Antagonistic effect of *Rhizobium leguminosarum*, *Pseudomonas fluorescens* and *Bacillus subtilis* in Soybean (*Glycine max* (L).Merill) under *in vitro* conditions

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**Abstract**

*Fusarium* wilt disease is caused by *Fusarium oxysporum* in Soybean plants. The present investigation deals with the treatment of seeds with different Plant growth promoting rhizobacteria (PGPR) such as *Rhizobium leguminosarum*, *Pseudomonasfluorescens* and *Bacillus subtilis* as single and dual inoculants over a period of 20, 40 and 60 days. The disease incidence and severity were reduced by the efficiency of the PGPR.

**Keywords**: Soybean, *Fusarium oxysporum*, PGPR, disease incidence and severity.

**Introduction**

Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production. They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure (Ahemad, 2012). Indeed, the bacteria lodging around/in the plant roots (Rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils (Hayat *et al.*, 2010). Hence, diverse symbiotic (*Rhizobium, Bradyrhizobium, Mesorhizobium*) and non-symbiotic (*Pseudomonas, Bacillus, Klebsiella, Azotobacter, Azospirillum, and Azomonas*), rhizobacteria are now being used worldwide as bio-inoculants to promote plant growth and development. Agrochemical treatment may result in environmental impact and pose a threat to humans and animals. As a result, there has been an increase in research on potential PGPR agents, aimed at finding a definitive solution or at least at reducing pesticide use in the treatment of phytopathogenic diseases. PGPR, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. Soybean is one of the most important crops and a source of vegetable protein and oil. Soybean represents half of the global legume crop area and 68% of global production and fixes 16.4 Tg N/year, it represents more than three forths of the N fixed by the crop legumes (Herridge *et al.*, 2008). This crop is also a good source of vitamins A, B and D. This crop is aptly called as “Golden Bean” or “Miracle Crop” of the 20th century, because of its multiple uses. In addition to the nutritional advantages, Soybean is also recognized for its benefits to human health such as the cholesterol lowering effect of protein as approved recently by the United States Food and Drugs administration (Wilmot, 2001).

*Fusarium* delete. and *Pythium* spp. are two fungal pathogens that heavily infect soybean and thus influence growth from germination to all stages of plant development. *Pythium* spp. infections are common to corn and soybean and cause damping-off diseases of crops. The damage caused by *Pythium* delete. can bring in major economic losses to plant growers. *Fusarium* delete, such as *Fusarium solani* f. sp. glycines infect the roots of seedling and cause leaf symptoms (necrosis, chlorosis, defoliation, etc.), which usually begin slightly
before or after flowering (Fig. 1). Severe early infections of this strain may reduce yield through seed and pod abortion (John et al., 2010). In view of the problems and potential solution stated above to work out suitable PGPR control of diseases of soybean and to evaluate the efficiency of three different microorganism in reducing disease incidence.

Materials and Methods

Seed materials

The seeds of Soybean [Glycine max (L.)Merill var. CO1 were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Bio-control agents

Different PGPR (R. leguminosarum, P. fluorescens and B. subtilis) were isolated from the rhizosphere of healthy Cowpea and Soybean plants from Vadakkumangudi village, Cuddalore district, Tamil Nadu.

Location of experimental site

Field experiments were conducted in Agricultural Land, Vadakkumangudi, Cuddalore district located within 4 km from the Annamalai University, Tamil Nadu, India. The studies were conducted during January 2014 to April 2014. The experimental site was situated at 11°24’N latitude and 79°41’E longitude with an altitude of 5°79’M above Mean Sea Level (MSL).

Mode of Action

A dual-culture test was conducted to examine, whether R. leguminosarum, P. fluorescens and B. subtilis could antagonize the growth of plant fungal pathogens. A mycelial disc, 0.5 cm in diameter of a pure culture of fungal pathogen was placed in the center of a PDA (Potato Dextrose Agar) plate and then R. leguminosarum, P. fluorescens and B. subtilis was inoculated in three symmetrical spots around the mycelial disc. The plates were incubated for 7-10 days at 28°C. The plates were scanned once a day to monitor the formation of an inhibition zone and the growth of fungal pathogens. The width of the inhibition zones were measured and then average was found. A cell-free supernatant of R. leguminosarum, P. fluorescens and B. subtilis was prepared by inoculating in the improved SM medium (Sodium Succinate medium) (Sayyed and Chincholkar, 2006) for 48 h at 37°C and then centrifuging at 12,000 rpm for 10 min. After mixing with PDA medium, the mixture was poured onto plates. After solidification, fungal mycelial disc of 0.5 cm was placed in the center of each petri dish. PDA medium containing 500µg mL⁻¹ of 75% chlorothalonil wettable powder was used as a positive control (CK⁺). The plates were incubated at 28°C and observed once a day to monitor the mycelial growth of the fungal pathogens. The fungal colony diameters were measured and then compared to the diameters of the same pathogens grown on the control plates. The radial growth of the pathogen and percentage reduction over control was calculated by using the formula (Vincent, 1947) as follows.

Percentage reduction over control = \( \frac{C - T}{C} \times 100 \)

Where,

C = Mycelial growth of the pathogen in control (mm) and

T = Mycelial growth of the pathogen in dual plate (mm)

Suppression of Fusarium wilt disease

To study the influence of PGPR agents, approximately after 55 days, the percent of diseased plants per treatment was scored on the basis of Fusarium Wilt disease symptoms. The experiments were conducted at least three times for all PGPR treatments.

Disease index (Wheeler, 1969)

The Soybean plants were analyzed for the incidence of disease during the 20, 40 and 60th days samples during the treatment. The percentage of the disease incidence was assessed by using the following formula.

Percentage of disease incidence = Number of infected plants/ Total number of plant X 100

Results and Discussion

Disease incidence of Soybean plants in field experiment (%)

In this study, higher disease incidence was recorded in control for Fusarium wilt (61.73 %). The incidence of Fusarium wilt diseases was less in dual inoculants than in single inoculant treated fields (Table 1; Fig.2).

Disease severity of Soybean plants in field experiment (%)

In Soybean, higher disease severity was recorded in control (10.58, 39.53 and 54.23 %) at 20, 40 and 60th days. The incidence of disease severity was less in dual inoculations particularly T₂ (28.67 and 38.96 %) followed by T₃ (28.99 and 37.39 %) and P. fluorescens+B. subtilis (28.03 and 35.74 %) when compared to single inoculations at 40 and 60th days (Table 2).
Table 1 Suppression of *Fusarium* wilt diseases in field treated with different PGPR agents

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Fusarium</em> wilt</th>
<th>Reduction over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease incidence (%)</td>
<td></td>
</tr>
<tr>
<td>$T_0$ - Control</td>
<td>62.67±2.52b</td>
<td>-</td>
</tr>
<tr>
<td>$T_1$ - <em>Rhizobium leguminosarum</em></td>
<td>33.13±5.588</td>
<td>34.11</td>
</tr>
<tr>
<td>$T_2$ - <em>Pseudomonas fluorescens</em></td>
<td>35.00±2.850</td>
<td>40.94</td>
</tr>
<tr>
<td>$T_3$ - <em>Bacillus subtilis</em></td>
<td>34.44±1.69</td>
<td>40.75</td>
</tr>
<tr>
<td>$T_4$ - <em>R. leguminosarum</em> + <em>P. fluorescens</em></td>
<td>29.92±2.50</td>
<td>51.79</td>
</tr>
<tr>
<td>$T_5$ - <em>R. leguminosarum</em> + <em>B. subtilis</em></td>
<td>31.28±1.27</td>
<td>49.41</td>
</tr>
<tr>
<td>$T_6$ - <em>P. fluorescens</em> + <em>B. subtilis</em></td>
<td>31.99±1.31</td>
<td>49.47</td>
</tr>
</tbody>
</table>

Table 2 Disease severity (%) of Soybean plants treated with different PGPR treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soybean</th>
<th>20th day</th>
<th>40th day</th>
<th>60th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_0$ - Control</td>
<td></td>
<td>10.58±0.317</td>
<td>39.53±1.006</td>
<td>54.23±1.627</td>
</tr>
<tr>
<td>$T_1$ - <em>Rhizobium leguminosarum</em></td>
<td></td>
<td>-</td>
<td>33.25±1.028</td>
<td>42.21±1.266</td>
</tr>
<tr>
<td>$T_2$ - <em>Pseudomonas fluorescens</em></td>
<td></td>
<td>-</td>
<td>34.00±1.020</td>
<td>44.35±1.330</td>
</tr>
<tr>
<td>$T_3$ - <em>Bacillus subtilis</em></td>
<td></td>
<td>-</td>
<td>33.10±1.173</td>
<td>43.97±1.319</td>
</tr>
<tr>
<td>$T_4$ - <em>R. leguminosarum</em> + <em>P. fluorescens</em></td>
<td></td>
<td>-</td>
<td>28.67±0.870</td>
<td>38.96±1.168</td>
</tr>
<tr>
<td>$T_5$ - <em>R. leguminosarum</em> + <em>B. subtilis</em></td>
<td></td>
<td>-</td>
<td>28.99±0.870</td>
<td>37.39±1.122</td>
</tr>
<tr>
<td>$T_6$ - <em>P. fluorescens</em> + <em>B. subtilis</em></td>
<td></td>
<td>-</td>
<td>28.03±0.841</td>
<td>35.74±1.072</td>
</tr>
</tbody>
</table>

Fig 1 *Fusarium* wilt

Fig 2 Soybean plants treated with different PGPRs
Conclusion

Results of the present study have shown that the disease severity caused by Fusarium oxysporum in soybean is reduced when treated with different PGPR agents as single and dual inoculants.

References


