Controlling the root and stem rot of cucumber, caused by *Pythium aphanidermatum*, using resistance cultivars and grafting onto the cucurbit rootstocks

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**ABSTRACT**

Cucumber damping off caused by *Pythium aphanidermatum* is the most important root and stem rot that limits greenhouse cultivations. In this study, relative susceptibility of grafting commercial cucumber cultivars including Alpha, Caspian 340, Storm 5910, Shalim 616, Delta scar, Janette 810, Festibal C5, Royal, Negyn, Soltan and Fadia on two Cucurbita rootstocks were evaluated against *P. aphanidermatum*. Disease severity, survival and seedling growth were used for the evaluation. The results showed significant differences between the studied cultivars \( p \leq 0.01 \). Caspian 340 and Alpha with 15.7% and 100% disease severity had more and less tolerant to *P. aphanidermatum*, respectively. Cucurbita maxima rootstock was more resistant than Cucurbita pepo to *P. aphanidermatum*. *C. pepo* had less compatibility with the cucumber and showed little resistance to the pathogen. The study revealed that grafting Caspian 340 on the resistant cucurbit rootstock i.e. *Cucurbita maxima* could be used as disease control strategies in greenhouses.

**Keywords:** Cucumber, Disease resistance, Grafting, Inoculation

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**INTRODUCTION**

Greenhouse cultivation has been extended significantly to produce vegetable products in Iran in the past two decades. Cucumber is one of the most important greenhouse products which annually grown widely. Continuous cultivation of this plant has been caused contamination to several soil-inhabiting pathogens. Damping-off, as the most seedling root and crown rot disease, is a limiting factor in cucumber greenhouse cultivation. Some species of *Pythium* and *Phytophthora* are the most important soil-borne pathogens that cause damping-off and root rot \( \text{(Behdad and Akhyani 1985; Stanghellini and Kornland, 1986; Farvin et al. 1988; Moulin et al. 1994; Menzies et al. 1996; Esmaili-Shirazifard and Banihashemi, 2008).} \) The disease has been reported to have up to 25% damage on cucumber \( \text{(Alavi 1973; Eetebarian 2002).} \) Root Rot disease of cucurbits caused by *Pythium aphanidermatum* (Eds.) Fitz. is one of the major factors limiting greenhouse cucumber culture in greenhouses \( \text{(Alavi 1973).} \) Due to the high cost of chemical control, environmental problems and sensitivity to salt of the cucumbers \( \text{(Roustaei et al. 2004) the use of resistant cultivars is very important. Using grafting on resistant rootstock can increase plant resistance against soil-borne diseases without use of pesticides and fungicides. The main objectives of grafting cucumber onto cucurbits are control of soil-borne diseases, nematodes, and achieve more and higher quality product (Edelstein 2004). Research on grafting vegetables began in Japan in 1920 with the introduction of *Cucurbita moschata* Duch., As the perfect rootstock to prevent *Fusarium* wilt of watermelon. In Japan, the use of cucurbit cultivars such Shirokikouza (*C. moschata*) and Shin-tosa (*C. maxima × C. moschata*) as the rootstock for cucumber production started in 1960 \( \text{(Iwasaki and Inaba 1988).} \) Today 80-100% of greenhouses are included rootstocks cultures in Japan and Korea. The main objective of this study is to investigate control of cucumber root rot using commercial cultivars resistant and grafting cucurbits on cucurbit. \( \text{1-Vali-e-Asr University of Rafsanjan, Faculty of Agriculture, Department of Plant Protection 2 Vali-e-Asr University of Rafsanjan, Faculty of Agriculture, Department of Horticultural Science 3-Zabol University of Zabol, Faculty of Agriculture, Department of Agronomy.} \)

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MATERIAL AND METHODS

Isolation, identification and pathogenicity of pathogens

Sampling was performed from commercial cucumber greenhouses in Rafsanjan. Plants with damping-off symptoms and the soil around them separately transported to the laboratory in the plastic bags. To isolate the pathogen, crowns and roots of infected plants were washed with tap water. Half-cm pieces were chosen, which included both healthy and infected tissue then washed again with sterile distilled water and were dried by the filter paper. Pieces were kept on corn meal agar medium (containing 17 g of powdered Corn Meal Agar (Merck), ampicillin 250 mg, 100 mg rifampicin and 50 mg Benomyl in one liter of distilled water) and cultured at 24±1°C. After the growth of fungus colonies, they were purified by using of hyphal tip method. Isolates were morphologically compared with keys and descriptions of known Pythium species (Van der Plaats-Niterink 1981; Mostowfizadeh-Ghalamfarsa and Banihashemi 2005).

Proving the pathogenicity

The alpha cucumber seedling was used for pathogenicity studies (stage of 2-3 leaves). Cucumber seeds with the same size, were grown in sterilized pots containing sterilized garden soil (three times in autoclaved in 121°C for 20 minutes each time) in a greenhouse (temperature 25±2°C and relative humidity at around 70 percent). To infect seedlings, cannabis seeds were placed for 48 hours in 20% alcohol, and then were maintained for 24 hours on young colonies of fungi at room temperature 25°C (Sanchez et al. 1999). Three seeds were placed beside the each plant’s root in the infected soil at 0.5 cm depth. To increase humidity, after inoculation the plants and the pots were sprayed with distilled water and cellophane plastic wrap stretched over them. Relative humidity was below 95% under the plastic cover. The plastic cover was removed after 72 hours and the seedlings were placed in normal temperature and humidity. After five days, the infected seedlings were collected and cultured on CMA (Corn Meal Agar) and the pathogen was re-isolated. The experiment was a completely randomized design with three replications.

Study of the relative resistance of cucumber to Pythium aphanidermatum by grafting on cucurbit

In this study two types of the rootstocks of the cucurbit, C. pepo and C. maxima were used and Alpha cucumber cultivar, as a control treatment, was considered as susceptible rootstock to disease.

Seeds of C. pepo and C. maxima germinated in vermicolitis then five seeds were planted in pots with 20 cm diameter. After appearance the initial cotyledons of cucurbit, grafting were performed (hole type). After 7 days, the plastic cover was removed and the seedlings were placed in normal temperature and humidity.

Inoculation was performed in the stage of 2 to 3 leaf by artificial infecting of cannabis seeds. Experiment was arranged based on completely randomized design with six treatments and three replications. The relative sensitivity of different treatments were examined until 40 days after inoculation with plant responses scored from “0” to “5” (“0” asymptomatic “5” complete plant death). That zero “0” healthy seedlings without symptoms, score of “1” wilting plant leaves and produces small necrotic spots at the crown, the “2” from the disappearance and death of 40% of the seedlings, score of “3” the disappearance and death of 60% of the seedlings, score of “4” the disappearance and death of 80% of the seedlings and the “5” is the loss of complete plantlets. Using a visible reaction between host and pathogen, a disease severity index was calculated for each replicate as follows: (Thomas et al. 1987; Alaei et al. 2008; De Backer et al. 2011).

\[
0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4 + 5 \times n_5 \\
5 \times (n_0 + n_1 + n_2 + n_3 + n_4 + n_5) \times 100
\]

Where \( n_i \) is the number of infected seedlings evaluated in the grading \( i \).

Each treatment was consisting three replications and each replication five seedlings that mean of pathogenicity was calculated for them. Sensitivity or resistance to treatment was identified on the basis of mean scores and the percentage of infection. Scoring for resistant (R), tolerant (T) and sensitive (S) to disease were considered (“0” and “1”), (“2” and “3”) and (“4” or “5”) respectively (table 1). After inoculation, fungus was isolated.

The relative resistance assessment commercial cultivars of cucumber to Pythium aphanidermatum

In this study, ten commercial cultivars of greenhouse cucumbers were chosen for evaluation resistance to damping-off disease. including Delta scar, Caspian 340, Storm 5910, Janette 810, the Royal, Fadia, Negyn, Shalim 616 and the Festibal C5. Alpha cultivar called as a control treatment was considered susceptible to the pathogen.

Seeds after 24 hours soaking, was sown in pots. Then five seeds of a cultivar were planted in each plot, irrigation was performed on a daily basis. Four weeks after germination, the seedlings infected by the fungal pathogen. Inoculation was done in the stage of 2 to 3 of leaf by method of
artificially contaminated cannabis seeds as described above. Response of different cucumber cultivars to fungus and disease severity was calculated until two weeks after the inoculation, zero "0" healthy and without signs or five "5" is totally polluted as described above.

**Data analysis**

All experiments were performed in a completely randomized design with eleven treatments (for determination of resistant cultivars) and three treatments (for grafting section) and three replications for each treatment. Analysis of data was done by ANOVA and Duncan’s comparison test using statistical software (SPSS Inc) SPSS, version 15.

**RESULT AND DISCUSSION**

A total of 18 infected soil and cucumber samples were collected and 23 isolates were isolated from infected soil and root and crown rot of cucumber. After two days culture of infected tissue, aseptate hyphae grew on the plate very fast, forming white colonies with loose and aerial mycelia (Fig. 1a). The daily mycelia growth rate at 25±1°C on CMA was 28 mm. The main hyphae were 6-9 µm wide. Sporangia were most terminal and consisting of complexes of swollen torulated hyphal branches; Vesicles were formed from the sporangia, which produced zoospores at room temperature. A large number of oogonia and antheridia also formed. Oogonia were terminal, globose, smooth and 18-29 µm in diameter. Antheridia were most intercalary, sometimes terminal, broadly sac-shaped and 9-12 µm in diameter. Oospores were globose, aplerotic, one per oogonium and 18-25 µm in diameter. The thickness of oospore wall ranged from 2-3 µm wide (Fig. 1b-d). Based on the morphological measurements and growth response to temperature of our isolates were identical to those of *Pythium aphanidermatum* (Edson) Fitzpatrick reported by Van der Plaats-Niterink (1981) and Mostofizadeh-Ghalamfarsa and Banihashemi (2005).

The results of the relative sensitivity of the cucurbit rootstock on root rot disease caused by *P. aphanidermatum* showed that *C. pepo* and *C. maxima* at the base of the seedling stage (20 days post inoculation) are quite resistant to the disease and did not show any symptoms, but at 20 days after inoculation by *C. pepo* showed greater sensitivity than the *C. maxima* (Table 1).

To assess the relative strength of commercial cucumber cultivars to the pathogen *P. aphanidermatum*, cucumber seedling in the suitable circumstances infection (95% moisture content and temperature, 24°C) were investigated. Based on the results the virulent pathogen among commercial cultivars of cucumber in a probably significant difference (p≤0.01). Results of mean comparison showed that the percentage of highly pathogenic Caspian 340, Delta Oscar and Negyn had the lowest percentage of contamination. Shalim 616, Royal, Fstybal C5, Janet810 and Fadya, with 22.2, 25.0, 27/8, 38/9 and 43/5 percent showed the lowest levels of infection respectively (Table 2 and Figure 1).

Several reports indicate that the base of the root system has high tolerance to soil-borne pathogens, including diseases caused by *Fusarium* spp. (Miguel et al. 2004) and *Verticillium* spp. (Paplomatas et al. 2000). Moreover, grafted plants may also produce higher quality and more product and on the other hand plants are more tolerance to environmental stresses such as low temperature, The relative sensitivity of some cucurbits to *Phytophthora* showed that *C. pepo* and *C. maxima* are resistant to the pathogen (Alavi 1973; Alavi and Strange 1982).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average of disease severity (%)</th>
<th>And assessment of the relative sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First 20 days</td>
<td>Second 20 days</td>
</tr>
<tr>
<td><em>Cucurbita pepo</em></td>
<td>6.7 (R)</td>
<td>36 (T)</td>
</tr>
<tr>
<td><em>Cucurbita maxima</em></td>
<td>0.0 (R)</td>
<td>9.2 (R)</td>
</tr>
<tr>
<td>Cucumber (cv. Alpha) on <em>C. pepo</em></td>
<td>26.7 (R)</td>
<td>56 (T)</td>
</tr>
<tr>
<td>Cucumber (cv. Alpha) on <em>C. maxima</em></td>
<td>13.3 (R)</td>
<td>44 (T)</td>
</tr>
<tr>
<td>Cucumber (cv. Alpha)</td>
<td>100 (S)</td>
<td>100 (S)</td>
</tr>
</tbody>
</table>

Resistant (R), Tolerant (T) and Susceptible (S)

Table 1. The reaction of stock and scion cucumber (cv. Alpha) and the base of the cucurbit to *Pythium aphanidermatum*
In another study (Mansoori and Banihashemi, 1982), the Ohio MR17 introduced more resistant to
P. drechsleri, and moderately resistant observed in
cultivars 3440 and 3436. In this study, using


cucurbit grafted with cucumber show good result
for control root rot caused by P. aphanidermatum.


So cucurbit C. maxima effectively showed

resistance to the pathogen, on the other hand alpha
cultivar (sensitive basis) on the basis of


C. maxima have good resistance to P. aphanidermatum.


Although rootstock C. pepo is tolera-
tant to the
disease, but the cucumber is shown incompatibility.


Of course, because cucumber and cucurbit do not
have a close affinity, it was not unexpected that
these results were consistent with the result of


Different reasons that may have an influence
on graft success: inherent system of cellular
incompatibility, formation of plasmodesmata,
vascular tissue connections, and the presence of
growth regulators and peroxidases. (Pina and Errea,
2005). Incompatibility between the scion/rootstock
and high costs are the problems for using of this
method, but nowadays the developments of science
and the presence of multiple grafting methods, this
method can be an appropriate method for enhance
the performance (Cohen et al. 2007).

Regarding the role of environmental stresses
such as increased temperature causes increased
susceptibility to root rot (Sutton et al. 2006). Other
studies show that in most areas of the world, it is
very difficult to achieve to resistant varieties with
desired characterized (Kalloo and Bergh. 1993).
Always varieties of cucumber show different
reaction against fungal. So far, 158 genes identified
in cucumber that 15 of them are compared as
resistance genes. (Wehner et al. 1997). Varieties
and genotypes of Iranian cucumbers can show
different responses to pathogen. Proper irrigation
and planting techniques can reduce the amount of
damage caused by this pathogen.


Table 2: The relative resistance assessment commercial cultivars of cucumber to Pythium aphanidermatum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average of disease severity (%)</th>
<th>And assessment of the relative sensitivity</th>
<th>Statistical Category**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>100.0 (S)</td>
<td>100.0 (S)</td>
<td>a</td>
</tr>
<tr>
<td>Storm 5910</td>
<td>74.1 (S)</td>
<td>62.0 (S)</td>
<td>b</td>
</tr>
<tr>
<td>Soltan</td>
<td>64.8 (S)</td>
<td>56.5 (S)</td>
<td>b</td>
</tr>
<tr>
<td>Fadia</td>
<td>43.5 (T)</td>
<td>31.5 (T)</td>
<td>c</td>
</tr>
<tr>
<td>Janette 810</td>
<td>38.9 (T)</td>
<td>21.3 (T)</td>
<td>cd</td>
</tr>
<tr>
<td>Festibal C5</td>
<td>27.8 (T)</td>
<td>18.5 (R)</td>
<td>de</td>
</tr>
<tr>
<td>Royal</td>
<td>25.0 (R)</td>
<td>13.9 (R)</td>
<td>e</td>
</tr>
<tr>
<td>Chalm 616</td>
<td>22.2 (R)</td>
<td>16.7 (R)</td>
<td>e</td>
</tr>
<tr>
<td>Delta scar</td>
<td>20.4 (R)</td>
<td>16.7 (R)</td>
<td>e</td>
</tr>
<tr>
<td>Negyn</td>
<td>19.4 (R)</td>
<td>17.6 (R)</td>
<td>e</td>
</tr>
<tr>
<td>Caspian 340</td>
<td>15.7 (R)</td>
<td>9.3 (R)</td>
<td>f</td>
</tr>
</tbody>
</table>

Resistant (R), Tolerant (T) and Susceptible (S) ** same letter are not significantly different (p≤0.01).

Figure 1: Pythium aphanidermatum: (a) colony in corn
meal agar medium; (b) oogonium with smooth walls and
antheridium (Scale 40x); (c) sporangium (scale 40 x); (d) sporangium rod and swollen (scale 40x).

Figure 2 The relative resistance assessment commercial
cultivars of cucumber to Pythium aphanidermatum
A: Storm 5910
B: Caspian 340, after 10 days post inoculation

CONCLUSION

The use of chemicals methods (such as methyl bromide) reduced due to environmental impact, other methods have been proposed including the use of resistant or tolerant cultivars and grafting on resistant rootstocks. Due to presence of new pathotypes of plant diseases or susceptibility of introduced cultivars to certain diseases, (Iwasaki and Inaba 1988) has raised the need to introduce new resistant cultivars. On the other hand, production of resistant cultivars is very time consuming and expensive, so use the rootstock-resistant hybridization technique is introduced as a
fast and relatively economical method (Trionfetti Nisini et al. 2002). In this study, symptoms after inoculation of the pathogen (in severe pollution) were yellowing of the cotyledon leaves, causing burning and fovea in the crown. In study of the resistance of commercial cultivars to *P. aphanidermatum*, Caspian 340 and Delta Oskar were observed to be a good option for cultivation in greenhouses. On the other hand, resistance genes can be detected by examining genetic and used in breeding systems.

**REFERENCES**


