Effect of Bio Fertilization on Growth and Constituents of Moringa oleifera Lam. Plants

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ABSTRACT

The objective of this study was to evaluate the effect of biofertilizers (control, Phosphorine, Rhizobactrine, Microbine and Nitrobine), and the effect on growth and chemical constituents of Moringa oleifera in the greenhouse during two successive seasons 2006 and 2007, at the Nubaria Research and Production Station. Effect of biofertilization on vegetative growth and chemical constituents of Moringa oleifera plants: The plants treated with Phosphorine and Nitrobine gave the highest values of plant height, stem diameter, root length, fresh and dry weight of roots, but branch number, leaf number and fresh and dry weight of shoots increased with application of Microbine in the two seasons. The chlorophyll a, b and carotenoid increased with Phosphorine, Nitrobine and Microbine as well as total carbohydrates; all biofertilizers treatments increased nutrients contents compared with the control.

Key words: Moringa oleifera, biofertilizers, Phosphorine, Rhizobactrine, Microbine, Nitrobine.

Introduction

Moringa oleifera, Lam. (syn. M. pterygosperma, Gaert.), is commonly known as horseradish and drumstick tree, belongs to the family Moringaceae which consists of the single genus Moringa, comprising 10-14 species. The best-known species is M. oleifera, indigenous to northwest India; and widely cultivated in Philippines, Thailand, Malaysia, Pakistan and other tropical and subtropical areas in Central Asia, America and Africa (Morton, 1991; Ramachandran et al., 1980). Moringa oleifera is often called "the miracle tree" because all its parts are useful. The seeds are used as a natural coagulant for raw water clarification, the powder of crushed seed kernels can leave waterclear with 90-99% of the bacteria removed (Sutherland et al., 1989). The seeds are also used for oil productions; this oil is used in art, cosmetics and medicine; and can be consumed as food. Bio-fertilizers are microbial inoculants used for application to either seeds or soil for increasing soil fertility with objective of increasing the number of such micro-organisms and to accelerate certain microbial processes. Such microbiological processes can change unavailable forms of nutrients into available ones that can be easily assimilated by plants (SubbaRao, 2001). Microbine, Phosphorine, Rhizobacterine and Nitrobine are bio-fertilizers which contain one or more of the previous nitrogen fixing bacteria. They are known for fixing atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. Biofertilizers play a crucial role in the reduction of inorganic fertilizers and its utilization. There has been considerable progress during the recent past in the development of biofertilizers production technology and has been demonstrated to some extent as an efficient tool for increasing the trees and plants productivity (Jamaluddin, 2002). Saher (2008) on jojoba seedlings indicated that the highest values were always obtained from spraying plants with high rate of Biomagic and inoculation with mixture of Azotobacter and Bacillus. The highest total carbohydrate in leaves, shoots and roots were recorded with spraying Biomagic at presence of mixture of Azotobacter and Bacillus.

The effect of Biomagic application and bacterial inoculation on leaves, shoots and roots content of mineral elements was significantly increased as compared with control.

The main purpose of this study is determine the effect of some biofertilizers on growth and chemical constituents of Moringa oleifera L. seedlings in order to increase its quantity and improve quality.

Materials and Methods

The present study was carried out at Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, and Research and Production Station at Nubaria region, National Research Center, Dokki, Giza, during two successive seasons (2005/2006) and (2006/2007). The effect of the biofertilizers on growth and chemical constituents of Moringa oleifera Lam was studied in this experiment. The seeds of Moringa oleifera Lam. were obtained from twenty-year-old trees, grown in the tropical garden of Kom-Ombo, Aswan, Egypt. The seeds were sown in seed beds on 1st April in each season and were held in a greenhouse until transplanting date. The germination of seeds was about to 90 %. Kristalon (NPK19:19:19), produced by Phayzon

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Bio-fertilizers were applied twice in a liquid form at a rate of 200 ml/pot after one month from transplanting and a second application was added 6 months later.

The treatments were: no inoculation (control), Phosphorine, Rhizobactrine, Microbine and Nitrobine.

The following data were recorded:

- Plant height (cm), stem diameter (mm), number of branches/plant, number of leaves/plant, root length (cm), shoots and Roots fresh weights (g) and shoots and Roots dry weights (g).
- The chemical analyses were carried out in laboratories of National Research Center and Faculty of Agriculture, Cairo University on both fresh and dry roots and shoots samples representing the different treatments. The dry weight of sample was determined after drying the samples in an oven at 70°C till a constant weight.

The following methods were used:

- Photosynthetic pigments: chlorophyll a, b and carotenoids were determined according to Nornai (1982). Total carbohydrates were determined according to Dubois et al. (1956). Nitrogen content was determined by modified micro-Kjeldahl method as described by Pregl (1945). Phosphorus content was estimated using ammonium molydate method according to Snell and Snell (1949). Potassium was determined using flame photometer according to Chapman and Pratt (1961). The results were recorded on young trees after 18 months from transplanting and were analyzed statistically according to Snedecor and Cochran (1980). The least significant difference test (L.S.D.) at 5% was used to compare between means of the different treatments.

Results and Discussion

Effect on growth

Data in Table (2) indicated that, in the first and second seasons fertilizing Moringa oleifera with phosphorine significantly increased the plant height. The increments were (86.12% and (14.20%) compared with control plants. Nitrobine produced a considerable increase of plant height, while Rhizobactrine and Microbine had no significant effect. Additively, in the first season, the application of Phosphorine or Nitrobine resulted in a significant increase in stem diameter. The increments were (72.25% and 55.58%), respectively compared with the control, in the second season, all biofertilizer treatments significantly increased the stem diameter. The increments were (40.35, 24.56, 26.32 and 28.07%), respectively compared with the control plants.

In this respect, the application of Microbine and Phosphorine significantly increased the number of branches / plant in the first and second season. The increments were (80%, 60%, 30.3% and 27.3%) compared with the control plants. Also, Leaf number ranged from 49.67 to 102.00 leaf / plant in the first season and from 46.75 to 83.75 in the second one. In both seasons, inoculating the plants with Microbine gave the highest significant the number of leaf / plant (102.00 and 83.75 in the first and second seasons, respectively) as compared with the control and the other treatments. In addition, using Phosphorine and Nitrobine produced a significant increase in

<table>
<thead>
<tr>
<th>Particle size distribution</th>
<th>Field capacity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand %</td>
<td>Silt %</td>
</tr>
<tr>
<td>70.8</td>
<td>25.6</td>
</tr>
<tr>
<td>EC dsm-1</td>
<td>pH (1:2.5)</td>
</tr>
<tr>
<td>1.26</td>
<td>7.9</td>
</tr>
<tr>
<td>Soluble Cations (meq l-1)</td>
<td>Soluble Anions (meq l-1)</td>
</tr>
<tr>
<td>Ca2+</td>
<td>Mg2+</td>
</tr>
<tr>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Total N (mg/100g)</td>
<td>Available (mg/100g)</td>
</tr>
<tr>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>15.1</td>
<td>13.0</td>
</tr>
<tr>
<td>4.0</td>
<td>Cu</td>
</tr>
</tbody>
</table>

Table 1: Physical and chemical properties of El-Nubaria soil
root length (43.00 and 41.00cm, respectively) as compared to the control and the other biofertilizers. In the second season a similar favourable effect was observed by applying Phosphorine and Nitrobe; also the response to Microbine inoculation changed from negative in the first season to positive and enhanced root elongation.

In the same Table data indicated that in the first season, using phosphorine and Nitrobe produced a significant increase in root length (43.00 and 41.00cm, respectively) as compared to the control and the other biofertilizers. In the second season a similar favourable effect was observed by applying phosphorine and Nitrobe; also the response to Microbine inoculation changed from negative in the first season to positive and enhanced root elongation.

The results which are presented in Table (3) showed that all the biofertilizer treatments caused a significant increase in the fresh and dry weight of shoots in the first and second season, except Rhizobactrine treatment had no significant effect. In the first season, Microbine showed to be the most effective biofertilizer for increasing shoot fresh and dry weight. The increments were (179.02% and 211.95%), respectively compared to the control. In the second season, a similar trend was observed. In this respect, in the first season, Phosphorine inoculation gave the highest root fresh and dry weight values (572.00g and 223.08g) as compared to the control (270.33g and 98.13g); Microbine and Nitrobe produced a considerable increase in fresh and dry weight of roots. In the second season, a similar trend was observed.

In view of the present results concerning the effect of biofertilizers on growth of Moringa oleifera, it may be concluded that biofertilizer inoculation produced a beneficial effect on all growth parameters. The efficiency in improving growth varied among the biofertilizers applied. Phosphorine showed to be the most effective biofertilizer for increasing root length, root fresh and dry weight. Similarly, previous researches on other tree species showed that phosphate solubulizing bacteria resulted in the best growth of Moringa oleifera during 2005 and 2006 and 2006 during 2005 and 2006.


<table>
<thead>
<tr>
<th>Bio-fertilizers</th>
<th>Plant height (cm)</th>
<th>Stem diameter (mm)</th>
<th>No of branch/ plant</th>
<th>No of leaves/ plant</th>
<th>Root length (cm)</th>
<th>Plant height (cm)</th>
<th>Stem diameter (mm)</th>
<th>No of branch/ plant</th>
<th>No of leaves/ plant</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.00</td>
<td>12.00</td>
<td>5.00</td>
<td>49.67</td>
<td>31.33</td>
<td>112.70</td>
<td>14.25</td>
<td>8.25</td>
<td>46.75</td>
<td>34.48</td>
</tr>
<tr>
<td>Phosphorine</td>
<td>124.70</td>
<td>20.67</td>
<td>8.00</td>
<td>58.00</td>
<td>43.00</td>
<td>128.70</td>
<td>20.00</td>
<td>10.50</td>
<td>62.25</td>
<td>44.91</td>
</tr>
<tr>
<td>Rhizobactrine</td>
<td>68.00</td>
<td>14.00</td>
<td>6.00</td>
<td>52.00</td>
<td>32.67</td>
<td>114.10</td>
<td>17.75</td>
<td>9.75</td>
<td>54.25</td>
<td>35.35</td>
</tr>
<tr>
<td>Microbine</td>
<td>74.33</td>
<td>14.67</td>
<td>9.00</td>
<td>102.00</td>
<td>33.33</td>
<td>114.20</td>
<td>18.00</td>
<td>10.75</td>
<td>83.75</td>
<td>43.49</td>
</tr>
<tr>
<td>Nitrobe</td>
<td>94.67</td>
<td>16.67</td>
<td>5.33</td>
<td>61.67</td>
<td>41.00</td>
<td>118.70</td>
<td>18.25</td>
<td>9.00</td>
<td>68.00</td>
<td>43.57</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>13.89</td>
<td>4.57</td>
<td>1.42</td>
<td>13.02</td>
<td>5.61</td>
<td>8.15</td>
<td>2.09</td>
<td>1.15</td>
<td>10.79</td>
<td>5.24</td>
</tr>
</tbody>
</table>

Effect on chemical constituents:

Table 3. Effect of biofertilizers on fresh and dry weight (g) of shoot and root of Moringa oleifera during 2005-2006 and 2006-2007.

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>46.95</td>
<td>270.33</td>
<td>12.39</td>
<td>98.13</td>
</tr>
<tr>
<td>Phosphorine</td>
<td>82.30</td>
<td>572.00</td>
<td>21.78</td>
<td>223.08</td>
</tr>
<tr>
<td>Nitrobe</td>
<td>131.00</td>
<td>509.00</td>
<td>38.65</td>
<td>193.42</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>7.98</td>
<td>16.75</td>
<td>4.10</td>
<td>11.21</td>
</tr>
</tbody>
</table>

Data presented in Table (4) show the effect of biofertilizers inoculation on the contents of total chlorophylls and carotenoids in the leaves of Moringa oleifera Lam. In the first season, Phosphorine, Microbine and Nitrobe produced the highest chlorophyll content (1.65 mg/g) compared to the control (1.47 mg/g), while Rhizobactrine produced a slight increase. In the second season a similar trend was observed. All the biofertilizers increased carotenoids content as compared with the control. The highest values of carotenoids content (0.34 and 0.36 mg/g in the first and second seasons, respectively) were recorded in plants inoculated with Microbine. Kumudha and Gomathinayagam (2007) on Albizia lebbek studied the effects of Rhizobium (18
g/pot), *phosphobacteria* (18 g/pot) and vesicular arbuscular mycorrhizal (VAM) fungi (45 g/pot) individually and in conjunction on the seedling biochemical parameters such as chlorophyll 'a', chlorophyll 'b', and total chlorophylls.

**Table 4.** Effect of biofertilizers on chlorophyll and carotenoids (mg/g FW) of *Moringa oleifera* during 2005-2006 and 2006-2007.

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>First season</th>
<th></th>
<th>Second season</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll mg/g FW</td>
<td>Carotenoids mg/g FW</td>
<td>Chlorophyll mg/g FW</td>
<td>Carotenoids mg/g FW</td>
</tr>
<tr>
<td>Control</td>
<td>1.47</td>
<td>0.24</td>
<td>1.57</td>
<td>0.27</td>
</tr>
<tr>
<td>Phosphorine</td>
<td>1.65</td>
<td>0.29</td>
<td>1.74</td>
<td>0.31</td>
</tr>
<tr>
<td>Rhizobactrine</td>
<td>1.53</td>
<td>0.26</td>
<td>1.65</td>
<td>0.29</td>
</tr>
<tr>
<td>Microbine</td>
<td>1.65</td>
<td>0.34</td>
<td>1.76</td>
<td>0.36</td>
</tr>
<tr>
<td>Nitrobine</td>
<td>1.65</td>
<td>0.31</td>
<td>1.75</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Total carbohydrates content (% DW)**

Data in Table (5) show the effect of biofertilizer applications on the content of the total carbohydrates in the different organs of *Moringa oleifera*. Carbohydrates content in leaves increased in response to all biofertilizer treatments in the first and second season. Nitrobose application produced significantly increased the carbohydrate content in the leaves and roots in the first season. The increments were (57.38 and 66.16%), respectively compared with control plants, the same trend was observed in the second season. Concerning the effect of different biofertilizers on total carbohydrates in stems of *Moringa oleifera*, the data showed that the application of Phosphorine resulted in the highest content of the carbohydrates (25.50 and 27.60%) in the first and second seasons, respectively. Saher (2008) on jojoba seedlings indicated that the highest total carbohydrate in leaves, shoots and roots were recorded with spraying Biomagic at presence of mixture of *Azotobacter* and *Bacillus*.

**Table 5.** Effect of biofertilizers on total carbohydrates % DW of *Moringa oleifera* during 2005-2006 and 2006-2007.

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>Total carbohydrates % DW</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First season</td>
<td>Second season</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>18.30</td>
<td>15.40</td>
<td>14.20</td>
</tr>
<tr>
<td>Phosphorine</td>
<td>23.70</td>
<td>25.30</td>
<td>18.20</td>
</tr>
<tr>
<td>Rhizobactrine</td>
<td>20.60</td>
<td>18.00</td>
<td>15.80</td>
</tr>
<tr>
<td>Microbine</td>
<td>25.30</td>
<td>20.10</td>
<td>21.20</td>
</tr>
<tr>
<td>Nitrobine</td>
<td>28.80</td>
<td>23.20</td>
<td>23.60</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.50</td>
<td>2.10</td>
<td>2.00</td>
</tr>
</tbody>
</table>

**Nutrient contents**

The response of nitrogen content in the leaves, stems and roots of *Moringa oleifera* to the different biofertilizer inoculations are presented in Table (6). All types of biofertilizers increased nitrogen content in leaves and stems as compared with the control in the two seasons. The most effective biofertilizer was Microbine which produced the highest nitrogen content in the first and second seasons (2.74, 2.66, 2.95 and 2.73%, respectively). The highest nitrogen content in roots (1.75% and 1.85%) in the first and second seasons were produced by Nitrobine as compared to the control.

**Table 6.** Effect of biofertilizers on nitrogen content % DW of *Moringa oleifera* during 2005-2006 and 2006-2007.

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>Nitrogen content (N) % DW</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First season</td>
<td>Second season</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
<td>Root</td>
</tr>
<tr>
<td>Control</td>
<td>2.60</td>
<td>2.47</td>
<td>1.63</td>
</tr>
<tr>
<td>Phosphorine</td>
<td>2.65</td>
<td>2.50</td>
<td>1.66</td>
</tr>
<tr>
<td>Rhizobactrine</td>
<td>2.63</td>
<td>2.53</td>
<td>1.68</td>
</tr>
<tr>
<td>Microbine</td>
<td>2.74</td>
<td>2.60</td>
<td>1.71</td>
</tr>
<tr>
<td>Nitrobine</td>
<td>2.70</td>
<td>2.55</td>
<td>1.75</td>
</tr>
</tbody>
</table>

The results presented in Table (7) show the effect of various biofertilizer types on *P* content in leaves, stem and root of *Moringa oleifera*. Phosphorus content in leaves, stems and roots increased markedly in the first season (0.20, 0.18, 0.25 and 0.17%, respectively) in response to Phosphorine inoculation, as compared to the control. In the second season, a similar response to the type of biofertilizer was recorded.

The results in Table (8) show the effect of biofertilizers on potassium content in leaves, stem and roots of *Moringa oleifera*. In the first season, K content in leaves and stems reached the highest value (1.73 and 1.50%) by applying Phosphorine, and in the second season, the highest values (1.91 and 1.62%) in case of K.
content in roots, it was observed that it responded differently to the type of biofertilizer; the highest content was obtained by Nitrobine in the first and second seasons (1.42 and 1.47%) compared with control (1.28 and 1.31%), respectively.

<table>
<thead>
<tr>
<th>Biofertilizers</th>
<th>Phosphorus content (% DW)</th>
<th>Leaves</th>
<th>Stems</th>
<th>Root</th>
<th>Leaves</th>
<th>Stems</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.07</td>
<td>0.07</td>
<td>0.10</td>
<td>0.12</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Phosphorine</td>
<td></td>
<td>0.20</td>
<td>0.18</td>
<td>0.17</td>
<td>0.25</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Rhizobactrine</td>
<td></td>
<td>0.12</td>
<td>0.10</td>
<td>0.12</td>
<td>0.15</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Microbine</td>
<td></td>
<td>0.13</td>
<td>0.13</td>
<td>0.12</td>
<td>0.18</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Nitrobine</td>
<td></td>
<td>0.18</td>
<td>0.14</td>
<td>0.14</td>
<td>0.21</td>
<td>0.19</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Generally, it might be concluded that supplying *Moringa oleifera* tree during its early stage of growth with biofertilizers, can be a better source of nutrients and less harmful than inorganic fertilizers. It can also be suggested to use a combined biofertilizer including Phosphorine, Nitrobine and Microbine; or a dual biofertilizer including Phosphorine and Nitrobine to produce a high quality *Moringa* tree. Several reports on biofertilizer utilization have emphasized that dual or combined inoculation showed higher productivity than single inoculation (Rajendran and Devaraj, 2004; and Shah et al., 2006).

**References**


