The studies on growth parameters of Rosa polyantha by the application of bio-fertilizer

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ABSTRACT

Azotobacter are dominant in the rhizosphere of plants and they help in phosphate solubilisation. They belong to the family Azotobacteraceae and they are used as broad spectrum biofertilizers. In the present study four samples were collected from the rhizosphere regions of the plants namely Raphanus sativus (radish), Daucus carota (carrot), Helianthus annuus (sun flower) and Allium cepa (onion). The collected samples were isolated and identified as isolates 1, 2, 3 and 4. Biochemical tests such as motility test, catalase test, oxidase test, starch hydrolysis, litmus milk and phosphate solubilisation were performed. Further pot experiments were conducted to determine the root length, shoot length and number of leaves in Rosa polyantha stems cuttings in the 10th, 30th and 60th day respectively. The results showed an increase in root length, shoot length and number of leaves in biofertilizer treated plant when compared with the control and inorganic fertilizer.

Keywords: Biofertilizer, Azotobacter, Rosa polyantha, Rhizosphere.

1. INTRODUCTION

In the early times, most of the agricultural practices used animal manure as the source of phosphorus to achieve enhanced plant growth. The essential compound for plant growth is phosphorus and it increases the productivity of plants. Phosphorus plays a key role in various physiological activities such as photosynthesis, cell division, enhanced root system and carbohydrate utilisation (Kannayyan et al. 2004). Deficiency of phosphorus leads to brown leaves, weak stem as well as slow development. During the 19th and 20th century inorganic compounds containing potassium, phosphorus and nitrogen were used as fertilizers. To feed the growing human population chemical fertilizers were used to increase crop production and to stabilise the demand for food. The increase in the manufacturing cost and hazardous nature of chemical fertilizers towards the environment, has led the mankind to show interest in the use of biofertilizer which leads to better crop yield, low production cost and enhanced environmental sustainability (Sachin, 2009).

Biofertilizers are the preparations of the living microbial cells (bacteria) which are used for improving the plant growth and productivity. Rhizobium spp are the first biofertilizers identified, which is being used commercially as inoculants for various plants (Kannayyan, 2002). Research in the field of biofertilizers has led to the development of different forms of biofertilizers which includes nitrogen fixing as well as phosphate solubilising microorganism. Most of the essential plant nutrients, including phosphorus remain in insoluble form in soil (Abd-Alla , 1994; Yadav and Dadarwal, 1997). A large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilised after application and becomes unavailable to plants, thus the release of insoluble and fixed forms of phosphorus in an important aspect of increasing soil phosphorus availability (Yadav and Dadarwal, 1997). Assimilation of phosphate takes place through the enzyme “phosphatase” which is found in various soil microorganisms. Inoculation of the phosphate solubilising bacteria along with seed or soil is found to improve the solubilisation of soil phosphorus resulting in the better crop production (Yosef et al. 1999). Phosphorous, facilitates flower formation and also helps in fruit production, quality of fruits, vegetables and grains drops may also be improved (Bisen and verma, 1996).

The normal phosphorus level in soil is found to be 400 - 1200 mg/kg of soil (Fernandez and novo, 1998). Microorganisms play a key role in the natural phosphorus cycle. This cycle does not interchange with the atmosphere and mainly takes place through cyclic oxidation and reduction of phosphorus compounds which involves the electron transfer reactions. Phosphate reserves of soil are found in most of the agricultural soils which is the result of continuous application of phosphate fertilizers (Richardson, 1994). Soluble inorganic phosphates applied as a chemical fertilizer gets immobilised as soon as applied and it becomes unavailable to the plants (Dey, 1988). Another major component of the soil phosphorus is organic matter which nearly constitutes to 30-50 % of the total phosphorus in soils, its range may be very as low as 5% to very high as 95%, it is found mostly in inositol phosphate form and prepared mainly by the microorganisms. Inositol phosphate is the most stable form of the organic phosphates in the soil (Dalal, 1977; Harley and Smith, 1983).

Many bacterial inoculants such as Azotobacter, Rhizobium, Azospirillum have been used in plants to increase its yield, among them Azotobacter is found to be more efficient and they are found in different places such as soil, water, surfaces of roots (rhizosphere) and leaves (phyllosphere). Few species of Azotobacter is also found in...
Table 1 Effect of biofertilizer, inorganic fertilizer on growth parameters of Rosa polyantha

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Root length</th>
<th>Shoot length</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1.10</td>
<td>11.20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Inorganic fertilizer</td>
<td>1.70</td>
<td>14.40</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Bio fertilizer</td>
<td>1.86</td>
<td>18.32</td>
<td>16</td>
</tr>
<tr>
<td>10th day</td>
<td>Control</td>
<td>2.61</td>
<td>13.65</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Inorganic fertilizer</td>
<td>2.85</td>
<td>17.28</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Biofertilizer</td>
<td>2.93</td>
<td>19.72</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.87</td>
<td>14.90</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Inorganic fertilizer</td>
<td>3.28</td>
<td>20.23</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Biofertilizer</td>
<td>3.90</td>
<td>23.24</td>
<td>30</td>
</tr>
<tr>
<td>30th day</td>
<td>Control</td>
<td>3.90</td>
<td>23.24</td>
<td>30</td>
</tr>
<tr>
<td>60th day</td>
<td>Control</td>
<td>3.90</td>
<td>23.24</td>
<td>30</td>
</tr>
</tbody>
</table>

The studies on growth parameters of Rosa polyantha by the application of biofertilizer and inorganic fertilizer have been reported (Divyashree et al., 2013). Many evidences which supports the ability of the microorganisms to supply phosphorus to plants as a consequence of phosphate solubilisation has been reported (Richardson, 1994; Krasilnikova, 1961; Lifshitz, 1987). Growth of the maize and lettuce was enhanced by the application of phosphate solubilising bacteria was reported by (Chabot et al. 1993). Some of the strains of Azotobacter also has the capability to produce little concentrations of IAA. In the present study four samples of Azotobacter were isolated from rhizosphere region of soil and identified as isolates 1, 2, 3 and 4. Biochemical tests such as motility test, catalase test, oxidase test, starch hydrolysis, litmus milk and phosphate solubilisation test were performed. Further pot experiments were conducted to determine the root length, shoot length and number of leaves in Rosa polyantha stems cuttings in the 10th, 30th and 60th day respectively. The results showed an increase in root length, shoot length and number of leaves in biofertilizer treated plant when compared with the control and inorganic fertilizer.

2. MATERIALS AND METHODS

2.1. Collection of soil samples
Soil samples were collected from different areas in Bangalore, in the month of January 2013. Following are the different samples collected from different regions.

1. Raphanus sativus (radish) collected from MVJ College of engineering, Channasandra, Bangalore.
2. Daucus carota (carrot) collected from the vegetable field, Channasandra, Bangalore.
3. Helianthus annuus (sun flower) from Cox town, Bangalore.
4. Allium cepa (onion) collected from the MVJ College of engineering, Bangalore.

Soils were collected from the rhizosphere region of the land. Roots were carefully dug out and the rhizospheric soil samples were taken into sterile plastic bags and stored at temperature of 4°C.

2.2. Isolation of bacteria
Collected samples were serially diluted and inoculated on the Jenson’s media. Sub culturing was done to obtain pure culture.

2.3. Characterisation based on colony morphology
Colony morphology of the isolated pure colonies on the streaked plates was examined. Different characteristics such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc were recorded.

2.4. Gram staining
Gram staining was performed to observe colony morphology.

2.5. Biochemical Tests
2.5.1. Motility test
Mobility of the bacterial cells were observed using stab agar method.

2.5.2. Catalase test
3% hydrogen peroxide was added to the 48 hr old bacterial colony. The effervescence revealed the positive catalase activity.

2.5.3. Oxidase test
Redox indicator reagent was added directly to the bacterial colony. It was checked for the development of purple colour.

2.5.4. Starch hydrolysis
Starch agar plates (without nitrogen source) were prepared and the plates were aseptically inoculated and kept for incubation. Amylase enzyme produced by the organism degraded the starch and hence the formation of zone was seen and the results were observed.

2.5.5. Litmus milk test
Litmus milk broth was prepared and inoculated. Peptinisation of the milk proteins reveals clearer solution which confirms the positive test.

2.6. Phosphate solubilisation test
NBRI P (National Botanical Research Institute’s Phosphate) media was used in the phosphate solubilisation by Azotobacter. Development of the zone surrounding the colony revealed the positive result.

2.7. Pot experiments
The stem cuttings of Rosa polyantha plants were planted in three different pots containing various fertilizers and watered regularly. The following parameters namely the root length, shoot length and the number of leaves were observed on 10th, 30th and the 60th day after planting. The length of the root was measured from the collar region to the growing tip of the root and expressed in cm. The length of the shoots was measured from the collar region, up to the tip of the shoot and expressed in cm. The total number of leaves in each plant was counted on 10th, 30th and 60th day after planting and expressed as number of leaves per plant for the purpose of statistical analysis. Duncan’s multiple range test was applied for comparing the treatments.

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of the collected sample
The samples were successfully collected from rhizosphere region of Raphanus sativus (radish), Daucus carota (carrot), Helianthus annuus (sun flower), Allium cepa (onion). The isolated samples were identified as isolates 1, 2, 3 and 4. All the isolates were gram negative rods. The results of motility, catalase, oxidase, starch hydrolysis and litmus tests were found to be positive for all four isolates.

3.2. Phosphate solubilization
When the isolates were grown in culture media supplemented with calcium phosphate all the isolates produced halo zone around the colonies indicating the solubilisation of the phosphate. Phosphate solubilisation is

- tropical and Polar Regions but the bacterial population varies in different soil conditions. They are very hardly found in acidic soil but easily found in neutral and alkaline soil conditions (Jensen, 2000). They are non-symbiotic bacteria which is widely being used in production of biofertilizers and it is capable of solubilising phosphate by the action of the enzyme phosphatase. Inoculation of the Azotobacter spp. with the barley grains resulted in increased plant growth, dry matter and nitrogen content in the soil (Monib et al. 1979). Many evidences which supports the ability of the microorganisms to supply phosphorus to plants as a consequence of phosphate solubilisation has been reported (Richardson, 1994; Krasilnikova, 1961; Lifshitz, 1987). Growth of the maize and lettuce was enhanced by the application of phosphate solubilising bacteria was reported by (Chabot et al. 1993). Some of the strains of Azotobacter also has the capability to produce little concentrations of IAA. In the present study four samples of Azotobacter were isolated from rhizosphere region of soil and identified as isolates 1, 2, 3 and 4. Biochemical tests such as motility test, catalase test, oxidase test, starch hydrolysis, litmus milk and phosphate solubilisation test were performed. Further pot experiments were conducted to determine the root length, shoot length and number of leaves in Rosa polyantha stems cuttings in the 10th, 30th and 60th day respectively. The results showed an increase in root length, shoot length and number of leaves in biofertilizer treated plant when compared with the control and inorganic fertilizer.
detected by the formation of clear halo zone around the colonies. The halo zone is produced due to the solubilization of insoluble phosphate which is mediated by the production of organic acids in the surrounding medium. Further confirmation of phosphate solubility was determined by phospomolybdate test. The results showed that isolate 2 showed greater solubility when compared with other isolates.

3.3. Pot experiments
In pot experiments, the growth of *Rosa polyantha* stem cuttings are tabulated (Table 1). There is an increase in root length, shoot length and number of leaf count when the cuttings (T3) were treated with biofertilizer. The stem cuttings showed maximum root length 3.90 cm, shoot length 23.34 cm and leaf count 30 in biofertilizer treated pot on 60th day after planting when compared to inorganic manure and control plants. Maximum growth in biofertilizer treated plant was mainly due to the ability of Azotobacter to solubilise phosphate and produce siderophores and hormones (Khan et al. 2009). Statistical data analyzed by DMRT resulted in significant differences between the mean of the treated and control plants.

4. CONCLUSION
From the present study it may be concluded that Azotobacter inoculants have significant growth promoting effect on various growth parameters like root length, shoot length and number of leaves in the potted plant. The scope of further study includes that Azotobacter can be used as an efficient microbial inoculants for enhancing the growth of plants when compared with inorganic fertilizers.

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REFERENCES
8. Jones DH, Darrah PR. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere *Plant soil*, 1994, 166, 247-257
16. Sachin DN. Effect of Azotobacter chroococcum (PGPR) on the Growth of Bamboo (Bambusa bamboo) and Maize (Zea mays) Plants. Department of Tissue Engineering, Allahabad agricultural institute-Deemed University, Allahabad, India, Biofrontiers, 2009, 24-31