IN VITRO AND IN VIVO BIOCONTROL OF ALTERNARIA TENUISSIMA BY USING RHIZOBIUM JAPONICUM

Kamthane Daiwshala C.

Department of Microbiology, S. G. B. College Purna Dist. Nanded, Maharashtra,

ABSTRACT

Alternaria Blight of soybean is caused by Alternaria tenuissima. Rhizobium japonicum are the symbiotic microorganisms which form root nodules on the roots of soybean. It was proved that R. japonicum is having growth promoting abilities. It was observed that R. japonicum was active against Alternaria tenuissima.

The antagonistic effect of R. japonicum against A. tenuissima was studied in vitro and in vivo in the present investigation. In vitro study was done by dual culture technique. The percent inhibition of R. japonicum against Alternaria tenuissima in case of soybean variety JS 71-5 was more than JS93-05 variety of soybean.

In vivo study was also done. The root and shoot length as well as number of root nodules were decreased when A. tenuissima alone was applied. The root and shoot length as well as number of root nodules were increased when R. japonicum in combination with A. tenuissima was applied.

KEY WORDS: ALTERNARIA TENUISSIMA, RHIZOBIUM JAPONICUM, IN VITRO, IN VIVO, ALTERNARIA BLIGHT, JS 71-5 AND JS93-05 SOYBEAN VARIETIES.

INTRODUCTION:

Soybean seed 100 gm edible portion include protein (34.1 gm), fat (17.7 gm), carbohydrate (33.3 gm), fiber (49 gm) and water (10 gm). Soybean constitutes a very nutritious food being very rich in proteins. Soybean oil is industrially used in manufacture of paints, oil cloth, printing ink, insecticides and disinfectants. Soybean milk extracted from seeds is used for cooking and is also prescribed for infants. Soybean also has different amino acids. The demand for soybean is increasing. Therefore efforts must be taken to increase the yield. Use of Soybean oil can reduce the heart problem, as it has low cholesterol content.

There is presence of symbiosis in between Rhizobium and legumes. This association results in the fixation of atmospheric nitrogen in the root nodules. This fixed nitrogen is used by the legume plant. This activity results in the increased productivity of the legume.

The underground as well as the foliar parts of soybean get affected by different micro-organisms causing various diseases. Among these, there are bacterial, fungal and viral diseases. The fungal disease Alternaria Blight is caused by Alternaria tenuissima. The Alternaria blight infected leaves show brown necrotic spots with concentric rings which coalesce and form large necrotic area. The lesions get surrounded by yellow zone. Infected leaves later in the season dry out and drop prematurely. This results in yield loss of soybean. Therefore the study of Alternaria Blight of soybean is essential one. In the present investigation R. japonicum was isolated from the root nodules and Alternaria tenuissima causing Alternaria blight in soybean was isolated from the leaf region. Biocontrol of A. tenuissima by using R. japonicum in vitro and in vivo was studied.

Control of fungal phytopathogens by using naturally occurring antagonistic microorganisms has been the focus of intense research throughout the world. This approach is popularly known as biological control of plant pathogens. Biological control is a bio-based, eco-friendly strategy which offers a practical and economical alternative. Therefore, control of plant diseases assumes greater importance.
MATERIAL AND METHODS:

1) Isolation of R. japonicum:

R. japonicum isolated from fresh, healthy, unbroken and pink nodules from the mature soybean plant roots from the mentioned varieties separately. The nodules were washed with sterile distilled water to remove attached soil particles. Nodules were externally sterilized by 1% Mercuric Chloride. Again water wash treatment was done to remove the Mercuric Chloride. Cleaned nodules were crushed in sterile distilled water. The five serial dilutions were prepared from this. The last dilution was selected and used for the isolation of R. japonicum. A loopful of the last dilution was streaked on modified sterile Yeast Extract Mannitol Agar (YEMA) medium containing 83 ìg/ml ZnCl\(_2\) and 500 ìg/ml CuSO\(_4\) (Ronald M. Atlas, 2005).

The streaked plates were incubated at 28°C for 3 days. After incubation, colony characteristics were taken (Aneja, 2002 and Dubey, 2012).

2) Collection of infected leaves:

For the present investigation, the crop selected for the study was JS 71-5 and JS 93-05 soybean varieties. In order to study biocontrol of fungus, samples of leaves showing “Alternaria Blight” disease were collected in sterile polythene bags from various places of Parbhani District (Patil and Kamble, 2009).

3) Isolation of Alternaria tenuissima from soybean:

The mentioned varieties infected leaves were cut in to small bits measuring about 2 mm and surface sterilized in 0.1 percent HgCl\(_2\) solution for 2 min. Such bits were transferred into petridishes containing modified Potato Dextrose Agar (PDA) with 1.5 % or MnCl\(_2\):4H\(_2\)O (Sorensen et al., 2009,) and incubated at 26°C for 7 days. Pure culture of the fungus was obtained by single spore isolation method.

A disc of 5mm fungal growth of respective pathogen was taken and kept on modified Potato Dextrose Agar medium. The respective plates containing fungal discs were kept at 26°C for 10 days. The radial growth during the incubation period was measured and recorded in millimeters. The growth was recorded by measuring colony diameter along with two diagonals axis passing through the centre of the colony. (Kamthane, 2002 and Meena & Ratnoo, 2013).

4) In vitro biocontrol testing of R. japonicum against the fungi isolated:

This was done by dual culture technique using King’s B agar. Now the isolated R. japonicum culture was spot inoculated on one edge of the plate. On the other edge of the plate, a 5mm disc of four day old pure, isolated and identified fungal pathogen was placed. The plates were prepared in triplicates along with the control plate. In the control plate, the purified fungus was inoculated and incubated only. The plates were prepared as mentioned above and were kept at room temperature for incubation to observe the interactions among the two organisms i.e. in between R. japonicum and the fungus. (Kamthane, 2002).

The percent inhibition was calculated using the following formula (Sandikar & Awasthi, 2010):

\[
\text{Percent inhibition} = \frac{(C-T)}{C} \times 100
\]

\(C=\text{Radial growth of fungus in mm in control plate.}\)

\(T=\text{Radial growth of fungus in mm on plate inoculated with Rhizobium japonicum.}\)
5) In vivo assay of antagonism:

The A.tenuissima causing Alternaria Blight of soybean varieties JS 71-5 and JS93-05 were isolated from the respected varieties.

The A. tenuissima antagonist causing Alternaria Blight of soybean was tested in vitro and later tested in vivo in pot culture experiment along with control for the comparison. The fungal spore suspension adjusted to 20x10^6 spores/ml was used for the study (Sunita Chandel, 2004).

Sterilized garden soil and sterilized sand in the ratio of 2:1 was used for the experiment (Kamthane and Rakh, 2013).

The seeds are treated with Rhizobium japonicum isolated from the two mentioned varieties as JS 71-5 and JS93-05 of soybean. This is done by mixing isolated rhizobia culture in 10 percent sugar and 40 percent gum arabic to form slurry. In this, seeds were added. With the result, a uniform coat of the R. japonicum is formed around the seeds. The treated seeds are dried in shade and sown immediately (Aneja, 2002). In the two test pots five coated seeds were sown. After a week, the suspension of A.tenuissima was applied (Chandel, 2004, Sangeeta and Shamarao, 2013 and Novina et al., 2013). In the first test pot, to the seedlings five cuts were made with a disinfected scissor. In the second test pot the seedlings are without cuts. In the control pot the uncoated five seeds were sown. After a weak, the suspension of A.tenuissima was applied.

The root nodules, roots and shoots were harvested on 28th day after soybean sowing. The roots were washed with water to remove the soil particles attached. Nodules formed, shoot length as well as root length and dry weight was recorded (Aneja, 2002 and Dhami & Prasad, 2006). Shoot length as well as root length was recorded in cm and the dry weight was measured in grams (Vaidya and Kelkar, 2008). The number of root nodules in each variety were also calculated.

RESULT AND DISCUSSION:

1. Isolation of R. japonicum

R. japonicum is isolated from fresh, healthy, unbroken and pink nodules were taken from the mature soybean (Glycine max) plant roots on modified YEMA medium containing 83ig/ml ZnCl₂ and 500ig/ml CuSO₄. The ZnCl₂ and CuSO₄ are antifungal compounds. After incubation, large, white translucent, glistening, gummy and elevated colonies having entire margin were appeared. The size of the colony was of 1.5mm in diameter.
2. Collection of infected leaves:

To study the biometric characters of fungus, the diseased leaves of different crops showing Alternaria Blight were collected in sterile polythene bags from various places of Parbhani District. From these leaves Alternaria species are isolated.

Figure 2- Infected leaves of Soybean

3. Isolation of Alternaria tenuissima from soybean:

Fig 3. Plate showing growth Alternaria tenuissima of from Variety JS 71-5

Figure 4-. Alternaria tenuissima spore from variety JS 71-5
The fungal pathogen i.e. A. tenuissima was isolated from soybean varieties, by single spore isolation method (Kamthane, 2013)

The pathogen was maintained on potato dextrose agar media. A 5mm disc of the respective fungal pathogen was inoculated in the centre of sterilized petriplate containing PDA medium. The radial growths during incubation were measured in millimeters up to 10th day of incubation and noted down as shown in the following table. In the same manner Meena and Ratnoo (2013) recorded the colony diameter of Alternaria spp. causing leaf spot on cotton.

4. In vitro biocontrol testing of Rhizobium japonicum against the fungi isolated:

Results expressed were means of percent inhibition of fungal pathogen growth in presence and absence of bacteria R. japonicum. The control plate is inoculated with A. tenuissima alone while the test plate was inoculated with both R.japonicum and A.tenuissima. The percent inhibition was calculated using the following formula (Sandikar & Awasthi, 2010)—

$$\text{Percent inhibition} = \frac{(C-T) \times 100}{C}$$

Where C: Radial growth of fungus in mm in control plate.

T: Radial growth of fungus in mm on plate inoculated with Rhizobium japonicum.

Table 1: The percent inhibition of Alternaria tenuissima

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Alternaria species</th>
<th>Dysof incubation</th>
<th>Percent inhibition</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 M nea</td>
<td></td>
</tr>
<tr>
<td>Soybean JS71-5</td>
<td>A rialternia ten auissim</td>
<td>110 1 23 28 30 32 34 3 3 41 2835 8.4</td>
<td>17.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T 7 1 12 18 22 28 32 3 3 36 2303 5.3</td>
<td></td>
</tr>
<tr>
<td>Soybean JS93-05</td>
<td>A rialternia ten auissim</td>
<td>1 14 18 24 29 31 3 3 38 2412 4.0</td>
<td>1 27.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T 9 11 14 16 21 25 2 3 33 199 2.7</td>
<td></td>
</tr>
</tbody>
</table>

The percent inhibition of Alternaria tenuissima in JS 71-5 soyabean variety was more as compared to JS93-05 variety.

5. In vivo assay of antagonisis:

The root nodules, roots and shoots were harvested on 28th day after soybean sowing.
The percent inhibition of Alternaria tenuissima in JS 71-5 soyabean variety was more as compared to JS93-05 variety.

5. In vivo assay of antagonism:

The root nodules, roots and shoots were harvested on 28th day after soybean sowing.

<p>| | | | |</p>
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<thead>
<tr>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>JS 71-5 variety</td>
<td>JS 71-5 variety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A=Control</td>
<td>B=Test with cut</td>
<td>C= Control</td>
<td>D= Test without cut</td>
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<thead>
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<tbody>
<tr>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>JS93-05 variety</td>
<td>JS93-05 variety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E= Control</td>
<td>F= Test with cut</td>
<td>G= Control</td>
<td>H= Test without cut</td>
</tr>
</tbody>
</table>

Figure 4: In vivo assay of antagonism in soybean varieties.
Table 2: In vivo assay of antagonism.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Soybean Variety</th>
<th>Length in cm.</th>
<th>Dry weight in grams</th>
<th>Number of root Nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>1</td>
<td>JS 71-5</td>
<td>Control</td>
<td>15</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>(With cut)</td>
<td>Test</td>
<td>14.83</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>JS 71-5</td>
<td>Control</td>
<td>15</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>16.875</td>
<td>11.33</td>
</tr>
<tr>
<td>3</td>
<td>JS93-05</td>
<td>Control</td>
<td>21.9740</td>
<td>8.4200</td>
</tr>
<tr>
<td></td>
<td>(With cut)</td>
<td>Test</td>
<td>22.14</td>
<td>8.92</td>
</tr>
<tr>
<td>4</td>
<td>JS93-05</td>
<td>Control</td>
<td>21.9740</td>
<td>8.4200</td>
</tr>
<tr>
<td></td>
<td>(Without cut)</td>
<td>Test</td>
<td>22.2200</td>
<td>9.75</td>
</tr>
</tbody>
</table>

The results are in agreement with the different workers. Aneja (2002) given the same time period for the development of nodules on the roots. The pathogenicity studies were conducted by Sangeeta and Shamarao. They inoculated the pathogenic fungi on soybean seedlings. They have measured the root length (root rot index) in the similar way.

Novina Shekhawat, Amit Trivedy, Ashok Kumar and Sandeep Kumar Sharma have conducted in vivo evaluation of fungicides and botanicals. Fungicides alone and in combination with Azadirictin against Alternaria burnsii as spray application for management of cumin blight under pot culture.

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REFERENCES:


