Ability of Some Antagonistic Fungi for Controlling Cucumber Downy Mildew Disease Caused by *Pseudoperonospora cubensis*

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ABSTRACT

The aim of this study is to find an effective alternative method for controlling cucumber downy mildew disease caused by *Pseudoperonospora cubensis* by using eco-frind by fungi such as *Trichoderma viride* and *Trichoderma harzianum* instead of chemical fungicide like copper acrobat under greenhouse conditions. Sprayed method of antagonistic fungi spore suspension was used on plants at a rate of 1 x 10^8 colony forming unit (cfu). By applying it before any symptoms of infection appear (in a protective method), and ten days after the appearance of symptoms of infection (in a curative method). The results of the obtained experiment here showed that *T. harzianum* was superior during different growth periods up to 90 days, as it gave 44.30% disease incidence and 19.26% disease severity compared to both control (100% disease incidence as well disease severity) and the chemical fungicide (66.67% disease incidence and 25.93% disease severity). The results of a curative trial also showed that *T. harzianum* was given during different growth periods up to 90 days, where 40% disease incidence and 31% disease severity compared to control (100% disease incidence and disease severity 74.44%) and chemical fungicide (20% disease incidence and 29.49% disease severity). While *T. viride* achieved moderate results as compared to control (60% disease incidence and 27.22% disease severity). From the results obtained, it is evident that *T. harzianum* gave better results than *T. viride*, and therefore we recommend using it in resisting downy mildew in cucumber.

**Keywords:** Downy mildew, *Pseudoperonospora cubensis*, *Trichoderma harzianum*, *Trichoderma viride*, biological control, greenhouses.

INTRODUCTION

Cucumber is one of the most important crops grown in both open field and the greenhouse for domestic consumption or export to Arab countries and European markets, and growing season ranging between 3-4 months. Inthis connection, cucumber crop suffers from many diseases in all stages of its growth from germination to maturity and harvesting stages. Downy mildew is one of the most important and most dangerous fungal diseases affecting cucumber in greenhouses.

Downy mildew is a foliage disease, caused by microscopic, fungus-like (Oomycete) organisms *Pseudoperonospora cubensis* (Berkeley and M. A. Curtis, 1868). This disease is a major foliar disease of cucumber, *Cucumis sativus* L. (Palti and Cohen, 1980). *P. cubensis* has a wide host range infecting approximately 20 genera of cucurbits, and it is an obligate biotrophic parasite that can’t grow on an artificial environment or plant waste(Cohen and Eyal 1977; Lebeda 1986).

The disease symptoms appear first on the lower leaves (the oldest), then, on the upper surface of the leaves and when conditions are ideal for the spread of the causative agent that is represented in high relative humidity of up to 100% on the surface of the leaves for at least 6 continuous hours and temperatures ranging from 16 to 22°C, downy mildew will spread rapidly, destroying leaf tissue without affecting stems or petioles, but the disease usually expand quickly. Downy mildew is difficult to be controlled.

Previous studies have indicated the use of different methods to resistant this disease, which is extremely dangerous to cucumber cultivation at the local and international level, and among these methods is the use of anti-fungi, anti-bacteria, essential oils, and also a chemical pesticide. The study during this research indicated the use of Trichoderma. As one of the ways to combat this disease. Which gave the best results in resisting downy mildew in cucumber.

The main objective during this study was to control this disease in safe and effective ways, as well as to reduce the risk of pesticides as they represent harm to society and the environment.

MATERIALS AND METHODS

1-Preparation of spore suspensions of *Trichoderma* sp:

*Trichoderma viride* and *Trichoderma harzianum* as antagonistic fungi were developed in 250 ml of a conical flasks of the autoclaved Potato Dextrose broth medium at (26±2°C) for two weeks. Spore suspensions conducted by blending the mycelial growth with 0.5 ml of solving tween-80 from 2weeks old pure culture of *T. viride* and *T. harzianum*. Spores were filtered through double layers of cheese cloth. The resulted spore suspensions were determined by using haemocytometer slide and were
were difference – 00% disease incidence and the 50% disease incidence followed by P. cubensis. T. harzianum on disease incidence% and disease severity% of between all treatments after 60 days of spraying appeared • disease incidence and 74.44% disease severity in the 70%. When compared with the untreated control (100% disease severity giving reduction rate ranged between 64.26%, followed by T. viride 67.89% disease severity. When compared with the controls (74.44% disease severity) and chemical control (33.82% disease severity).

After 90 days from planting:
The disease incidence after 90 days reduced in case of T. harzianum treatment giving reduction of 40% followed by T. viride giving 60% disease incidence, while, the control treatment gave 100% disease incidence.

Disease severity reduction in case of T. viride was 27.22% followed by T. harzianum 31% when 100% disease incidence and 74.44% disease severity was in the infested control and chemical control 20%, and 29.49% respectively.

Table 1. Effect of some antagonistic fungi on disease incidence and disease severity under the curative experiment conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30day</th>
<th>60day</th>
<th>90day</th>
<th>30day</th>
<th>60day</th>
<th>90day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. harzianum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100.00a</td>
<td>50.00c</td>
<td>40.00c</td>
<td>64.46b</td>
<td>64.26c</td>
<td>31.00b</td>
<td></td>
</tr>
<tr>
<td>T. viride</td>
<td>100.00a</td>
<td>70.00b</td>
<td>60.00b</td>
<td>60.56b</td>
<td>67.89b</td>
<td>27.22c</td>
</tr>
<tr>
<td>Acrobat copper</td>
<td>100.00a</td>
<td>40.00d</td>
<td>20.00d</td>
<td>50.19d</td>
<td>33.82d</td>
<td>29.49c</td>
</tr>
<tr>
<td>Control</td>
<td>100.00a</td>
<td>100.00a</td>
<td>100.00a</td>
<td>74.44a</td>
<td>74.44a</td>
<td>74.44a</td>
</tr>
</tbody>
</table>

*DI: Disease Incidence  DS: Disease severity
Values within a column followed by the same letter are not significantly different according to Duncan’s multiple range test (P<0.05).

2-Effect of some antagonistic fungi on disease incidence and disease severity under protective experiment condition:
• After 30 day from planting:

Data presented in Table-2 showed that high effect for T. viride in reducing disease severity giving 66.67% disease incidence, when compared with the natural control giving 88.89% and the chemical control giving 88.56% incidence. While, T. harzianum had a moderate effect on disease incidence giving 77.67%.

The same results show that; T. viride and T. harzianum gave 57.41% disease severity. When compared with the control and the chemical control that recorded 55.56% and 57.13% respectively.

Table 2. Effect of some antagonistic fungi on disease incidence and disease severity under protective experiment conditions:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>30day</th>
<th>60day</th>
<th>90day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. harzianum</td>
<td>77.67b</td>
<td>65.82c</td>
<td>44.30d</td>
</tr>
<tr>
<td>T. viride</td>
<td>66.67c</td>
<td>66.67c</td>
<td>57.13c</td>
</tr>
<tr>
<td>Acrobat copper</td>
<td>88.56a</td>
<td>66.67c</td>
<td>57.13c</td>
</tr>
<tr>
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<td>100.00a</td>
<td>55.56d</td>
</tr>
</tbody>
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• After 60 day from planting:

T. harzianum and T. viride showed high reduction in disease incidence giving 65.82% and 66.67%, respectively. T. harzianum and T. viride treatments showed moderate reduction when compared with the natural control which gave 100% disease incidence and the chemical control gave 66.67%.

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results and discussion

1-Effect of some antagonistic fungi on cucumber downy mildew P. cubensis disease incidence and disease severity under conditions of the curative experiment:

• After 30 days from planting:

Data presented in Table-1 showed that there were no significant differences between all treatments after 30 days from treatment on disease incidence with P. cubensis. Results at all treatments gave non-significant differences in disease severity giving reduction rate ranged between 50-70%. When compared with the untreated control (100% disease incidence and 74.44% disease severity in the infested control and in the chemical control 100% and 50.19%, respectively).

• After 60 days from planting:

Data showed that there is a significant difference between all treatments after 60 days of spraying appeared on disease incidence% and disease severity% of P. cubensis. T. harzianum was the best treatment which gave 50% disease incidence followed by T. viride 70%.

While, disease severity observed in case of T. harzianum treatment giving reduction of 64.26%, followed by T. viride 67.89% disease severity. When compared with the controls (74.44% disease severity) and chemical control (33.82% disease severity).
About disease severity, T. viride treatment proved high reduction in disease severity giving 49.67% followed by T. harzianum 57.41%. When compared with the natural control which gave 100% disease severity, while the chemical control gave 42.60%.

• After 90 days from planting:

T. harzianum and T. viride were still achieving high reduction in disease incidence by giving 44.30% and 66.67% respectively, when compared with the natural control which gave 100% and the chemical control by giving 66.67%. While, T. harzianum gave 19.26% disease severity followed by T. viride 40.83% disease severity. When compared with the natural control which gave 100% and the chemical control which gave 25.93% reduction.

About T. harzianum obtained results, these results agree with Elsharkawy et al., 2014, they used T. harzianum to control cucumber downy mildew under greenhouse conditions, and found that T. harzianum reduced disease incidence over than 50%. Also Michele et al., 2011 their results showed that T. harzianum decreased downy mildew harshness on susceptible grapevines under controlled greenhouse conditions and also the stronger local than systemic modulation of defense-related genes corresponded to an higher local than systemic disease control in T. harzianum treated plants.

About T. viride results of Szczecz et al., (2017), found that when treated seed with T. viride improved cucumber germination, enhanced vegetative plant growth and reduced downy mildew infection, compared to untreated plants.

Through the research it can be concluded that the treatment using T. harzianum was one of the best results obtained in the resistance of downy mildew in cucumbers under the greenhouse.

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