Controlling of Fusarium Wilt of Cucumber by Antagonistic Bacteria

Gamal Ashour Ahmed

Plant Pathology Branch, Agric. Botany Dept., Fac. Agric. at Moshtohor, Benha University, Benha 13736, Egypt

Received: May 31, 2010 / Accepted: August 07, 2010 / Published: December 30, 2010.

Abstract: Studying about the effect of four pseudomonas and two serratia isolates on growth of Fusarium oxysporum showed that, Pseudomonas fluorescens No.2 & No.3 and Serratia marcescens No.2 gave highest inhibition zones which were 37.33, 35.00 and 31.33 mm, respectively. Evaluating about the effect of pseudomonas and serratia culture filtrates at three concentrations (10, 25, 50%) on the linear growth and spore germination of Fusarium oxysporum revealed that, all filtrates of the tested isolates reduced the mycelial growth and spore germination of F. oxysporum. All filtrates of the tested isolates at 50% concentration completely inhabited spore germination of F. oxysporum. Culture filtrates of Pseudomonas putida and Serratia marcescens No.2 at 50% concentration reduced the mycelial growth of F. oxysporum except Pseudomonas fluorescens No.3. Effect of treating cucumber seeds with cell suspension of pseudomonas and serratia isolates on incidence of Fusarium wilt disease revealed that all isolates were effective in reducing disease incidence and disease severity and increasing the percentage of healthy plants compared to the control. Pseudomonas fluorescens No.3 and Serratia marcescens No.2 were the best isolates and completely prevented the disease incidence.

Key words: Cucumber, fusarium wilt, pseudomonas, serratia and greenhouses.

1. Introduction

Cucumber (Cucumis sativus L.) is one of the most important economical crops, which belongs to family cucurbitaceae. Cucumber is grown either in the open field or under protected houses. The purpose of growing crops under protected house conditions is to extend their cropping season and to protect them from adverse conditions as well as diseases and pests [1]. The causal agent of wilt disease in cucumber Fusarium oxysporum f. sp. cucumerinum is economically important wilting pathogen of cucumber and causing significant yield losses in greenhouse cucumber.

Mechanisms of biological control of Fusarium wilt by beneficial microorganisms are complex. Most studies conducted previously have focused on using nonpathogenic fusaria or other antagonists [2-5] that exert biological control through mechanisms such as competition for nutrients or iron [6-8] competition for infection sites on roots [9], or production of antibiotics [10-13]. Investigations of plant growth-promoting rhizobacteria (PGPR) as agents of induced systemic resistance (ISR) against various pathogens, including F. oxysporum, have been conducted recently [14]. Fluorescent pseudomonads and nonpathogenic isolates of F. oxysporum were effective in inducing suppressiveness to Fusarium wilt of cucumber (F.o. f.sp. cucumerinum) [15].

The purpose of this study is to examine the in vitro and in greenhouses inhibition and suppression of antagonistic bacteria toward Fusarium oxysporum f. sp. cucumerinum (FOC).

2. Materials and Methods

2.1 Studying the Effect of Antagonistic Bacteria against F. Oxysporum

Studying the effect of four pseudomonas isolates (3
Controlling of Fusarium Wilt of Cucumber by Antagonistic Bacteria

Pseudomonas fluorescens & Pseudomonas putida) and 2 isolates of Serratia marcensens on growth of Fusarium oxysporum were conducted as following, individual plates contained PDA medium were streaked at one side 1 cm apart from the plate edge with a given isolate of antagonistic bacteria with a loop full of the antagonistic bacteria (48 hrs old) grown on liquid NG medium and incubated for 24 hrs at 28 °C. Thereafter the same plate was inoculated at the opposite side 1 cm apart from the plate edge with 5 mm disc of 4 days old plain agar culture of an isolate of F. oxysporum. All plates were incubated at 25 °C for 5 days. The inhibition zone (mm) between bacteria and the pathogen was measured [16].

2.2 Evaluating the Effect of Antagonistic Bacteria Culture Filtrates on the Linear Growth and Spore Germination of F. Oxysporum

The tested antagonists bacteria were inoculated separately into conical flasks 125 cc each containing 50 ml of liquid NG media. The inoculated flasks were incubated at 25 °C under complete darkness conditions to stimulate toxin production [17]. The culture filtrates for antagonistic bacteria were collected after 3 days after incubation. The obtained filtrates were centrifuged for 15 mins at 4000 rpm to separate the bacterial growth, sterilized by filtration through centered glass filter (G5).

2.2.1 Evaluating the Effect of Antagonistic Bacteria Culture Filtrates on the Linear Growth of F. Oxysporum

The sterilized filtrates were added to warm sterilized Czapek's agar medium at rate of 10, 25 and 50% and poured before solidification into Petri dishes (10 mL/plate). Each of the treated plates was inoculated at the center with equal discs obtained from the periphery of 7 days old cultures of F. oxysporum. Plates contained media without culture filtrates and inoculated with F. oxysporum was served as control treatment. All plates were incubated at 25 °C. The experiment was terminated when mycelial mats covered medium surface in control treatment, all plates were examined and growth reduction (X) was calculated.

\[ X = \frac{G_1 - G_2}{G_1} \times 100 \]  

Where: \( G_1 \) = linear growth of the pathogen inoculated alone; \( G_2 \) = linear growth of the pathogen inoculated against the antagonistic fungus.

2.2.2 Evaluating the Effect of Antagonistic Bacteria Culture Filtrates on Spore Germination of F. Oxysporum

The antifungal activity bacteria culture filtrates 10, 25 and 50% was investigated by using the method of spore counting. Spore suspension was prepared from a 15 days old culture of the fungus in sterile distilled water, and 100 μL fungal suspension was added to 100 μL bacteria culture filtrates, of concentration 10, 25 and 50%, in glass vials and incubated at 25 ± 2 °C for 24 hours. The control vials contained sterile distilled water in place of bacteria culture filtrates. After incubation, the content of the vials was stained with cotton blue and mounted in lactophenol. The spores were observed under a microscope for their germination status. Percentage inhibition was calculated by using the established formula according [18].

\[ \% \text{ Spore inhibition} = \frac{A - B}{A} \times 100 \]  

A: Spore germination in control; B: Spore germination in treatment.

2.3 Effect of Treating Cucumber Seeds with Cell Suspension Antagonistic Bacteria Isolates on Incidence of Fusarium Wilt Disease

Cucumber seeds were treated with antagonistic bacteria according to Ref. [19]. Any of the tested antagonistic bacterial isolates was grown for 48 hrs at 26 °C was grown on nutrient agar medium, (NA), then bacterial growth was scraped and the re-suspended in mixture of 1.5 mL of 1.0% methyl cellulose (MC) and 1.5 mL of 0.1 M MgSO4. Surface sterilized Cucumber seeds were thoroughly mixed with 2 mL of bacterial suspension for 5 minutes then left for 2 hrs to air dried in a laminar-flow before planting. Bacterial population determined per seed was 1x10^8 cfu/seed according to dilution plate assay described by Ref. [20]. Disease
incidence was recorded using a scale containing 6 grades suggested by Ref. [21]:

- Grade 0: no symptoms.
- Grade 1: Plants with < 25% of leaves with symptoms.
- Grade 2: Plants with 25-50% of leaves with symptoms.
- Grade 3: Plants with 50-75% of leaves with symptoms.
- Grade 4: Plants with 75-100% of leaves with symptoms.
- Grade 5: Plants with complete death.

Disease severity percent was determined according to equation:

\[
\text{Disease severity} (\%) = \frac{\sum (\text{rating}) \times \text{(no. plants in rating category)(100)}}{\text{(Total no. plants)(highest rating value)}}
\]

Reduction (%) = \[\frac{\text{Control - Treatment}}{\text{Control}}\] \times 100

3. Results

3.1 Studying the Effect of Antagonistic Bacteria Against F. oxysporum

Data presented in Table 1 show that, *Pseudomonas fluorescens* No.2, *Pseudomonas fluorescens* No.3 and *Serratia marcensens* No.2 were the best antagonistic bacteria for limiting growth of *F. oxysporum*, they caused the highest inhibition zone (37.33, 35.00 and 31.33 mm, respectively). *Pseudomonas fluorescens* No.1 (30.67 mm) came next whereas *Pseudomonas putida* was the lowest effective one that caused the narrowest inhibition zone (27.33 mm).

3.2 Evaluating the Effect of Antagonistic Bacteria Culture Filtrates on the Linear Growth and Spore Germination of *F. oxysporum*

The results in Table 2 and Fig. 1 stated that, all filtrates of the tested isolates reduced the mycelial growth and spore germination of *F. oxysporum*. All filtrates of the tested isolates at 50% concentration completely inhibited spore germination of *F. oxysporum*. Culture filtrates of *Pseudomonas putida* and *Serratia marcensens* No.2 at 50% concentration were more effective and reducing the mycelial growth of *F. oxysporum* by 80.74 and 80.37%, respectively. *Pseudomonas fluorescens* No.2 (77.41%) came next whereas *Pseudomonas fluorescens* No.1 was the lowest effective one that caused inhibition (69.23%).

In this respect all tested isolates made lysis to mycelial of *F. oxysporum* except *Pseudomonas fluorescens* No.2.

3.3 Effect of Treating Cucumber Seeds with Cell Suspension of Antagonistic Bacteria Isolates on Incidence of Fusarium Wilt Disease

The results in Table 3 & Fig. 2 revealed that all isolates were effective in reducing disease incidence and disease severity compared with the control. *Pseudomonas fluorescens* No.3 and *Serratia marcensens* No.2 were the best isolates and completely prevented the disease incidence. *Serratia marcensens* No.1 came next and reducing disease incidence and disease severity by 83.33 and 97.33%, respectively while *Pseudomonas putida* was the least effective isolate and reducing disease incidence and disease severity by 50.00 and 83.33%, respectively.

4. Discussion

Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetable crops. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* is economically important wilting pathogen of cucumber and causing significant yield losses in greenhouse cucumber.

The obtained results showed that, *Pseudomonas fluorescens* and *Serratia marcensens* inhibited the growth of *F. oxysporum* and were inhibiting the linear growth and spore germination. The present results are...
### Table 2  Effect of some antagonistic bacteria culture filtrate at different concentrations on the growth and spore germination of *Fusarium oxysporum*.

<table>
<thead>
<tr>
<th>Antagonistic bacteria</th>
<th>Dilutions (%)</th>
<th>growth (mm)</th>
<th>% of spore germination</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Growth</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 1</td>
<td>10</td>
<td>58.33</td>
<td>17.67</td>
<td>35.19</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>42.67</td>
<td>10.33</td>
<td>52.59</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>27.33</td>
<td>0.00</td>
<td>69.63</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 2</td>
<td>10</td>
<td>55.33</td>
<td>11.00</td>
<td>38.52</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>34.33</td>
<td>8.00</td>
<td>61.86</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20.33</td>
<td>0.00</td>
<td>77.41</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 3</td>
<td>10</td>
<td>57.67</td>
<td>12.67</td>
<td>35.92</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>41.67</td>
<td>10.00</td>
<td>53.70</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25.00</td>
<td>0.00</td>
<td>72.22</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>10</td>
<td>34.33</td>
<td>9.67</td>
<td>61.86</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>22.67</td>
<td>5.00</td>
<td>74.81</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>17.33</td>
<td>0.00</td>
<td>80.74</td>
</tr>
<tr>
<td><em>Serratia marcensens</em> 1</td>
<td>10</td>
<td>38.33</td>
<td>11.33</td>
<td>57.41</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>35.33</td>
<td>8.67</td>
<td>60.74</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21.67</td>
<td>0.00</td>
<td>75.92</td>
</tr>
<tr>
<td><em>Serratia marcensens</em> 2</td>
<td>10</td>
<td>35.33</td>
<td>10.33</td>
<td>60.74</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.00</td>
<td>5.33</td>
<td>72.22</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>17.67</td>
<td>0.00</td>
<td>80.37</td>
</tr>
<tr>
<td>Control</td>
<td>90.00</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Fig. 1**  Effect of some antagonistic bacteria culture filtrate at different concentrations on the growth of *Fusarium oxysporum*.  
The concentrations of bacterial culture filtrate from left to right are 10, 25 and 50%, respectively.  
Controlling of Fusarium Wilt of Cucumber by Antagonistic Bacteria

Table 3  Effect of treating cucumber seeds with cell suspension of antagonistic bacteria isolates on incidence of *Fusarium* wilt disease.

<table>
<thead>
<tr>
<th>Antagonistic bacteria</th>
<th>Disease incidence</th>
<th>Disease severity</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 1</td>
<td>50.00</td>
<td>10.00</td>
<td>50.00</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 2</td>
<td>33.33</td>
<td>6.67</td>
<td>66.67</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> 3</td>
<td>00.00</td>
<td>00.00</td>
<td>100</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>50.00</td>
<td>16.67</td>
<td>50.00</td>
</tr>
<tr>
<td><em>Serratia marcensens</em> 1</td>
<td>16.67</td>
<td>3.33</td>
<td>83.33</td>
</tr>
<tr>
<td><em>Serratia marcensens</em> 2</td>
<td>00.00</td>
<td>00.00</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>00.00</td>
</tr>
</tbody>
</table>

Fig. 2  Effect of treating cucumber seeds with cell suspension antagonistic bacteria isolates on incidence of *Fusarium* wilt disease.


in parallel to those reported by Ref. [22-27]. In fact, the action by which the antagonistic microorganism(s) could suppress the activity of a plant pathogen was quite varied. Ref. [28] noted variable types of antagonism of which, the formation of free zone between the fungi; lytic phenomena or complete covering of the pathogen by the antagonist.

Under greenhouse conditions, the obtained results revealed that all isolates were effective in reducing disease incidence and disease severity and increasing the percentage of healthy plants compared to the control. *Pseudomonas fluorescens* No.3 and *Serratia marcensens* No.2 were the best isolates and completely prevented the disease incidence. *Serratia marcensens* No.1 came next and reducing disease incidence and disease severity by 83.33% and 97.33%, respectively. The present results are agreed with the author of Ref. [15] who found that Fluorescent pseudomonads and nonpathogenic isolates of *F. oxysporum* were effective in inducing suppressiveness to *Fusarium* wilt of cucumber (*F.o. f.sp. cucumber*) when added to soil together (pH 6.7) but ineffective when added separately.

PGPR significantly reduced Fusarium wilt of cucumber when applied as root treatments [29, 30] this reduction in disease development appeared to be related to delayed movement of the pathogen within PGPR-treated plants compared to the nonbacterized disease control.

References

[7] M.I. Frommel, G.S. Pazos, J. Nowak, Plant-growth stimulation and biocontrol of Fusarium wilt (*Fusarium* oxysporum) in parallel to those reported by Ref. [22-27]. In fact, the action by which the antagonistic microorganism(s) could suppress the activity of a plant pathogen was quite varied. Ref. [28] noted variable types of antagonism of which, the formation of free zone between the fungi; lytic phenomena or complete covering of the pathogen by the antagonist.

Under greenhouse conditions, the obtained results revealed that all isolates were effective in reducing disease incidence and disease severity and increasing the percentage of healthy plants compared to the control. *Pseudomonas fluorescens* No.3 and *Serratia marcensens* No.2 were the best isolates and completely prevented the disease incidence. *Serratia marcensens* No.1 came next and reducing disease incidence and disease severity by 83.33% and 97.33%, respectively. The present results are agreed with the author of Ref. [15] who found that Fluorescent pseudomonads and nonpathogenic isolates of *F. oxysporum* were effective in inducing suppressiveness to *Fusarium* wilt of cucumber (*F.o. f.sp. cucumber*) when added to soil together (pH 6.7) but ineffective when added separately.

PGPR significantly reduced Fusarium wilt of cucumber when applied as root treatments [29, 30] this reduction in disease development appeared to be related to delayed movement of the pathogen within PGPR-treated plants compared to the nonbacterized disease control.
oxysporum f. sp. lyco-persici) by co-inoculation of tomato seeds with Serratia plymuthica and Pseudomonas sp., Fitopatologia 26 (1991) 67-73.


