Synergistic Effects of AMF and Bacillus lehensis Strain MLB2 on Ocimum sanctum Grown under Fluoride Stress

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Abstract: The aim of the present study was to notice the synergistic effect of both, Arbuscular Mycorrhizal fungi (AMF) and Bacillus lehensis, on the growth of Ocimum sanctumvar. CIM-AYU grown under 40 ppm of Sodium fluoride stress. Glomus mossae and Bacillus lehensis strain MLB2 increased plant weight by 41% and 9% respectively. Consortium of Glomus mossae and Bacillus lehensis strain MLB2 resulted in 44% increase in Fresh herbage yield of Ocimum sanctum as compared to control. Plant growth promoting rhizobacteria when grown in association with various mycorrhizal fungi showed a remarkable increase in plant height, leaf fresh weight, and total fresh biomass. Best consortium result was shown by G.mossae followed by G. fasciculatum G. aggregatum and G.intraradices. Inoculation of tulsi seedlings with mycorrhizal fungi and Bacillus lehensis is increased the fluoride tolerance level of the herb.

Keywords: Arbuscular Mycorrhizal Fungi, Consortium, Sodium fluoride, Ocimum sanctum

1.Introduction

From ancient time, Tulsi is used as a traditional remedy for wound healing and microbial infections. Ocimum sanctum is a medicinal and aromatic plant (MAP), belonging to family Lamiaceae (Grayer et al. 1996). It is an annual herb native to India and other parts of Asia (Klimankova et al. 2008). The use of plant parts like root, stem and leaves has been maintained traditionally (Leonti et al. 2003). Medicinal herbs have been used from pre-historic times (Dragland et al. 2003). Mycorrhizal fungi are found everywhere. These symbionts attach and become part of the plant. AM fungi enhance the nutrient uptake and crop yield by solubilizing phosphate. If there is high concentration of phosphorous in the soil, mycorrhiza never die rather they control the phosphorus content of the soil (Smith et al. 1997).

Bacillus lehensis strain MLB2 is a gram positive, alkali-tolerant and endospore forming bacteria (Ghosh et al. 2007). Fluorine is the most electronegative atom, and therefore has the ability to make strong hydrogen bonds. Fluoride accumulation, in even low concentrations can cause an abnormal change in biochemical and physiological parameters in plants and animals. In higher concentrations, it causes dental and skeletal fluorosis in humans (Lakshmi 2013).

2.Material and Method

Sample Collection

A bacterium, Bacillus lehensis strain MLB2, was isolated from the fluoride affected soil of Sirsahakhera region of Unnao district in Uttar Pradesh, India and was tested against different Sodium fluoride (NaF) concentrations: 100ppm, 200ppm, 300ppm, 400ppm and 500ppm.

Experimental Site

The experimental site, Lucknow (Uttar Pradesh), with a warm humid subtropical climate is situated in the north-eastern part of Uttar Pradesh, India. Latitude: 26°50′21″ N, Longitude: 80°55′23″ E and Elevation above sea level: 126 m = 413 ft. The average annual rainfall of this area is 313 mm, which is evenly distributed from June to October and August is the wettest month of the year. The average temperature ranges from 26°C to 39°C and actual temperature ranges from 29°C to 47°C. The relative humidity fluctuate between 34 % and 92%.

Seed treatment

Seeds of Ocimum sanctum were obtained from the National Gene Bank for Medicinal and Aromatic Plants at the CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. Ocimum sanctum seeds were surface sterilized with 10% NaOCl for 5 minutes and properly rinsed with distilled water for 5 minutes before sowing.

Experimental set-up

Seeds of O.sanctum were sown in the polyethylene bags filled with sterile soil. 15 days old seedlings were then transferred to the earthen pots containing 1kg soil by using 5 g of inoculum of different AM fungi per seedling respectively placed at 5cm depth in pots. The seedlings were dipped in the bacterial inoculum solution of Bacillus lehensis for 30 min and were transferred to the pots having AM Fungi. The experimental set up was in completely randomized block design with three replicates of each, i.e. control and treatments in a glass house. The inoculum of different glomus fungi (Glomus mossae, Glomus fasciculatum, Glomus intraradices and Glomus.
aggregatum) were obtained from CSIR-Central institute of medicinal and aromatic plants, Lucknow (U.P)

**Determination of Biochemical Parameters**

After 2 months, the crop was harvested to determine the physical parameters, chlorophyll content and enzymatic activity.

**Spore Count and Percent Root Colonisation**

The spores produced by G. mossae, G. fasciculatum, G. aggregatum and G. intraradices were counted by following the wet sieving and decanting method (Gerdemann & Nicolson, 1963). Percentage colonization of roots by arbuscules and vesicles was done by McGonigle et al. 1990 method.

**Statistical Analysis**

The collected data was subjected to statistical analysis for analysis of variance method (ANOVA), suitable to completely randomized design (CRD) for pot experiment with the help of software ASSISTAT 7.7 beta version. Microsoft excel was used for calculating Standard deviation and Standard error. The means were calculated using Duncan’s multiple range tests under a significance level of $P \leq 0.05$.

**3. Result**
Graphs showing effect of various AM fungi and Bacillus lehensis strain MLB2 on various plant parameters of Ocimum sanctum. Where F: Sodium fluoride, St: 6: Bacillus lehensis strain MLB2, Gm: Glomus mosseae, Ga: Glomus aggregatum, Gi: Glomus fasciculatum, Gf: Glomus intraradices.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Shoot fresh weight(gm)</th>
<th>Leaf fresh weight (gm)</th>
<th>Total dry weight(gm)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CONTROL</td>
<td>85.913de</td>
<td>10.016e</td>
<td>16.246f</td>
<td>5.663fg</td>
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<tr>
<td>2</td>
<td>C + F</td>
<td>57.700g</td>
<td>5.256f</td>
<td>9.1433h</td>
<td>3.106h</td>
</tr>
<tr>
<td>3</td>
<td>St 6</td>
<td>82.840f</td>
<td>12.526bc</td>
<td>16.67ef</td>
<td>5.926ef</td>
</tr>
<tr>
<td>4</td>
<td>St 6 + F</td>
<td>82.460f</td>
<td>9.4855e</td>
<td>16.002g</td>
<td>5.500g</td>
</tr>
<tr>
<td>5</td>
<td>Gm</td>
<td>100.20ab</td>
<td>14.410a</td>
<td>22.533ab</td>
<td>8.220a</td>
</tr>
<tr>
<td>6</td>
<td>Gf</td>
<td>94.760ab</td>
<td>12.110bc</td>
<td>22.186ab</td>
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<tr>
<td>7</td>
<td>Ga</td>
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<td>11.636cd</td>
<td>21.486b</td>
<td>7.173bc</td>
</tr>
<tr>
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<td>Gi</td>
<td>90.273bc</td>
<td>11.58cd</td>
<td>17.063c</td>
<td>6.22de</td>
</tr>
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<td>9</td>
<td>Gm+St 6</td>
<td>101.533a</td>
<td>14.60a</td>
<td>23.177a</td>
<td>8.386a</td>
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<tr>
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<td>Gm+St 6+F</td>
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<td>11.21d</td>
<td>19.101cd</td>
<td>6.53d</td>
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<td>13</td>
<td>Ga+St 6</td>
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<td>11.650cd</td>
<td>19.263cd</td>
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<tr>
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<td>Ga+St 6+F</td>
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<td>5.81ef</td>
</tr>
</tbody>
</table>

Values denoted by same letter are not significantly different at P<0.05level

4. Discussion

Sodium fluoride has inhibitory effect on plant growth. Neutral to alkaline pH favors germination of G. mosseae. Plants inoculated with AM fungi growing under saline conditions resulted in an increase in root length, fresh and dry weights of shoot and increased photosynthesis (Shekoofeh and Sepideh 2011). Root length and shoot length, decreased in seedlings under fluoride stress (Gadi et al. 2012). Synergistic effect of AM fungi and B. lehensis showed a remarkable coping effect of the herb against fluoride stress. The best coping effect was shown by G. mosseae followed by G. fasciculatum, G. aggregatum and G. intraradices respectively.

5. Conclusion

Being a medicinally important plant, shoot of Ocimum sanctum is rich in essential oil. Synergistic effect of AM fungi (AMF) inoculation with Bacillus lehensis under fluoride stress resulted in increase in leaf & shoot fresh weights by coping up with stress effects of fluoride. In this way, AM fungi and Bacillus lehensis used synergistically can prove to be a promising note for better yielding of natural herbs in stress conditions.

6. Acknowledgement

Authors are sincerely thankful to the Head of Department of Botany, University of Lucknow, for providing the necessary laboratory facilities, Dr. Alok Kalra (senior Scientist, CSIR-CIMAP, Lucknow) for bacterial isolation & identification and CSIR-CIMAP, Lucknow for encouragement and facilities.

References