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Cladosporium tenuissimum Cooke (Deuteromycotina: Hyphomycetes) as a causal organism of new disease on cucumber fruits

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Abstract

The present research describes the symptoms of infection of *Cladosporium tenuissimum* on cucumber fruits. This infection induced formation of numerous small circular swellings (3.3 mm diameter by 1.8 mm high) on the surface of infected fruits rendering them unfit for marketing purposes. No previous description of these symptoms on cucumber fruits has been reported. Results obtained on susceptibility of the locally common-cultivated cucumber cultivars to the infection indicated significant differences in cultivar susceptibility. Cultivar Nour was the most susceptible whereas Rocket and Ringo were the least susceptible. Other tested cultivars were intermediate. Treatment of the infection on cultivar Nour with fungicides indicated significant reduction in number of *C. tenuissimum* swellings per cm^2 of infected fruit surface in comparison with the untreated control. Score® (difenoconazole) applied at a rate of 0.35% v/v and Merpan® (captan) applied at a rate of 0.35% w/v demonstrated as the most effective among tested fungicides with 83% to 86% reduction in the number of disease swellings relative to the control.

Introduction

Cucumber (*Cucumis sativum*) is one of the major cash crops in Palestine (West Bank and Gaza Strip). The cultivated area with cucumbers reached 4517 ha in 2003 (21.6% of the total cultivated area with vegetable crops). The total production of cucumbers reached 160,077 tons in 2003, and approximately one half of this production was exported (MFO, 2003). Cucumber production in Palestine is mainly under protected cultural conditions such as in plastic houses and tunnel houses. Subtropical conditions (high temperatures and relative humidities) usually prevail during this type of production and these conditions may create a favorable climate for development of diseases.

Here, we report the first case of infection caused by *Cladosporium tenuissimum* on cucumber fruits. Infection was detected on fruits produced by cucumbers grown under plastic houses in Tulkarm area – West Bank, Palestine. On other crops, *C. tenuissimum* has been reported as a pathogen of dry rot disease of tomato fruits in India and Nigeria (Fajola, 1979; Narain and Rout, 1981), leaf spot disease of banana in Almora district, India (Pandey and Gupta, 1983), pre- and post-emergence seed rot of moth bean (*Vigna aconitifolia*) (Jain et al., 1983), leaf blight and fruit rot of watermelon (Narain et al., 1985), leaf blight of *Pongamia pinnata* Pierre (Bhat et al., 1989). It has also been isolated as a fungal endophyte in association with other fungi from leaf blades, leaf sheaths and roots of rice (Fisher and Petrini, 1992), from rotted ber fruits (*Ziziphus mauritiana*) collected from markets in Rajasthan, India (Sharma and Majumdar, 1993), from blossom-end rotted tomato fruits (Dhal et al., 1997).

Cladosporium tenuissimum has also been reported as a mycoparasite of uredia of *Melampsora laricipopulina* in vitro (Sharma and Heather, 1988), of aeciospores of two needle pine rusts caused by *Cronartium flaccidum* and *Peridermium pini* (Moricca and Ragazzi, 1997 and 1998; Moricca et al., 1999). Also, it has been shown that *C. tenuissimum* is able to proliferate in mice tissues (Okeke et al., 1991). Ellis (1976) stated that *C. tenuissimum* is a saprotroph and is commonly isolated from a wide range of substrata especially in tropical areas. Some investigators have reported that certain species of *Cladosporium* are harmful in the indoor environment causing health problems in central and eastern european countries (Horak et al., 1996; Gorny and Dutkiewicz, 2002).

The objectives of the present research were: (i) to characterize the strain of *C. tenuissimum* obtained from cucumber fruits and to describe the symptoms of infection caused by this strain; (ii) to test susceptibility of the locally common-cultivated cucumber cultivars to infection with *C. tenuissimum*; and (iii) to assess efficacy of the common-used fungicides against *C. tenuissimum* infection.

Materials and methods

Cucumber cultivars

Healthy fruits of five cucumber cultivars were used in the susceptibility test. The cultivars were: Nour, Jana, Rocket, Ringo and NV_2. The fruits were obtained from cucumber plants grown after sowing certified seeds of each cultivar under protected cultural conditions. The certified seeds were obtained from local distributors of foreign producing companies of these cultivars.

Cladosporium tenuissimum *strain*

Strain PD_3 of *C. tenuissimum* was used in the inoculation of cucumber fruits during bioassays. It was first isolated from naturally infected cucumber fruits (cv: Rocket) produced by plants grown locally under plastic house conditions in Tulkarm area, West Bank, Palestine. Pure cultures of 10-day old on potato dextrose agar (PDA) medium were used for preparing the conidial suspensions of the fungus to be used in the tests of inoculation. These cultures were also used for studying the morphological characteristics of the fungal colony, the dimensions, shape and colour of the conidia and conidiophores. Microscopic preparations of conidia and conidiophores were made using lactophenol and examined under the light microscope (200 and 400x magnifications). Identification of the fungal strain as *Cladosporium tenuissimum* was done according to the morphological characteristics of the fungus and its culture (Barnett and Hunter, 1998). The identification was confirmed by Dr. J.C. David from CABI-Bioscience Identification Services, CAB International, UK-Center, Egham, UK (Ref. No of CABI-Bioscience: H251/03/YP3;IMI No 391334).

Susceptibility of cucumber cultivars to infection by Cladosporium tenuissimum

Equal-sized mature fruits of five cucumber cultivars (Nour, Jana, Rocket, Ringo, and NV_2) were used. Fruits were washed with tap water then disinfected with sodium hypochlorite (0.025% v/v) for 30 s. Inoculation was carried out by spraying unwounded fruits with a standardized volume of 2 ml conidial suspension (7.75×10^6 conidia ml^{-1}) of *C. tenuissimum* (strain PD_3) per fruit using a small calibrated hand sprayer (1.5 l capacity). Inoculated fruits that were held in closed plastic containers (90 mm diameter by 100 mm deep) with moistened filter paper in the bottom for 2 weeks at 20 ± 2 °C. Evaluation of susceptibility was done by counting the number of swellings characteristic to the fungus on each fruit surface after 2 weeks of inoculation. The mean number of swellings was calculated per fruit and per cm^2 of fruit surface. The experiment contained five treatments representing five cultivars and three replicates representing three fruits per treatment.

Efficacy of fungicidal treatment against C. tenuissimum *infection*

Four fungicides were tested against *C. tenuissimum* infection. The recommended rates of application during the test were 0.30% (w/v) for metalaxyl + mancozeb (Ridomil® MZ 63.5 WP, a.i = 7.5% metalaxyl + 56% mancozeb), 0.35% (v/v) for difenoconazole (Score® 250 EC, a.i = 250 g l^{-1}), 0.35% (w/v) for captan (Merpan® 50 WP,

a.i = 50%), and 0.20% (w/v) for cyprodinil + flodioxonil (Switch® 62.5 WG, a.i = 375 g kg^{-1} cyprodinil + 250 g kg^{-1} flodioxonil). In addition to the above-mentioned fungicidal treatments, an untreated control was included in the experiment. Each fungicidal treatment was applied in two ways: (i) by depositing 25-µl droplet at the same site of inoculation with *C. tenuissimum* on the fruit surface when localized infection technique was used, and (ii) by fruit dip in each fungicidal preparation for 30 s before fruit inoculation with the conidial suspension of *C. tenuissimum* when spraying technique of infection was used.

Two techniques of inoculation with *C. tenuissimum* were applied to the fruit surface of the susceptible cultivar Nour during the fruit assessment of fungicidal treatment efficacy: (i) by localized infection using 25-µl droplet per fruit of conidial suspension (7.75×10^6 conidia ml^{-1}), (ii) by spraying 2 ml/fruit of the same conidial suspension as in the first technique. No wounds were induced on the fruits during inoculation in both techniques. Spraying and incubation methods were done in the same manner as in test of cultivars susceptibility. Evaluation of fungicidal treatment efficacy in both types of inoculation techniques was carried out by counting the number of typical *C. tenuissimum* swellings/fruit in each fungicidal treatment 2 weeks after inoculation. The mean number of the swellings per fruit and per cm^2 of fruit surface was calculated. Experimental treatments representing four fungicidal treatments and the control were distributed and then analyzed according to completely randomized design with four replicates representing four fruits per treatment.

Statistical analyses

Standard error of the means (SE) was calculated and presented in the tables with the mean number of *C. tenuissimum* swellings per fruit and per cm^2 of fruit surface in the two tests of susceptibility and fungicidal treatments. The percentage of reduction in number of *C. tenuissimum* swellings per cm^2 of fruit surface relative to control due to fungicidal treatments was calculated. ANOVA and Tukey-HSD test were used in the analysis to test significant differences in means throughout the results.

Results

Characterization of Cladosporium tenuissimum *strain and description of its symptoms of infection*

The pure culture of *C. tenuissimum* (strain PD_3) on PDA medium has a typical dark appearance of mycelia and conidia. The conidiophores are tall, dark brown in colour, upright, branched variously near the apex, clustered or single. The conidia (blastospores) are also dark brown in colour, they are variable in shape and size. Some of them are ovoid and 1-celled, others are cylindrical and 2-celled, often in simple or branched acropetalous chains. The dimensions of the conidia are 5–10 µm (mean 8.5 µm) in length and 2–5 µm (mean 3.5 µm) in width for the ovoid shape conidia, and 20–45 µm (mean 29.5 µm) in length and 2.5–5.0 µm (mean 3.75 µm) in width for the cylindrical shape conidia. All dimensions were taken as an average of 100 conidia for each shape type.

Symptoms of infection with *C. tenuissimum* (strain PD_3) on cucumber fruit surface appeared as numerous small circular swellings with 3–4 mm (mean 3.3 mm) diameter and 1–2 mm (mean 1.8 mm) high (average of 100 swellings). Such swellings were observed scattered on the infected fruit surface, but they may coalesce when close together (Figure 1(A) and (B)). The swelling surface was, at first, green in colour then become gradually slightly chlorotic and then finally become cracked with very small cracks before being dried rendering infected fruits unsuitable for marketing (Figure 1(C)). Microscopic examination of a cross section in the typical swelling of *C. tenuissimum* on cucumber fruit before cracking and dryness of its surface indicated the presence of hypertrophied and thick-walled subcuticular and epidermal cells (Figure 1(D)).

Susceptibility of cucumber cultivars to C. tenuissimum *infection*

Significant differences (at $P < 0.05$) were obtained in the mean number of *C. tenuissimum* swellings per infected fruit of tested cucumber cultivars: Nour, Jana, Rocket, Ringo and NV_2 (Table 1). Cultivar Nour was the most susceptible (mean 107.7 swellings per fruit), cultivars Rocket and Ringo were the

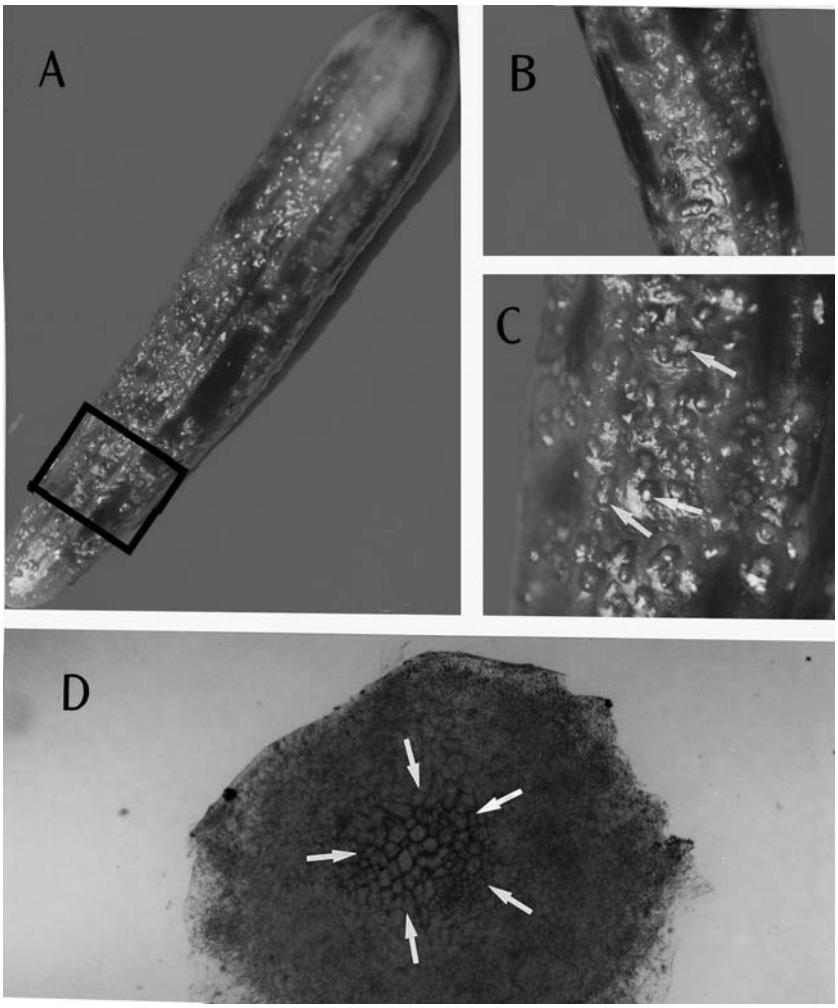

Figure 1. Symptoms of infection with *Cladosporium tenuissimum* (strain PD_3) on cucumber fruits. (A) General view of swellings resulted from *C. tenuissimum* infection on fruits of susceptible cultivar Nour. (B) Enlarged area of A showing the characteristics of typical *C. tenuissimum* swellings. (C) Upper view of enlarged typical swellings (arrows) showing its cracked and dried upper surface layers (cuticle and epidermis) due to fungal attack. (D) Thin cross section in young swelling showing hypertrophied thick-walled cells (arrows) in the subcuticular and epidermal layers (magnification 200×).

least susceptible (means 46.3 and 50.0 swellings per fruit, respectively). Cultivars Jana and NV_2 were intermediate in their susceptibility (mean 69.0 and 75.7 swellings per fruit, respectively). Similar trends were seen when the mean number of *C. tenuissimum* swellings per cm^2 of infected fruit surface was used as the measure of susceptibility (Table 1). Cultivar Nour was always the most susceptible, and cultivars Rocket and Ringo were the least susceptible. Cultivar Nour was thus selected for the assessment of fungicidal treatment efficacy against *C. tenuissimum* infection.

Efficacy of fungicidal treatment against C. tenuissimum *infection*

The treatment with four types of fungicides resulted in a significant reduction of the number of *C. tenuissimum* swellings per cm^2 of infected fruit surface compared to untreated control (Table 2). No significant differences were obtained between the four fungicidal treatments when the technique of localized inoculation and treatment was used. However, significant differences (at $P < 0.05$) were obtained between the same fungicidal treatments

Table 1. Susceptibility of the locally common-cultivated cucumber cultivars to infection with *Cladosporium tenuissimum* (strain PD_3) 2 weeks after inoculation of the fruits and incubation at 20 ± 2 °C

Cucumber cultivars	Mean number (±SE) of *C. tenuissimum* swellings per infected fruit	Mean number (±SE) of *C. tenuissimum* swellings per cm^2 of infected fruit surface[a]
Nour	107.7 ± 4.2 c[b]	1.37 ± 0.05 d[b]
Jana	69.0 ± 4.3 b	0.64 ± 0.04 b
Rocket	46.3 ± 2.9 a	0.50 ± 0.03 a
Ringo	50.0 ± 4.1 a	0.52 ± 0.04 a
NV_2	75.7 ± 5.4 b	0.75 ± 0.05 c

[a]Mean number of swellings/cm^2 of fruit surface = $\frac{\text{Mean number of swellings/fruit}}{\text{Lateral surface area of fruit in cm}^2}$.

[b]Means within each column followed by different letters are significantly different (at $P < 0.05$) according to ANOVA and Tukey-HSD test.

Table 2. Efficacy of fungicidal treatment against infection with *Cladosporium tenuissimum* (strain PD_3) on cucumber fruits (cv: Nour) 2 weeks after inoculation and treatment at 20 ± 2 °C

Fungicides	Localized infection on fruit surface with conidial suspension of *C. tenuissimum*		Infection by spraying conidial suspension of *C. tenuissimum* on whole fruit surface	
	Mean number (±SE) of swellings/cm^2 of fruit surface[a]	% reduction in swelling number/cm^2 of fruit surface relative to control	Mean number (±SE) of swellings/cm^2 of fruit surface[a]	% reduction in swelling number/cm^2 of fruit surface relative to control
Ridomil® (metalaxyl + mancozeb)	0.17 ± 0.13 a[b]	52.8	0.76 ± 0.22 c[b]	45.3
Merpan® (captan)	0.06 ± 0.02 a	83.3	0.22 ± 0.02 a	84.2
Switch® (cyprodinil + flodioxonil)	0.09 ± 0.04 a	75.0	0.39 ± 0.09 b	71.9
Score® (difenoconazole)	0.05 ± 0.01 a	86.1	0.20 ± 0.02 a	85.6
Control (untreated)	0.36 ± 0.15 b		1.39 ± 0.04 d	

[a]Mean number of swellings/cm^2 of fruit surface = $\frac{\text{Mean number of swellings/fruit}}{\text{Lateral surface area of fruit in cm}^2}$.

[b]Means within each column followed by different letters are significantly different (at $P<0.05$) according to ANOVA and Tukey-HSD test.

when the spraying technique of inoculation was used (Table 2). Score® and Merpan® were the most effective in reducing the number of *C. tenuissimum* swellings cm^{-2} of infected fruit surface (means 0.20 and 0.22 swellings cm^{-2}, respectively). Ridomil® was the least effective (mean 0.76 swellings cm^{-2}), and Switch® was intermediate (mean 0.39 swellings cm^{-2}) (Table 2). The percentage of reduction in the number of *C. tenuissimum* swellings relative to control due to fungicidal treatment was up to 86% in both types of inoculation (Table 2). The highest percentage of reduction was obtained due to treatment with Score® and Merpan® (86.1% and 83.3%, respectively, for localized inoculation, and 85.6% and 84.2%, respectively, for spraying inoculation) (Table 2).

Discussion

Symptoms of infection with *C. tenuissimum* (strain PD_3) on cucumber fruits were described. They were always reproduced after inoculation either during the symptom description or during assays carried out for studying susceptibility or fungicidal efficacy. This reproduction of symptoms clearly demonstrated pathogenicity of the fungal strain to cucumber fruit tissues. This pathogenicity was confirmed by completing Koch's postulates when the repeated inoculation and subsequent isolation of *C. tenuissimum* from cucumber fruits were done successfully. Also, repeated subculturing of the fungal strain on PDA medium during the above mentioned assays always yielded the same fungus

with identical morphological characteristics confirming its identification as *C. tenuissimum*. The identification was also confirmed by Dr. J.C. David from CABI-Bioscience Identification Services, Egham, UK. Our results on optimum growth of *C. tenuissimum* on PDA medium at 20 ± 2 °C coincide with those obtained by Xiang et al. (1989).

The present research clearly demonstrated its pathogenicity to cucumber fruits. Although *C. tenuissimum* is a common saprophyte and has been isolated from a wide range of substrata (Ellis, 1976), it has also been reported to be an opportunistic pathogen on other crops rather than cucumber (e.g., tomato, banana, and watermelon) causing dry rot of tomato fruits (Fajola, 1979; Narain and Rout, 1981), leaf spot of banana (Pandey and Gupta, 1983), leaf blight and fruit rot of watermelon (Narain et al., 1985). The opportunistic behavior of some fungi has been also reported by many other investigators for other filamentous fungi such as *Phaeoisaria clematidis* that has been reported as soilborne saprophyte and pathogenic to plants and animals including human beings by causing Keratomycosis (Deighton, 1974; Guarro et al., 2000).

Cladosporium tenuissimum may have been introduced into cucumber plants grown under plastic houses at harvesting time or just before it, and then favorable conditions to infection (high temperatures and relative humidities) that were prevailing in these cultures facilitated its penetration into fruits. Moreover, occurrence of this penetration was not dependent on wounds in fruits. In addition, results obtained here have indicated that the treatment of the fruits just before harvesting with an effective fungicide such as captan (applied at 0.35% w/v of Merpan®) or difenoconazole (applied at 0.35% v/v of Score®) reduced significantly the infection with the fungus at postharvest stage. This would indicate that the infection with *C. tenuissimum* on cucumber fruits could be controlled effectively by fungicidal treatment at proper time and rate. The efficacy of treatment with captan against pre- and post-emergence seed rot of moth bean (*Vigna aconitifolia*) caused by *C. tenuissimum* was also demonstrated by Jain et al. (1983).

In conclusion, symptoms of infection with *C. tenuissimum* on cucumber fruits are reported here for the first time. The control of infection with this opportunistic fungus on cucumber fruits is possible by application of an effective fungicide (difenoconazole or captan). Further studies such as the effect of temperatures, relative humidities, and other environmental factors on the infection, modality of infection with the fungus and swelling formation (e.g. reason of the cell hypertrophy seen in the infected cells of swelling tissues and enzymes involved) are recommended to be carried out or investigated in order to establish the host–parasite relationship that governs this disease.

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