CHAPTER IV

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RESULTS AND DISCUSSION

The results recorded in the present investigation are furnished and discussed as follows.

4.1.0 Field survey and enumeration of Frankia population from major Casuarina growing areas of Kerala

Three major Casuarina plantations in Kerala were selected for the assessment of Frankia population. They were Punaloor, Thrissur and Alapuzha. The species of Casuarina grown in all the fields under study were Casuarina equisetifolia. The age of trees of Punaloor plantation were of 4 years, Thrissur 6 years and Alapuzha plantation were of 20 years. Physicochemical properties of soil samples collected from these locations were analysed and the isolation and enumeration of Frankia population from soil samples and root nodules of Casuarina plants were conducted.

4.1.1 Physicochemical properties of soil samples under study

Physical and chemical factors of soil samples such as soil colour, texture, pH, electrical conductivity and available nutrient contents were analysed and presented in Table 1. Soils of all the three locations were brown in colour. Texture of Punaloor and Thrissur soils were clay loam where as that of Alapuzha was sand.
only. pH values of Punaloor soil and Thrissur soil were found to be acidic with pH 5.96 and 5.43 respectively, whereas that of Alapuzha soil was tending to be alkaline with a pH value of 7.88. Electrical conductivity was slightly higher in Alapuzha soil (0.11 mmhos/cm) when compared to Punaloor (0.07mmhos/cm) and Thrissur (0.04 mmhos/cm). With regard to the available nutrient contents of soil samples, Thrissur soil rated higher in available nitrogen content (16.8mg/100g) when compared to Punaloor (12.5mg/100g) and Alapuzha samples (8.0mg/100g). The soil phosphorus content values were observed as Alapuzha 0.9mg/100g, Punaloor sample 0.3 mg/100g and Thrissur sample 0.15mg/100g. With respect to soil available potassium content, the Punaloor soil and Thrissur soil recorded almost same range with values 11.80mg/100g and 11mg/100g respectively whereas Alapuzha soil recorded a low level of potassium indicating 2.0mg/100g. The available calcium (4.80mg/100g) and magnesium content (1.60mg/100g) were observed to be more in Alapuzha sample. This was followed by Thrissur soil with calcium 4.65mg/100g and magnesium 1.55mg/100g. When compared to Alapuzha and Thrissur samples, the values were low in Punaloor samples with calcium content 4.05mg/100g and magnesium content 1.35mg/100g.
It was observed from Table 1 that the soil samples collected from Punaloor and Thrissur were brown clay loam where as it was brown sandy in Alapuzha. The pH of soils of Punaloor and Thrissur clearly indicated acidic nature whereas in Alapuzha soil it was slightly tending to alkaline registering a pH value of 7.88. Smolander and Sundman (1987) observed a strong positive correlation between pH and *Frankia* population. The result is in accordance with the suggestion of Lechevalier and Lechevalier (1989) that most *Frankia* grow best at a slightly acid to neutral pH. The EC values in all the locations showed harmless salt content. Thrissur soil recorded a higher available nitrogen status, whereas both Punaloor and Alapuzha soils were registering medium level of available nitrogen content. The available phosphorus content was low in all the soils. The available potassium was medium in status in Punaloor and Thrissur whereas it was low in Alapuzha soils. The available calcium was low in all the soils similar to that of available phosphorus. The available magnesium value was medium l in all the soils.
Table 1

Physico-Chemical properties of soil samples under study.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Locations</th>
<th>Depth (cm)</th>
<th>Soil colour</th>
<th>Soil texture</th>
<th>Soil pH</th>
<th>EC mmhos/cm</th>
<th>Available nutrients (mg/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>Punaloor</td>
<td>0-30</td>
<td>Brown</td>
<td>Clay loam</td>
<td>5.96</td>
<td>0.07</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>Thrissur</td>
<td>0-30</td>
<td>Brown</td>
<td>Clay loam</td>
<td>5.43</td>
<td>0.04</td>
<td>16.8</td>
</tr>
<tr>
<td>3</td>
<td>Alapuzha</td>
<td>0-30</td>
<td>Brown</td>
<td>Sand</td>
<td>7.88</td>
<td>0.11</td>
<td>8.0</td>
</tr>
</tbody>
</table>

4.1.2 Isolation, enumeration and identification of Frankia from soil samples under study

Frankia colonies from soil samples were isolated in DPM medium by modified phenol-sucrose fractionation method. The mean values were recorded in Table 2 and Figure 1. (Plate 6) It was observed from Table 2 that the highest population of Frankia was recorded in Punaloor soils with a mean value of 86cfugm⁻¹ followed by Thrissur soils with a mean value of 69cfugm⁻¹. In Alapuzha the population of Frankia recorded was 49cfugm⁻¹. When the three locations under study were compared it was shown that the Punaloor soil samples recorded maximum number of colonies. Therefore it is observed that the Punaloor soil samples had the highest population of Frankia colonies among all the locations under study.
**Table 2**  
Isolation and enumeration of *Frankia* from soil samples under study

<table>
<thead>
<tr>
<th>Locations</th>
<th>No of <em>Frankia</em> colonies (cfu/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replications</td>
</tr>
<tr>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>Punaloor</td>
<td>88</td>
</tr>
<tr>
<td>Thrissur</td>
<td>70</td>
</tr>
<tr>
<td>Alapuzha</td>
<td>50</td>
</tr>
</tbody>
</table>

**Figure 1**  
Population of *Frankia* species in soil under study (cfu/gm)
Characteristic features of *Frankia* colonies isolated from soil samples were presented in Table 3. The colonies obtained from all the locations were compact in nature. They were Gram positive and catalase positive. Colonies from Punaloor soil presented an excellent growth where as the isolates from Thrissur and Alapuzha recorded a normal growth pattern. The colonies were composed of hyphae, sporangia and vesicles embedded in mucilage. Hyphae were filamentous, septate and branched. Sporangia were in intercalary positions within hyphae and was club shaped. Vesicles were bulb shaped, attached to substrate mycelium by a stalk.

**Table 3**

**Characteristic features of *Frankia* isolated from soil samples**

<table>
<thead>
<tr>
<th>No.</th>
<th>Locations</th>
<th>Growth</th>
<th>Colony</th>
<th>Hyphae (Size)</th>
<th>Sporangia (Nos)</th>
<th>Vesicles (Nos)</th>
<th>Gram reaction</th>
<th>Catalase test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Punaloor</td>
<td>Excellent</td>
<td>Compact</td>
<td>Normal</td>
<td>Numerous</td>
<td>Numerous</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Thrissur</td>
<td>Normal</td>
<td>Compact</td>
<td>Normal</td>
<td>Few</td>
<td>Few</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Alapuzha</td>
<td>Normal</td>
<td>Compact</td>
<td>Small</td>
<td>Few</td>
<td>Rare</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
4.1.3 Isolation and enumeration of *Frankia* colonies from the root nodules of *Casuarina equisetifolia* under study

Differential filtration technique was adopted to isolate *Frankia* colonies from root nodules. Three different media were used for the isolation and the observations were recorded in Table 4 and Figure 2. It was observed that the highest population were recorded in Punaloor sample with a mean value of 72 cfugm$^{-1}$ followed by Thrissur sample with 60 cfugm$^{-1}$ and the lowest population were recorded in Alapuzha with a mean value 40 cfugm$^{-1}$ of *Frankia* colonies.

**Table 4**

Isolation of *Frankia* colonies from the root nodules of *Casuarina equisetifolia* in different media.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Locations</th>
<th>No. of <em>Frankia</em> colonies(cfu/gm) in different media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Q MOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>1</td>
<td>Punaloor</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Thrissur</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Alapuzha</td>
<td>7</td>
</tr>
</tbody>
</table>

DPM= Defined Propionate Minimal Medium
4.1.3.1. Selection of the most suitable medium for the isolation and growth of *Frankia*

Qmod medium, BuCT medium and Defined propionate minimal medium were tested to select the favourable medium for the culturing of *Frankia* species (Table 4). The growth were found to be maximum in the BuCT medium and minimum in Qmod medium. DPM medium exhibited medium growth.
Presence or absence of *Frankia* population in soil and root nodules could be correlated with soil pH, soil texture, moisture content, chemical composition, plant factor or the site history (Akkermans *et al.*, 1991). Similar findings were also recorded in the present study. The slightly acidic pH of the Punaloor and Thrissur soil may favour the higher *Frankia* population when compared to slightly alkaline Alapuzha soil. According to Kant and Narayana (1978), the symbiotic nitrogen fixation, nodule formation and hence *Frankia* growth are affected by both deficiency and excess water content (soil moisture) in the root zone of host plants. This may be a reason for the higher number of *Frankia* colonies in the clay loam soil of Punaloor and Thrissur plantation when compared to sandy soil of Alapuzha, as the moisture content is supposed to be more in clay loam textured soil samples. Direct soil isolations will give a more accurate reflection of soil *Frankia* population because they do not involve symbiotic selection by a host plant (Baker and O'Keefe, 1984). From the above reasons, it was observed that the genetic make up of plants, plant age, physiochemical properties of soils of the studies and composition of isolation medium might be the various factors that determine the *Frankia* population.
The results revealed that the *Frankia* population in root nodules of Punaloor samples recorded the highest value. This might be due to the general opinion that the plant age can be one of the factors that regulate *Frankia* population in *Casuarinas*. The results also revealed that the media components have an effect on the growth of *Frankia* colonies. BuCT medium on which successful isolation were attained, composed of sodium propionate as carbon source and casaminoacids as the nitrogen source. Sodium propionate is the major component of DPM medium also. The results indicated that propionate present in BuCT medium and DPM medium might have favoured better growth of *Frankia* when compared to Qmod medium which consisted of yeast extract, bactopeptone and lecithin as the major constituents. Blom (1981); Shipton and Burggraaf (1982b) reported that propionate is the most effective carbon source for various strains of *Frankia*. Also the presence of “Tween 80”as another carbon source along with propionate in BuCT medium made it most suitable for the growth of this strain of *Frankia*. The effective growth of *Frankia* on media containing Tween 80 as carbon source was reported by Blom *et. al.*, (1980). From the results it is concluded that BuCT medium containing sodium propionate and Tween 80 as carbon source together with casamino acid as nitrogen source should
have provided an effective nutritional requirement for the growth of *Frankia*. This result is in contrast to the findings of Diem and Dommergues (1983); Debadin Bose and Arnabsen (2006) who reported that Qmod medium provided better growth of *Frankia* colonies. The present studies also support the view of Shipton and Burggraaf (1982a) that there is no universally superior medium for *Frankia* strains.

### 4.1.3.2 Morphological and cultural characteristics of *Frankia* isolated from root nodules

The growth pattern of *Frankia* in different media is depicted in Table 5. Colonies were developed after 4 weeks. They were compact cushion like in BuCT medium and DPM medium with a dense networks of branched septate hyphae bearing clubshaped sporangia and few bulb shaped vesicles. Sporangia were found at the hyphal tips. Whereas the colonies developed in Qmod agar medium exhibited white diffuse colonies having loose networks of hyphae around the centre bearing typical spore bearing sporangia with a fluffy periphery. Sporangia was club shaped and colonies appeared to be embedded in a mucilageneous material. With regard to growth rate and sporulation, BuCT medium exhibited excellent growth and high sporulation. In DPM medium normal growth rate was observed and
poor growth was recorded in Qmod medium. Therefore for further isolation, BuCT medium was selected as the most favourable medium. Nodule sections, when stained with trypan blue in lactophenol, and observed microscopically, typical *Frankia* colonies with bulb shaped vesicles and club shaped sporangia were found.

The isolates developed on BuCT and DPM medium were compact in nature. The strains developed in Qmod medium were diffuse type. This is a clear evidence for the polymorphism exhibited by *Frankia* colonies on different solid media as reported by Subba Rao (1998).

From the above facts it is concluded that the isolates obtained from the nodules and rhizosphere soil of *Casuarina equisetifolia* from the locations under study belongs to *Frankia* as it possessed septate branched hyphae, vesicles and sporangia which was similar to the previously described *Frankia* isolate (Diem and Dommergues; 1983; St. Laurent and Lalonde (1987) and Diem *et al.* (1982b).
Table 5

Growth pattern of *Frankia* in different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Colony</th>
<th>Sporulation</th>
<th>Sporangia</th>
<th>Vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuCT</td>
<td>Excellent</td>
<td>Compact</td>
<td>High</td>
<td>Club shaped</td>
<td>Few, bulb shaped</td>
</tr>
<tr>
<td>DPM</td>
<td>Normal</td>
<td>Compact</td>
<td>Low</td>
<td>Club shaped</td>
<td>Few, bulb shaped</td>
</tr>
<tr>
<td>Qmod</td>
<td>Poor</td>
<td>Diffuse</td>
<td>low</td>
<td>Club shaped</td>
<td>Rare, bulb shaped</td>
</tr>
</tbody>
</table>

4.2.0 Pot culture experiments

Garden soil of clay loam texture with pH 6.85 and E.C 0.13 mmhos/cm were selected for pot culture studies. The chemical properties of soil were also observed and recorded in Table 6.

Table 6

Physico chemical properties of test soil sample

<table>
<thead>
<tr>
<th>Soil parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Brown</td>
</tr>
<tr>
<td>Texture</td>
<td>Clay loam</td>
</tr>
<tr>
<td>pH</td>
<td>6.85</td>
</tr>
<tr>
<td>E.C. (mmhos/cm)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Available nutrients (mg/100g)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>8.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.60</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.6</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Table 7

Features of *Casuarina* Seedlings during Transplanting

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>Casuarina equisetifolia</em></th>
<th><em>Casuarina junghuhniana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of seedlings</td>
<td>49 days</td>
<td>49 days</td>
</tr>
<tr>
<td>Shoot Characters</td>
<td>Thick, dark green colour</td>
<td>Shoot slender, light</td>
</tr>
<tr>
<td></td>
<td>with numerous side</td>
<td>green colour with fewer</td>
</tr>
<tr>
<td></td>
<td>branches having a mean</td>
<td>side branches having a</td>
</tr>
<tr>
<td></td>
<td>2.36 cm.</td>
<td>mean length 1.88 cm.</td>
</tr>
<tr>
<td>Root Characters</td>
<td>Long highly branched</td>
<td>Moderately branched .</td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>7.8 (Mean value)</td>
<td>8.5 (Mean value)</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>3.39 (Mean value)</td>
<td>2.33 (Mean value)</td>
</tr>
<tr>
<td>Total length(cm)</td>
<td>11.19 (Mean value)</td>
<td>10.83 (Mean value)</td>
</tr>
</tbody>
</table>

4.2.1 Effect of treatments on the seedling height of *Casuarina*

49 days old seedlings of *Casuarina equisetifolia* and *Casuarina junghuhniana* having an average shoot length of 7.8cm and 8.5cm respectively were transplanted (Table 7). After 15 days treatments were imposed to the respective pots. Seedling height was recorded at one month interval upto 10 months till harvesting. Influence of treatments on the shoot length of *Casuarina* plants was assessed. Results indicated that *Frankia* inoculation had significant influence on the seedling height of *Casuarina equisetifolia* over other treatments. (Table 8, Figure 3 and Plates 8 and 9).
Table 8
Effect of treatments on the height of Casuarina plants at different intervals (Mean values in cm)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>One month</th>
<th>Two months</th>
<th>Three months</th>
<th>Four months</th>
<th>Five months</th>
<th>Six months</th>
<th>Seven months</th>
<th>Eight months</th>
<th>Nine months</th>
<th>Ten months</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>10.13</td>
<td>15.4</td>
<td>22.6</td>
<td>28.0</td>
<td>36.0</td>
<td>48.0</td>
<td>60.8</td>
<td>90.5</td>
<td>120</td>
<td>147</td>
</tr>
<tr>
<td>T2</td>
<td>11.05</td>
<td>14.65</td>
<td>24.2</td>
<td>32.0</td>
<td>41.0</td>
<td>67.0</td>
<td>88.0</td>
<td>112.0</td>
<td>132</td>
<td>163</td>
</tr>
<tr>
<td>T3</td>
<td>10.28</td>
<td>17.6</td>
<td>30.25</td>
<td>43.0</td>
<td>75.3</td>
<td>107.0</td>
<td>133.0</td>
<td>151.6</td>
<td>170</td>
<td>205</td>
</tr>
<tr>
<td>T4</td>
<td>9.73</td>
<td>13.75</td>
<td>25.0</td>
<td>38.0</td>
<td>57.0</td>
<td>86.0</td>
<td>99.0</td>
<td>120.0</td>
<td>140</td>
<td>188</td>
</tr>
<tr>
<td>T5</td>
<td>7.18</td>
<td>11.5</td>
<td>23.23</td>
<td>33.3</td>
<td>52.0</td>
<td>84.0</td>
<td>94.0</td>
<td>128.0</td>
<td>148</td>
<td>182</td>
</tr>
<tr>
<td>T6</td>
<td>9.07</td>
<td>14.75</td>
<td>29.8</td>
<td>40.0</td>
<td>59.0</td>
<td>88.0</td>
<td>98.0</td>
<td>131.0</td>
<td>152</td>
<td>190</td>
</tr>
<tr>
<td>CD 5% level</td>
<td>0.89</td>
<td>2.31</td>
<td>1.25</td>
<td>1.83</td>
<td>3.19</td>
<td>4.2</td>
<td>3.9</td>
<td>2.97</td>
<td>3.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>
4.2.2 Effect of treatments on the yield attributing characters of *Casuarina* plants

Yield attributing character of experimental plants such as root length, number of nodules per plant, basal girth and number of connates were measured and recorded (Table 9).
Table 9

Effect of treatments on the yield attributing characters of 

*Casuarina* plants (Mean values)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root length (cm)</th>
<th>Number of nodules/plant</th>
<th>Basal girth (cm)</th>
<th>Number of connates per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>28</td>
<td>20</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>T2</td>
<td>33</td>
<td>30</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>T3</td>
<td>70</td>
<td>172</td>
<td>5.6</td>
<td>52</td>
</tr>
<tr>
<td>T4</td>
<td>40</td>
<td>70</td>
<td>4.1</td>
<td>40</td>
</tr>
<tr>
<td>T5</td>
<td>35.6</td>
<td>60</td>
<td>3.9</td>
<td>39</td>
</tr>
<tr>
<td>T6</td>
<td>80</td>
<td>137</td>
<td>4.2</td>
<td>44</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td>1.84</td>
<td>2.5</td>
<td>0.44</td>
<td>1.57</td>
</tr>
</tbody>
</table>

With regard to root length, *Frankia* inoculated plants recorded higher values over the uninoculated ones. Among all the treatments, *Casuarina junghuhniana* dual inoculated treatments recorded a significantly higher value followed by *Casuarina equisetifolia* inoculated with *Frankia* alone. When observations on root length is considered, the ranking is as follows: In the case of *Casuarina*
equisetifolia $T_3 > T_5 > T_1$ and that of Casuarina junghuhniana $T_6 > T_4 > T_2$. (Figure 4 and Plates 12 and 13)

**Figure 4**

**Effect of treatments on the root length of Casuarina plants**

<table>
<thead>
<tr>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
<tr>
<td>T4</td>
</tr>
<tr>
<td>T5</td>
</tr>
<tr>
<td>T6</td>
</tr>
</tbody>
</table>

Among the characters such as the number of nodules per plant, (Figure 5) basal girth and number of connates, Casuarina equisetifolia inoculated with Frankia alone recorded a significantly higher values when compared to all other treatments. This was followed by dual inoculated Casuarina junghuhniana. The controls recorded the minimum values.
Figure 5

Effect of treatments on the number of nodules of *Casuarina* plants

The influence of treatments on the growth and yield attributing characters have been investigated in the present study. Significant increase in seedling height was observed in *Casuarina equisetifolia* inoculated with *Frankia* alone. This might be due to the existence of host specific symbiosis between *Frankia* and *Casuarina equisetifolia* and the contribution, of greater amount of nitrogen by *Frankia* as suggested by Thanun Nathan *et al.*, (2002). This confirms previous observations of a wide range of symbiotic effectiveness of *Casuarina* -
Frankia associations that were observed in studies using crushed nodule inocula (Sellestedt, 1995).

Among the yield attributing characters, the root length were significantly higher in *Casuarina junghuhniana* inoculated with both Frankia and Azotobacter. This may be one of the reasons for the fact that *Casuarina junghuhniana* is more drought resistant and tolerant to salinity stress than *Casuarina equisetifolia* (Kondas 1988). The results also supported the report of Lippmann et al. (1995) that Azotobacter inoculation enhances the root development.

Other yield attributing parameters such number of nodules/plant, basal diameter and number of connates per plant were higher in *Casuarina equisetifolia* inoculated with Frankia alone. This was followed by *Casuarina junghuhniana* inoculated with both Frankia and Azotobacter. Nodulation frequency is dependent upon soil and plant factors regulating infection and nodule development (Huss Danell & Kristin Frej 1986). Environmental factors, bacterial factors, inoculum dose and culture age of Frankia affects nodule development and distribution, but not the degree of nodulation (Wall, 2002). Nodule formation depends on host as well as Frankia, plant genotype and growing condition (Quispel, 1958). The results indicated that different species of *Casuarina* respond differently to
similar treatments. In *Casuarina equisetifolia* inoculation with *Frankia* alone enhanced growth and yield whereas in *Casuarina junghuhniana* combined inoculation proved successful. This conforms the absence of a competition between *Frankia* and *Azotobacter* for nodulation in *Casuarina junghuhniana*. This might be due to different biochemical adjustments and altered physiological state of plants. This corroborates with the report of Reddy and Desingh (2001). The interaction of *Azotobacter* along with *Frankia* increased the yield of *Casuarina junghuhniana*, but not so effective on *Casuarina equisetifolia*. Thus a positive interaction was found to exist between *Azotobacter* and *Casuarina junghuhniana*. This fact makes it necessary to find the compatibility of different microbes in the inoculant, cropplants, environment, natural soil ecosystem and their overall interaction. Therefore, it is assumed that the performance varies with species of *Casuarina* and for the successful inoculation and high productivity of *Casuarina* the compatibility of a species with *Frankia* should be taken into consideration (Lihua, 1996). This support the view of Reddell and Bowen (1985) that *Frankia* highly effective on one species may be poorly effective on another. Therefore, it is concluded that no competition exists between
Frankia and Azotobacter in Casuarina junghuhniana where as effect of Frankia dominate over Azotobacter in Casuarina equisetifolia.

4.2.3 Effect of treatments on the Agronomical Parameters of Casuarina Plants

Agronomical parameters such as shoot dry weight, root dry weight, total biomass, root: shoot ratio and quality index were analyzed. (Table 10) The present results showed that higher and similar shoot dry weight were found in Casuarina equisetifolia single and dual inoculated treatments and in Casuarina junghuhniana dual inoculated treatments. Controls recorded lowest shoot dryweight whereas the root dry weight was found to be significantly higher in Casuarina equisetifolia inoculated with Frankia alone followed by Casuarina equisetifolia inoculated with both Frankia and Azotobacter. With regard to Casuarina junghuhniana both single inoculated and dual inoculated treatments recorded similar root dry weight. Lower values were observed in uninoculated plants. Increased uptake and accumulation of nutrients due to Frankia and Azotobacter inoculation might be responsible for higher shoot and root dry weight. Similar findings was reported by Sanginga et al., (1991) and Masuka and Makoni (1995).
Table 10
Effect of treatments on the agronomical parameters of *Casuarina* plants (Mean values in oven dry basis)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot dry weight (gm)</th>
<th>Root dry weight (gm)</th>
<th>Total biomass content (gm)</th>
<th>Root : shoot ratio</th>
<th>Quality index</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>50</td>
<td>5</td>
<td>55</td>
<td>0.10</td>
<td>3.64</td>
</tr>
<tr>
<td>T2</td>
<td>75</td>
<td>10</td>
<td>85</td>
<td>0.13</td>
<td>7.36</td>
</tr>
<tr>
<td>T3</td>
<td>125</td>
<td>38</td>
<td>163</td>
<td>0.30</td>
<td>23.44</td>
</tr>
<tr>
<td>T4</td>
<td>113</td>
<td>13</td>
<td>126</td>
<td>0.11</td>
<td>9.49</td>
</tr>
<tr>
<td>T5</td>
<td>125</td>
<td>25</td>
<td>150</td>
<td>0.19</td>
<td>15.53</td>
</tr>
<tr>
<td>T6</td>
<td>125</td>
<td>13</td>
<td>138</td>
<td>0.09</td>
<td>9.5</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td>0.33</td>
<td>1.56</td>
<td>2.04</td>
<td>0.16</td>
<td>5.86</td>
</tr>
</tbody>
</table>

Figure 6

Effect of treatments on the total biomass of *Casuarina* plants
*Casuarina equisetifolia* inoculated with *Frankia* alone recorded highest total biomass content (Figure 6), root: shoot ratio and quality index (Figure 7). According to Gurumurthi and Rawat (1989), *Casuarina equisetifolia* accumulates 80 percent of biomass in the main stem and it can improve soil nitrogen and has inherent morphological characters to withstand water and nutrient stress when compared to other varieties of *Casuarina*. The biomass accumulation ratio is positively related to plant age (Srivastava, 1995). Studies of Thanunathan *et al.*, (2002) supports the present result that *Frankia* inoculation can improve the nitrogen fixation, total biomass production and quality index in *Casuarina equisetifolia*. Whereas a contrasting report from Mansour and Baker (1994) suggests that biomass productivity was more in a *Frankia* inoculated hybrid of *Casuarina cunninghamiana* and *Casuarina glauca* than *Casuarina equisetifolia*. 
4.2.4 Influence of treatment on the physico chemical parameters of post harvest soil samples

Changes in the soil properties due to the treatments were assessed and were compared with the preharvest soil parameters (Table 11). The soil pH value 6.85 observed in preharvest sample were found to be changed due to the treatments. When the treatments of *Casuarina equisetifolia* was considered those inoculated with *Frankia* alone recorded a pH value of 7.06, near neutrality and combined inoculated treatment with pH 7.14 tending slightly to alkalinity. Whereas in *Casuarina junghuhnniana* inoculated with both
Frankia and Azotobacter recorded a pH value of 7.19 and the treatment with Frankia alone recorded a pH value of 6.90. The electrical conductivity of soil before experiment was 0.13 mmhos/cm. EC was found to be highest in Casuarina junghuhniana inoculated with both Frankia and Azotobacter with a value of 0.17 mmhos/cm whereas the Casuarina junghuhniana inoculated with Frankia alone show no change in EC. In the Casuarina equisetifolia also the single inoculated treatments showed increased EC value when compared to the dual inoculated treatments. With regard to the chemical properties, the available nitrogen value were observed to be higher in Casuarina equisetifolia inoculated with Frankia alone (15.9mg/100g) followed by Casuarina junghuhniana inoculated with both Frankia and Azotobacter (13.6 mg/100g). Casuarina equisetifolia, dual inoculated treatment recorded a value of 12.4mg/100g and Casuarina junghuhniana inoculated with Frankia alone with a value of 11.2 mg/100g. A change in available phosphorus content were observed due to the effect of treatment. Before treatment the soil phosphorous value were recorded to be 0.6mg/100g. A significant change in available phosphorus content were observed in Casuarina junghuhniana inoculated with Frankia alone (0.99mg/100mg) immediately followed by Casuarina equisetifolia inoculated
with *Frankia* alone (0.97mg/100g). The available potassium, calcium and magnesium content rated higher in *Casuarina junghuhniana* inoculated with *Frankia* alone with the values K-6.55mg/100g, Ca-6.90mg/100g and Mg-0.93mg/100g respectively. This is followed by *Casuarina equisetifolia* with *Frankia* treatment with potassium (6.43mg/100g) and Mg (0.88mg/100g) content where as with regard to the calcium content, *Casuarina equisetifolia* combined inoculation ranked next with a value of 6.34mg/100g.

**Table 11**

**Influence of treatments on the physico chemical parameters of post harvest soils under study (Mean values)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil colour</th>
<th>Soil texture</th>
<th>Soil pH</th>
<th>EC mmhos/cm</th>
<th>Available nutrients (mg/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Brown</td>
<td>Clay loam</td>
<td>6.82</td>
<td>0.15</td>
<td>N 8.0  P 0.65  K 5.92  Ca 5.67  Mg 0.56</td>
</tr>
<tr>
<td>T2</td>
<td>Brown</td>
<td>Clay loam</td>
<td>6.98</td>
<td>0.11</td>
<td>N 10.2  P 0.85  K 6.04  Ca 5.98  Mg 0.78</td>
</tr>
<tr>
<td>T3</td>
<td>Brown</td>
<td>Clay loam</td>
<td>7.06</td>
<td>0.15</td>
<td>N 15.9  P 0.97  K 6.43  Ca 5.90  Mg 0.88</td>
</tr>
<tr>
<td>T4</td>
<td>Brown</td>
<td>Clay loam</td>
<td>6.90</td>
<td>0.13</td>
<td>N 11.2  P 0.99  K 6.55  Ca 6.90  Mg 0.93</td>
</tr>
<tr>
<td>T5</td>
<td>Brown</td>
<td>Clay loam</td>
<td>7.14</td>
<td>0.12</td>
<td>N 12.4  P 0.89  K 6.32  Ca 6.34  Mg 0.66</td>
</tr>
<tr>
<td>T6</td>
<td>Brown</td>
<td>Clay loam</td>
<td>7.19</td>
<td>0.17</td>
<td>N 13.6  P 0.93  K 6.21  Ca 6.21  Mg 0.75</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N 0.024  P 0.0012  K 0.42  Ca 0.02  Mg 1.95</td>
</tr>
</tbody>
</table>

CD (5% level)
In the present study soil pH changed from slightly acidic nature of pre harvest soil to neutral and/or is tending to be alkaline due to the effect of treatments. Low soil pH adversely affects the Frankia growth and its infection processes in host plants. Optimum pH for Casuarina-Frankia symbiosis is near neutrality (Bond, 1967). pH has its direct effect on nodulation, nitrogen fixation and in the availability of several plant nutrients. When pH decreases, nodulation in expected to be inhibited (Quispel 1958). The electrical conductivity of soil samples was increased due to inoculation. This shows that the inoculation has increased the soluble salt concentration of soil. The available nitrogen content of soil has been increased significantly due to the effect of Frankia. It rated highest in Casuarina equisetifolia inoculated with Frankia alone which was followed by dual inoculated Casuarina junghuhniana. This is due to the ability of Casuarina to fix nitrogen through Frankia and improve the nitrogen content of the soil. Frankia nodulated N-fixing plants can assimilate mineral nitrogen through the nitrate reductase present in nodules, leaves and roots (Pizelle and Thiery, 1986) and can utilize mineral nitrogen as well as biologically fixed nitrogen. A high mineral nitrogen content of soil may result in partial or total elimination of nitrogen fixing capacity of nodules (Ingestad 1980) High levels of soil available
nitrogen limits *Frankia* growth and activity (Rodriguez-Barrueco, 1972). Plants prefer to assimilate combined nitrogen when it is available in the soil, rather than symbiotically fixed nitrogen (Arnone and Gordon, 1990). The ability of *Casuarina* to fix atmosphere nitrogen through *Frankia* and cluster roots make it a choice for plantation programmes. (Kumar and Matharoo, 2001). A synergetic effect between *Frankia* and *Azotobacter* is observed in *Casuarina junghuhniana* in this regard.

A significant increase in soil available phosphorus level in soil was observed due to the inoculation of *Frankia* alone. The results were on par with each other in both varieties of *Casuarina*. Phosphorus is a strong stimulator for nodule growth and suppression of phosphorus supply reduced nodule enzymes such as glutamine synthetase but nitrogenase is not affected (Valverde and Wall, 2003). Reddell (1990) reported that phosphorus deficiency is a major limiting factor for growth and nitrogen fixation by *Casuarina*. Yang (1995) investigated the effect of phosphorus on nodule formation and function in the *Casuarina – Frankia* symbiosis.
4.2.5 Assessment of *Frankia* population in post harvest rhizosphere soil and root nodules of *Casuarina* plants

*Frankia* colonies from soil samples and root nodules were isolated, enumerated and identified by the methods previously described and were depicted in Table 12 and Table 13 (Figure 8). With respect to *Frankia* population in soil samples, combined inoculation recorded a significantly higher colony count when compared to the individual inoculated and uninoculated treatments. *Casuarina junghuhniana* inoculated with both *Frankia* and *Azotobacter* recorded a higher mean colony count of 25 cfu/gm followed by *Casuarina equisetifolia* inoculated with both *Frankia* and *Azotobacter* with a mean value of 23 Cfu/gm. *Casuarina equisetifolia* inoculated with *Frankia* alone recorded a mean population count of 18 Cfu/gm and *Casuarina junghuhniana* single inoculated with *Frankia* recorded a value of 16 Cfu/gm.
Table 12

Effect of treatments on the *Frankia* population in post harvest soil samples (Mean values in cfu/gm)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Replications</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
<td>T1</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>T2</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>T3</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>T4</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>T5</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>T6</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Isolation of *Frankia* colonies from post harvest root nodules were carried out in the BuCT medium, which was selected as the better growth medium for the *Frankia* isolates. (Plate 7)When the growth pattern and colony count was considered, *Casuarina equisetifolia* inoculated with *Frankia* alone recorded a significantly higher mean value of 20 Cfu/gm followed by combined inoculated
Casuarina junghuhniana with a mean value of 16 CfU/gm. Single inoculated Casuarina junghuhniana recorded a mean value of 13 CfU/gm and dual inoculated Casuarina junghuhniana with a mean value of 11 CfU/gm controls recorded the minimum values.

**Table 13**

**Enumeration of Frankia colonies from root nodules of Casuarina plants at post harvest stage (cfu/gm)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Replications</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R₁</td>
<td>R₂</td>
<td>R₃</td>
</tr>
<tr>
<td>T₁</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>T₂</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>T₃</td>
<td>21</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>T₄</td>
<td>12</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>T₅</td>
<td>10</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>T₆</td>
<td>15</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
4.2.5.1 Culture behaviour of *Frankia* isolates

Compact colonies with branched filamentous septate hyphae bearing club shaped sporangia and bulb shaped vesicles (Table 14). They were Gram positive and catalase positive.

**Table 14**

**Characteristic features of *Frankia* isolates from post harvest samples (BuCT Medium)**

<table>
<thead>
<tr>
<th>Colony type</th>
<th>Colour</th>
<th>Sporangia</th>
<th>Vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compact</td>
<td>Grey</td>
<td>Club shaped</td>
<td>Bulb shaped</td>
</tr>
</tbody>
</table>
The characteristic features of *Frankia* isolates were similar to the strains isolated previously in BuCT medium. It is similar to the findings of Diem *et. al.*, (1982b); St. Laurent and Lalonde (1987).

In the present investigation it is found that *Frankia* population in soil is greater significantly higher in dual inoculated treatments. The interaction effect of various soil microorganisms with *Frankia* have been reported. But co-inoculation of *Frankia* with *Azotobacter* has not yet studied. Pakkar *et al.*, (1977) reported that presence of *Azotobacter* has tremendously increased the population density of *Rhizobia* in the root nodule niche on legume roots. The result obtained in the present study with respect to soil *Frankia* population can be correlated with the above report.

With regard to the *Frankia* population in root nodules, *Casuarina equisetifolia* inoculated with *Frankia* alone, recorded significantly higher colony count followed by dual inoculated *Casuarina junghuhniana*. This is strengthened by the report of Wheeler *et al.*, (1981) that the genotypic variability of host plant species have been found to affect the nodulation and nitrogen fixation rates. Studies of Huss Danell and Myrold (1994) states that Different plant species could produce compounds that stimulate or inhibit the growth and infectivity of *Frankia*. 
The results of the present study indicated a positive interaction between *Frankia* and *Azotobacter* in *Casuarina junghuhniana* whereas for *Casuarina equisetifolia*, treatment with *Frankia* alone were more effective. The positive effect of dual inoculation on growth, and nitrogen fixation by *Frankia* with VAM have been reported by Sempavalan *et al.*, (1998) in *Casuarina equisetifolia*, Tian *et al.*, (2002) in *Hippophae tibetana* and Lumini *et al.*, (1994) in *Alnus cordata*.

The growth pattern of *Frankia* colonies on BuCT medium again support the fact that the components such as propionate and Tween 80, the carbon source and casaminoacids, the nitrogen source provided a better nutritional requirement for the isolates. This indicates that these factors influence the metabolic behaviour of *Frankia* (Sempavalan, 1998).

4.2.6 **Effect of treatments on *Azotobacter* population in post harvest soil samples.**

It is observed from Table 15 that the *Azotobacter* population was significantly higher in dual inoculated treatments at different dilutions. *Casuarina equisetifolia* inoculated with both *Frankia* and *Azotobacter* recorded a higher mean values that ranged from 13-31 CfU/gm at different concentrations, followed by *Casuarina*
junghuhniana inoculated with both Frankia and Azotobacter having mean values that ranged from 10-26 Cf/u/gm at different dilutions. Moderate count were observed in uninoculated Casuarina equisetifolia with values ranging from 9-12 Cf/u/gm where as minimum growth were observed in Casuarina equisetifolia inoculated with Frankia alone (1-6 Cf/u/gm), Casuarina junghuhniana inoculated with Frankia alone (1-3 Cf/u/gm) and uninoculated Casuarina junghuhniana (3-5 Cf/u/gm) at different concentrations (Figure 9 and Plate 14)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Azotobacter population at different dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$1 \times 10^{-5}$</td>
</tr>
<tr>
<td>T1</td>
<td>12</td>
</tr>
<tr>
<td>T2</td>
<td>5</td>
</tr>
<tr>
<td>T3</td>
<td>6</td>
</tr>
<tr>
<td>T4</td>
<td>3</td>
</tr>
<tr>
<td>T5</td>
<td>31</td>
</tr>
<tr>
<td>T6</td>
<td>26</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td>1.30</td>
</tr>
</tbody>
</table>
4.2.6.1 Characteristic features of *Azotobacter* species isolated from post harvest soil samples

The characteristic features of *Azotobacter* isolated were similar to that described previously (Narula, 2000; Bhandari and Somani 2000). They were Gram negative slimy raised colony, light brown in colour. They were roughly spherical in nature motile and a catalase producer. They were positive for indole, citrate and NO₃ reduction tests.
The environmental factors like pH, salinity, and soil characteristics are known to affect the growth and activities of *Azotobacter* (Chaudhary *et al.*, 1996). They are capable of synthesizing and secreting various plant growth promoters in addition to nitrogen fixation (Verma *et al.*, 2001). *Azotobacter chroococcum* can tolerate environmental stress and could remain active in soil even in adverse conditions (Ahmad and Ahmad, 2004). The positive interaction of *Azotobacter* with *Frankia* and test crop might be due to the synthesis of growth promoting substances by the *Azotobacter* and also by the suppression of the phytopathogens in the soil by the *Azotobacter* interaction. Gadgil and Bhide (1960) reported that some actinomycetes stimulate nitrogen fixation by *Azotobacter*. The importance of symbiotic and non-symbiotic nitrogen fixation must be stressed, together with the advantages of bio-fertilizers in sustaining agricultural production without deleterious effect on the environment.
Table 16

Characteristic features of *Azotobacter* species isolated from the post harvest rhizosphere soil of *Casuarina* plants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony</td>
<td>Slimy raised light brown coloured colonies</td>
</tr>
<tr>
<td>Gram reaction</td>
<td>Gram negative</td>
</tr>
<tr>
<td>Cell type</td>
<td>Spherical cells</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Present</td>
</tr>
<tr>
<td>Mobility</td>
<td>Positive</td>
</tr>
<tr>
<td>Catalase test</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole test</td>
<td>Positive</td>
</tr>
<tr>
<td>Nitrite reduction test</td>
<td>Positive</td>
</tr>
</tbody>
</table>

4.2.7 Influence of treatments on the total nutrient contents of *Casuarina* plants

The data on changes in total nutrient contents of *Casuarina* plants due to the influence of treatments are depicted in Table 17. (Figure 10) When compared to other treatments, *Casuarina equisetifolia* inoculated with *Frankia* alone recorded a higher total nitrogen value (2.62%). This was followed by other treatments except
controls with same response towards inoculation (2.52%). Lower levels were observed in controls (1.96%). The total phosphorus contents were observed to be higher in single inoculated and uninoculated *Casuarina junghuhniana* (0.10%). This was followed by dual inoculated *Casuarina junghuhniana*, *Casuarina equisetifolia* and single inoculated *Casuarina equisetifolia* with same phosphorus content (0.05%). Very low phosphorus content were observed in uninoculated *Casuarina equisetifolia* (0.005%). When the total potassium content were assessed, *Casuarina equisetifolia* inoculated with *Frankia* alone and *Casuarina junghuhniana* inoculated with both *Frankia* and *Azotobacter* recorded significantly higher value and they were statistically on par with each other (0.96%). This was followed by uninoculated and single inoculated *Casuarina junghuhniana* and dual inoculated *Casuarina equisetifolia* with a value of 0.84%. Lower levels were observed in uninoculated *Casuarina equisetifolia* (0.75%). When the total calcium and total magnesium content in *Casuarina* plants were evaluated, *Casuarina junghuhniana* inoculated with *Frankia* alone recorded a much higher value of 0.41% and 0.10% respectively. Followed by this *Casuarina junghuhniana* uninoculated and dual inoculated treatments with a calcium content 0.38% and magnesium content (0.08%) were observed and *Casuarina*
equisetifolia single and dual inoculated treatments recorded a calcium content of 0.36% and magnesium content 0.07%. Lower levels were observed in uninoculated Casuarina equisetifolia.

**Table No.17**

Influence of treatments on the total nutrient contents of Casuarina shoots (Mean value in percentage)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>T1</td>
<td>1.96</td>
</tr>
<tr>
<td>T2</td>
<td>1.96</td>
</tr>
<tr>
<td>T3</td>
<td>2.62</td>
</tr>
<tr>
<td>T4</td>
<td>2.52</td>
</tr>
<tr>
<td>T5</td>
<td>2.52</td>
</tr>
<tr>
<td>T6</td>
<td>2.52</td>
</tr>
<tr>
<td>CD (5% level)</td>
<td>0.13</td>
</tr>
</tbody>
</table>
A significantly high total nitrogen content were observed in *Casuarina equisetifolia* inoculated with *Frankia* alone. The result shows that a positive relation exists between nodule number and nitrogen content and thereby the growth of *Casuarina equisetifolia* upon inoculation with *Frankia*. A high phosphorus content were observed in *Casuarina junghuhniana* inoculated with *Frankia* alone. Valverde *et al.*, (2002) reported that phosphorus nutrition has an
important role in the regulation of nodulation in actinorhizal plants. In presence of phosphorus higher flux of nitrogenous compounds to the xylem sap occurs because the proportion of symbiotic tissue was augmented by phosphorus.

Decrease in phosphorus reduces the nodule glutamine synthetase, malate dehydrogenase, glutamate synthase and asparagine synthase. This fact is strengthened by the report that even though nodules can fix atmospheric nitrogen, its efficiency is determined mostly by the phosphorus nutrient condition of host plant since appropriate phosphorus nutrient support is indispensable for the process of nitrogen fixation (Tian et al (2002). With respect to other nutrients like K, Ca and Mg also the Frankia inoculation enhanced their concentrations in plant shoot.

4.2.8. Nitrogen and Phosphorus Uptake Studies

A significantly higher nitrogen uptake was observed in Casuarina equisetifolia inoculated with Frankia alone registering a value of 327.5mg/plant followed by combined inoculated Casuarina equisetifolia and Casuarina junghuhniana which recorded a nitrogen uptake of 315 mg/plant. Lower N₂ uptake was detected in controls. Phosphorus uptake was also found to be changed due to the effect of treatments. The results indicated that among the treatments
Casuarina junghuhniana inoculated with Frankia alone was found to be superior over others with a value of 11.3 mg/plant. This was followed by uninoculated Casuarina junghuhniana (7.5 mg/plant). Single and dual inoculated Casuarina equisetifolia and dual inoculated Casuarina junghuhniana recorded a phosphorus uptake of 6.25 mg/plant. Lowest phosphorus uptake was observed in uninoculated Casuarina equisetifolia (0.25 mg/plant) (Table 18 and Figure 11).

Table 18

Effect of treatments on the nutrient uptake by Casuarina plants (Mean values)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nitrogen uptake (mg/plant)</th>
<th>Phosphorus uptake (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>98</td>
<td>0.25</td>
</tr>
<tr>
<td>T2</td>
<td>147</td>
<td>7.5</td>
</tr>
<tr>
<td>T3</td>
<td>327.5</td>
<td>6.25</td>
</tr>
<tr>
<td>T4</td>
<td>284.7</td>
<td>11.3</td>
</tr>
<tr>
<td>T5</td>
<td>315</td>
<td>6.25</td>
</tr>
<tr>
<td>T6</td>
<td>315</td>
<td>6.25</td>
</tr>
<tr>
<td>CD</td>
<td>28</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Uptake of nitrogen and phosphorus were increased significantly due to treatments and higher nitrogen uptake was observed in *Casuarina equisetifolia* inoculated with *Frankia* alone and higher phosphorus uptake in *Casuarina junghuhniana* treated with *Frankia* alone. This might be due to the increase in the number of root hairs and lateral roots that increases the absorptive capacity of
the root system for nutrients due to enhanced root growth by *Frankia*. This result also support the fact that *Frankia* strains are also able to utilise N\textsubscript{2} as nitrogen source in addition to organic and inorganic nitrogen compounds. The increased uptake of nutrients through increased root volume, increased nodule number, plant height and greater nutrient accumulation might be responsible for higher root dry matter production. The uptake studies shows that nutrient uptake by both varieties of *Casuarina* is solely dependent on *Frankia* than *Azotobacter*. Similar findings were made by Mansour and Baker (1994) that the inoculation of *Frankia* contributed to a greater amount of nitrogen uptake for the tree growth. It is inferred that soil and rhizosphere bacteria can affect the mineral nutrition of plants by changing root-uptake characteristics, due to a modification of root morphology or alteration of uptake mechanisms, relative growth rate or internal composition of plants.

### 4.2.9. Effect of treatments on the biochemical properties of *Casuarina* plants

Changes in the biochemical properties of *Casuarina* plants due to inoculation and uninoculation were investigated. Biochemical parameters analysed were nitrogenase enzyme activity, ACP and ALP activity, total carbohydrate content, total protein content, total free
amino acid and proline content, total phenol and Indole Acetic Acid. The observations are as follows.

4.2.9.1. Influence of treatments on Nitrogenase activity of Casuarina root nodules

Maximum nitrogenase activity of 2.84 μ moles C₂H₄/g nodule/hr was recorded in root nodules of Casuarina equisetifolia inoculated with Frankia alone followed by Casuarina equisetifolia inoculated with both Frankia and Azotobacter (1.89 μ moles C₂H₄/g nodule/hr.) With regard to Casuarina junghuhniana nitrogenase activity was more in those treatment dual inoculated with Frankia and Azotobacter registering a value of 1.78 μ moles C₂H₄/g nodule/hr followed by single inoculation with a value of 1.15 μ moles C₂H₄/g nodule/hr. Uninoculated Casuarina equisetifolia recorded an activity of 1.78 μ moles C₂H₄/g nodule/hr. When both Casuarina varieties were compared Casuarina equisetifolia recorded a significantly higher nitrogenase activity over Casuarina junghuhniana (Table 19 and Figure 12).
Table 19
Effect of treatments on the nitrogenase activity of root nodules of *Casuarina* plants (µ moles C2H4/gm nodule/hr)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1.77</td>
<td>1.78</td>
<td>1.78</td>
<td>1.79</td>
<td>7.12</td>
<td>1.78</td>
</tr>
<tr>
<td>T2</td>
<td>1.66</td>
<td>1.69</td>
<td>1.67</td>
<td>1.7</td>
<td>6.72</td>
<td>1.68</td>
</tr>
<tr>
<td>T3</td>
<td>2.83</td>
<td>2.85</td>
<td>2.84</td>
<td>2.84</td>
<td>11.36</td>
<td>2.84</td>
</tr>
<tr>
<td>T4</td>
<td>1.15</td>
<td>1.15</td>
<td>1.14</td>
<td>1.16</td>
<td>4.6</td>
<td>1.15</td>
</tr>
<tr>
<td>T5</td>
<td>1.90</td>
<td>1.90</td>
<td>1.89</td>
<td>1.87</td>
<td>7.56</td>
<td>1.89</td>
</tr>
<tr>
<td>T6</td>
<td>1.78</td>
<td>1.79</td>
<td>1.78</td>
<td>1.77</td>
<td>7.12</td>
<td>1.78</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Figure 12
Effect of treatments on the nitrogenase activity of root nodules of *Casuarina* plants

![Diagram showing nitrogenase activity for T1 to T6 treatments](image-url)
Nitrogen fixation in all diazotrophs is performed by nitrogenase and nitrogenase activity was determined by acetylene reduction. *Casuarina equisetifolia* exhibited a higher nitrogenase activity than *Casuarina junghuhniana*. The result reveals that in *Casuarina equisetifolia* inoculation with *Frankia* alone has increased the effectivity whereas in *Casuarina junghuhniana* the effectivity was increased due to dual inoculation. This indicates that considerable variation exists in effectivity of the symbiosis in *Casuarina* species.

An effective *Casuarina - Frankia* association is characterized by high nitrogen fixation activity (Reddell and Bowen 1985). It is concluded that the activity or synthesis of nitrogenase enzyme in diazotrophs is inhibited by an abundance of fixed nitrogen compounds in the environment. In plentiful supplies of ammonium, nitrogenase activity may be inhibited (Colnaghi *et al.*, 1997).

The results indicates a positive correlation between seedling biomass and nitrogenase activity. This confirms the view that symbiotic nitrogen fixation is dependent also on host plant photosynthesis (Yamanaka *et al.*, 2003).
4.2.9.2. Changes in phosphatase enzyme activities of *Casuarina* root nodules

A significantly higher acid phosphatase activity was noticed in *Casuarina equisetifolia* inoculated with *Frankia* alone which registered 10mM/mint/mg protein followed by dual inoculated *Casuarina equisetifolia* with a value of 8mM/mint/mg protein. When compared to *Casuarina junghuhniana*. The results indicate that ACP activity were observed to be higher in *Casuarina equisetifolia* treatments. The Alkaline phosphatase activity were found to be higher in *Casuarina junghuhniana* inoculated with both *Frankia* and *Azotobacter* with 38.2mM/mint/mg protein activity followed by *Casuarina equisetifolia* inoculated with *Frankia* alone (34mM/mint/mg protein.) The results indicated that the ALP activity recorded in single inoculated *Casuarina equisetifolia* were higher than dual inoculated *Casuarina equisetifolia* (22mM/mint/mg protein). Whereas in the *Casuarina junghuhniana* treatments dual inoculation recorded a much higher ALP activity in root nodules than single inoculation. Controls recorded lower activities (Tables 20, 22 and Figures 13, 14).
Table 20
Changes in Acid phosphatase activity of root nodules of *Casuarina* due to treatments (mM/mg protein/mg protein)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>T2</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>T3</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>8</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>T5</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>T6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Table 21
Changes in Acid Phosphatase activity of post harvest soil samples due to treatments (m M/mg protein/mg protein)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>T2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>T3</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>T5</td>
<td>24</td>
<td>25</td>
<td>23</td>
<td>24</td>
<td>96</td>
<td>24</td>
</tr>
<tr>
<td>T6</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.4</td>
</tr>
</tbody>
</table>
4.2.9.3. Changes in phosphatase enzyme activities of rhizosphere soil samples due to treatments

Dual inoculated *Casuarina equisetifolia* recorded a significantly higher ACP activity in soil samples registering an activity of 24mM/mint/mg protein over other treatments, followed by *Casuarina equisetifolia* inoculated with *Frankia* alone (12mM/mint/mg protein). With regard to *Casuarina junghuhniana* related treatments also dual inoculation recorded a higher ACP activity (8mM/mint/mg protein) over individually inoculated treatment (4mM/mint/mg protein). ACP activity in rhizosphere soil of *Casuarina equisetifolia* treatments were higher than *Casuarina junghuhniana* treatments. When the ALP activity of soil samples were evaluated, *Casuarina junghuhniana* inoculated with both *Frankia* and *Azotobacter* recorded a higher activity of 46mM/mint/mg protein followed by dual inoculated *Casuarina equisetifolia* with 38 mM/mint/mg protein activity. Single inoculated *Casuarina equisetifolia* recorded an activity of 34mM/mint/mg protein and *Casuarina junghuhniana* with 32.4mM/mint/mg protein activity. (Tables 21, 23 and Figures 13,14).
### Table 22
Changes in Alkaline Phosphatase activity of root nodules of *Casuarina* due to treatments (mM/mint/mg protein)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>13.5</td>
<td>13.7</td>
<td>13.6</td>
<td>13.6</td>
<td>54.4</td>
<td>13.6</td>
</tr>
<tr>
<td>T2</td>
<td>17</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>T3</td>
<td>32</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>136</td>
<td>34</td>
</tr>
<tr>
<td>T4</td>
<td>27</td>
<td>28</td>
<td>30</td>
<td>27</td>
<td>112</td>
<td>28</td>
</tr>
<tr>
<td>T5</td>
<td>21</td>
<td>23</td>
<td>22</td>
<td>22</td>
<td>88</td>
<td>22</td>
</tr>
<tr>
<td>T6</td>
<td>38.5</td>
<td>38.0</td>
<td>38.1</td>
<td>38.2</td>
<td>152.8</td>
<td>38.2</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.40</td>
</tr>
</tbody>
</table>

### Table 23
Changes in Alkaline Phosphatase activity in post harvest soil samples due to treatments (mM/mint/mg protein)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>9.3</td>
<td>9.0</td>
<td>9.5</td>
<td>9.0</td>
<td>36.8</td>
<td>9.2</td>
</tr>
<tr>
<td>T2</td>
<td>12.5</td>
<td>12.2</td>
<td>12.4</td>
<td>12.5</td>
<td>49.6</td>
<td>12.4</td>
</tr>
<tr>
<td>T3</td>
<td>33</td>
<td>33</td>
<td>36</td>
<td>34</td>
<td>136</td>
<td>34</td>
</tr>
<tr>
<td>T4</td>
<td>32.5</td>
<td>32.3</td>
<td>32.4</td>
<td>32.4</td>
<td>129.6</td>
<td>32.4</td>
</tr>
<tr>
<td>T5</td>
<td>39</td>
<td>37</td>
<td>37</td>
<td>39</td>
<td>152</td>
<td>38</td>
</tr>
<tr>
<td>T6</td>
<td>47</td>
<td>47</td>
<td>45</td>
<td>45</td>
<td>184</td>
<td>46</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.32</td>
</tr>
</tbody>
</table>
The acid phosphatase enzyme activity was maximum in *Casuarina equisetifolia* associated treatments, both in soil and in root nodules and the alkaline phosphatase activity was higher in *Casuarina junghuhniana*. Phosphorus concentration strongly influence the phosphatase enzyme activity Yang *et al.*, (1997) reported that *Frankia* strains differ in their responses to increasing phosphorus supply and ACP and ALP activities was higher when phosphorus was omitted and the activity was decreased with increasing P supply.

Figure 13

**Effect of treatments on the acid phosphatase activity of rhizosphere soils and root nodules of *Casuarina* plants**

![Bar chart showing the effect of treatments on ACP activity](chart)

- T1: Rhizophere soil, T2: T3: T4: T5: T6: Root nodule
- ACP Activity (mM/mint/mg protein)
- Treatments: T1, T2, T3, T4, T5, T6
- Values: 4, 2, 4, 4, 4, 6, 8, 8, 24, 4
4.2.9.4 Effect of treatments on the total carbohydrate content of *Casuarina* root nodules

Data on changes in total carbohydrate content of *Casuarina* root nodules are depicted on Table 24 and Figure 15. It was noticed that higher carbohydrate value of 1.7 mg/100mg was shown *Casuarina equisetifolia* inoculated with both *Frankia* and *Azotobacter* followed by *Casuarina junghuhniana* inoculated with *Frankia* alone which registered a value of 1.1mg/100mg. *Casuarina equisetifolia* inoculated with *Frankia* alone recorded a value of 0.9mg/100mg and dual inoculated *Casuarina junghuhniana* recorded 0.7mg/100mg.
Table 24
Changes in total carbohydrate content of root nodules of *Casuarina* plants due to treatments (mg/100mg)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8</td>
<td>2.8</td>
<td>0.7</td>
</tr>
<tr>
<td>T2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>T3</td>
<td>0.85</td>
<td>0.9</td>
<td>0.9</td>
<td>0.95</td>
<td>3.6</td>
<td>0.9</td>
</tr>
<tr>
<td>T4</td>
<td>1.1</td>
<td>1.15</td>
<td>1.05</td>
<td>1.1</td>
<td>4.4</td>
<td>1.1</td>
</tr>
<tr>
<td>T5</td>
<td>1.7</td>
<td>1.7</td>
<td>1.8</td>
<td>1.6</td>
<td>6.8</td>
<td>1.7</td>
</tr>
<tr>
<td>T6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
<td>0.7</td>
<td>2.8</td>
<td>0.7</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Figure 15
Effect of treatments on the total carbohydrate content of root nodules of *Casuarina* plants (mg/100mg)
The results indicated that total nodule carbohydrate content was increased significantly due to inoculation and maximum level was observed in *Casuarina equisetifolia* dual inoculated treatment. Wheeler (1971) suggested that a substantial part of the nodule carbohydrate is unavailable for nitrogen fixation and that maximum rates of fixation are only attained when new photosynthetates are entering the nodules. According to Reddy and Desingh (2001), *Casuarina equisetifolia* accumulated more starch and *Casuarina junghuhniana* maintained high levels of soluble sugars under salinity stress. *Frankia* fixes molecular nitrogen and the product ammonia is excreted into nodule cytoplasm while in return *Frankia* receives carbohydrates from host plants (Hus Danell et al., 1982). Trehalose, a common reserve carbohydrates present in *Frankia* helps *Casuarina* to overcome desiccation and other environmental stress (Burleigh and Dawson (1994).

### 4.2.9.5 Effect of treatment on the total protein content of *Casuarina* root nodules

*Casuarina equisetifolia* inoculated with *Frankia* alone exhibited a higher total protein value of 51 mg/100g followed by *Casuarina junghuhniana* inoculated with *Frankia* alone with a value of 41mg/100g. Dual inoculated *Casuarina junghuhniana* recorded a
total protein content of 29mg/100g and that of *Casuarina equisetifolia* 21mg/100g. Uninoculated treatments recorded lower protein content (Table 25 and Figure 16).

**Table 25**

**Changes in total protein content of root nodules of *Casuarina* plants due to treatments (mg/100g)**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>14</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>T2</td>
<td>16</td>
<td>16</td>
<td>13</td>
<td>15</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>T3</td>
<td>50</td>
<td>51</td>
<td>51</td>
<td>52</td>
<td>204</td>
<td>51</td>
</tr>
<tr>
<td>T4</td>
<td>40</td>
<td>41</td>
<td>42</td>
<td>41</td>
<td>164</td>
<td>41</td>
</tr>
<tr>
<td>T5</td>
<td>20</td>
<td>22</td>
<td>22</td>
<td>20</td>
<td>84</td>
<td>21</td>
</tr>
<tr>
<td>T6</td>
<td>30</td>
<td>29</td>
<td>29</td>
<td>28</td>
<td>116</td>
<td>29</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.48</td>
</tr>
</tbody>
</table>

The results indicated that individual inoculation with *Frankia* increased the total protein content when compared to dual inoculated treatments. It is known that proteins of *Frankia* play a major role in actinorhizal infection (Taveres and Sellstedt, 2000).
4.2.9.6 Effect of treatments on the total free amino acid content of Casuarina root nodules

The total free amino acid content was found to be maximum in *Casuarina junghuhniana* inoculated with *Frankia* alone (56mg/100g) followed by dual inoculated *Casuarina junghuhniana* (46mg/100g). *Casuarina equisetifolia* individually inoculated treatments recorded a
total free amino acid level of 38mg/100g and dual inoculated *Casuarina equisetifolia* recorded 28mg/100g. The results indicated that *Casuarina junghuhniana* exhibited a higher total free amino acid content when compared to *Casuarina equisetifolia*. The results are presented in Table 26 and Figure 17.

**Table 26**

*Changes in total free amino acids of root nodules of *Casuarina* plants due to treatments (mg/100g)*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>T2</td>
<td>26</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>T3</td>
<td>37</td>
<td>37</td>
<td>40</td>
<td>38</td>
<td>152</td>
<td>38</td>
</tr>
<tr>
<td>T4</td>
<td>58</td>
<td>55</td>
<td>54</td>
<td>57</td>
<td>224</td>
<td>56</td>
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<tr>
<td>T5</td>
<td>28</td>
<td>28</td>
<td>29</td>
<td>27</td>
<td>112</td>
<td>28</td>
</tr>
<tr>
<td>T6</td>
<td>45</td>
<td>47</td>
<td>45</td>
<td>47</td>
<td>184</td>
<td>46</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.8</td>
</tr>
</tbody>
</table>
The present investigation reveals that the *Casuarina junghuhniana* associated treatments recorded higher total free amino acid content. This is attributed to the ability of *Casuarina junghuhniana* to tolerate salinity stress when compared to *Casuarina equisetifolia*. This is due to the enhanced protein degradation which yield high concentration of free amino acids needed to maintain
osmotic pressure in salt stressed plants when metabolites in high concentration leads to water retention and/or have the ability to take up water when the osmotic pressure of external medium is high (Reddy and Desingh, 2001). Total free amino acids composition changes in disease conditions in plants and hence its measurement gives the physiological health status of plants.

4.2.9.7 Influence of treatments on the proline content of root nodules of Casuarina

A significant increase in proline content was observed in Casuarina junghuhniana inoculated with both Frankia and Azotobacter (30mg/g tissue) followed by Casuarina junghuhniana inoculated with Frankia alone (21mg/g tissue). Casuarina equisetifolia single inoculation with Frankia and uninoculated Casuarina junghuhniana recorded a proline content of 17 mg/g tissue. Uninoculated and dual inoculated Casuarina equisetifolia recorded lowest proline content of 12mg/g tissue (Table 27 and Figure 18).
Table 27
Changes in proline content of root nodules of *Casuarina* plants due to treatments (mg/g tissue)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>T2</td>
<td>15</td>
<td>18</td>
<td>19</td>
<td>16</td>
<td>68</td>
<td>17</td>
</tr>
<tr>
<td>T3</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>19</td>
<td>68</td>
<td>17</td>
</tr>
<tr>
<td>T4</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>84</td>
<td>21</td>
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<td>12</td>
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<tr>
<td>T6</td>
<td>29</td>
<td>30</td>
<td>31</td>
<td>30</td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Figure 18
Influence of treatments on the proline content in root nodules of *Casuarina* plants (mg/g)
Proline content was also found to be higher in *Casuarina junghuhniana* associated treatments. This corroborates with the works of Reddy and Desingh (2001). According to their work *Casuarina junghuhniana* proved support with high salinity tolerance and drought resistance when compared to *Casuarina equisetifolia*. This is attributed to the high proline content in *Casuarina junghuhniana* Reddy *et al* (2001). Proline act as an osmolyte and protect the plant from osmotic shock under dry conditions and avoid severe drought effects. Proline avoid salinity stress by the stabilization of enzymes active sites or by replacing water in the hydration shell of proteins or by stabilizing enzyme complexes or membranes.

### 4.2.9.8 Influence of treatments on the total phenol content in *Casuarina* root nodules

As shown in Table 28 (Figure 19) the total phenol content was found to be significantly higher in dual inoculated *Casuarina junghuhniana* (8mg/100g) followed by *Casuarina equisetifolia* inoculated individually with *Frankia* having a phenol content of 7.1mg/100g. Dual inoculation on *Casuarina equisetifolia* recorded a value of 6.4mg/100g.
Table 28

Changes in the total phenol content of root nodules of *Casuarina* plants due to treatments (mg/100g)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>5.5</td>
<td>5.5</td>
<td>5.4</td>
<td>5.6</td>
<td>22</td>
<td>5.5</td>
</tr>
<tr>
<td>T2</td>
<td>4.8</td>
<td>4.8</td>
<td>4.6</td>
<td>4.6</td>
<td>18.8</td>
<td>4.7</td>
</tr>
<tr>
<td>T3</td>
<td>7.1</td>
<td>7.0</td>
<td>7.2</td>
<td>7.1</td>
<td>28.4</td>
<td>7.1</td>
</tr>
<tr>
<td>T4</td>
<td>5.6</td>
<td>5.4</td>
<td>5.4</td>
<td>5.6</td>
<td>22</td>
<td>5.5</td>
</tr>
<tr>
<td>T5</td>
<td>6.4</td>
<td>6.5</td>
<td>6.3</td>
<td>6.4</td>
<td>25.6</td>
<td>6.4</td>
</tr>
<tr>
<td>T6</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>CD</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Figure 19

Effect of treatments on the Total Phenol content in root nodules of *Casuarina* plants (mg/100gm)
The results indicate that dual inoculation on *Casuarina junghuhniana* increased the total phenol content and in *Casuarina equisetifolia* individual inoculation were more effective with regard to total phenol content. In plant pathogen interactions, phenols serve as pathogen barrier forming a part of defence response of the plant. (Pawalowski and Sirrenberg 2003). Therefore the results indicate that inoculation with *Frankia* has a considerable effect on the defense mechanism of the plants by increasing the phenol concentration. However, there are reports that *Frankia* is sensitive to plant phenol like the ferulic acid (Perradin *et al.*, 1983). Whereas Baker and O’Keefe (1984) found that phenol inoculation (0.7%) of soil enabled isolation of *Frankia* from soils, while a phenol incubation of crushed alder nodules increased both the number and robustness of developing *Frankia* colonies. Vogel and Dawson (1986) suggested that phenolic substances can also enhance the growth of *Frankia*. According to their study plant phenolics significantly affect the invitro growth and morphology of *Frankia* isolates and may play an important role in *Frankia* growth and development in soil and within host tissue. Whereas Wolters *et al.* (1999) reported that ineffective
Frankia strains in Alnus glutinosa exhibited characteristic of a mild pathogen instead of being a symbiont and higher amount of phenolic compounds were present in the infected cells of these ineffective Frankia nodules. But such an accumulation was not observed in effective Frankia nodules.

4.2.9.9 Influence of treatment on the Indole Acetic Acid content in root nodules of Casuarina

A significant increase in Indole Acetic Acid content were observed in dual inoculated Casuarina junghuhniana (53μg/ml) followed by Casuarina equisetifolia, both single inoculated and dual inoculated treatments with a value of 48 μg/ml which are statistically on par with each other. Single inoculated Casuarina junghuhniana registered a value of 44μg/ml. Uninoculated ones recorded minimum contents. (Table 29 and Figure 20).
Table 29
Changes in Indole Acetic Acid content of root nodules of *Casuarina* plants due to treatments (μg/ml)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>Total</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
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<td>23</td>
<td>21</td>
<td>24</td>
<td>88</td>
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<tr>
<td>T3</td>
<td>47</td>
<td>46</td>
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<td>192</td>
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</tr>
<tr>
<td>T4</td>
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<td>44</td>
<td>43</td>
<td>176</td>
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<td>49</td>
<td>49</td>
<td>47</td>
<td>47</td>
<td>192</td>
<td>48</td>
</tr>
<tr>
<td>T6</td>
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<td>54</td>
<td>53</td>
<td>52</td>
<td>212</td>
<td>53</td>
</tr>
<tr>
<td>CD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Figure 20

Effect of treatments on the Indole Acetic Acid content in root nodules of *Casuarina* plants (μgm/ml)

![Graph showing the effect of treatments on IAA content](image-url)
The results revealed that dual inoculation increased Indole Acetic Acid contents. Wheeler et al. (1984) reported that *Frankia* can produce low levels of Indole Acetic Acid. Arahou et al., (1998) reported that *Frankia* can produce indoles and siderophores that facilitates *Frankia* to survive under non symbiotic conditions. Information regarding the production of plant hormones by *Frankia* are less available. There are reports regarding the production of plant growth regulators viz, IAA, gibberellins, cytokinins by *Azotobacter* (Verma et al., 2001); Chahal and Chahal (2000), Abbas and Okon (1993). These reports suggest that *Azotobacter* probably influences the development of plants by producing growth regulating substances. The results of present study confirms with the above reports. Findings of Ahmad and Ahmad (2004) indicated that IAA production is a common characteristics of *Azotobacter chroococcum* and level of its production was strain dependent. Wheeler et al., (1979) suggested that occurrence of IAA and cytokinines in root nodules controls growth of mature nodules. Silver et al., (1966) reported that the positive geotropism, characteristic of nodulated roots of *Alder, Myrica* and *Casuarina* is due to the auxin content in the roots.
Plate 3
seeds of *Casuarina junghuhniana*

Plate 4
*Casuarina equisotifolia*-germination in tray
Plate 5
Casuarina junghuhniana-germination in tray

Plate 6
Frankia Colonies in DPM medium
Plate 7
*Frankia colonies* in BuCT medium

Plate 8
Pot Culture Studies with *Casuarina equisetifolia* on third month

T1-Casuarina equisetifolia control
T3-Casuarina equisetifolia+Frankia
T5-Casuarina equisetifolia+Frankia+Azotobacter
Plate 9
Pot culture Studies with *Casuarina junghuhniana* on third month

T2-Casuarina junghuhniana control
T4-Casuarina junghuhniana+Frankia
T6-Casuarina junghuhniana+Frankia+ Azotobacter

Plate 10
Overall view of pot culture experiment on sixth month
Plate 11
Overall view of pot culture experiment on ninth month

Plate 12
Root formation in *casuarina equisetifolia* plants

T1-Casuarina equisetifolia control
T3-Casuarina equisetifolia+Frankia
T5-Casuarina equisetifolia+Frankia+Azotobacter
Plate 13
Root formation in *Casuarina junghuhniana* plants

T2-Casuarina junghuhniana control
T4-Casuarina junghuhniana+Frankia
T6-Casuarina junghuhniana+Frankia+ Azotobacter

Plate 14
*Azotobacter* colonies in Jensen's medium