EFFECT OF RHIZOBACTERIAL INOCULATION ON ALKALOID ALOIN CONTENT OF ALOEVERA

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ABSTRACT
A group of natural soil microbial flora acquire dwelling in the rhizosphere and on the surface of the plant root which impose beneficial effect on the overall wellbeing of the plant are categorized as plant growth promoting rhizobacteria (PGPR). Rhizobacterial isolates such as Azospirillum, Azotobacter, Bacillus and Pseudomonas were isolated from 40 different locations. These isolates were further screened for their plant growth promoting traits. Based on the performance of PGPR strain were selected for pot culture studies inoculum used as single, dual and consortium. The aloin content was estimated by high performance liquid chromatography. The alkaloid content of Aloin peak eluted at about 14.2 minutes. Inoculation of rhizobacteria either alone or in various combinations increased Aloin content in Aloe vera. Maximum Aloin content was recorded in the treatment receiving T11 PGPR inoculation (0.825 mg 100 g⁻¹) followed by dual inoculation of T7 (0.796 mg 100 g⁻¹ root), T6 (0.795 mg 100 g⁻¹ root), T5 (0.794 mg 100 g⁻¹ root), T10 (0.793 mg 100 g⁻¹ root), T9 (0.792 mg 100 g⁻¹ root) and T8 (0.791 mg 100 g⁻¹ root) and it was followed by single inoculation treatments T1, T4, T3 and T2 respectively. The control treatment recorded lowest Aloin content in Aloe vera.

Keywords: Azospirillum, Azotobacter, Bacillus, pseudomonas sp, aloin content, Aloe vera.

INTRODUCTION:
Medicinal plant has specific property and specific use owing to their biological group of compounds. Indian Systems of Medicine (ISM) use around 2500 plant species belonging to more than 1000 genera. About 800 species are used by industry of which approximately 25 per cent are cultivated. Several species of the genus alone have been used under the common name viz., Aloe vera, aloe barbadensis, Aloe ferox, Aloe chinesis, Aloe indic, Aloe peyrii etc. A group of natural soil microbial flora acquire dwelling in the rhizosphere and on the surface of the plant root which impose beneficial effect on the overall wellbeing of the plant are categorized as plant growth promoting rhizobacteria (PGPR). Rhizobacterial isolates such as Azospirillum, Azotobacter, Bacillus and Pseudomonas were isolated from 40 different locations. These isolates were further screened for their plant growth promoting traits. Based on the performance of PGPR strain were selected for pot culture studies inoculum used as single, dual and consortium. Bioinoculants are a vital component for the long-term sustainable agriculture system of any crop (Tilak et al., 2005). Considering the potential of Plant growth promoting rhizobacteria the knowledge on the association of PGPR with medicinal herbs will be of immense help for standardizing microbial technique to enhance the crop yield and alkaloid quality of Aloe Vera. Further, the use of biofertilizer technology for sustainable production of medicinal plants is lacking. Hence, the present work carried with the aim of developing a suitable PGPR consortium, to enhance the crop yield and alkaloid quality of Aloe Vera.

MATERIALS AND METHODS:
Estimation of alkaloid (Aloin)
The analysis of the alcoholic extracts of the tuber sample were carried out by the methodology given by Inamdar et al. (1984) by using the High performance liquid chromatographic (HPLC) available at Department of Microbiology, Annamalai University.

Standard Aloin

Aloe Vera leaf samples
Fresh leaf of Aloe Vera was collected and dried at room temperature and then ground into uniform powder. Solvent methanol of HPLC grade purchased from E-Merck for extraction and analysis.

Preparation of standard and samples for HPLC analysis
Standard
Aloin standard (98%) was obtained from National Institute of Standards and Technology (NIST) Library. Exactly 25 mg Aloin was placed into a 25 ml volumetric flask, dissolved, and diluted to volume with HPLC-grade methanol.

Sample preparation
One gram of each samples was weighed into a 100 ml flask, and 50 ml methanol was added and refluxed for 30 min. the process was performed three times with 50 ml of methanol each time. All of the methanolic extracts were combined and diluted to 250 ml with methanol, mixed, and filtered through Whatman no.42 filter paper to obtain a clear solution.

Mobile phase
Hexane, ethyl acetate and methylene chloride were mixed in the proportion of 70:20:10 (v/v/v) and degassed.

Chromatographic system
The liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) was equipped with a 210-nm UV detector and C_{18} column (250 x 4.6 mm; M/s Spinco Biotech Pvt. Ltd., Bangalore, India). The mobile phase was pumped at the rate of 0.8 ml min^{-1} with a back pressure of 200 psi. The injector and the detector were flushed with the mobile phase. The refractive index of the detector was set at 4x and the potentiometer chart speed was set at 0.5 cm min^{-1}.The column was equilibrated for 0.5 h. with a liquid flow rate of about 1.0 ml min^{-1}. The standard preparation (10 µl) was chromatographed, and the peak response for aloin was recorded. Equal volumes (10 µl) of the sample preparations were injected, the chromatograms were recorded, and the peaks corresponding to aloin were measured.

Statistical analysis
The experimental data were analysed by following the methods of Panse and Sukhatme (195).

RESULTS AND DISCUSSION:
The Aloin alkaloid content was estimated in all treatments. The Aloin Alkaloid content of Aloe Vera ranged between 0.690-0.825 mg 100 g^{-1} of roots in various treatments as measured by High performance liquid chromatography (HPLC).

Aloin content
The alkaloid content of Aloin peak eluted at about 14.2 minutes. Inoculation of rhizobacteria either alone or in various combinations increased Aloin content in Aloe vera. Maximum Aloin content was recorded in the treatment receiving T_{11} PGPR inoculation (0.825 mg 100 g^{-1}) Followed by dual inoculation of T_{7} (0.796 mg 100 g^{-1} root), T_{5} (0.795 mg 100 g^{-1} root), T_{9} (0.794 mg100 g^{-1}root), T_{10} (0.793 mg 100 g^{-1} root), T_3 (0.792 mg 100 g^{-1} root) and T_{9} (0.791 mg 100 g^{-1} root) and it was followed by single inoculation treatments T_{1}, T_{5}, T_{3} and T_{2} respectively. The control treatment recorded lowest Aloin content in Aloe Vera. Effect of rhizobacterial inoculation on Aloin content of Aloe vera by HPLC

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Aloin content (mg100g^{-1} of roots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>T_1 - Azospirillum (AVAzs-3)</td>
<td>0.775</td>
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<tr>
<td>2.</td>
<td>T_2 - Azotobacter (AVAzt-9)</td>
<td>0.764</td>
</tr>
<tr>
<td>3.</td>
<td>T_3 - Bacillus (AVPb-11)</td>
<td>0.770</td>
</tr>
<tr>
<td>4.</td>
<td>T_4 - Pseudomonas (AVPF-23)</td>
<td>0.773</td>
</tr>
<tr>
<td>5.</td>
<td>T_5 - Azospirillum (AVAzs-3) Azotobacter (AVAzt-9)</td>
<td>0.794</td>
</tr>
<tr>
<td>6.</td>
<td>T_5-Azospirillum (AVAzs-3) + Bacillus (AVPb-11)</td>
<td>0.795</td>
</tr>
<tr>
<td>7.</td>
<td>T_7-Azospirillum (AVAzs-3) + Pseudomonas (AVPF-23)</td>
<td>0.796</td>
</tr>
<tr>
<td>8.</td>
<td>T_9 - Azotobacter (AVAzt-9) + Bacillus (AVPb-11)</td>
<td>0.791</td>
</tr>
<tr>
<td>9.</td>
<td>T_9 - Azotobacter (AVAzt-9) + Pseudomonas (AVPF-23)</td>
<td>0.792</td>
</tr>
<tr>
<td>10.</td>
<td>T_{10} - Bacillus (AVPb-11) + Pseudomonas (AVPF-23)</td>
<td>0.793</td>
</tr>
<tr>
<td>11.</td>
<td>T_{11} - PGPR Inoculation</td>
<td>0.825</td>
</tr>
<tr>
<td>12.</td>
<td>T_{12} - control</td>
<td>0.690</td>
</tr>
</tbody>
</table>

SED = 0.01

\( \bar{D} = (0.05) \)
Effect of rhizobacterial inoculation on Aloin content of *Aloe vera* by HPLC

Effect of rhizobacterial inoculation on Aloin content of *Aloe vera* by HPLC

- **T₁** - *Azospirillum (AVAzs-3)*
- **T₂** - *Azotobacter (AVAzt-9)*
- **T₃** - *Bacillus (AVPb-11)*
- **T₄** - *Pseudomonas (AVPf-23)*
- **T₅** - *Azospirillum (AVAzs-3) + Azotobacter (AVAzt-9)*
- **T₆** - *Azospirillum (AVAzs-3) + Bacillus (AVPb-11)*
- **T₇** - *Azospirillum (AVAzs-3) + Pseudomonas (AVPf-23)*
REFERENCES: