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

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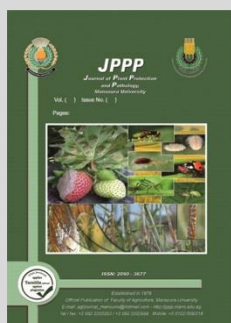


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ABSTRACT

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-friendly 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 1×10^7 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 *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. In this 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 ($26 \pm 2^\circ\text{C}$) for two weeks. Spore suspensions conducted by blending the mycelial growth with 0.5 ml of solving tween-80 from 2 weeks 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

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adjusted to be approximately 1×10^7 spores/ml. according to the method applied from El-Sheshtawi, (2009).

2- Efficacy of antagonistic fungi on cucumber downy mildew *P.cubensis*.

Plants were grown under greenhouse conditions and six successive sprays were carried out. The first one (a curative) was applied on 30-day-old plants after infection of the disease just appeared and the second (protective) on the plants before the natural infection started within 2 days, while in case of the preventive trial the spraying happened before noticing any infection. Suspensions of bioagents (*Trichoderma harzianum*, *Trichoderma viride*) were evaluated for their efficiency under the greenhouse conditions as follows: plants were sprayed of each bio-control agents, six times with the initiation of the disease at an interval of 7 days and plants sprayed with water served as control. Completely Randomized Design (CRD) was followed to layout the experiment along with three replications in each treatment. The data on disease severity were recorded after every 7 days up to the last spray and subjected to statistical analysis.

The data on disease severity was recorded by following a disease rating based on 0–6 scale developed for the estimation of rose downy mildew by Sahni (1987). The percent disease index (PDI) was calculated by using the formula given by McKinney (1923). where 0: no disease, 1: 5–5% infected, 2: 10–10%, 3: 25–25%, 4: 50–50%, 5: more than 50%, 6: more than 75%. The disease severity of the plot was expressed by the following formula: The percent disease index (PDI) was calculated by using the formula given by McKinney (1923).

A one-way analysis of variance (ANOVA) was conducted to analyze the data, by completely randomized design (CRD). Data collected from all experiments were statistically analyzed using the Statistical Analysis System package (SAS institute, Cary, NC, USA). Differences between treatments were determined using Fisher's least significant difference (LSD) test by Duncan's multiple range test (Duncan, 1955). All comparisons were performed at $P \leq 0.05$.

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).

• 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.

Table1. Effect of some antagonistic fungi on disease incidence and disease severity under the curative experiment conditions.

Treatment	*DI			DS		
	30day	60day	90day	30day	60day	90day
<i>T. harzianum</i>	100.00a	50.00c	40.00c	64.46b	64.26c	31.00b
<i>T. viride</i>	100.00a	70.00b	60.00b	60.56c	67.89b	27.22d
Acrobat copper	100.00a	40.00d	20.00d	50.19d	33.82d	29.49c
Control	100.00a	100.00a	100.00a	74.44a	74.44a	74.44a

*DI: Disease Incidence DS: Disease severity
 Values within a column followed by the same letter are not significantly different according to Duncun'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 condition:

Treatment	*DI			DS		
	30day	60day	90day	30day	60day	90day
<i>T. harzianum</i>	77.67b	65.82c	44.30d	57.41c	57.41b	19.26g
<i>T. viride</i>	66.67c	66.67c	66.67b	57.41c	49.67c	40.83b
Acrobat copper	88.56a	66.67c	66.67b	57.13c	42.60d	25.93e
Control	88.89a	100.00a	100.00a	55.56d	100.00a	100.00a

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

• 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%

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 by 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 thier 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 Szczech *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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قدرة بعض فطريات التضادة الحيوي في السيطرة على مرض البياض الزغبي في الخيار المتسبب عن الفطر *Pseudoperonospora cubensis*

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تمت هذه الدراسة بهدف الحصول على طريقة فعالة لمقاومة مرض البياض الزغبي في الخيار المتسبب عن الفطر *cubensis* *Pseudoperonospora* باستخدام بعض فطريات التضاد الحيوي مثل *Trichoderma viride* و *Trichoderma harzianum* مقارنة بالمبيد الكيماوي اكروبات النحاس تحت ظروف الصوبة وتم استخدام معلق من الفطريات المضادة رشا علي النباتات بمعدل $10^7 \times 1$ وحدة/مل وذلك بتطبيقه بصورة وقائية قبل ظهور اي اعراض من الاصابة ومرحلة اخري بتطبيقه بصورة علاجية بعد ظهور أعراض الاصابة بعشرة أيام. وأظهرت النتائج في التجربة الوقائية تفوق فطر *T. harzianum* خلال فترات النمو المختلفة حتي 90 يوم حيث اعطي 44.30% نسبة اصابة و 19.26% شدة اصابة بالمقارنة بالكنترول 100% نسبة اصابة وشدة اصابة والمبيد الكيماوي اعطي 66.67% نسبة اصابة و 25.93% شدة اصابة. بينما جاءت ال *T. viride* في المرتبة الثانية وحقت نتائج متوسطة في المقاومة. كما أظهرت نتائج التجربة العلاجية تفوق *T. harzianum* خلال فترات النمو المختلفة حتي 90 يوم حيث اعطي 40% نسبة اصابة و 31% شدة اصابة بالمقارنة بالكنترول 100% نسبة اصابة وشدة اصابة و 74.44% والمبيد الكيماوي اعطي 20% نسبة اصابة و 29.49% شدة اصابة. بينما جاءت ال *T. viride* في المرتبة الثانية وحقت نتائج متوسطة في المقاومة و 60% نسبة اصابة و 27.22% شدة اصابة.