising bacteria five each of *Pseudomonas* spp. designated as P17, P21, P29, P33 and P74 and *Bacillus* spp. as B40, B61, B114, B116 and B118 showed that the significant concentration of N (2.268%) and K(2.0%) was observed in P29. The highest P (0.28%) concentration was noted in B40 treated plants as compared to other treatments. P29 significantly enhanced the Zinc content (278.8 ppm) in plant tissue.

Miransari (2013) studied that the soil fungi Arbuscular Mycorrhiza (AM) were able to establish a mutual symbiosis with their host plant in which the necessary carbon for the use of fungi is exchanged with water and nutrients. He demonstrated that for the initiation of the symbiotic association, there must be some kind of biochemical dialogue between the fungi and the host plant, which results in morphological and physiological changes in two symbionts. The unique properties of AM fungi make them suitable symbiont for its host plant under different conditions including stress where the uptake of nutrients and water is enhanced by the AM fungi.

Fellbaum *et al.* (2012) evaluated that Arbuscular mycorrhizae (AM) fungi considerably enhance plant phosphorus (P) acquisition by extending hyphae beyond the nutrient depletion zones, but their role in acquiring nitrogen (N)-the primary limiting nutrient in most of the temperate ecosystems - has only been recognized recently.

Afzal and Bano (2011) revealed that dual inoculation of Rhizobium and PSB without fertilizer (P) improved grain yield of wheat upto 20% as compared to sole P fertilizer application in pot experiment.

Smith and Smith (2011) studied that Arbuscular-Mycorrhizal-hyphae very rapidly colonize soil aggregates rich in organic nitrogen and take up both inorganic and organic Nitrogen forms. As most of the AM fungi have limited saprotrophic abilities and inorganic N forms are relatively more mobile in soils where AM plants are dominant, it is therefore believed that these plants primarily utilize inorganic nutrient forms.

Yousefi *et al.* (2011) demonstrated that phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) alone and their combination led to an increase shoot dry matter yield (both Shoot Dry Weight and Root Dry Weight), grain spike number and grain yield of wheat. Highest shoot dry weight and root dry weight recorded were justified in terms of increment of the root and shoot length as well as phosphorus uptake by roots following PSB and AMF application compared over the control.

Hegde and Sudhakara (2009) reported that dual inoculation of *Azospirillum* and *Azotobacter* could be able to substitute up to 50 per cent of the N requirement in sunflower under rainfed conditions.

Raja *et al.* (2009) conducted a field experiment to study the effect of VAM fungi and its interaction with other beneficial microbial inoculants, *Azospirillum spp., Azotobacter spp.* and phosphate solubilizing bacteria on plant biomass, nutrients and biochemical constituents in *Jatropha curcas*. Application of combined microbial inoculants has significantly enhanced the fresh biomass, total soluble protein and phenols as well as relative water content over other treatments and un-inoculated control.

Archana *et al.* (2008) conducted an experiment to evaluate the effect of potassium solubilizing bacteria on growth and yield of maize. Efficient potassium solubilizing bacteria *Bacillus* spp. were used and the result showed that there was a further increase in growth, nutrition including Nitrogen, Potassium, Phosphorous along with various micronutrients. Also the yield of maize was more than the control.

Aseri *et al.* (2008) conducted field experiments and assessed the effectiveness of Plant Growth Promoting Rhizobacteria (*Azatobacter chroococcum* and *Azotobacter brasilence*) and Arbuscular Mycorrhizal Fungi (*Glomus mosseae* and *G. fasciculatum*) on the growth, nutrient uptake and biomass production of pomegranate (*Punica granatu*m L.). Strains were applied individually or in combinations. Results showed that dual inoculation of PGPR and AMF led to higher biomass production and increase in the uptake of N as well as P, K, Ca and Mg in pomegranate seedling.

Gupta and Agarwal (2008) studied the effect of integrated use of farmyard manure and microbial inoculants on wheat productivity for two consecutive years (1999-2000 and 2000-2001). The grain and straw yield of wheat was found significantly enhanced in both the years due to inoculation of *Azotobacter chroococcum* (msx-9), *Azotobacter brasilense* (sp-7) and *Azotobacter lipoferum* (a-5) compared to un-inoculated controls.

Sreeramulu and Srikantaiah (2003) studied the effect of N_2 fixers and Phosphorous solubilizer individually and in combination on growth and yield of popularly grown varieties of banana (Yalakki and Robusta) under field conditions. Among the nitrogen fixers tested, *Azotobacter chroococcum* proved better in improving the yield compared to *Azotobacter diazotrophicus*. However, combined inoculation of *A. chroococcum* and *Bacillus megaterium* var. *phosphaticum* was found best in improving the yield.

Sukumar *et al. (*2003) reported that inoculation of *Bacillus megaterium and Azospirillum* has increased the yield by 1.77 and 1.69 folds respectively in mulberry. They found that dual inoculation is better than individual inoculation. The results has revealed that a synergistic interaction exists between the inoculants, which helped in saving 25 per cent of N and P chemical fertilizers with significant increase in leaf yield and nutrient uptake in mulberry plant.

Muthukumar *et al. (*2001) inoculated neem (*Azadirachta indica* A.Juss) seedlings with Arbuscular Mycorrhizal Fungi (*Glomus intraradices* and *G.geosporum*), *Azospirillum barsilence* and Phosphate Solubilizing Bacteria (PSB) individually or in various combinations in unsterile soil under nursery conditions. Seedlings were harvested at 60 and 120 days of transplanting. Microbial inoculation resulted in increased mycorrhizal colonization, greater plant height, leaf area and number, root collar diameter, biomass, phosphorus, nitrogen and potassium content and seedling quality. Microbial inoculation effects were greatest when seedlings were inoculated in combination of microbes rather than individually. The results emphasize the importance of microbial inoculations for the production of robust, rapidly growing seedlings in nurseries and illustrate the advantages of inoculating soils of low microbial population with indigenous microbes through synergistic effect of various microbes.

Liu *et al. (*2000), Bianciotto and Bonfante (2002) reported that the Arbuscular Mycorrhizal Fungi have a high-affinity phosphorous-uptake mechanism that enhances phosphorous nutrition in plants. The AMF are able to scavenge the available P through their hyphae that have large surface areas on which the extra-radical hyphae act as a bridge between the soil and plant roots.

Maharudrappa *et al*. (2000) reported that plant potassium content in case of Himalayan cypress seedlings could be attributed to its efficient nutrient uptake capacity and rapid mineralization of organic matter and nutrient release.

Kim *et al. (*1998) studied the interaction of Phosphate Solubilizing Bacteria (PSB) and vesicular-arbuscular mycorrhiza (VAM) on plant growth, soil microbial activities and the production of organic acids were studied in non-sterile soil containing hydroxyl-apatite and glucose. Three treatments and a control were used-inoculation with *Enterobacter agglomerans* (treatment E), inoculation with *Glomus etunicatum* (treatment G), inoculation with *Enterobacter agglomerans* and *Glomus etunicatum* (treatment E+G) and the control (C). A significantly higher soluble phosphorus (P) concentration was observed in treatments E and E+G on day 55 compared with C. Total N and P uptake in plants from treatments E and G were higher compared with the control. However, the highest N and P uptake was observed in treatment E+G. This study suggests a synergistic interaction between *Enterobacter agglomerans* and *Glomus etunicatum.*

Nielsen (1990) experimented that when seeds of lucerne were sown in pots containing different levels of P with or without VAM fungi, the dry matter yield increased with higher P levels and at each P level when VAM were present, although this VAM effect decreased with higher fertilizer rates. The N content of the dry matter increased with VAM fungi, but the K content was not affected. VAM increased the uptake of P, Ca, Mg, Cu and Zn, but decreased the dry matter content of Zn.

Sharma *et al*. (1988) experimentally showed that inoculation of potted greenhouse-grown rice plants (cultivars Jaya and Ratna) with *Glomus caledonium* led to increases in shoot and root dry weight and in P and Zn levels. Shoots and roots of mycorrhizal plants contained 2-3 times more Zn than in non-mycorrhizal control plants and suggested that VAM inoculation may prove promising for the management of Khaira disease of rice caused by Zn deficiency.

2.3 Nutrient status and microbial population of experimental medium as influenced by the application of microbial inoculants

2.3.1 Nutrient status of experimental medium as influenced by the application of microbial inoculants

Goteti *et al*. (2013) through their experiment using 10 strains of Zinc solubilising bacteria five each of *Pseudomonas spp*. designated as P17, P21, P29, P33 and P74 and *Bacillus* spp. as B40, B61, B114, B116 and B118. Among 10 strains screened for Zn solubilization P29, P33 and B40 produced 22.0 mm clear haloes on solid medium amended with ZnCO3. Similarly, P17 and B40 showed 31.0 mm zone in Zinc Oxide (ZnO) incorporated medium. P29 and B40 showed significant release of Zn in broth amended with $ZnCO_3$ (17and16.8ppm) and ZnO (18and17 ppm) respectively.

Krizilkaya (2008) studied that seeds of wheat (*Triticum aestivum*) when inoculated with 11 bacterial strains of *Azotobacter chroococcum* had positive effect on the yield and nitrogen concentrations of wheat.

Chen *et al. (*2006) studied 36 bacterial strains, with the capacity to solubilize mineral phosphate, were characterized from Taiwan after screening them with tri-calcium phosphate medium. The principal mechanism for their solubilization capacity was reported as production of organic acids.

Han and Lee (2006) conducted an experiment to evaluate the potential of phosphate solubilising bacteria (PSB) *Bacillus megaterium* var. phosphaticum and potassium solubilising bacteria (KSB) *Bacillus mucilaginosus* inoculated in nutrient limited soil planted with pepper and cucumber. Results showed that rock P and K applied either singly or in combination did not significantly enhance soil availability of P and K, indicating their unsuitability for direct application. PSB was a more potent P-solubilizer than KSB and co-inoculation of PSB and KSB resulted in consistently higher P and K availability than in the control without bacterial inoculum and without rock material fertilizer.

Lozano and Bonfante (1999) investigated the role of *Burkholderia* sp. in Arbuscular Mycorrhizal Fungi phosphorous metabolism and its possible shunting off in Phosphorous transfer from fungus to the plant. *Burkholderia* is an intracellular bacteria present throughout the life cycle of many AMF species of Gigasporaceae. They cloned and characterized an operon for a Pst-like system. The open reading frames in the operon and the protein they encode (PstA, PstB, PstC, PstS and PhoU) were studied. They concluded that *Burkholderia* contains a genomic region similar to the pst operon of *E. coli* in sequence, order and number of genes and has the potential to take up P from the environment and affect P uptake by the AMF host (*Glomus margarita*) and the plant symbiont. With the possession of a DNA region having the nitrogenase-coding genes (nif operon), the intracellular Burkholderia can also affect N uptake.

Harrison and Buuren (1995) conducted an experiment relating to inorganic phosphate (Pi) transporter and its expression in the external hyphae of Arbuscular Mycorrhizal Fungi, which is important in the uptake of Pi and transfer from the Arbuscular Mycorrhizal Fungi to plants.

2.3.2 Microbial population of experimental medium as influenced by the application of microbial inoculants

Awasthi *et al.* *(*2011) reported that application of PSB as inoculation in soil increases the P uptake by plants and thus increasing the yield. PSB plays an important role in enhancing phosphorous availability to plants by lowering the soil pH and by microbial production of organic acids and mineralization of organic P by acid phosphatases.

Raj and Sharma (2009) reported that treatment combination comprising of *Azotobacter chroococcum* and *Glomus vesiculatum* of Arbuscular-Mycorrhizae recorded 64 and 53 per cent higher spore count per 100 g of soil in comparison with untreated plots of apple seedlings. Higher spore count in higher per cent root colonization of 35 and 36.4 per cent during both the years of study.

Sharda and Rodrigues (2009) opined that *Carica papaya* L. is known to exhibit a strong growth response to colonization by Arbuscular Mycorrhizal Fungi; yet it is generally believed that, mycorrhizal growth effects are primarily nutritionally mediated and are inversely related to improved soil fertility, especially available soil P, which affects the fungus symbiotic effectiveness.

Sharma and Sharma (2009) studied spore population per cent root colonization and *Azotobacter* count of citrus orchards. They recorded maximum number of AM fungi from Jach (3947 kg^{-1} of soil), maximum root colonization (10.50%) samples collected from Daad and maximum *Azotobacter* count (4.3 × 106 cfu/100 g soil) from Jhamrari.

Aseri *et al.* *(*2008) showed that the degree of root colonization and sporulation in rhizosphere soils of pomegranate was higher in dual inoculation with *Azotobacter chroococcum* + *Glomus mosseae* as compared to single inoculation treatment.

Sharma and Sharma (2006) recorded maximum number of AM fungi from Jach, whereas minimum were recorded from Jhamari. They also concluded that maximum root colonization (16.2%) was recorded in the samples collected from Nihari and minimum (4.4%) from Kurswan. They have found positive correlation between mycorrhizal spore populations with roots of apple seedling.

Mahmood *et al.* (2001); Tawaraya *et al.* (2006) demonstrated that inoculants, PGPR and AMF, are playing significant roles in the solubilization of inorganic phosphate and mineralization of organic phosphates.

Chapter 3

MATERIAL AND METHOD

The present investigation entitled “Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* G. Don) and Himalayan Cypress (*Cupressus torulosa* D. Don) under nursery conditions” was conducted during spring 2018-19 with the following objectives:

1. To study the effects of microbial inoculants on growth of Deodar and Himalayan Cypress.
2. To assess the concentration of nutrients in Deodar and Himalayan Cypress seedlings as influenced by microbial inoculants.
3. To ascertain the nutrient status and microbial population of the experimental medium as influenced by the application of microbial inoculants.

The details of materials used and methods adopted to achieve the aforementioned objectives during the course of present investigations are summarized in this chapter.

3.1 General description

3.1.1 Location of experimental field

The experiment was conducted under nursery conditions in polybags at Faculty of Agriculture (FOA) & Regional Research Station, Wadura Sopore. The research station is located in the North of Kashmir about 15 kms from Sopore town. The location is 1, 524 metres above mean sea level and situated at 34.28° N of latitude and 74.55° E longitude. The climate of Wadura, Sopore is temperate with moderately hot summers and very cold winters. The mean maximum and minimum temperature of the study site varied from-4.87 to 30.74 ^{0} C with January and August as coldest and hottest months, respectively. The precipitation was about 794.56 mm most of which was received in the form of snow during winter.

The laboratory studies were undertaken in the Division of Basic Sciences and Humanities and Soil Science, SKUAST-Kashmir, Wadura, Sopore.

3.1.2 Climate and weather conditions during crop season

The climate in Kashmir is temperate, characterized by mild summers. The meteorological data pertaining to the period of crop season is given in Appendix-I.

3.1.3 Soil properties of the experimental material

Table 1: Chemical composition of experimental soil before microbial inoculation

Chemical composition	Value	Method of Analysis
Soil pH (1:2.5)	7.53	Glass electrode method (Jackson, 1973)
Available Nitrogen (kg ha^{-1})	156.63	Alkaline permanganate method (Subbiah and Asija, 1956)
Available Phosphorous (kg ha^{-1})	11.35	Olsen's method (Olsen *et al.,* 1954)
Available potassium (kg ha^{-1})	112.51	Neutral Normal Ammonium Acetate (Jackson, 1967)
Available Zinc (kg ha^{-1})	7.08	(Lindsay and Norvell, 1978)

3.2 Experimental details

The details of the experiment with respect to crop variety, treatment, design, plot size, are given below.

3.2.1 Experimental material

The materials used in the present investigation consisted of conifer seeds of Deodar and Himalayan Cypress raised in polybags and different microbial inoculants, that were obtained from Bio-fertilizer Laboratory (Basic Sciences and Humanities), FoA/RSS, Wadura Sopore.

3.2.2 Treatments

The experiment consisted of 10 treatments including single inoculation and also combinations as detailed below:

T_1	:	Control
T_2	:	*Azotobacter chroococcum*
T_3	:	Phosphorous Solubilising Bacteria (PSB)
T_4	:	Potassium Solubilising Bacteria (KSB)
T_5	:	Zinc Solubilising Bacteria (ZnSB)
T_6	:	Vesicular Arbuscular Mycorrhiza (VAM)
T_7	:	VAM + PSB
T_8	:	VAM + KSB
T_9	:	VAM + ZnSB
T_{10}	:	VAM + *Azotobacter chroococcum*

3.3 Technical details

1. Species : **02**

   S_1 = *Cedrus deodara* and **S_2** = *Cupressus torulosa*

2. No. of treatments : 10
3. No. of replications : 04
4. No. of seedling / replicate : 10
5. Design : CRD
6. Season : Spring 2018
7. Date of Sowing : March, 2018
8. Date of Transplanting : May, 2018

3.4 Cultural practices

The details of various operations carried out during the field experiment are as under:

3.4.1 Preparation of plot

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.

3.4.2 Transplanting

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.

3.4.3 After care

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.

3.5 Collection of experimental data

3.5.1 Soil properties

3.5.1.1 pH of soil

The pH of the soil was measured in 1:2.5 soil water suspensions with the help of glass electrode pH meter (Jackson, 1973).

3.5.1.2 Available nitrogen (kg ha^{-1})

Available nitrogen was determined by liberating NH_3. N from soil using alkaline permanganate and absorbing the ammonia liberated by boric acid (4%)

which is collected as a distillate. Titration of this distillate against standard acid (0.2 N H_2SO_4) gives the quantity of ammonia absorbed (Subbiah and Asija, 1956).

3.5.1.3 Available Phosphorous (kg ha^{-1})

Soil phosphorus was extracted by 0.5M sodium bicarbonate ($NaHCO_3$) at pH 8.5 as described by Olsen *et al.* *(*1954).

3.5.1.4 Available Potassium (kg ha^{-1})

The available potassium was extracted with neutral normal ammonium acetate and determined on flame photometer as described by Jackson (1967).

3.5.1.5 Available Zinc (kg ha^{-1})

The available zinc was estimated with DTPA and determined on atomic absorption spectrophotometer (Lindsay and Novell, 1978).

3.5.2 Growth parameters

3.5.2.1 Plant height (cm)

The height of the main shoot was recorded from the ground level to the apex of the leading shoot by using measuring scale.

3.5.2.2 Collar diameter (mm)

The diameter of the seedlings at the collar region was recorded before and after inoculation at the end of one complete growing season using a digital caliper and expressed in millimeters.

3.5.2.3 Seedling survival (%) at the end of growing season

The total number of survived seedlings at the end of growing season was compared with the number of transplanted seedlings and expressed in percentage.

3.5.2.4 Root length (cm)

Seedlings from both the species were randomly selected from every treatment and measurements were recorded. It is expressed in centimeters.

3.5.2.5 Fresh root and shoot biomass (g)

Randomly selected seedlings of both the species from each treatment were taken to the laboratory and weighed using weighing balance.

3.5.2.6 Dry root and shoot biomass (g)

Seedlings of Himalayan cypress and Deodar selected randomly were oven-dried to a constant weight and weighed by a balance.

3.5.2.7 Root shoot ratio

The root length and shoot length of randomly selected seedlings of both the species was measured for calculating root-shoot ratio.

3.5.3 Plant Analysis

Randomly selected seedlings as representative plants were collected from each replication and analyzed for nutrient status.

3.5.3.1 Digestion of plant samples

For the estimation of nitrogen 0.5 g plant sample was digested in 25 ml of sulphuric acid after adding digestion mixture comprising of potassium sulphate and copper sulphate in the ratio of 1:5 and heating the mixture till the $FeSO_4$ changes to crystal green.

For determination of phosphorus and potassium the samples were digested in di-acid mixture comprising of nitric acid and perchloric acid in the ratio 9:4 in a 100 ml conical flask till crystal clear. The digested material was transferred to 50 ml volumetric flask and 6 washings were given to each conical flask to ensure complete transfer of digested material. After making up the volume to 50 ml using double distilled water, the material was filtered through Wattman No.1 filter paper and stored in sterilized bottles for further analysis.

3.5.3.2 Total nitrogen (%)

The nitrogen was estimated by Micro-kjeldahl's distillation method by adding a sufficient amount of 40% NaOH to digested plant material and then titrating against boric acid followed by back titration with 0.1 N HCl as described by Jackson (1973).

3.5.3.3 Total phosphorus (%)

Phosphorus was determined by treating the digested plant extract (5 ml) with Breton's reagent (ammonium metavanadate + ammonium molybdate + nitric acid) in 25 ml volumetric flask. The transmittance after volume make up is determined using spectrophotometer at 470 nm Jackson (1973).

3.5.3.4 Total potassium (%)

Potassium in the extract was determined on flame photometer described by Jackson (1973).

3.5.5 Microbial characteristics of experimental soil

The microbial properties of experimental soil of both the species were determined before and after inoculation using serial dilution pour plate method (Aneja, 1996). The microbial population in soil after harvest of the crop was determined by serial dilution plate count technique. Soil sample in each treatment was collected replication wise. Ten grams soil of each treatment was mixed separately in 100 ml sterilized water blank to give 10^{-1} dilution and subsequent dilutions were made up to 10^{-6} by transferring serially 1 ml of the dilution into 9 ml sterilized water blanks. The population of fungi and bacteria were estimated by transferring 1 ml of appropriate dilutions (10^{-5}) and (10^{-6}) to sterilized Petri-plates and poured with appropriate media (i.e. Potato Dextrose Agar for fungi and Nutrient Agar for bacteria. For total viable bacteria, plates were kept for incubation at 25-30°C ± 2°C and for total viable fungi, plates were incubated at 26 ± 2°C and emerged colonies were counted.

3.6 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.

Chapter 4

EXPERIMENTAL FINDINGS

The experimental findings of the present investigation entitled "Influence of Microbial Inoculation on Growth of Deodar and Himalayan Cypress under Nursery Conditions" carried out at Faculty of Agriculture and Regional Research Station, Wadura, Sopore during spring 2018-2019 are given in this chapter under the following headings as per the objectives of the study:

1. To study the effects of microbial inoculants on growth of Deodar and Himalayan Cypress.
2. To assess the concentration of nutrients in Deodar and Himalayan Cypress seedlings as influenced by microbial inoculants.
3. To ascertain the nutrient status and microbial population of the experimental medium as influenced by the application of microbial inoculants.

The data has been statistically analyzed, presented in the tables wherever necessary.

4.1 Plant growth characteristics

4.1.1 Plant height (cm)

Perusal of the data presented in Table 2 and 3 represent that the application of various microbial inoculants significantly enhanced the plant height of Himalayan Cypress and Deodar compared to control. Among various treatments, VAM + *Azotobacter chroococcum* was found to be significantly superior over all other treatments in respect of plant height in both the species.

The maximum plant height (23.48 cm) amounting to a growth of 19.48 cm in Himalayan Cypress after transplanting in one complete growing season was recorded with VAM + *Azotobacter chroococcum* followed by VAM + Zinc Solubilsing Bacteria (22.02 cm) which amounted to 18.22 cm growth, after application of microbial inoculants. In control growth of only 9.38 cm was recorded during growing season after transplanting.

Maximum growth after transplanting and application of microbial inoculants in Deodar was recorded under VAM + *Azotobacter chroococcum* 6.87 cm followed by 6.60 cm in VAM + ZnSB. Control recorded a growth of only 2.84 cm after transplanting.

Table 2: Impact of microbial inoculation on plant height of Himalayan Cypress

Treatments	**Height at the time of transplanting (cm)**	**Height after one year (cm)**	**Growth in one season (cm)**
Control	4.28	13.67	9.38
Azotobacter chroococcum	4.52	20.65	16.12
Phosphorus Solubilising Bacteria (PSB)	5.11	18.60	13.48
Potassium Solubilising Bacteria (KSB)	4.86	15.35	10.48
Zinc Solubilising Bacteria (ZnSB)	5.16	19.35	14.18
Vesicular Arbuscular Mycorrhiza (VAM)	4.92	21.45	16.52
VAM + PSB	4.96	21.80	16.85
VAM + KSB	4.75	20.14	15.38
VAM +ZnSB	3.82	22.02	18.22
VAM + *Azotobacter chroococcum*	4.02	23.48	19.48
C.D ($p \leq 0.05$)	**0.017**	**0.140**	**0.014**
SE(m)	**0.006**	**0.052**	**0.005**

Plate 1: **Difference in plant heights of Himalayan Cypress seedlings by treatments VAM + *Azotobacter chroococcum* (T$_{10}$), VAM + ZnSB (T$_9$), VAM + PSB (T$_7$) and Control (T$_1$)**

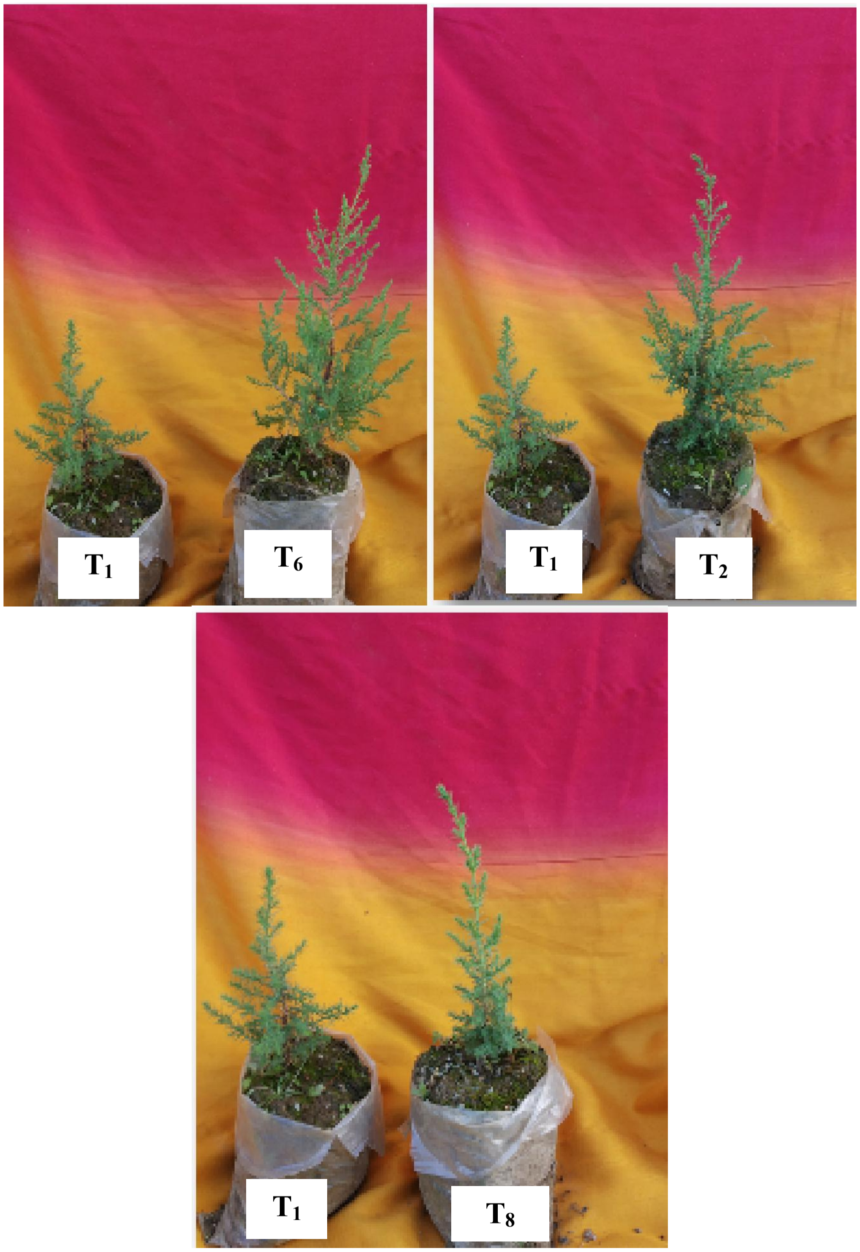


Plate 2: **Difference in plant heights of Himalayan Cypress seedlings by treatments VAM (T_6), *Azotobacter chroococcum* (T_2), VAM + KSB (T_8) and Control (T_1)**

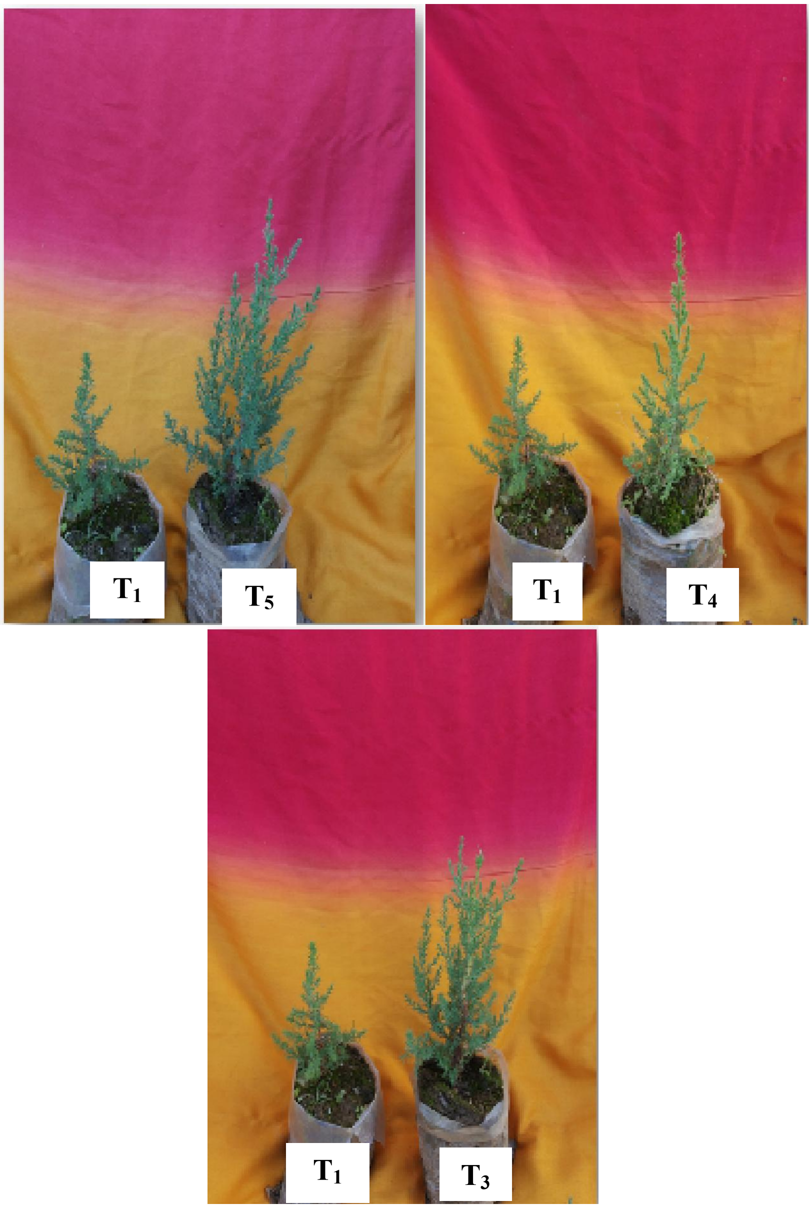


Plate 3: Difference in plant heights of Himalayan Cypress seedlings by treatments ZnSB (T_5), KSB (T_4), PSB (T_3) and Control (T_1)

Plate 4: **Difference in plant heights of Deodar seedlings by treatments VAM + *Azotobacter chroococcum* (T_{10}), VAM + ZnSB (T_9), VAM + PSB (T_7) and control (T_1)**

Plate 5: **Difference in plant heights of Deodar seedlings by treatments VAM (T_6), *Azotobacter chroococcum* (T_2), VAM + KSB (T_8) and Control (T_1)**

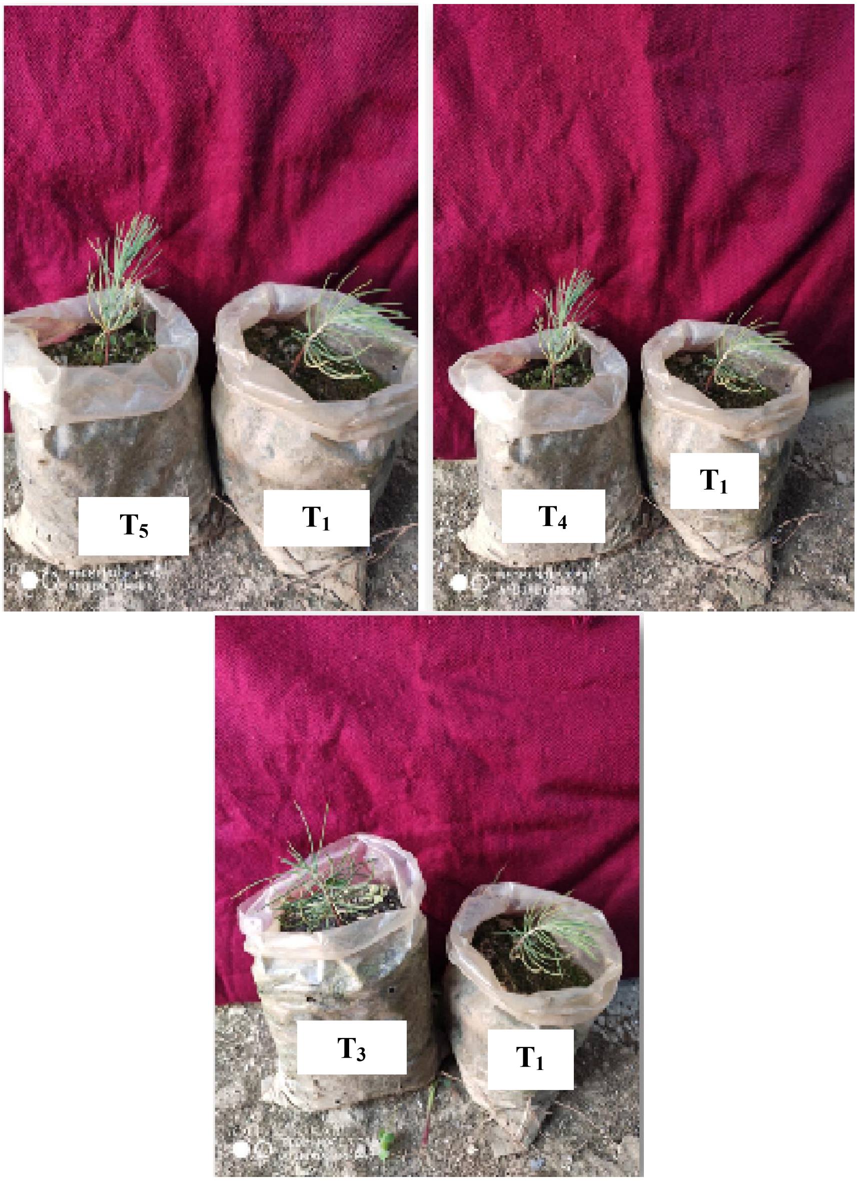


Plate 6: **Difference in plant heights of Deodar seedlings by treatments ZnSB (T_5), KSB (T_4), PSB (T_3) and Control (T_1)**

Table 3: Impact of microbial inoculation on plant height of Deodar

Treatments	Height at the time of transplanting (cm)	Height after one year (cm)	Growth in one season (cm)
Control	2.33	5.22	2.84
Azotobacter chroococcum	2.46	8.77	6.28
Phosphorus Solubilising Bacteria (PSB)	3.04	8.40	5.34
Potassium Solubilising Bacteria (KSB)	2.71	8.17	5.47
Zinc Solubilising Bacteria (ZnSB)	2.26	8.50	6.23
Vesicular Arbuscular Mycorrhiza (VAM)	2.61	8.90	6.30
VAM + PSB	2.81	9.05	6.16
VAM + KSB	2.30	8.57	6.28
VAM +ZnSB	2.96	9.12	6.60
VAM +*Azotobacter chroococcum*	2.89	9.50	6.87
C.D ($p \leq 0.05$)	**0.039**	**0.081**	**0.034**
SE(m)	**0.013**	**0.028**	**0.12**

4.1.2 Collar diameter (mm)

The data presented in Table 4 and 5 reveals a significant increase in the collar diameter of Himalayan Cypress and Deodar seedlings due to application of various microbial inoculants under nursery conditions. Among the microbial inoculants maximum increase in collar diameter after application of microbial inoculants in Himalayan Cypress was 4.57 mm which was exhibited by the application of VAM + *Azotobacter chroococcum* followed by 3.28 mm and 3.27 mm by the application of VAM + ZnSB and *Azotobacter chroococcum* respectively which were statistically at par. In control growth was only 2.67 mm during the same period.

In Deodar seedlings, the maximum increase of 1.11 mm in collar diameter was exhibited by the application of VAM + *Azotobacter chroococcum* followed by the application of VAM + ZnSB that recorded 1.02 mm increase in collar diameter in one complete growing season. In control diameter growth was only 0.49 mm during the same periods.

Table 4: **Impact of microbial inoculation on collar diameter of Himalayan Cypress**

Treatments	**Diameter at the time of transplanting (mm)**	**Diameter after a year (mm)**	**Growth in one season (mm)**
Control	1.01	3.66	2.67
Azotobacter chroococcum	1.00	4.28	3.27
Phosphorus Solubilising Bacteria (PSB)	1.00	4.06	3.05
Potassium Solubilising Bacteria (KSB)	1.30	3.76	2.45
Zinc Solubilising Bacteria (ZnSB)	1.21	4.26	3.05
Vesicular Arbuscular Mycorrhiza (VAM)	1.10	4.34	3.23
VAM + PSB	1.75	4.37	2.61
VAM + KSB	1.30	4.27	2.97
VAM +ZnSB	1.26	4.51	3.28
VAM +*Azotobacter chroococcum*	1.19	5.76	4.57
C.D ($p \leq 0.05$)	**0.018**	**0.080**	**0.016**
SE(m)	**0.006**	**0.028**	**0.005**

Table 5: Impact of microbial inoculation on collar diameter of Deodar

Treatments	**Diameter at the time of transplanting (mm)**	**Diameter after one year (mm)**	**Growth in one season (mm)**
Control	0.36	0.85	0.49
Azotobacter chroococcum	0.31	1.23	0.92
Phosphorus Solubilising Bacteria (PSB)	0.38	1.12	0.74
Potassium Solubilising Bacteria (KSB)	0.41	1.04	0.62
Zinc Solubilising Bacteria (ZnSB)	0.49	1.14	0.64
Vesicular Arbuscular Mycorrhiza (VAM)	0.47	1.31	0.84
VAM + PSB	0.32	1.33	0.98
VAM + KSB	0.45	1.19	0.74
VAM +ZnSB	0.39	1.37	1.02
VAM +*Azotobacter chroococcum*	0.41	**1.52**	**1.11**
C.D ($p \leq 0.05$)	**0.017**	**0.036**	**0.011**
SE(m)	**0.006**	**0.12**	**0.04**

Plate 7: Measuring collar diameter with a digital caliper

4.1.3 Root length (cm)

The data presented in Table 6, reveals a significant increase in root length of Himalayan Cypress seedlings due to application of various microbial inoculants under nursery conditions. Among the inoculants maximum root length was exhibited by VAM + *Azotobacter chroococcum* (45.66 cm) followed VAM + Zinc Solubilising Bacteria (ZnSB) (43.55 cm) and among microbial inoculants minimum root length of 38.91 cm was recorded in KSB while control plot recorded the least root length of 18.82 cm.

Table 6: Impact of microbial inoculation on root length of Himalayan Cypress

Treatments	Root length (cm)
Control	18.82
Azotobacter chroococcum	40.40
Phosphorus Solubilising Bacteria (PSB)	39.70
Potassium Solubilising Bacteria (KSB)	38.91
Zinc Solubilising Bacteria (ZnSB)	39.58
Vesicular Arbuscular Mycorrhiza (VAM)	42.90
VAM + PSB	43.13
VAM + KSB	40.05
VAM +ZnSB	43.55
VAM +*Azotobacter chroococcum*	45.66
C.D ($p \leq 0.05$)	**0.442**
SE(m)	**0.152**

Plate 8: **Root length of Himalayan Cypress seedlings in treatments Control (T_1), *Azotobacter chroococcum* (T_2), PSB (T_3), KSB (T_4) and ZnSB (T_5)**

Plate 9: **Root length of Himalayan Cypress seedlings in treatments VAM (T_6), VAM + PSB (T_7), VAM + KSB (T_8), VAM + ZnSB (T_9) and VAM + *Azotobacter chroococcum* (T_{10})**

The data presented in Table 7, reveals a significant increase in root length of Deodar seedlings also due to application of various microbial inoculants under nursery conditions. Among the inoculants maximum root length was exhibited by VAM + *Azotobacter chroococcum* (12.95 cm) followed VAM + Zinc Solubilising Bacteria (ZnSB) (12.64cm) and minimum root length of 9.95 cm was recorded by the application of KSB while lowest root length of 6.40 cm was observed in control plot.

Table 7: Impact of microbial inoculation on root length of Deodar

Treatments	Root length (cm)
Control	6.40
Azotobacter chroococcum	11.35
Phosphorus Solubilising Bacteria (PSB)	10.65
Potassium Solubilising Bacteria (KSB)	9.95
Zinc Solubilising Bacteria (ZnSB)	10.80
Vesicular Arbuscular Mycorrhiza (VAM)	11.8s7
VAM + PSB	12.53
VAM + KSB	11.26
VAM +ZnSB	12.64
VAM +*Azotobacter chroococcum*	12.95
C.D ($p \leq 0.05$)	**0.128**
SE(m)	**0.044**

Plate 10: **Root length of Deodar seedlings in treatments Control (T_1), *Azotobacter chroococcum* (T_2), PSB (T_3), KSB (T_4) and ZnSB (T_5)**

T_6 T_7 T_8 T_9 T_{10}

Plate 11: Root length of Deodar seedlings in in treatments VAM (T_6), VAM + PSB (T_7), VAM + KSB (T_8), VAM + ZnSB (T_9) and VAM + *Azotobacter chroococcum* (T_{10})

4.1.4 Seedling survival (%) at the end of growing season

The data presented in Table 8, reveals a significant increase in seedlings survival after one growing season due to application of various microbial inoculants under nursery conditions. Among various treatments, ZnSB exhibited maximum survival of 100% and 95.0% in Himalayan Cypress *(Cupressus torulosa)* and Deodar *(Cedrus deodar)* respectively. Minimum percentage of survived seedlings recorded 88.80% by the application of VAM which was significantly at par with VAM + PSB in Himalayan Cypress seedlings. In Deodar seedlings minimum percentage of survived seedlings recorded 88.20% and 88.80% by the application of *Azotobacter chroococcum* and VAM respectively which were significantly at par. While control plot recorded only 80.0% and 78.50% of survived seedlings in Himalayan Cypress and Deodar respectively.

Table 8: Impact of microbial inoculation on seedling survival percentage

Treatments	**Survival %age of *Cupressus torulosa***	**Survival %age of *Cedrus deodar***
Control	80.00	78.50
Azotobacter chroococcum	94.40	88.20
PSB	91.60	94.10
KSB	97.20	94.50
ZnSB	100.00	95.00
VAM	88.80	88.80
VAM + PSB	88.80	94.40
VAM + KSB	86.10	93.25
VAM + ZnSB	92.30	93.40
VAM + *Azotobacter chroococcum*	90.00	89.52
CD ($p \leq 0.05$)	**0.335**	**0.236**
SE(m)	**0.115**	**0.081**

4.1.5 Total fresh (root and shoot) biomass (g)

Root and shoot biomass was estimated at the end of one complete growing season. The data on fresh total (root and shoot) biomass of Himalayan Cypress seedlings in response to different treatments is presented in Table 9.

The treatments showed significant variations in fresh total biomass. It was observed that VAM + *Azotobacter chroococcum* recorded the highest fresh total biomass of 9.54 g followed by VAM + Zinc solubilising Bacteria (ZnSB) (8.97g), VAM + PSB recorded 7.75g, VAM recorded 7.63g, *Azotobacter chroococcum* showed 7.56g, VAM + KSB as 7.50g, ZnSB with 7.12g, PSB with 6.51g and in KSB treated seedlings fresh total biomass was recorded low as 5.25 g while the lowest (2.53g) was recorded in control plot.

Table 9: Impact of microbial inoculation on total fresh (root and shoot) biomass of Himalayan Cypress

Treatments	Total fresh (root and shoot) biomass (g)
Control	2.53
Azotobacter chroococcum	7.56
Phosphorus Solubilising Bacteria (PSB)	6.51
Potassium Solubilising Bacteria (KSB)	5.25
Zinc Solubilising Bacteria (ZnSB)	7.12
Vesicular Arbuscular Mycorrhiza (VAM)	7.63
VAM + PSB	7.75
VAM + KSB	7.50
VAM +ZnSB	8.97
VAM +*Azotobacter chroococcum*	9.54
C.D ($p \leq 0.05$)	**0.073**
SE(m)	**0.025**

From the data presented in Table 10 regarding Deodar, the treatments showed similar response as that of Himalayan cypress and varied significantly in total fresh (root and shoot) biomass. VAM + *Azotobacter chroococcum* had the highest fresh total biomass of 1.53 g, followed by VAM + Zinc solubilising Bacteria (ZnSB) that recorded 1.41g of fresh total biomass. Control recorded only 0.31 g of fresh total biomass.

Table 10: Impact of microbial inoculation on total fresh (root and shoot) biomass of Deodar

Treatments	Total fresh (root and shoot) biomass (g)
Control	0.31
Azotobacter chroococcum	0.97
Phosphorus Solubilising Bacteria (PSB)	0.74
Potassium Solubilising Bacteria (KSB)	0.71
Zinc Solubilising Bacteria (ZnSB)	0.92
Vesicular Arbuscular Mycorrhiza (VAM)	1.13
VAM + PSB	1.36
VAM + KSB	1.08
VAM +ZnSB	1.41
VAM +*Azotobacter chroococcum*	1.53
C.D ($p \leq 0.05$)	**0.027**
SE(m)	**0.009**

4.1.6 Total dry (root and shoot) biomass (g)

It was recorded at the end of one complete growing season. The data on total dry (root and shoot) biomass of oven-dried Himalayan Cypress seedlings in response to different treatments is presented in Table 11.

The treatments showed significant variations in total dry (root and shoot) biomass. It was observed that VAM + *Azotobacter chroococcum* had the highest total dry biomass of 5.29 g, followed by VAM + Zinc solubilising Bacteria (ZnSB) of 4.67 g, VAM + PSB recorded 4.01g, VAM only recorded dry total biomass of 3.88g, 3.80g was recorded in *Azotobacter chroococcum* treatments, VAM + KSB recorded 3.65g, ZnSB recorded 3.61g, PSB recorded 3.45g and in KSB total dry biomass was recorded as 2.35g while the lowest of 1.24 g was recorded in control plot.

Table 11: Impact of microbial inoculation on total dry (root and shoot) biomass of Himalayan cypress

Treatments	total dry (root and shoot) biomass (g)
Control	1.24
Azotobacter chroococcum	3.80
Phosphorus Solubilising Bacteria (PSB)	3.45
Potassium Solubilising Bacteria (KSB)	2.35
Zinc Solubilising Bacteria (ZnSB)	3.61
Vesicular Arbuscular Mycorrhiza (VAM)	3.88
VAM + PSB	4.01
VAM + KSB	3.65
VAM +ZnSB	4.67
VAM +*Azotobacter chroococcum*	5.29
C.D ($p \leq 0.05$)	**0.024**
SE(m)	**0.008**

Table 12 regarding impact on Deodar showed similar response as that of Himalayan cypress and varied significantly in total dry (root and shoot) biomass. It was observed that VAM + *Azotobacter chroococcum* recorded the highest total dry biomass of 0.79 g, followed by VAM + Zinc solubilising Bacteria (ZnSB) of 0.73 g, VAM + PSB recorded 0.67g, VAM treated seedlings recorded dry total biomass as 0.58g, VAM + KSB recorded 0.52 g, ZnSB recorded 0.48 g, PSB recorded 0.49 g and in KSB total dry biomass was recorded as 0.42 g while the lowest value of 0.17 g was recorded in control plot.

Table 12: Impact of microbial inoculation total dry (root and shoot) biomass of Deodar

Treatments	total dry (root and shoot) biomass (g)
Control	0.17
Azotobacter chroococcum	0.49
Phosphorus Solubilising Bacteria (PSB)	0.42
Potassium Solubilising Bacteria (KSB)	0.35
Zinc Solubilising Bacteria (ZnSB)	0.48
Vesicular Arbuscular Mycorrhiza (VAM)	0.58
VAM + PSB	0.67
VAM + KSB	0.52
VAM +ZnSB	0.73
VAM +*Azotobacter chroococcum*	0.79
C.D ($p \leq 0.05$)	**0.019**
SE(m)	**0.007**

4.1.7 Root-shoot ratio

Root-shoot ratio was estimated at the end of one complete growing season. The data on root-shoot ratio of Himalayan Cypress and Deodar seedlings in response to different treatments was estimated by measuring root and shoot lengths of seedlings for both the species.

The data presented in Table 13, reveals that the treatments differed significantly in their response towards root-shoot ratio of Himalayan Cypress seedlings. It was observed that VAM + *Azotobacter chroococcum* recorded highest root-shoot ratio of 2.53 followed by VAM + Zinc solubilising Bacteria (ZnSB) with 2.13, VAM recorded 2.04, VAM+ PSB (2.02), VAM + KSB (1.98), *Azotobacter chroococcum* (1.91), ZnSB (1.85), PSB (1.73), KSB (1.64) while the lowest value of root-shoot of 1.37 was found in control plot.

Table 13: Impact of microbial inoculation on root-shoot ratio of Himalayan Cypress

Treatments	Root-shoot ratio
Control	1.37
Azotobacter chroococcum	1.91
Phosphorus Solubilising Bacteria (PSB)	1.73
Potassium Solubilising Bacteria (KSB)	1.64
Zinc Solubilising Bacteria (ZnSB)	1.85
Vesicular Arbuscular Mycorrhiza (VAM)	2.04
VAM + PSB	2.02
VAM + KSB	1.98
VAM +ZnSB	2.13
VAM +*Azotobacter chroococcum*	2.53
C.D ($p \leq 0.05$)	**0.023**
SE(m)	**0.008**

The data presented in Table 14, reveals that the treatments showed significant variations in root-shoot ratio. It was observed that VAM + *Azotobacter chroococcum* recorded root-shoot ratio of 1.40 followed by 1.38 in VAM + Zinc Solubilising Bacteria (ZnSB), 1.36 in VAM + Phosphorous solubilising Bacteria (PSB), 1.33 in VAM. VAM + KSB recorded 1.31, *Azotobacter chroococcum* recorded 1.29. Lowest root-shoot ratio of 1.19 was recorded in control.

Table 14: Impact of microbial inoculation on root-shoot ratio of Deodar

Treatments	Root-shoot ratio
Control	1.19
Azotobacter chroococcum	1.29
Phosphorus Solubilising Bacteria (PSB)	1.26
Potassium Solubilising Bacteria (KSB)	1.24
Zinc Solubilising Bacteria (ZnSB)	1.27
Vesicular Arbuscular Mycorrhiza (VAM)	1.33
VAM + PSB	1.36
VAM + KSB	1.31
VAM +ZnSB	1.38
VAM +*Azotobacter chroococcum*	1.40
C.D ($p \leq 0.05$)	**0.005**
SE(m)	**0.002**

4.2 Plant analysis

4.2.1 Total nitrogen (%)

The data presented in Table 15, depicts that total nitrogen in the plant extract of Himalayan Cypress seedlings with respect to microbial inoculation of VAM + *Azotobacter chroococcum* exhibited a significant increase over control and other treatments. At the end of one complete growing season, total nitrogen in Himalayan Cypress seedlings was observed in VAM + *Azotobacter chroococcum* (1.42%), followed by VAM +ZnSB (1.38%) while as KSB recorded the least amount of total nitrogen of 1.10% among microbial treatments though significant over the control (1.03%).

The data presented in Table 16, shows a similar response in Deodar seedlings, with respect to microbial inoculation. VAM + *Azotobacter chroococcum* exhibited a significant increase over control and other treatments. At the end of one complete growing season, total nitrogen in Deodar seedlings was observed in VAM + *Azotobacter chroococcum* (1.41%), followed by 1.38% in VAM +ZnSB where as KSB showed the least amount of total nitrogen (1.08%) among microbial treatments. Control recorded 1.05% of plant nitrogen.

4.2.2 Total phosphorous (%)

The data presented in Table 15, reveals that in Himalayan Cypress seedlings, total phosphorous with respect to microbial inoculation of VAM + PSB exhibited a significant increase over control and other treatments. After one complete growing season, the highest level of total phosphorous was observed in VAM + PSB as 0.09%, followed by 0.08% in VAM + KSB which was significantly at par with VAM with 0.08% of plant phosphorous. Also, treatments VAM + *Azotobacter chroococcum* and VAM + ZnSB were significantly at par with 0.07% of total phosphorous. In control it was only 0.01%.

The data presented in Table 16 for Deodar reveals a similar trend where total phosphorous with respect to microbial inoculation of VAM + PSB exhibited a significant increase over control and other treatments. After one complete growing season, the highest level of total phosphorous of 0.24%was observed in VAM + PSB followed by 0.18% in VAM + KSB and control recorded 0.02% of total phosphorous.

4.2.3 Total potassium (%)

The data presented in Table 15, shows that total potassium in the plant extract of Himalayan Cypress seedlings with respect to microbial inoculation of VAM + Potassium Solubilizing Bacteria (KSB) exhibited a significant increase over control and other treatments. At the end of growing season, the highest level of total potassium was observed in VAM + Potassium Solubilizing Bacteria (KSB) (0.53%), followed by 0.47% in VAM + *Azotobacter chroococ*cum, 0.43% in VAM + ZnSB, 0.41% in VAM + PSB, 0.38% in KSB, 0.35% in VAM while lowest was recorded as 0.25% in control plot.

With respect to the Deodar seedlings, the data presented in Table 16 reveals that the highest level of total potassium (0.92%) was observed in VAM + Potassium Solubilizing Bacteria (KSB), followed by 0.82 % as in VAM + ZnSB. Control recorded 0.47% of total potassium.

Table 15: Impact of microbial inoculation on nutrient concentration of Himalayan Cypress

Treatment	Total Nitrogen (%)	Total Phosphorous (%)	Total Potassium (%)
Control	1.03	0.01	0.25
Azotobacter chroococcum	1.36	0.04	0.33
Phosphorus Solubilising Bacteria (PSB)	1.15	0.06	0.29
Potassium Solubilising Bacteria (KSB)	1.10	0.04	0.38
Zinc Solubilising Bacteria (ZnSB)	1.19	0.03	0.30
Vesicular Arbuscular Mycorrhiza (VAM)	1.20	0.08	0.35
VAM + PSB	1.25	0.09	0.41
VAM + KSB	1.20	0.08	0.53
VAM +ZnSB	1.38	0.07	0.43
VAM +*Azotobacter chroococcum*	1.42	0.07	0.47
C.D ($p \leq 0.05$)	**0.063**	**0.008**	**0.016**
SE(m)	**0.022**	**0.003**	**0.006**

Table 16: Impact of microbial inoculation on nutrient concentration of Deodar

Treatment	Total Nitrogen (%)	Total Phosphorous (%)	Total Potassium (%)
Control	1.05	0.02	0.47
Azotobacter chroococcum	1.28	0.09	0.58
Phosphorus Solubilising Bacteria (PSB)	1.11	0.12	0.53
Potassium Solubilising Bacteria (KSB)	1.08	0.12	0.65
Zinc Solubilising Bacteria (ZnSB)	1.12	0.08	0.55
Vesicular Arbuscular Mycorrhiza (VAM)	1.15	0.16	0.61
VAM + PSB	1.25	0.24	0.68
VAM + KSB	1.21	0.18	0.92
VAM +ZnSB	1.38	0.13	0.73
VAM +*Azotobacter chroococcum*	1.41	0.14	0.82
C.D ($p \leq 0.05$)	**0.099**	**0.019**	**0.132**
SE(m)	**0.034**	**0.006**	**0.046**

4.3 Soil properties

4.3.1 Soil pH

The initial and final pH of experimental soil was tested in order to see the level of the acidity and alkalinity. The initial soil pH test was done before transplanting of the seedlings. The initial pH of soil was 7.53 to be considered as neutral as shown in Table 1(in previous chapter).

The data on effect of microbial inoculants on soil pH in Himalayan Cypress is presented in Table 17, pH differed significantly an d maximum pH 7.49 was recorded in VAM + PSB which is significantly at par with VAM that recorded pH of 7.48 while as minimum pH of 7.19 was recorded in VAM + *Azotobacter chroococcum* treatments. Control showed pH of 7.51.

The data in Table 18 regarding Deodar recorded maximum pH of 7.48 in VAM + PSB treatment while minimum pH of 7.18 was recorded in VAM + *Azotobacter chroococcum* and 7.53 was recorded in control.

Table 17: Impact of microbial inoculation on pH of soil under Himalayan Cypress

Treatment	Soil pH
Control	7.51
Azotobacter chroococcum	7.40
Phosphorus Solubilising Bacteria (PSB)	7.45
Potassium Solubilising Bacteria (KSB)	7.31
Zinc Solubilising Bacteria (ZnSB)	7.41
Vesicular Arbuscular Mycorrhiza (VAM)	7.48
VAM + PSB	7.49
VAM + KSB	7.35
VAM +ZnSB	7.33
VAM +*Azotobacter chroococcum*	7.19
C.D (p≤0.05)	**0.029**
SE(m)	**0.010**

Table 18: Impact of microbial inoculation on soil pH of Deodar seedlings

Treatment	Soil pH
Control	7.53
Azotobacter chroococcum	7.30
Phosphorus Solubilising Bacteria (PSB)	7.42
Potassium Solubilising Bacteria (KSB)	7.38
Zinc Solubilising Bacteria (ZnSB)	7.40
Vesicular Arbuscular Mycorrhiza (VAM)	7.47
VAM + PSB	7.48
VAM + KSB	7.31
VAM +ZnSB	7.44
VAM +*Azotobacter chroococcum*	7.18
C.D (p≤0.05)	**0.058**
SE(m)	**0.020**

4.3.2 Available nitrogen

The data presented in Table 19 depicts that available nitrogen in soil medium of Himalayan Cypress seedlings with respect to microbial inoculation of VAM + *Azotobacter chroococcum* exhibited a significant increase over control and other treatments. Before transplanting available nitrogen in medium was recorded to be 156.63 kg ha^{-1} (Table 1) which was considerably low. After one growing season, the highest level of available nitrogen was observed in VAM + *Azotobacter chroococcum* (522.4 kg ha^{-1}) followed by *Azotobacter chroococcum* (514.1 kg ha^{-1}). Control plot (157.6 kg ha^{-1}) showed the least amount of available nitrogen.

The data presented in Table 20 depicts that available nitrogen in soil medium of Deodar seedlings with respect to microbial inoculation of VAM + *Azotobacter chroococcum* exhibited a significant increase over control and other treatments. After one complete growing season, the highest level of available nitrogen was observed in VAM + *Azotobacter chroococcum* (521.8 kg ha^{-1}) followed by *Azotobacter chroococcum* (517.9 kg ha^{-1}). Control plot (134.6 kg ha^{-1}) showed the least amount of available nitrogen.

4.3.3 Available phosphorous

The data presented in Table 19 shows that available phosphorous in experimental soil medium of Himalayan Cypress seedlings with respect to microbial inoculation of VAM + Phosphorous Solubilizing Bacteria (PSB) exhibited a significant increase over control and other treatments. Before transplanting available phosphorous was recorded to be 11.35 kg ha^{-1} (Table 1) which was considerably low. After one complete growing season, the highest level of available phosphorous was observed in VAM + PSB (23.0 kg ha^{-1}) followed by Phosphorous Solubilizing Bacteria (PSB) (19.3 kg ha^{-1}). Control plot (10.4 kg ha^{-1}) showed the least amount of available phosphorous.

The data presented in Table 20 shows that available phosphorous in experimental soil medium of Deodar seedlings with respect to microbial inoculation of VAM + Phosphorous Solubilizing Bacteria (PSB) exhibited maximum and significant increase over control and other treatments. After one complete growing season, the highest level of available phosphorous was observed in VAM + PSB (24.1 kg ha^{-1}) followed by Phosphorous Solubilizing Bacteria (PSB) (21.6 kg ha^{-1}). Control plot (10.0 kg ha^{-1}) showed the least amount of available phosphorous.

4.3.4 Available potassium

The data presented in Table 19 depicts that available potassium in the experimental soil medium of Himalayan Cypress seedlings with respect to microbial inoculation of VAM + Potassium Solubilizing Bacteria (KSB) exhibited a significant increase over control and other treatments. Before transplanting available potassium was recorded to be 112.51 kg ha^{-1} (Table 1) which was considerably low. After one complete growing season, the highest level of available potassium of 194.6 kg ha^{-1} was observed in VAM + KSB followed by KSB (193.1 kg ha^{-1}). Control plot (112.9 kg ha^{-1}) showed the least amount of available Potassium.

The data presented in Table 20 depicts that available potassium in the experimental soil medium of Deodar with respect to microbial inoculation of VAM + Potassium Solubilizing Bacteria (KSB) exhibited a significant increase over control and other treatments. After one complete growing season, the highest level of available potassium was observed in VAM + Potassium Solubilizing Bacteria (KSB) (186.8 kg ha^{-1}) followed by KSB (179.0 kg ha^{-1}). Control plot (109.1 kg ha^{-1}) showed the least amount of available potassium.

4.3.5 Available Zinc

The data presented in Table 19 depicts that available zinc in experimental soil medium of Himalayan Cypress seedlings with respect to microbial inoculation of Zinc Solubilizing Bacteria (ZnSB) exhibited maximum and a significant increase over control and other treatments. Before transplanting Available Zinc was recorded to be 7.08 kg ha^{-1} (Table 1) which was considerably low. After one complete growing season the highest level of available zinc was observed in VAM + Zinc Solubilizing Bacteria (ZnSB) (20.4 kg ha^{-1}) followed by Zinc Solubilising Bacteria (18.2 kg ha^{-1}). Control plot (7.2 kg ha^{-1}) showed the least amount of available zinc.

The data presented in Table 20 depicts that available zinc in experimental soil medium of Deodar seedlings with respect to microbial inoculation of Zinc Solubilizing Bacteria (ZnSB) exhibited a significant increase over control and other treatments. After harvesting the highest level of available potassium was observed in VAM + Zinc Solubilizing Bacteria (ZnSB) (22.3 kg ha^{-1}) followed by Zinc Solubilising Bacteria (19.3 kg ha^{-1}). Control plot (7.2 kg ha^{-1}) showed the least amount of available zinc.

Table 19: Impact of microbial inoculation on nutrient status of soil under Himalayan Cypress (kg ha^{-1})

Treatment	Available N	Available P	Available K	Available Zn
Control	157.6	10.4	112.9	7.2
Azotobacter chroococcum	514.1	14.8	141.7	13.4
Phosphorus Solubilising Bacteria (PSB)	420.5	19.4	143.5	11.7
Potassium Solubilising Bacteria (KSB)	252.5	15.5	193.1	14.3
Zinc Solubilising Bacteria (ZnSB)	391.5	15.2	141.4	18.2
Vesicular Arbuscular Mycorrhiza (VAM)	439.0	18.5	141.9	15.6
VAM + PSB	442.4	23.0	161.8	15.2
VAM + KSB	438.0	17.4	194.6	17.3
VAM +ZnSB	428.4	16.2	170.9	20.4
VAM +*Azotobacter chroococcum*	522.4	18.3	172.3	15.6
C.D ($p \leq 0.05$)	**2.392**	**0.372**	**1.572**	**0.186**
SE(m)	**0.824**	**0.128**	**0.542**	**0.064**

Table 20: Impact of microbial inoculation on nutrient status of soil under Deodar (kg ha^{-1})

Treatment	Available N	Available P	Available K	Available Zn
Control	134.6	10.0	109.1	7.2
Azotobacter chroococcum	517.9	14.3	134.6	13.0
Phosphorus Solubilising Bacteria (PSB)	416.6	21.6	144.3	12.3
Potassium Solubilising Bacteria (KSB)	253.8	16.4	179.0	13.8
Zinc Solubilising Bacteria (ZnSB)	384.5	15.2	123.1	19.3
Vesicular Arbuscular Mycorrhiza (VAM)	434.7	18.2	142.6	15.6
VAM + PSB	446.5	24.1	155.2	14.3
VAM + KSB	423.7	16.3	186.8	17.5
VAM +ZnSB	428.6	15.9	157.1	22.3
VAM +*Azotobacter chroococcum*	521.8	17.1	162.4	16.5
C.D ($p \leq 0.05$)	**3.523**	**0.297**	**2.502**	**0.232**
SE(m)	**1.214**	**0.102**	**0.862**	**0.080**

4.4 Microbial analysis

Before microbial inoculation the experimental soil medium was tested to obtain the value of microbial population in it. The data obtained is presented in Table 21.

Table 21: Microbial population before soil inoculation

Total viable bacteria in a composite soil sample × 10^{-6} CFUg^{-1} soil	Total viable Fungi in a composite soil sample ×10^{-5} CFUg^{-1} soil
30	2

4.4.1 Total viable bacteria

The data presented in Table 22 gives the impact of microbial inoculation on total viable bacterial population in experimental soil under Himalayan Cypress. Before transplanting total viable bacteria were recorded to be 30×10^6 $CFUg^{-1}$ (Table 20) which was considerably low. At the end of the growing season, the highest level of total viable bacteria were observed in VAM + *Azotobacter chroococcum* (158.0×10^6 $CFUg^{-1}$), followed by 145.50×10^6 $CFUg^{-1}$ under VAM + PSB while seedlings without treatment showed least population of bacteria of 55.0×10^6 $CFUg^{-1}$. Other microbial inoculations also recorded significantly higher $CFUg^{-1.}$

Table 22: Impact of microbial inoculation on total viable bacteria ($CFUg^{-1}$ soil) over experimental medium under Himalayan Cypress

Treatment	Total viable bacteria × 10^{-6} ($CFUg^{-1}$ soil)
Control	55.00
Azotobacter chroococcum	110.25
Phosphorus Solubilising Bacteria (PSB)	107.00
Potassium Solubilising Bacteria (KSB)	103.75
Zinc Solubilising Bacteria (ZnSB)	87.50
Vesicular Arbuscular Mycorrhiza (VAM)	92.50
VAM + PSB	145.50
VAM + KSB	123.75
VAM +ZnSB	140.50
VAM +*Azotobacter chroococcum*	158.00
C.D ($p \leq 0.05$)	**1.051**
SE(m)	**0.362**

The data presented in Table 23 regarding impact on Deodar shows that the highest level of total viable bacteria in experimental soil was observed in VAM + *Azotobacter chroococcum* (115.50×10^6 $CFUg^{-1}$) followed by 104.50×10^6 $CFUg^{-1}$ in VAM+ PSB treated seedlings while the least count of bacteria of 32.75×10^6 $CFUg^{-1}$ was recorded under control.

Table 23: Impact of microbial on total viable bacteria ($CFUg^{-1}$ soil) over experimental medium under Deodar

Treatment	**Total viable bacteria × 10^{-6} ($CFUg^{-1}$ soil)**
Control	32.75
Azotobacter chroococcum	83.25
Phosphorus Solubilising Bacteria (PSB)	81.51
Potassium Solubilising Bacteria (KSB)	80.50
Zinc Solubilising Bacteria (ZnSB)	70.75
Vesicular Arbuscular Mycorrhiza (VAM)	76.50
VAM + PSB	104.50
VAM + KSB	93.12
VAM +ZnSB	96.25
VAM +*Azotobacter chroococcum*	115.50
C.D ($p \leq 0.05$)	**0.116**
SE(m)	**0.040**

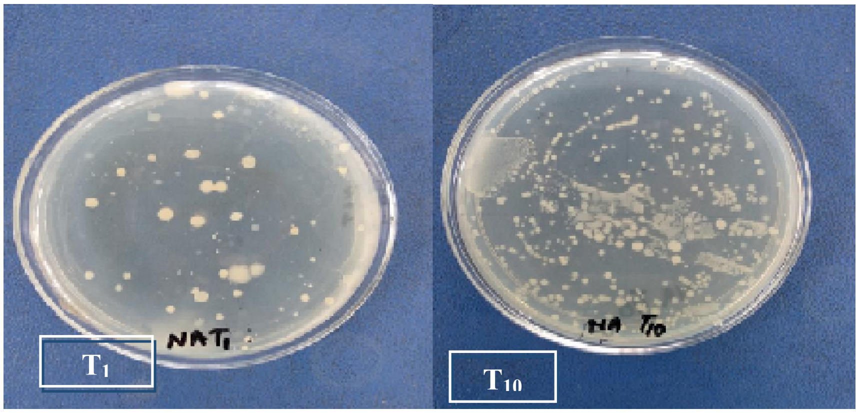


Plate 12: **Total viable bacteria in the experimental soil under Himalayan Cypress**

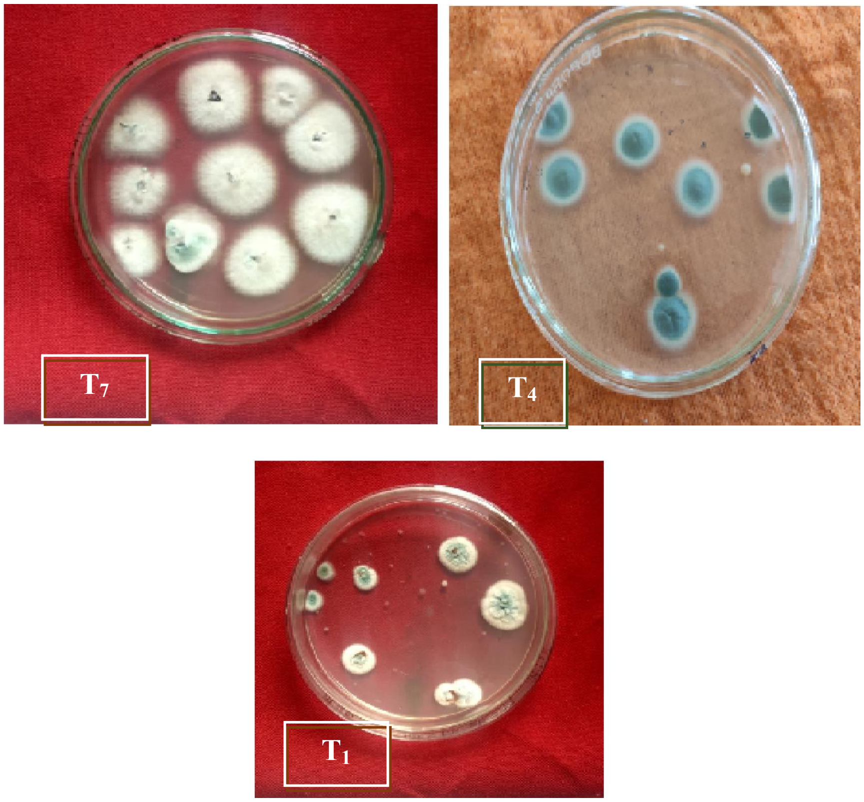


Plate 13: **Total viable fungi in the experimental soil under Himalayan Cypress**

4.4.2 Total viable fungi

Total viable fungal count in the experimental soil under Himalayan Cypress, before transplanting was recorded to be (2.0 × 10^5 $CFUg^{-1}$) which were considerably low. After one complete growing season, the highest level of Total viable fungi was observed in VAM + Phosphorous Solubilizing Bacteria (PSB) of 11.00 × 10^5 $CFUg^{-1}$ soil followed by VAM + *Azotobacter chroococcum* (10.51× 10^5 $CFUg^{-1}$). *Azotobacter chroococcum* showed the lowest population of total viable fungal count of 8.05 × 10^5 $CFUg^{-1}$ among treatments while untreated soils showed the least total viable fungal count of 6.50 ×10^5 $CFUg^{-1}$ of soil.

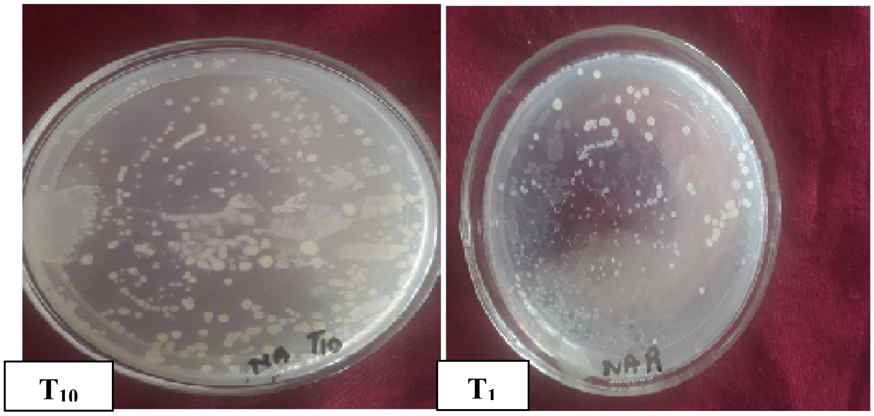
Table 24: Impact of microbial inoculation on total viable fungi ($CFUg^{-1}$ soil) over experimental medium under Himalayan Cypress

Treatment	Total viable fungi × 10^{-5} ($CFUg^{-1}$ soil)
Control	6.50
Azotobacter chroococcum	8.05
Phosphorus Solubilising Bacteria (PSB)	9.20
Potassium Solubilising Bacteria (KSB)	8.87
Zinc Solubilising Bacteria (ZnSB)	8.51
Vesicular Arbuscular Mycorrhiza (VAM)	9.90
VAM + PSB	11.00
VAM + KSB	9.50
VAM +ZnSB	9.81
VAM +*Azotobacter chroococcum*	10.51
C.D ($p \leq 0.05$)	**0.275**
SE(m)	**0.095**

The data presented in Table 25 reveals that highest total viable fungal count in the experimental soil under Deodar after one complete growing season was observed in VAM + Phosphorous Solubilizing Bacteria (PSB) (11.00 × 10^5 CFUg^{-1}) followed by VAM + *Azotobacter chroococcum* that recorded as 10.00 × 10^5 CFUg^{-1} soil. *Azotobacter chroococcum* showed significantly less fungal population of 5.50 × 10^5 CFUg^{-1} soil amongst all microbial treatments. Control recorded the least fungal population as of 4.50 × 10^5 CFUg^{-1} soil only.

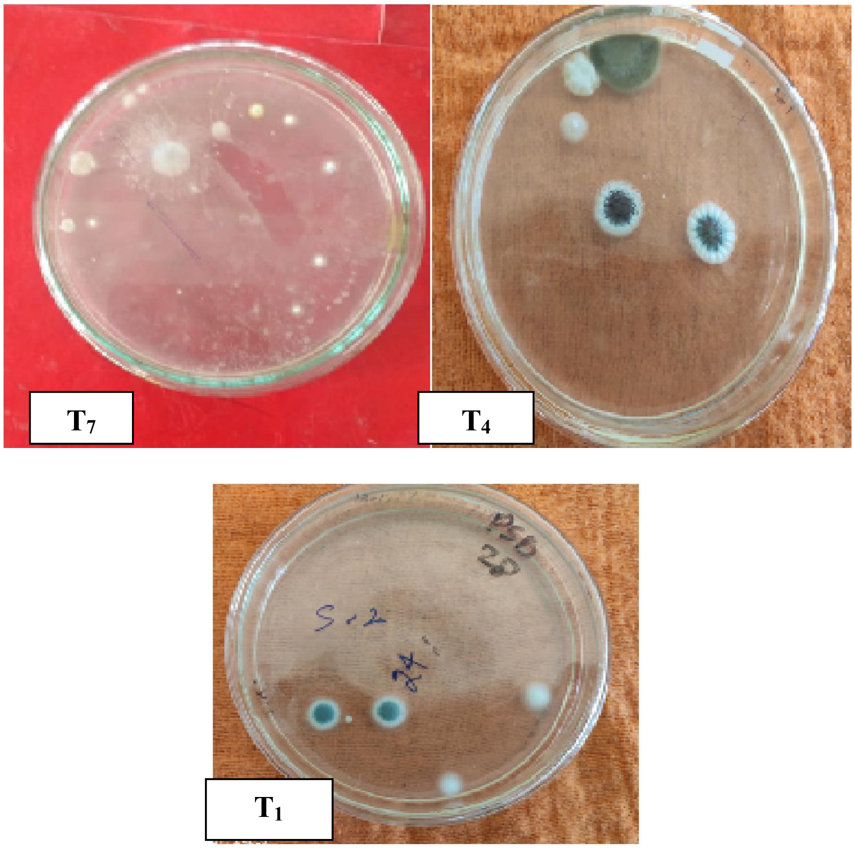
Table 25: Impact of microbial inoculation on total viable fungi (CFUg^{-1} soil) over experimental medium under Deodar

Treatment	**Total viable fungi × 10^{-5} (CFUg^{-1} soil)**
Control	4.50
Azotobacter chroococcum	5.50
Phosphorus Solubilising Bacteria (PSB)	6.25
Potassium Solubilising Bacteria (KSB)	6.00
Zinc Solubilising Bacteria (ZnSB)	5.25
Vesicular Arbuscular Mycorrhiza (VAM)	8.25
VAM + PSB	11.00
VAM + KSB	7.00
VAM +ZnSB	8.50
VAM +*Azotobacter chroococcum*	10.00
C.D ($p \leq 0.05$)	**0.557**
SE(m)	**0.199**



T_{10} T_1

Plate 14: Total viable bacteria in the experimental soil under Deodar



T_7 T_4

T_1

Plate 15: Total viable fungi in the experimental soil under Deodar

Chapter 5

DISCUSSION

The relationship between fungi and roots of higher plants commonly termed as Mycorrhiza along with other rhizo-bacteria present in the rhizosphere is well known. In the present study inoculation of nitrogen-fixing *Azotobacter chroococcum*, Phosphorous Solubilising Bacteria (PSB), Potassium Solubilising Bacteria (KSB), Zinc Solubilising Bacteria (ZnSB), Arbuscular Vesicular Mycorrhiza (VAM) and their combinations were monitored for their respective contributions on nutrient concentration and plant growth of Deodar and Himalayan Cypress seedlings. Impact of microbial inoculants on nutrient status as well as microbial population of the experimental medium was also studied. *Azotobacter chroococcum* is a nitrogen fixing microorganism present abundantly in the rhizosphere benefiting the plants by frequently enhancing shoot height as well as root growth through the production of growth promoting phyto-hormones like Indole-Acetic Acid (IAA), Gibberellins-like substances and cytokinins (Azcon and Barea, 1975). VAM fungi promotes nutrient and water uptake especially phosphorous from the soil, protection from the pathogens, increases root surface area and root elongation for nutrient absorption, thus stimulating the rooting, growth and survival. Arbuscular mycorrhiza produces plant hormones and increases the activity of nitrogen fixers in root zone (Bagyaraj, 1984).

5.1 Plant growth (in height and diameter)

There was a significant variation in plant height due to different microbial inoculations. The plant height increased in all the treatments. Among the microbial inoculations, mycorrhizal inoculations showed significant increase. 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 Deodar as well as Himalayan Cypress seedlings. Maximum growth (height) of 19.48 cm in

Himalayan Cypress seedlings (Table 2) in one growing season after the application of VAM + *Azotobacter chroococcum* followed by 18.22 cm was recorded by the application of VAM + ZnSB. Control recorded a growth of only 9.38 cm during the same period after application of microbial inoculants. In Deodar seedlings (Table 3), after one complete growing season maximum growth in height was 6.87 cm by the application of VAM + *Azotobacter chroococcum* followed by 6.60 cm the application of VAM + ZnSB while control recorded height of 2.84 cm only. 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 both the species under study. After one complete growing season, maximum growth in diameter 4.57 mm was recorded by the application of VAM + *Azotobacter chroococcum* followed by 3.28 mm and 3.27 mm in *Azotobacter chroococcum* and VAM + ZnSB respectively although both were at par. Control recorded only 2.67 mm of growth at the collar region in seedlings of Himalayan Cypress (Table 4). In deodar seedlings (Table 5) after one growing season maximum growth in diameter by the application of microbial inoculants was recorded as 1.11 mm in VAM + *Azotobacter chroococcum* followed by 1.02 mm by the application of VAM + ZnSB while control recorded 0.49 mm of growth in diameter. The findings are in accordance with the finding of Seema *et al*. (2000) who reported that AM fungi and *Azotobacter chroococcum* inoculation on *Gmelina arborea, Bambusa arundinacea, Tectona grandis* and *Dilbergia sissoo* resulted in improved growth and biomass.

The positive correlation of VAM fungi with *Azotobacter chroococcum* with growth (Shoot extension, leaf area and fruit yield) in mango orchards was shown by Sharma and Kumar (2008). Furthermore, the enhancement in shoot extension growth is also attributed to an increased nutrient uptake particularly, phosphorus apart from an increased uptake of micronutrients (Mathews *et al*., 2003). Additionally, the findings on *Pongamia pinnata* are in agreement with present results, seedlings inoculated with AMF combined with nitrogen fixing microorganisms significantly enhanced shoot length better than Arbuscular Mycorrhizal Fungi or nitrogen fixing microorganisms separately (Singh and Jamaluddin, 2010).

5.2 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 45.66 cm in VAM + *Azotobacter chroococcum* treated seedlings of Himalayan Cypress followed by 43.55 cm in seedlings inoculated with VAM + ZnSB. Control recorded as 18.82 cm of root length (Table 6). Similarly, in Deodar seedlings, the maximum root length was recorded 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 7). This positive correlation of VAM and *Azotobacter chrococcum* is attributed to the production of growth substances (IAA) by the *Azotobacter* sp. (Azcon and Barea, 1975). Concerning VAM effects, the increment in growth of inoculated seedlings may be due to the ability of VAM fungi to produce some growth promoting substances, organic, inorganic acids and CO_2 which lead to increase in soil acidity, consequently convert the insoluble forms of nutrients into soluble ones. VAM hyphae also help in retaining moisture around the root zone of plants which reflect efficient mobilization and absorption of nutrients and water, so AM influence on optimizing the availability and uptake of mineral nutrients in the rhizosphere is one such important regulator of plant growth and development. 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) (Ghazi, 2013) and (Merwad *et al.,* 2014). However, Barea and Azćon-Aguilar (1982) proved that VAM synthesized at least two gibberellin-like substances and four substances with the properties of cytokinins and their effects to enhance plant growth. Further, Azćon and associates (1978) mentioned that plant hormones synthesized by *Azotobacter* sp. could interact with endomycorrhizal fungi, hence it seemed to play role in the formation and function of VAM. The results in the present are in conformity with Bagyaraj and Menge (1978) and 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.

5.3 Seedling survival %age

The percentage survival of seedlings (both species) 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. For seedlings of Himalayan Cypress, 100% of seedlings survived when treated with ZnSB followed by 97.20 % in KSB, 94.40% in *Azotobacter chroococcum*, 91.60% in PSB, 92% in VAM + ZnSB, 90% in VAM + *Azotobacter chroococcum*, 88.80% in VAM and VAM + PSB although both are significantly at par and 86.10% in VAM + KSB treatment (Table 8). Control recorded only 80% of survived seedlings at the end of one growing season. For Deodar seedlings, at the end of one growing season 95% of seedlings survived in treatment 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 8).

5.4 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 9.54 g was recorded in Himalayan Cypress seedlings treated with VAM + *Azotobacter chroococcum* obviously because of their more growth followed by 8.97 g in VAM + ZnSB treated seedlings while control recorded 2.53 g only (Table 9). In Deodar seedlings, maximum total fresh biomass was recorded as 1.53 g in VAM + *Azotobacter chroococcum* followed by 1.41 g in VAM + ZnSB treated seedlings and control plot recorded 0.31g of fresh total biomass (Table 10).

Total dry biomass in Himalayan Cypress seedlings was recorded highest 5.29 g in VAM + *Azotobacter chroococcum* because of highest growth parameters followed by 4.67 g in dual inoculation of VAM and ZnSB after one growing season. Control recorded 1.24 g of total dry biomass (Table 11). Similarly, the maximum total dry biomass was recorded 0.79g in VAM + *Azotobacter chroococcum* treated Deodar 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 12). These results are in conformity with the findings of Raheem *et al*. (1988) who showed increase in overall plant growth and nutrient uptake by the inoculation of VAM fungus and *Azotobacter chroococcum* in tomato plants. 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 results of the present studies were also in accordance with those of (Rana and Srivastava, 1984), who also reported positive and significant relationship of AM spore number with growth and yield of litchi orchards. 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.

5.5 Root-shoot ratio

The root and shoot ratio varied significantly among all the treatments. In seedlings of Himalayan Cypress, maximum r/s ratio of 2.53 was recorded in VAM + *Azotobacter chroococcum*, followed by 2.13 in VAM + ZnSB and 2.04 in VAM. Control recorded only 1.37 root-shoot ratio after one growing season by the application of microbial inoculants (Table 13). In Deodar seedlings, 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 14). The non-inoculated seedlings had relatively less root-shoot ratio than the inoculated ones. A less vigorous but non-destructive, index is the 'sturdiness quotient' which compares height (in cm) over root collar diameter (in mm). A small quotient indicates a sturdy plant with a higher expected chance of survival, especially on windy or dry sites. A sturdiness quotient higher than 6 is undesirable (Jaenicke, 1999; Gregorio *et al*., 2007). From the study of Sreedhar and Mohan (2016), *Neolamarckia cadamba* seedlings had desirable sturdiness quotient values (<6.0) at two ages of observation. However, in both the cases, the inoculation of seedlings with AM fungi and PGPR has resulted in improvement of sturdiness quotient values towards <6. Our findings are in accordance with the findings of Sumana and Bagyaraj (2002) who reported that dual inoculation of *Glomus mossae* and *Azotobacter chroococcum* resulted in maximum volume index and quality index of neem seedlings.

5.6 Nutrient concentration

The results showed that in both the species, the highest plant nitrogen (1.42% in Himalayan Cypress and 1.41% in Deodar seedlings) was observed in VAM +*Azotobacter chroococcum* inoculated seedlings followed by 1.38% in VAM + ZnSB treated Cupressus as well as Deodar seedlings while the least was observed in KSB (1.10% in Himalayan Cypress and 1.08% in Deodar seedlings) [Table 15&16]. Only 1.03% and 1.05% of total nitrogen was recorded under control plots of Himalayan Cypress and Deodar seedlings respectively. The study of nitrogen accumulation by Himalayan Cypress and Deodar seedlings concluded with the finding that nitrogen accumulation in both the species was increased by inoculation with VAM + *Azotobacter chroococcum*. The possible mechanisms which facilitated more nitrogen uptake by crops are N_2 fixation; delivering combined nitrogen to the plant and the production of phytohormone-like substances that alter plant growth and morphology and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Mrkovacki and Milic, 2001). This finding was in close agreement with Raheem *et al. (*1988) who reported that presence of *Azotobacter chroococcum* and VAM fungus increased N content in tomato plants. Bagyaraj and Menge (1978); (Redente and Reeves (1981) also recorded a synergistic effect of mycorrhizal fungi *Glomus fasciculatum* and a nitrogen fixing bacteria (*Azotobacter* or *Rhizobium*) with respect to nitrogen fixation rates of tomato and sweetvetch.

The results showed that the highest plant phosphorous in Himalayan Cypress seedlings (0.09%) was found in VAM + PSB inoculated plants while the least was found in control (0.01%) [Table 15]. In Deodar seedlings, the highest plant phosphorous 0.24% was found in VAM + PSB inoculated plants while the least was observed in control with 0.02% of total phosphorous [Table 16]. Other microbial treatments also recorded more phosphorous than control in both the species. This increase in phosphorus content might be due to increased availability of soil phosphorus because of solubilizing effect of organic acids, which are produced from decomposing organic manures. Further, PSB helps in solubilizing the insoluble phosphorus into soluble form and also reduce the fixation of phosphorus, hence, the availability and the absorption of phosphate by the plant was more. Similar, results have been reported by Reddy (1999) and Herencia *et al. (*2011).

The results showed that in Himalayan Cypress seedlings, the highest plant potassium of 0.47% was estimated in VAM +KSB inoculated plants while the least was recorded in control 0.27% [Table 15]. In Deodar seedlings, the highest plant potassium 0.92% was observed in VAM +KSB inoculated seedlings while the least was recorded in control of 0.49% [Table 16]. This increase in potassium content in both the seedlings might be due to the release of some phosphatic ions from otherwise sparingly soluble phosphorous sources (Barea *et al.,* 1983) which are trapped and translocated by the arbuscular mycorrhizal fungal hyphae to the plant. The results are in conformity with the findings of Piccini and Azcon (1987) and Bagyaraj (1984).

5.7 Soil pH

During the present study, it is evident from the data (Table 1) that the soil properties like pH deviated from their initial status after the completion of one growing season in most of the cases due to different treatments in both the species (Table 17 & 18). The results indicate that application of biofertilizers caused relative decrease in pH, which is attributed to the production of organic acids (during mineralization of organic material) and for solubilization of bound elements. These results are in conformity with Patel *et al. (*2014) who showed that microbes lower the soil pH by releasing of organic acids.

5.8 Nutrient status of experimental medium

In Himalayan Cypress, data shows that the soil properties like available nitrogen, phosphorous potassium and available zinc content increased when compared with their initial status after the completion of one growing season due to application of different bio-fertilizer treatments. It was seen that there was an increase in soil available nitrogen. Highest soil nitrogen 522.4 kg ha^{-1} was recorded in soil inoculated with VAM + *Azotobacter chroococcum* followed by 514.1 kg ha^{-1}in *Azotobacter chroococcum* while the least 157.6 kg ha^{-1} was estimated in control plot of Himalayan Cypress seedlings (Table 19). In case of Deodar (Table 20) available nitrogen was observed highest 531.8 kg ha^{-1} in VAM + *Azotobacter chroococcum* followed by 517.9 kg ha^{-1} in seedlings treated with *Azotobacter chroococcum.* Least amount i.e., 134.6 kg ha^{-1} available nitrogen was recorded in control plot. The increase in soil available nitrogen was due to bacterization. Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism (Monib *et al.,* 1979).

Available phosphorous also resulted in increase from the initial status. In Himalayan Cypress seedlings, the highest value for available phosphorus 23.0 kg ha^{-1} was recorded in soils inoculated with VAM + Phosphate solubilizing bacteria (VAM + PSB) followed by 19.4 kg ha^{-1} in PSB, 18.5 kg ha^{-1} in VAM treated seedlings while the least 10.4 kg ha^{-1} of available nitrogen was recorded in control plot (Table 19). A similar pattern followed in Deodar seedlings where highest value for available phosphorus 24.1 kg ha^{-1} was estimated in soils inoculated with VAM+ Phosphate solubilizing bacteria followed by 21.6 kg ha^{-1} in PSB, 18.2 kg ha^{-1} in VAM treated plants while the least 10.0 kg ha^{-1} was observed in control plot (Table 20). Phosphate ions in soil become rapidly bound with cations, forming unsoluble complexes that are unavailable to plants. It is known that the presence of mycorrhizal fungi in the soil improves phosphate solubility. VAM is able to alter mobilization of soil phosphorous of its host plant through mycorrhizal symbiosis (Priyadarshni and Muthukumar, 2016; Kumar *et al.,* 2017; Raghavendra *et al.,* 2016; Zahedi, 2016; Dominguez-Numez *et al*., 2016; Dotaniya *et al*., 2016). The increased availability of soil phosphorous due to phospho-bacterial inoculation was also observed by Sundara *et al.* (1999).

An increase in available potassium was also seen after one growing season by the inoculation of different microbial inoculants. In Himalayan Cypress seedlings, the highest soil potassium 194.6 kg ha^{-1} was estimated in soils inoculated with VAM + Potassium solubilizing bacteria (KSB) followed by 193.1 kg ha^{-1} in KSB, 172.3 kg ha^{-1} in VAM + *Azotobacter chroococcum* while the least 112.9 kg ha^{-1} was observed in control plot (Table 19). In Deodar seedlings, the highest soil potassium 186.8 kg ha^{-1} was estimated in soils inoculated with VAM + Potassium solubilizing bacteria (KSB) followed by 179.0 kg ha^{-1} in KSB, 162.4 kg ha^{-1} in VAM + *Azotobacter chroococcum* while the least 109.1 kg ha^{-1} was observed in control plot (Table 20).The influence of biofertilizers in increasing nutrient availability have been reported by (Deshmukh, 1994) in cucumber and (Mahendran and Kumar, 1997) in cabbage.

Available zinc also showed a significant increase in experimental soil after one growing season by the application of microbial inoculants. In Himalayan Cypress seedlings highest available zinc 20.4 kg ha^{-1} was found in soils inoculated with VAM + zinc solubilizing bacteria (ZnSB) followed by 18.2 kg ha^{-1} in ZnSB, 17.3 kg ha^{-1} in VAM + KSB while the least7.2 kg ha^{-1} was observed in control plot (Table 19). A similar pattern followed in Deodar seedlings where highest available zinc of 22.3 kg ha^{-1} was estimated in soils inoculated with VAM + zinc solubilizing bacteria followed by 19.3 kg ha^{-1} in ZnSB, 17.5 kg ha^{-1} in VAM + KSB while the least value of available zinc 7.2 kg ha^{-1} was recorded in control plot (Table 20).

5.9 Microbial population of experimental medium

All the microbial inoculants have significant and marked influence on total viable bacteria (Table 22) in experimental soil under Himalayan Cypress seedlings over control. VAM + *Azotobacter chroococcum* inoculants gave higher viable bacterial count of 158× 10^6 CFUg^{-1}. Similarly, in soils under Deodar seedlings VAM + *Azotobacter chroococcum* inoculants recorded higher viable bacterial count of 115.50× 10^6 CFUg^{-1} (Table 23).

The results showed that dual inoculation obtained more population than the individual ones. The domination of microbial populations in the rhizospheric area of inoculated species of Himalayan Cypress and Deodar seedlings with combined *Azotobacter chroococcum* and Mycorrhiza is attributed to hyphae as symbiotic fungi extend surface area for interaction with other microorganisms and provide an important pathway for the translocation of energy rich plant assimilates to soil. Free living *Azotobacter* with potential to fix nitrogen has been discovered growing endosymbiotically within arbuscular mycorrhizal hyphae. This explained the increase of total microbial population in general and *Azotobacter* in particular more than uninoculated ones. Our findings are well matched with the findings obtained by (Johansson *et al.,* 2004).

Furthermore, all the microbial inoculants have significant and marked influence on total viable fungi (Table 24) in soil under Himalayan Cypress seedlings over control. VAM + Phosphorous Solubilising Bacteria inoculants gave higher viable fungal count of 11×10^5 $CFUg^{-1}$ of soil. In soils under Deodar seedlings, highest total viable fungi (Table 25) was observed in VAM + PSB treatments (11×10^5 $CFUg^{-1}$).The increase in fungal count could be attributed to the production of growth promoting substances secreted by soil microbes and secretion of certain root exudates. Further the presence of suitable soil moisture and temperature conditions might have increased its population.

Chapter 6

SUMMARY AND CONCLUSION

The present investigation on "Influence of Microbial Inoculation on Growth of Deodar and Himalayan Cypress Seedlings" was conducted during spring 2018-2019 with following objectives:

1. To study the effects of microbial inoculants on growth of Deodar and Himalayan Cypress.
2. To assess the concentration of nutrients in Deodar and Himalayan Cypress seedlings as influenced by microbial inoculants.
3. To ascertain the nutrient status and microbial population of the experimental medium as influenced by the application of microbial inoculants.

The above objectives were achieved by laying field experimental trial in which ten treatments involving different microbial inoculants *viz*; *Azotobacter chroococcum*, Phosphorous Solubilizing Bacteria (PSB), Potassium Solubilizing Bacteria (KSB), Zinc Solubilizing Bacteria (ZnSB), Arbuscular Mycorrhiza and co-inoculation of VAM + PSB, VAM + KSB, VAM + ZnSB and VAM + *Azotobacter chroococcum* were tried under four replicates. The findings of the investigation are summarized as under:

6.1 Effect of microbial inoculants on growth of Deodar and Himalayan Cypress seedlings

6.1.1 Plant height

All the microbial inoculants improved the plant height significantly over control in both the coniferous species during one growing season. In Himalayan Cypress seedlings VAM + *Azotobacter chroococcum* resulted in maximum increase in plant height (19.48 cm) among all the microbial inoculations, followed by VAM+ Zinc solubilising Bacteria (18.22 cm). In Deodar seedlings also

VAM + *Azotobacter chroococcum* resulted in maximum increase in plant height (6.87 cm) among all the microbial inoculations, followed by VAM + Zinc solubilising Bacteria (6.60 cm).

6.1.2 Collar diameter

Application of various microbial inoculants enhanced collar diameter in both the species significantly over control. In Himalayan Cypress seedlings, among various treatments VAM+ *Azotobacter chroococcum* resulted in maximum increase in collar diameter (4.57 mm) and was superior to all the inoculants followed by VAM + Zinc Solubilising Bacteria (3.28 mm) which is significantly at par with *Azotobacter chroococcum* that recorded growth of 3.27 mm in diameter. In case of deodar seedlings, among various treatments VAM + *Azotobacter chroococcum* resulted in maximum increase in collar diameter (1.11 mm) and was superior to all the inoculants followed by VAM + Zinc Solubilising Bacteria with 1.02 mm increase in diameter.

6.1.3 Root length

Application of various microbial inoculants enhanced the root length significantly over control. Among various treatments VAM + *Azotobacter chroococcum* (45.66 cm) resulted in maximum increase in root length and was superior to all the inoculants followed by VAM+ ZnSB which recorded a length of 43.55 cm in Himalayan cypress seedlings. In Deodar seedlings, among various treatments VAM + *Azotobacter chroococcum* resulted in maximum increase in root length of 12.95 cm and was superior to all the inoculants followed by VAM + ZnSB that recorded root length of 12.64 cm.

6.1.4 Total fresh (root and shoot) biomass

Application of various microbial inoculants enhanced the total fresh (root and shoot) biomass significantly over control. In Himalayan cypress seedlings, among various treatments VAM + *Azotobacter chroococcum* (9.54 g) resulted in maximum increase in total fresh (root and shoot) biomass obviously because of more growth and was superior to all the inoculants followed by VAM + ZnSB (8.97 g). In Deodar seedlings also VAM + *Azotobacter chroococcum* resulted in maximum increase in total fresh (root and shoot) biomass of 1.53 g and was superior to all the inoculants followed by VAM + ZnSB (1.41 g).

6.1.5 Total dry (root and shoot) biomass

Application of various microbial inoculants enhanced total dry (root and shoot) biomass significantly over control. In Himalayan cypress seedlings, among various treatments VAM + *Azotobacter chroococcum* resulted in maximum total dry (root and shoot) biomass of 5.29 g and was superior to all the inoculants followed by VAM + ZnSB (4.67 g). In Deodar seedlings also VAM + *Azotobacter chroococcum* resulted in maximum increase in total dry (root and shoot) biomass of 0.79 g and was superior to all the inoculants followed by VAM + ZnSB (0.73 g).

6.1.6 Root-shoot ratio

Applications of various microbial inoculants enhanced plant root and shoot ratio significantly over control. VAM + *Azotobacter chroococcum* resulted in maximum increase in root and shoot ratio of 2.53 and was superior to all the inoculants followed by VAM + ZnSB (2.13) in Himalayan cypress seedlings. A similar trend followed in Deodar seedlings where VAM + *Azotobacter chroococcum* resulted in maximum increase in root and shoot ratio of 1.40 and was superior to all the inoculants followed by VAM + ZnSB (1.38).

6.1.7 Seedling survival (%)

Applications of various microbial inoculants increased seedling survival over control. Among various treatments ZnSB recorded maximum survival percentage of 100% and was superior to all the inoculants followed by KSB (97.2 %) in Himalayan cypress seedlings. A similar trend followed in Deodar seedlings where ZnSB recorded maximum survival percentage of 95% and was superior to all the inoculants followed by KSB (94.5 %).

6.2 Effect of microbial inoculants on nutrient concentration in Deodar and Himalayan Cypress seedlings

6.2.1 Total nitrogen (%)

Application of different microbial inoculants resulted in increase of plant nitrogen. VAM + *Azotobacter chroococcum* showed highest plant nitrogen of 1.42% while KSB showed the least plant nitrogen of 1.10% in one year old Himalayan Cypress seedlings. A similar trend followed in one year old Deodar seedlings where VAM + *Azotobacter chroococcum* showed highest plant nitrogen of 1.41% while KSB showed the least plant nitrogen of 1.08%.

6.2.2 Total phosphorous (%)

Different microbial inoculants resulted in increase of plant phosphorous. Among various treatments VAM + PSB showed highest plant phosphorous of 0.09% while ZnSB showed the least plant phosphorous of 0.03% in one year old Himalayan Cypress seedlings. A similar trend followed in one year old Deodar seedlings where VAM + PSB showed highest plant phosphorous of 0.24% while ZnSB recorded the least plant phosphorous of 0.08%.

6.2.3 Total potassium (%)

Inoculation of various microbial inoculants resulted in increase of plant potassium. Among various treatments VAM + KSB recorded highest plant potassium of 0.47% while PSB showed the least plant potassium of 0.29% in one year old Himalayan Cypress seedlings. A similar trend followed in one year old Deodar seedlings where VAM + KSB recorded highest plant potassium of 0.92% while PSB showed the least plant potassium of 0.53%.

6.3 Effect of microbial inoculants on nutrient status of Deodar and Himalayan Cypress seedlings

6.3.1 Soil pH

Application of various microbial inoculants resulted in the deviation of soil pH from that of initial state but there was not much difference between the treatments as soil pH tended to be neutral in all the treatments.

6.3.2 Available nitrogen

Various microbial inoculants resulted in increase of soil nitrogen. Among various treatments VAM + *Azotobacter chroococcum* recorded highest available nitrogen of 522.4 kg ha^{-1} while control plot showed the least available nitrogen of 157.6 kg ha^{-1} in soils of Himalayan Cypress seedlings. A similar pattern followed in the soils of Deodar seedlings where VAM + *Azotobacter chroococcum* recorded highest available nitrogen of 521.8 kg ha^{-1} while control plot showed the least available nitrogen of 134.6 kg ha^{-1}.

6.3.3 Available phosphorous

Various microbial inoculants resulted in increase of soil phosphorous. Among various treatments VAM + PSB recorded highest available phosphorous of 23.0 kg ha^{-1} while control plot showed the least available phosphorous of 10.4 kg ha^{-1} in soils of Himalayan Cypress seedlings. A similar pattern followed in the soils of Deodar seedlings where VAM + PSB recorded highest available phosphorous of 24.1 kg ha^{-1} while control plot showed the least available phosphorous of 10.0 kg ha^{-1}.

6.3.4 Available potassium

Application of various microbial inoculants resulted in increase of soil potassium. Among various treatments VAM + Potassium Solubilizing Bacteria recorded highest available potassium of 194.6 kg ha^{-1} while control recorded the least available potassium of 112.9 kg ha^{-1}. A similar pattern followed in the soils of Deodar seedlings where VAM + KSB recorded highest available potassium of 186.8 kg ha^{-1} while control plot recorded the least available potassium of 109.1 kg ha^{-1}.

6.3.5 Available zinc

Application of various microbial inoculants resulted in increase of soil zinc. Among various treatments VAM + Zinc Solubilizing Bacteria recorded highest available zinc of 20.4 kg ha^{-1} while control recorded the least available zinc of 7.2 kg ha^{-1}. A similar pattern followed in the soils of Deodar seedlings where VAM + ZnSB recorded highest available zinc of 22.3 kg ha^{-1} while control plot recorded the least available zinc of 7.2 kg ha^{-1}.

6.4 Effect of microbial inoculants on microbial status of experimental soil under Deodar and Himalayan Cypress

6.4.1 Total Viable Bacteria

Application of various microbial inoculants resulted in increase of bacterial count in soil. Among various treatments, 158 × 10^6 CFUg^{-1} bacterial population was recorded maximum in VAM + *Azotobacter chroococcum* while 55.0 × 10^6 CFUg^{-1} of total bacterial count was recorded in control plot under Himalayan Cypress seedlings. Similarly, in the soil medium of Deodar seedlings 115.50× 10^6 CFUg^{-1} of total bacterial count were observed in VAM + *Azotobacter chroococcum* while only 32.75 × 10^6 CFUg^{-1} of bacterial population were observed in control plot.

6.4.2 Total Viable Fungi

Application of various microbial inoculants resulted in increase of fungal count in soil. Among various treatments 11 × 10^5 CFUg^{-1} of fungal count was recorded maximum in treatment VAM + Potassium Solubilizing Bacteria while only 6.50 × 10^5 CFUg^{-1} of fungal population was recorded in control plot under Himalayan Cypress seedlings. Similarly, in the soil medium of Deodar seedlings 11 × 10^5 CFUg^{-1} of fungal count was recorded highest in VAM + Potassium Solubilizing Bacteria while only 4.50 × 10^5 CFUg^{-1} of fungal count was recorded in control plot.

CONCLUSION

In light of the present investigation it could be concluded that:

- Among all the microbial treatments in both the species, 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.

- Plant N, P and K was recorded highest in the dual inoculations of VAM + *Azotobacter chroococcum*, VAM + PSB and VAM + KSB respectively over control.

- In case of nutrient status of experimental medium, a significant increase was observed among all the treatments over control. Available nitrogen was recorded highest in case of VAM + *Azotobacter chroococcum*. Available phosphorus was recorded maximum in treatment VAM + PSB. Available potassium was recorded highest in dual inoculation of VAM +KSB and available Zinc was recorded highest in treatment VAM + ZnSB. Control recorded least of available soil nutrients. Soil pH decreased in every treatment and was observed slightly neutral in all the treatments.

- Total viable bacteria were recorded highest in dual treatment of VAM + *Azotobacter chroococcum* over control. Total viable fungi were highest in treatment combination of VAM + PSB and least was observed in the control for both the species.

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APPENDIX – I

Agro-metrological data for the year 2018

Months (2018)	Mean Temperature (^{0}C)		Rainfall (mm)	Relative Humidity (%)		Sunshine Record
	Max.	Min.		Max.	Min.	
January	10.73	-4.87	0.03	93.03	51.19	3.95
February	12.30	-0.91	1.88	88.92	48.60	3.51
March	18.45	3.32	1.36	74.54	43.35	5.50
April	21.41	6.81	5.34	77.5	51.46	6.20
May	24.64	9.00	1.63	70.54	52.03	7.25
June	28.33	13.44	2.05	70.76	58.16	7.23
July	28.83	17.16	4.32	84.96	60.03	5.73
August	30.74	16.58	3.90	81.64	51.87	7.77
September	27.98	11.92	0.76	81.20	50.33	7.00
October	21.74	2.90	0.33	84.06	55.54	6.16
November	12.06	0.11	4.60	88.76	70.30	1.06
December	9.37	-4.56	0.18	93.67	60.77	1.66

Source: Division of Agronomy, FoA Wadura, SKUAST-K

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Upma Dutta, Assistant Professor, Division of Microbiology, SKUAST-Jammu during Viva-Voce examination held on 27-02-2020 have been incorporated in the manuscript entitled **"Influence of Microbial Inoculation on Growth of Deodar (*Cedrus deodara* D. Don) and Himalayan Cypress (*Cupressus torulosa* G. Don) under Nursery Conditions"** submitted by **Ms. Asifa Yousuf Wani (Regd. No. 2017-A-1165-M)**.

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