RESEARCH ARTICLE

INFLUENCE OF MICROBIAL INOCULANTS ON NUTRIENT UPTAKE OF HIMALAYAN CYPRESS (CUPRESSUS TORULOSA DON) SEEDLINGS UNDER TEMPERATE NURSERY CONDITIONS

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ARTICLE INFO

Article History:
Received 18th October, 2012
Received in revised form 10th, November, 2012
Accepted 20th November, 2012
Published online 29th December, 2012

Key words:
Himalayan Cypress, Microbial inoculant, Nutrient uptake, Nursery.

ABSTRACT

The present study investigated the influence of microbial inoculants on nutrient uptake of Himalayan Cypress seedlings under temperate nursery conditions. The experiment was tested under Completely Randomised Design with three replications which comprised of 7 inoculants viz; Azotobacter sp, Azospirillum sp, Pseudomonas fluorescens, Bacillus subtilis, Pisolithus tinctorius, Laccaria laccata and control. The research findings show that the various microbial inoculants enhanced the plant N, P, K, Ca and Mg significantly than control. Further, the two ectomycorrhizae viz, Pisolithus tinctorius and Laccaria laccata used in the studies proved superior over rest of the inoculants and control. It was followed by Azotobacter sp, Azospirillum sp, Pseudomonas fluorescens and Bacillus subtilis respectively. Therefore, it can be concluded from the study that application of microbial inoculants at nursery stage improve the nutrient uptake of Himalayan cypress.

INTRODUCTION

The Himalayan cypress belonging to the family Coniferae is a large evergreen tree with a pyramidal crown and dropping branchlets. Trees upto 47 m height and 7.15 m in girth have been measured in Tehsil Garhwal (Troup, 1921). Bark greyish brown, peeling off in long thin strips; leaves small, scale like; seeds compressed with an orbicular wing, light reddish brown. The tree has a local distribution in the western Himalayas from Chamba to Nepal between 1800-2750 m elevations. The tree is naturally found on limestone. In its natural habitat the absolute maximum shade temperature is probably about 90°F, the absolute minimum about 15°F and the normal rainfall varies from 1000 to 2400 mm per annum. The heartwood is light brown with dark streaks, moderately hard, suitable for making furniture and building materials. It is an excellent timber for making railway sleepers. The timber of cypress varies from 1000 to 2400 mm per annum. Due to limited availability, its uses are not explored fully.

The indiscriminate use of inorganic fertilizers and pesticides is neither environmentally safe nor economically feasible. There is pressing demand for microbial inoculants for quality seedling production in nursery and also the establishment of plantation to increase the forest productivity. Bio-inoculants are cost effective, ecofriendly, cheaper and renewable sources of plant nutrients and play a vital role in maintaining long-term soil fertility and sustainability. Thus, to meet the challenges like poor regeneration, deforestation and spread of wastelands, introduction of microbial inoculants at the nursery stage of forest trees has become inevitable. Although various aspects of mycorrhizal impact of the forest trees have been studied, no work has been done on the impact of other microbial inoculants on the regeneration of forest trees. Therefore, the present study was undertaken to ascertain the influence of microbial inoculation on nutrient uptake of Himalayan Cypress (Cupressus torulosa Don) Seedlings under nursery conditions.

MATERIALS AND METHODS

The present investigations were undertaken at the Forest Nursery of Department of Forestry, Faculty of Agriculture and Regional Research Station, SKUAST-Kashmir, Wadura, Sopore during 2009-2010. Microbial inoculants isolated from rhizosphere of Himalayan cypress forest stands were used in the studies.

Mass production of microbial inoculants

The two free living aerobic nitrogen fixing bacteria viz., Azotobacter sp. and Azospirillum sp. were mass cultured using nutrient medium enriched with glucose and peptone. Plant growth promoting rhizobacteria (PGPR) viz., Pseudomonas fluorescens and Bacillus subtilis were mass propagated in King’s B nutrient broth. The two ectomycorrhizae viz., Pisolithus tinctorius and Laccaria laccata were mass multiplied in Melin Norkran’s nutrient broth and Potato Dextrose Agar, respectively.
Field operations
For the microbial inoculation, one year old seedlings of Himalayan cypress of uniform heights and collar diameter growing in polyethylene bags (9” x 7”) containing 1 kg potting material of soil and sand mixture in the ratio of 1:1 were selected.

Microbial inoculation
For inoculation, the different broth cultures of N-fixers, P-solubilizers and ectomycorrhizal inoculants isolated from local forest stands were applied to the potting material (25 ml/seedling) in the month of March, 2010, without disturbing the root system of the seedlings.

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

Plant analysis
The following procedures were adopted in the plant analysis:

Preparation of plant samples
The plant samples collected at different stages, were first washed in running tap water to remove adhering soil and other foreign material followed by dipping in dilute hydrochloric acid (0.1 N HCl). Washing was repeated with single and double distilled water. After washing, the samples were air dried on filter paper and then oven dried at 60±5°C for 24 hours. The dry matter weight for each plant sample was recorded before crushing the material. The crushed sample material was passed through 2 mm mesh sieve and stored in airtight polythene bags for chemical analysis. The initial plant nutrient status of both the species was determined at the beginning of the experiment.

Estimation of nitrogen
Oven dried plant material (0.5 g) was taken in Kjeldhal’s digestion flask and added 25 ml of concentrated H2SO4, 10 g of digestion mixture (K2SO4 + CuSO4 + FeSO4 in the ratio of 10 : 0.5 : 1) and 1 g of selenium powder was added. The samples were digested till solution became clear. Just after cooling of digested contents, volume was made up to 100 ml with distilled water. Then 10 ml of the aliquot was transferred to micro-Kjeldhals distillation flask and to 10 ml of 40 per cent NaOH solution was also added. The condenser-outlet of distillation apparatus was dipped into 4 per cent boric acid solution containing bromocresol green and methyl red indicator. After completion of distillation, boric acid was titrated against 0.005 N H2SO4 to the original shade (pink). Blank was also run for the final calculation.

Estimation of phosphorus
The plant material (0.5 g) was digested with 20 ml of triacid mixture (HNO3: HClO4: H2SO4 in the ratio of 9:4:1). The contents were heated until volume was reduced to 3-5 ml. The completion of digestion was confirmed when liquid became colourless. The volume was then made up to 100 ml by adding distilled water. Digested extract (20 ml) was taken in 50 ml volumetric flask and to it 10 ml of ammonium molybdate-vanadate solution was added. After thorough mixing, the volume was made up to 50 ml with distilled water and mixture allowed to stand for 30 minutes for blue colour development. The colour was then read at 470 nm on spectrophotometer (Bhargava and Raghupatti, 1993).

Estimation of potassium
Total potassium in the extract was estimated by flame photometer at 548 nm as per the procedure outlined by Jackson (1973).

Estimation of calcium and magnesium
Total calcium and magnesium in the plant extract was estimated as per the procedure outlined by Jackson (1973) 0.5 g of plant sample was taken in a 150 ml Erlenmeyer flask, 25 ml of normal ammonium acetate (pH = 7) was added to it. The solution was shaken for 5 minutes on a mechanical shaker and then filtered through Whatman No. 1 filter paper. 25 ml aliquot was pipetted into a 125 ml Erlenmeyer flask and it was diluted to a volume of 25 ml. 10 drop (0.5 ml) ammonium chloride buffer and 4 drops of Eriochrome black T-indicator was added. The solution was titrated with 0.01 N EDTA using 10 ml microburette. The colour changed from wine red to blue or green.

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

RESULTS AND DISCUSSION
Plant nitrogen
Perusal of the data presented in Table-1, reveals that plant nitrogen of Himalayan cypress with microbial inoculation exhibited a significant increase over control. Maximum plant nitrogen content was recorded with respect to P. tinctorius inoculation which was 26.85 per cent more then control. It was followed by L. laccata (22.54%). Azotobacteria and Azospirillum inoculations which resulted in 18.55 and 15.05 per cent more nitrogen then control. Similarly inoculation with Pseudomonas fluorescens and Bacillus subtilis gave 13.18 and 9.19 per cent more nitrogen over control, respectively. Moreover, there was an increasing trend in plant nitrogen from April to October and thereafter it showed a declining trend from December to February. The higher plant nitrogen due to application of inoculants lies in the fact that they fix atmospheric nitrogen and improve soil environment (Singh et al., 1998). Further, microbial inoculants are reported to increase the root proliferation, root volume and weight, thereby facilitating higher plant nitrogen by increasing roof surface area (Parvatham et al., 1989). Ectomycorrhizae through improved root system help to counteract the effects of nitrogen depletion zones around roots (Finlay et al., 1988) and through their mycelial network increase the uptake of NH4-N (Reid et al., 1983). Moreover, our results corroborate with the findings of several other workers (Lehri and Mehrotta, 1972; Chattoo et al., 1997; Skvorna et al., 2002). The decrease in total plant nitrogen content in the later half of study may be attributed to relatively rapid increase in leaf dry matter which caused dilution (Meclung and Lott, 1956; Emmert, 1959; Smith, 1962; Evans, 1979; Verma and Mishra, 1989) and back translocation of nitrogen from leaves to woody components during late autumn and winter (Alban, 1985). Similarly the higher plant nitrogen in case of fast growing Himalayan cypress seedlings could be attributed to its efficient nutrient
uptake capacity and rapid mineralization of organic matter and nutrient release (Maharudrappa et al., 2000). Pisolithus tinctorius inoculation which was 58.33 per cent more than control. It was followed by L. laccata (50%).

Table 1 Impact of microbial inoculation on total plant N (%) of Himalayan cypress (Cupressus torulosa Don) at nursery stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>April</th>
<th>June</th>
<th>August</th>
<th>October</th>
<th>December</th>
<th>February</th>
<th>2009 Mean</th>
<th>2010 Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.50</td>
<td>0.68</td>
<td>0.77</td>
<td>0.95</td>
<td>0.94</td>
<td>0.92</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.63</td>
<td>0.84</td>
<td>1.05</td>
<td>1.13</td>
<td>1.12</td>
<td>1.10</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>0.60</td>
<td>0.81</td>
<td>1.02</td>
<td>1.08</td>
<td>1.07</td>
<td>1.05</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0.57</td>
<td>0.80</td>
<td>0.98</td>
<td>1.05</td>
<td>1.04</td>
<td>1.03</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.52</td>
<td>0.78</td>
<td>0.93</td>
<td>1.01</td>
<td>1.00</td>
<td>0.99</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Pisolithus tinctorius</td>
<td>0.73</td>
<td>0.95</td>
<td>1.12</td>
<td>1.25</td>
<td>1.24</td>
<td>1.20</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.60</td>
<td>0.82</td>
<td>0.99</td>
<td>1.09</td>
<td>1.08</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Impact of microbial inoculation on total plant P (%) of Himalayan cypress (Cupressus torulosa Don) at nursery stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>April</th>
<th>June</th>
<th>August</th>
<th>October</th>
<th>December</th>
<th>February</th>
<th>2009 Mean</th>
<th>2010 Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.04</td>
<td>0.07</td>
<td>0.08</td>
<td>0.10</td>
<td>0.09</td>
<td>0.07</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>0.02</td>
<td>0.05</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0.07</td>
<td>0.09</td>
<td>0.11</td>
<td>0.13</td>
<td>0.12</td>
<td>0.10</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.05</td>
<td>0.07</td>
<td>0.09</td>
<td>0.11</td>
<td>0.10</td>
<td>0.08</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Pisolithus tinctorius</td>
<td>0.10</td>
<td>0.11</td>
<td>0.13</td>
<td>0.15</td>
<td>0.14</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.08</td>
<td>0.08</td>
<td>0.11</td>
<td>0.13</td>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Impact of microbial inoculation on total plant K (%) of Himalayan cypress (Cupressus torulosa Don) at nursery stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>April</th>
<th>June</th>
<th>August</th>
<th>October</th>
<th>December</th>
<th>February</th>
<th>2009 Mean</th>
<th>2010 Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.04</td>
<td>0.14</td>
<td>0.26</td>
<td>0.38</td>
<td>0.37</td>
<td>0.35</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.12</td>
<td>0.23</td>
<td>0.35</td>
<td>0.45</td>
<td>0.43</td>
<td>0.41</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>0.10</td>
<td>0.21</td>
<td>0.33</td>
<td>0.43</td>
<td>0.41</td>
<td>0.39</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0.09</td>
<td>0.19</td>
<td>0.32</td>
<td>0.42</td>
<td>0.40</td>
<td>0.38</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.07</td>
<td>0.17</td>
<td>0.30</td>
<td>0.40</td>
<td>0.39</td>
<td>0.37</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Pisolithus tinctorius</td>
<td>0.15</td>
<td>0.27</td>
<td>0.38</td>
<td>0.48</td>
<td>0.47</td>
<td>0.45</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.13</td>
<td>0.25</td>
<td>0.36</td>
<td>0.45</td>
<td>0.44</td>
<td>0.43</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

Plant phosphorus

Table-2, presents the data on impact of microbial inoculation on total plant phosphorus of Himalayan cypress at nursery stage. The data indicate that the plant phosphorus was increased with the application of various inoculants. Maximum plant phosphorus was recorded in response to Pseudomonas fluorescens (50%), Bacillus subtilis (37.50%), Azotobacter (28.57%) and Azospirillum (16.66%), respectively. Moreover the two treatments viz. L. laccata and Pseudomonas fluorescens were at par with each other. Further an increasing trend in plant phosphorus content was noticed from April to October and thereafter a decreasing trend was observed. Similar observations have been reported by various
other workers (Raj et al., 1981; Bopaiah and Khader, 1989; Mukherji and Sharma, 1993). Hattingh (1975) attributed the increased P uptake to extensive and continued growth of mycorrhizal roots extending beyond the vicinity of root surface. Further solubilization effect on native phosphorus and release of several organic acids might have enhanced the availability of phosphorus (Singh et al., 1998). Further ectomycorrhizal fungi catalyse hydrolysis of inorganic phosphorus and thereby enhance phosphorus uptake by roots (Gerlitz and Work, 1994). Moreover, the physiological basis for decline in plant phosphorus content in last months of experiment are probably the same as documented for nitrogen content fluctuations. These findings are in agreement with findings of Guha and Mitchell (1966). Similarly the reasons for higher plant phosphorus in Himalayan cypress seedlings are probably the same as documented for plant nitrogen.

Table 4 Impact of microbial inoculation on plant exchangeable Ca (%) of Himalayan cypress (Cupressus torulosa Don) at nursery stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2009</th>
<th>2010</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
<td>June</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.50</td>
<td>0.66</td>
<td>0.85</td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.67</td>
<td>0.90</td>
<td>1.09</td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>0.66</td>
<td>0.88</td>
<td>1.17</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0.62</td>
<td>1.10</td>
<td>1.36</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.63</td>
<td>0.84</td>
<td>1.13</td>
</tr>
<tr>
<td>Pisolithus tinctorius</td>
<td>0.71</td>
<td>1.12</td>
<td>1.34</td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.69</td>
<td>0.96</td>
<td>1.26</td>
</tr>
<tr>
<td>Mean</td>
<td>0.64</td>
<td>(0.039)</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Initial plant exchangeable Ca = 0.48 %
Figures in parenthesis indicate CD of individual months

Table 5 Impact of microbial inoculation on total plant Mg (%) of Himalayan cypress (Cupressus torulosa Don) at nursery stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2009</th>
<th>2010</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
<td>June</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.04</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Azotobacter sp.</td>
<td>0.13</td>
<td>0.25</td>
<td>0.45</td>
</tr>
<tr>
<td>Azospirillum sp.</td>
<td>0.12</td>
<td>0.35</td>
<td>0.44</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>0.09</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.07</td>
<td>0.31</td>
<td>0.43</td>
</tr>
<tr>
<td>Pisolithus tinctorius</td>
<td>0.16</td>
<td>0.35</td>
<td>0.49</td>
</tr>
<tr>
<td>Laccaria laccata</td>
<td>0.15</td>
<td>0.27</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean</td>
<td>0.10</td>
<td>(0.024)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Initial total plant Mg = 0.02 %
Figures in parenthesis indicate CD of individual months

Plant potassium

As evident from Table-3, the potassium content of Himalayan cypress at nursery stage got improved by the application of bioinoculants as compared to control. Inoculation with Pisolithus tinctorius resulted in maximum plant potassium content which was 30.55 per cent more over control and proved best over other microbial inoculants. It was followed by L. laccata (26.47%), Azotobacter (24.24%), Azospirillum (19.35%), Pseudomonas fluorescens (16.12%) and Bacillus subtilis (10.71%), respectively over control. Further plant potassium content demonstrated an increasing trend from April to October and a decreasing trend from October to February. The increase in plant potassium may be related to the production of hormones by microbial inoculants (Tien et al., 1979). Further soil consists of vast array of microorganisms and many of them might have been favoured by growth promoting substances released by microbial inoculants. These organisms with their complex enzymatic activities might have improved the plant K. Similar results have also been reported by other researchers (Willy et al., 1983; Samui et al., 1987; Ahlawat et al., 1995). Further, the decline in total plant potassium content in the later half of study may be attributed to relatively rapid increase in leaf dry matter which caused potassium initially present in high concentration to be diluted (Mcclung and Lott, 1956) and back translocation of potassium from leaves to woody components during late autumn and early winter (Sampson and Samisch, 1935).

Plant exchangeable calcium

Table-4, presents data on impact of microbial inoculation on plant exchangeable calcium of Himalayan cypress at nursery stage. The data indicate that the plant calcium content was improved by the application of various microbial inoculants over control. Amongst various microbial inoculants Pisolithus...
tinctorius was superior and resulted in 24.10 per cent more plant calcium content over control. It was followed by Laccaria laccata which gave 22.72 per cent increase over control. Further there was an increasing trend in plant calcium content from April to February. Hattingh (1975) attributed the increased calcium uptake due to extensive and continued growth of mycorrhizal roots extending beyond the vicinity of root surface. Further, the increase in plant calcium content with the maturity of leaves may be due to the fact that calcium being an immobile element therefore the rate of its translocation from leaf to other sinks might be slow (Insley et al., 1981). The present findings are in line with the observation made by Negi et al. (1980); Barrueco et al. (1984) and Tolksma et al. (1987) who reported increased calcium enrichment with advancement of the seasons.

### Plant magnesium

As evident from Table-5, the plant magnesium content of Himalayan cypress at nursery stage was enhanced with the application of microbial inoculants. Amongst the various microbial inoculants Pisolithus tinctorius and Laccaria laccata proved superior over rest of the inoculants. Inoculation with Pisolithus tinctorius and Laccaria laccata resulted in 52.63 and 50.00 per cent more magnesium content as compared to control. Plant magnesium content demonstrated an increasing trend from April to October and a decreasing trend in the last months of December and February. The enhancement in plant magnesium could be ascribed to atmospheric fixation of N by microbial inoculants, which itself increases the magnesium content of plants, which is the main constituent of chlorophyll a and b. Further the general decline in mean content of the element with the advancement of leaf maturity was basically due to dry matter accumulation leading to dilution effect. Our results are in well conformity with the findings of Ohmann et al. (1978) who concluded that foliar magnesium concentration decreased with the passage of time.

### Acknowledgements

We thank to Mr. Younus Ahmad bhat and Mr. Rafiq Ahmad of M/s Universal Computers for their help in preparing the manuscript. This research project was supported by Division of Env. Science and Faculty of Forestry, SKUAST-Kashmir-India.

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1017


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