

Alternaria Leaf Spot Disease on Cucumber: Susceptibility and Control Using Leaf Disk Assay

مرض تبقع الأوراق الالترناري على الخيار: الحساسية والمكافحة باستعمال اختبار الأقراص الورقية

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Abstract

Results obtained in the present research using leaf disk assay indicated significant differences in susceptibility of tested cucumber cultivars to *Alternaria cucumerina*-infection. Hasan cultivar was the most susceptible, but Rocket cultivar was the least susceptible. Assessment of treatment efficacy with fungicides and *Trichoderma harzianum* against the disease using leaf disk assay indicated the presence of significant differences between the treatments with 4 types of new, low-residual fungicides or 2 forms of *T. harzianum* and the control treatments with a blank formulation of invert emulsion or sterile distilled water. Treatments with fungicides just before the disease inoculation demonstrated that Score® and Switch® completely inhibited appearance of the disease-lesion on treated cucumber leaf-discs when applied at a rate of 0.35% (V/V) and 0.20% (W/V), respectively. Treatments with the same fungicides at the same application rates 24h after the disease inoculation also completely inhibited the disease-lesion appearance when Score® was applied. It suppressed the disease-lesion diameter to 2.5mm or reduced it by 83.5% relative to control when Switch® was applied. Moreover, application of formulated conidia of *T. harzianum* in invert emulsion at a concentration of 2.0×10^8 conidia/ml significantly suppressed the disease-lesion diameter on treated cucumber leaf-discs. Such application decreased the lesion diameter to 4.5 and 6.5mm or reduced it by 70.4 and 57.2% relative to control when used just before or 24h after the disease inoculation, respectively. It is, thus, concluded that the results obtained on susceptibility of cucumber cultivars to the disease infection and its control should be confirmed on whole plants under field conditions before being recommended to be applied by the farmers and extension agents.

Keywords: Cucumber leaf spot, *Alternaria cucumerina*, Fungicides, *Trichoderma harzianum*, Cultivars' susceptibility.

ملخص

أوضحت النتائج التي تم الحصول عليها في هذا البحث باستعمال اختبار الأفراس الورقية وجود فروق معنوية في حساسية الأصناف التي تم اختبارها للاصابة بالمرض. كان الصنف حسن هو أكثر الأصناف حساسية في حين كان الصنف روكت هو أقلها حساسية. أظهر اختبار فعالية مكافحة باستعمال المبيدات الفطرية أو فطر *Trichoderma harzianum* ضد المرض عن طريق اختبارات الأفراس الورقية وجود فروقات معنوية بين المعاملات التي تمت باستخدام أربعة أنواع من المبيدات الفطرية الجديدة وذات الأثر المتبقي القليل أو شكلين من فطر *T. harzianum* والشاهد. المعاملات التي تم استعمالها قبل العدوى بالمرض أظهرت أن استخدام المبيد سكور والمبيد سويتش قد منعت بشكل كلي ظهور بقع المرض على الأفراس الورقية التي تمت معاملتها بمعدل (0.35% V/V) و (0.20% W/V) للمبيدين السابقين على الترتيب. أما عند استخدام نفس المبيدات الفطرية وفطر *T. harzianum* بعد 24 ساعة من العدوى بالمرض، فإن المبيد الفطري سكور والذي استعمل بمعدل (0.35% V/V) قد منع أيضا بشكل كلي ظهور بقع المرض ولكن المبيد الفطري سويتش والذي استعمل بمعدل (0.20% W/V) قد خفّض قطر بقع المرض إلى 2.5 ملم أو إلى 83.5% أقل بالمقارنة مع الشاهد. إن استعمال كونيديا الفطر *T. harzianum* والذي تم بشكل مستحلب منعكس وبتركيز مقداره (2.0×10^8 conidia/ml) قد خفّض وبشكل معنوي قطر بقعة المرض إلى 4.5 و 6.5 ملم أو إلى 70.4 و 57.2% أقل بالمقارنة مع الشاهد عند استعماله ضد المرض قبل العدوى بقليل أو 24 ساعة بعدها على الترتيب. يُستنتج مما سبق أن النتائج التي تم الحصول عليها في هذا البحث حول حساسية أصناف الخيار للاصابة بالمرض ومكافحته يجب ان يتم تأكيدها على نباتات كاملة تحت ظروف الحقل قبل أن يتم تعميمها على المزارعين والمرشدين الزراعيين لتطبيقها بشكل عملي.

1. Introduction

Cucumber (*Cucumis sativum* L.) is one of the major vegetable crops produced in Palestine. Estimated area cultivated with cucumber was 23,100 dunums which constitutes 18.2% of the total area planted with vegetable crops in the West Bank⁽¹⁸⁾.

Alternaria leaf spot disease caused by *Alternaria cucumerina* (Ell. & Ev.) Elliot was reported to infect cucumbers during the growing season in many countries^(7,12,14). The other types of cucurbits especially watermelons, muskmelons, pumpkins, and cantaloupes were also reported to be infected with the disease^(4-6, 12-14, 16, 19, 20, 26-27). The disease attacks the leaves especially lower leaves of various cucurbitous crops causing large necrotic areas at favourable conditions and sporulation of the disease-causing fungus in form of brown to dark-brown growth could be observed on the upper surface of necrotic areas^{(4,12-}

^{14, 19-20}). Yield losses due to the leaf necrosis and foliage loss are variable according to the type of cucurbitous crop and its susceptibility, reaching at 80% on pumpkins and 88% on watermelons in India⁽⁴⁾. When the disease-causative fungus establishes itself in an area, it overseasons on infected seeds or in infected host-plant debris ^(11, 14-15).

Under local conditions, in spite of the lack of reports on the disease attack or spread on cucumbers or other cucurbits, preliminary field observations conducted in the last few years especially after introduction of a large number of hybrid cucumbers of parthenocarpic-type into the Palestinian cultures, demonstrated the presence of high incidence of the disease under plastic-house or in the open field conditions. Environmental conditions especially temperatures (20-32°C) prevailing during the growing season and high relative humidities or dew presence favor the disease-development^(14, 19, 26).

In this research, the causative-fungus of the disease was, at first, isolated from infected cucumber leaves then typical pure culture of the fungus was obtained to be used in the assays. The objectives of the present research were: i) to test the susceptibility of common cucumber cultivars grown under local conditions to infection with the disease, and ii) to assess the efficacy of the disease-treatment with the new, low-residual fungicides applied under local conditions, in addition to formulated and non-formulated *T. harzianum*-conidia.

2. Materials and Methods

2.1 Cucumber cultivars

Three cultivars of parthenocarpic cucumbers: I.V-40, Hasan, and Rocket were used in assessing cultivars' susceptibility to *A. cucumerina* and in its control. These cultivars are commonly cultivated under local conditions. Certified seeds of the three cultivars provided by local distributors of the foreign producing companies were sown in pots with a mixture of peatmoss, vermiculite, coarse and fine perlite (2:1:1:1 ratio) in order to obtain healthy leaves of cultivated cultivars for being used in the different tests.

2.2 Cultures of fungi used in the inoculation and treatment

The causative-organism of the disease (*A. cucumerina*: strain Alt-cu) was isolated from diseased cucumber leaves on plants grown under plastic-houses in Tulkarm area (cultivar: I.V-40) then, cultured on potato dextrose agar (PDA) medium to be used in the disease inoculation after being subcultured on PDA plates. The antagonistic fungus: *Trichoderma harzianum* (strain Th₂) obtained

from Professor P. Lepoivre, Faculty of Agriculture in Gembloux (Belgium) was subcultured on plates with oat meal agar medium to be used in the treatments against *A. cucumerina*. Young pure cultures (15-day old) of both fungi were used to conduct the different tests. The concentrations of the conidial suspensions prepared from these cultures were 4.0×10^5 conidia/ml for the strain Alt-cu and 2.0×10^8 conidia/ml for the strain Th₂. Hemacytometer was used for carrying out the counts.

2.3 Technique of cultivars' susceptibility assessment

Leaf-discs (20mm diameter) of the three cucumber cultivars mentioned above cut by using 20mm cork borer from healthy leaves were used in assessing the susceptibility of the cultivars to disease-infection. Inoculation with causative-organism *A. cucumerina* was accomplished by depositing 25- μ l droplet of conidial suspension (containing 10,000 conidia using a suspension with 4.0×10^5 conidia/ml) on the leaf-disc center which had been superficially wounded using sterile needle. The leaf-discs were, then, incubated at $22 \pm 2^\circ\text{C}$ in petri-dishes under humid conditions (moistened filter paper) for 6 days before being evaluated by measuring the disease-lesion diameter on each leaf-disc. The experiment contained 3 treatments (3 cultivars) and 7 replicates per treatment represented by 7 leaf-discs and distributed in a completely randomized design.

2.4. Types of fungicides and *T. harzianum* treatments used to control the disease

Four types of treatments with fungicides were applied against the disease. The recommended rates during application of these fungicides were: 0.30% (W/V) for metalaxyl + mancozeb (Ridomil® MZ 63.5WP, a.i.=7.5% metalaxyl + 56% mancozeb); 0.35% (V/V) for difenoconazole (Score® 250 EC, a.i.=250 g/l); 0.35% (W/V) for captan (Merpan® 50 WP, a.i.=50%); and 0.20% (W/V) for cyprodinil + fludioxonil (Switch® 62.5 WG, a.i.=375 g/Kg cyprodinil + 250 g/Kg fludioxonil).

Two forms of *T. harzianum* (strain Th₂) were applied for treatment of the disease: conidial suspension in sterile distilled water and formulated conidia in invert emulsion. Concentration of the fungus conidia in the two forms was 2.0×10^8 conidia/ml. Ingredients of the invert emulsion (water-in-oil type) used in the experiment are composed of the following (W/W): sterile distilled water (45.25%), glycerin (4.00%), water-soluble wax or Dehymuls K® (0.75%), Tween 20 (2.50%), and a mixture of 19.00% coconut oil + 28.50% soybean

oil⁽³⁾. Conidia of *T. harzianum* (strain Th₂) were introduced into the invert emulsion according to the technique developed in the previous research⁽³⁾. The concentration of the introduced conidia was 2.0X10⁸ conidia/ml.

Two additional control treatments were applied in the experiment: the first with sterile distilled water and the second with the blank formulation of invert emulsion. The eight experimental treatments were distributed and then, analyzed according to completely randomized design with 6 replicates represented by 6 leaf-discs per treatment held in one Petri-dish with moistened filter paper. Standard error of the mean (SEM) was calculated and then added to the tables as mean ±SEM.

2.5 Technique of assessing effect of the treatment with fungicides and *T. harzianum* on the disease-control

For this, treatment with fungicides or *T. harzianum* was applied either just before inoculation with the disease-causative organism or 24 h after the inoculation. Leaf-discs (20 mm diameter) of the cultivar Hasan were used in carrying out this assessment. Inoculation with *A. cucumerina* was done in both types of treatment by depositing a 25-μl droplet of conidial suspension contained 10,000 conidia taken from a suspension with 4.0X10⁵ conidia/ml. Each droplet was deposited on the leaf-disc center after being superficially disinfected, and then wounded. A similar droplet size (25μl) of fungicides solution or formulated *T. harzianum* in invert emulsion or its conidial suspension in sterile distilled water was used. The latter type of droplets was deposited at the same site of inoculation just before the disease inoculation or 24 h after the inoculation. The leaf-discs were, then, incubated in petri-dishes with moistened filter papers at 22±2°C for 6 days before measuring the resulted disease-lesion diameter.

2.6. Evaluation of the effect of disease-control treatments and tests of cultivars' susceptibility

Efficacy of the treatments with fungicides or with *T. harzianum* to control the disease was evaluated according to the capacity of each fungicide or *T. harzianum* to reduce the disease-lesion development on leaf-discs compared to the control treatments with sterile distilled water or blank formulation of invert emulsion. The disease-lesion diameter was, thus, measured in all replicates 6 days after inoculation and treatment, and the mean lesion-diameter of each treatment was then calculated to be used in the efficacy comparison. Similarly, the disease-lesion diameter measured on the leaf-discs of tested cultivars was

used to compare their susceptibility to disease-infection 6 days after inoculation. The mean lesion-diameter was calculated and then used in the susceptibility comparison. Standard error of the mean (SEM) was calculated. Statistical analyses of means was carried out using Duncan's multiple range test. Mean percent reduction in the disease-lesion diameter relative to water control was also calculated for all treatments conducted before and after the disease-inoculation.

3. Results and Discussion

3.1 Susceptibility of cucumber cultivars to *A. cucumerina*-infection

Significant differences (at $p \leq 0.05$) were obtained in the means of *A. cucumerina*-lesion diameter measured on leaf-discs of tested cucumber cultivars (Table 1). Hasan cultivar was the most susceptible to disease-infection, whereas Rocket cultivar was the least susceptible and I.V-40 was intermediate. Hasan cultivar was, thus, selected as a susceptible cucumber cultivar for being used in the assessment of treatment with fungicides and *T. harzianum* to chose the proper treatment against *A. cucumerina*. To the best of our knowledge, no studies have been conducted to test the susceptibility of cucumber cultivars especially parthenocarpic types to infection with *A. cucumerina* at least, under local conditions. However, Jackson (1959)⁽¹⁴⁾ reported that sunscalded muskmelons were highly susceptible to infection by *A. cucumerina*, whereas Thomas et al. (1990)⁽²⁷⁾ attributed the resistance of muskmelons to *Alternaria* infections to several factors of which resistant reaction was the most important. This reaction was characterized by small necrotic lesions which didn't expand to support abundant sporulation. This hypothesis could explain the results obtained on small disease-lesion diameter measured on leaf-discs of the resistant cucumber cultivar Rocket.

3.2 Assessment of the effect of fungicides and *T. harzianum* treatments on control of *A. cucumerina*

The application of 4 types of fungicidal treatment and 2 forms of *T. harzianum* treatment resulted in significantly decreasing the development of *A. cucumerina*-lesions on cucumber leaf-discs compared to the control (Table 2). Consequently, Score® completely inhibited the development of *A. cucumerina*-lesion when used just before or 24h after the disease inoculation. The mean lesion-diameter of *A. cucumerina* in both types of effect was zero (Table 2). Such total inhibition was also obtained when Switch® was applied just before

disease inoculation, but not after 24h of disease inoculation (Table 2). Moreover, significant reduction in *A. cucumerina*-lesion diameter relative to water control was also obtained when treatments with *T. harzianum* were applied either just before or 24h after disease inoculation. Therefore, treatment with formulated *T. harzianum* in invert emulsion gave 4.5 and 6.5mm as means of lesion-diameter or 70.4 and 57.2% as means of percent reduction in disease-lesion diameter relative to water control treatment when applied either just before or 24h after disease inoculation, respectively. Treatment with conidial suspension of *T. harzianum* in sterile distilled water gave 8.2 and 11.2mm as means of lesion-diameter when applied just before or 24h after disease inoculation, respectively. All comparisons made in the above-mentioned treatments were made in proportion with the mean lesion-diameter in control treatments (13.8mm diameter for treatment with the blank formulation of invert emulsion and 15.2mm diameter for treatment with sterile distilled water) (Table 2).

According to the previous results, the general classification of treatment efficacy with fungicides and *T. harzianum* when applied just before disease inoculation was as follows: Score®, Switch®, Merpan®, Formulated *T. harzianum* in invert emulsion, Ridomil®, Conidial suspension of *T. harzianum* in sterile distilled water, Blank formulation of invert emulsion or sterile distilled water only (respectively, in a descending order). For the treatment applied 24h after disease inoculation, the descending order of treatment efficacy was as follows: Score®, Switch®, Formulated *T. harzianum* in invert emulsion or Merpan®, Ridomil®, Conidial suspension of *T. harzianum* in sterile distilled water, Blank formulation of invert emulsion or sterile distilled water only, respectively (Table 2).

The efficacy of applying Score® at a concentration of 0.25% and 0.35% (V/V) was previously reported against *Alternaria alternata* on fig leaves⁽¹⁾ and persimmon fruits⁽²⁾, respectively. Moreover, effective application of *Trichoderma harzianum* at a concentration of 2.0×10^7 and 2.4×10^7 conidia/ml was also reported against *A. alternata* on fig leaves and persimmon fruits, respectively⁽¹⁻²⁾. Difenoconazole [1-(2-{4-(4-chlorophenoxy)-2 chlorophenyl}-4-methyl-1,3 dioxolan-2,2 yl-methyl)-1H-1,2,4 triazole] as the a.i of Score® is a new triazole fungicide with a slight toxicity (oral LD₅₀ to rats = 2125 mg/Kg), and acts through inhibition of ergosterol biosynthesis in treated fungi⁽²⁸⁾. This mode of action explains its treatment effect before disease inoculation by preventing conidial germination and/or development of the germ tube if

germination takes place. So far all infections with *A. alternata* and *A. macrospora* on different hosts were reduced through treatment with different types of fungicides including those of systemic-type such as iprodione^(17, 21, 23-25).

Certain authors reported the antagonistic effect of *T. harzianum* to many fungi causing soil borne diseases, so that it may be used as an effective bioagent for controlling diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium aphanidermatum*^(8-10, 22). *T. harzianum* exercises its antagonistic effect to the above mentioned phytopathogenic fungi through secretion of an antifungal antibiotic called viridin or through production of an antifungal substance called gliotoxin where both of them inhibit germination of phytopathogenic fungal conidia⁽²⁸⁾. Recently, the antagonistic effect of *T. harzianum* to *Alternaria alternata* on fig leaves⁽¹⁾, persimmon fruits⁽²⁾, and *Botrytis cinerea* on apples at postharvest stage⁽³⁾ was also reported. This may lead to the possibility of using *T. harzianum* in biocontrol of leaf and fruit infecting fungi especially *B. cinerea* and *Alternaria* spp. The probable mode of action of *T. harzianum* against these fungi might be through production of viridin and gliotoxin. Further experiments are needed to test the efficacy of *T. harzianum* against *Botrytis* and *Alternaria* especially under field conditions before being used as bioagent against these phytopathogenic fungi at a large scale.

4. Conclusion

Confirmation of the results obtained on the efficacy of tested fungicides should be carried out on whole plants under field conditions before being recommended to be applied by the farmers and extension agents. Also, once the results are confirmed at field level, integration of treatments with fungicidal spray and formulated *T. harzianum* could be done to reduce the disease severity on susceptible cultivars of cucumber grown under field conditions. Application of unformulated or formulated *T. harzianum* in invert emulsion to control *A. cucumerina* needs also to be confirmed on whole plants under field conditions before being applied by the farmers and extension agents at a large scale.

Table (1): Means of disease-lesion diameter developed on leaf-discs of local common cucumber cultivars 6 days after inoculation with *Alternaria cucumerina* (strain Alt-cu) and incubation at $22 \pm 2^\circ\text{C}$ and humid conditions

Cucumber cultivars	Mean disease-lesion diameter in mm \pm SEM
Hasan	14.1 \pm 2.4 c
I.V-40	8.1 \pm 1.3 b
Rocket	3.4 \pm 2.3 a

Means followed by different letters are significantly different at 5% level of probability according to Duncan's multiple range test.

Table (2): Means of disease-lesion diameter 6 days after treatment with fungicides and *Trichoderma harzianum* (strain: Th₂) either just before or 24h after inoculation with *Alternaria cucumerina* (strain Alt-cu) on leaf-discs of cucumber (variety: Hasan) incubated at $22 \pm 2^\circ\text{C}$ and humid conditions

Fungicides and <i>T. harzianum</i> treatments	Treatment just before the disease inoculation		Treatment 24h after the disease inoculation	
	Mean disease-lesion diameter in mm \pm SEM	Mean percent reduction in disease-lesion diameter relative to water control	Mean disease-lesion diameter in mm \pm SEM	Mean percent reduction in disease-lesion diameter relative to water control
Ridomil®	6.0 \pm 1.9 c	60.5 c	7.7 \pm 1.0 c	49.3 c
Score®	0 \pm 0 a	100 e	0 \pm 0 a	100 e
Merpan®	2.2 \pm 1.4 b	85.5 d	7.5 \pm 0.8 c	50.6 c
Switch®	0 \pm 0 a	100 e	2.5 \pm 1.8 b	83.5 d
Formulated conidia of <i>T. harzianum</i> in invert emulsion	4.5 \pm 1.3 c	70.4 c	6.5 \pm 1.4 c	57.2 c
Blank formulation of invert emulsion (control)	13.8 \pm 1.2 e	9.2 a	13.8 \pm 1.2 e	9.2 a
Conidial suspension of <i>T. harzianum</i> in sterile distilled water	8.2 \pm 1.1 d	46.1 b	11.2 \pm 2.1 d	26.3 b
Sterile distilled water (control)	15.2 \pm 3.2 e		15.2 \pm 3.2 e	

Means of disease-lesion diameter within each column (in each treatment type) followed by different letters are significantly different at 5% level of probability according to Duncan's multiple range test.

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