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Evaluation of resistance to seedling damping-off caused by *Phytophthora drechsleri* in cucumber cultivars under greenhouse conditions

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ABSTRACT: Resistance of greenhouse cucumber cultivars was assessed against damping-off caused by *Phytophthora drechsleri* in Persian Gulf University during 2014-15. Cucumber varieties showed different reactions to disease. Regarding death at 2-3 leaves stage, Barracuda was grouped as very sensitive with 95.87% seedling decay; Sultan, Aseal, Fenomeno, Storm, Number 1 and Kashmir with 77.77, 74.99, 74.99, 73.05, 72.21, 69.43% death respectively were sensitive and PS 547 with 60.83 percent death was semi-sensitive. In the 5-6 leaves stage, Barracuda, Sultan and Number 1 with respectively 71.66, 70.83, 65.41% death were susceptible, and PS 547, Kashmir, Storm, Aseal and Fenomeno with 59.59, 59.41, 55.27 , 55.08, 49.25% percent death, respectively, were moderately susceptible. During flowering stage, Barracuda with 63.28% death was susceptible, Number 1 with 44.22% death was moderately susceptible, Storm, Sultan, Kashmir, Aseal and Fenomeno with respectively 38.88, 29.72, 28.04, 27.77, 24.44% percent death were moderately resistant and PS 547 was resistant with 12.3% death. Cluster analysis at different developmental stages classified cultivars in different groups as well.

Keywords: Cucumber, Damping-off, Phytophthora, Resistance, Soilborne

INTRODUCTION

Cucumber (*Cucumis sativus* L.), is a cross-pollinated, annual, bi-sexual, one base and two cotyledonous crop. This herbaceous plant belongs to cucurbitaceae family and consists of 118 genera and 825 species. Cucumber (*Cucumis sativus* L.), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), Zucchini-Courgette (Cucurbita pepo) and Buttercup squash (*Cucurbita maxima*) are some of the most important species in this family (Salehi and Bonaj., 2013).

Cucumber is cultivated in the broader culture comparing to other vegetables of cucurbitaceae. The origin of cucumber is attributed to India or South Asia. Although the plant is not high in calories and nutrients and its nutritional value is due to different types of sugars and vitamins, but is one of the important mineral resources; fruit carbohydrates are of the secondary importance, and water consists the mail part of the fruits (Poustchi, 2001).

Cucurbits like other crops are suffering from various diseases; damping-off is one of the most important diseases of these plants and causes extensive damage to the greenhouse and also farm crops. *Phytophthora drechsleri* is the causal agent of cucumber damping-off and was first isolated, identified and named by Tucker (Tucker, 1931).

Damping-off disease of cucurbit plants is common including cucumber, all kinds of melon, watermelon and zucchini. The most important plant parts that will be attacked by the pathogen are roots and crowns (Behdad, 1984).

In Iran, damping off disease of the melons was first reported in 1332 by Sharif and Ershahd and his colleagues carried out broad studies in 1342 on it (Ershad *et al.*, 1969).

Alavi and Strange., (1982) isolated Ph. drechsleri from roots, stems and fruit of cucurbits (kitchen garden). Pathogen attacks host plants at all plant growth stages. The pathogen is soilborne and therefore attacks plant root and crown. In the seedling stage fungus makes roots thin, soft and threadlike, and this leads to the plant wilt or death (Hwang and Beneson., 2005). Nasrollahnezhad., (2004), isolated 88 fungal isolates from infected cucumber plants; 58 strains belonged to the genus *Phytophthora* and *P. drechsleri* had the greatest abundance of the species (Nasrollahnezhad., 2004). *Phytophthora* root and crown rot is one of most important and destructive diseases of cucumber in the world (Ristaino and Johnston., 1999).

Therefore, in this study, the reaction of eight different cucumber cultivars to the damping-off disease was examines under greenhouse conditions for three different growth stages and eventually cluster analysis was carried out to evaluate the degree of similarity in different varieties.

MATERIALS AND METHODS

A. Preparation of the pathogen isolate and cucumber seeds

To perform this study, the Ph. drechsleri isolate was received from Plant Pathology lab, Tehran University and was sub-cultured on corn meal agar (CMA) medium. Seeds of eight varieties of cucumbers with the name of Kashmir, Storm, PS 547, Fenomeno, Number 1, Barracuda, Aseal and Sultan were received from Falat-e-Iran Company.

Seed and soil preparation

Seed varieties were transferred to pots containing sterile soil. Soil in the pots was consisted of three equal parts of soil, peat and sand formed. Three seeds were planted in each pot and the pots were irrigated on a daily basis.

Preparation of pathogen inoculom. To prepare the inoculom, 100 g of millet seed with 100 grams of wet sand were poured into 500 ml Erlenmeyer flask and after blocking the door, it was autoclaved at 121 ° C, then the millets were inoculated with mycelium of seven-day old pathogen and incubated in growth chamber, at 25 °C with 12 hours of light and 12 hours of darkness for three weeks. Millet grains contaminated with the pathogen were used as inoculants.

Inoculation method. In independent tests, seedlings reached to the certain growth stages, two grams of inoculom was placed next to the plant crown. In control plants, two gram of un-infested millet seeds was used and covered with soil. The pots were kept flooding for 48 hours and then watered normally. Experiments were carried out as factorial in a completely randomized design with eight treatments and four blocks as replications. Inoculation of seedlings was performed at three timess of 2-3 leaves, 5-6 leaves and flowering stages in independent experiments.

In all three tests, after inoculation, plant growth were assessed daily, the number of dead seedlings was recorded and the percentage of death were calculated (Rezaei and Alizadeh., 1999; Hayat Moghaddam *et al*., 2011). To ensure the presence of pathogen zoospore in the soil around the seedling crowns, leaves of sour orange were placed in drain-water of the pots and after 48 hours were transferred to Carnation Leaf Agar medium and the presence of the pathogen in it was studied (Nasr-e- Esfahani *et al*., 2012)

B. Determining cultivar reaction and statistical analysis

Cultivar reaction was evaluated based on the number of dead plants as semi-sensitive, sensitive, very sensitive, semi-resistant and resistant. Percentages of dead seedlings in three stages were transformed by ArcSin before statistical analysis and were analyzed using SAS software and means were compared on the basis of Duncan at one percent probability. Finally, cluster analysis of cultivars was done to determine similar genotype groups using of SPSS software.

RESULTS AND DISCUSSION

The results of greenhouse studies showed substantial variation in the percentage of cultivar damping- off in various growth stages. Seedling mortality was observed in cucumber cultivars in different growth stages of 2-3 leaves, 5-6 leaves and flowers showed due to the presence of the pathogen, but control plants showed no infection or damping-off. According to the analysis of variance, a significant difference observed in the percentage of seedling mortality between cucumber cultivars in three growth stages (Table 1). In a survey conducted on susceptibility of eight potato varieties to infection by *Phytophthora erythroseptica* and *Pythium ultimum* it was revealed that Russet Norkotah variety is susceptible to *P. erythroseptica*, while Pike and Atlantic cultivars were resistant to the disease.

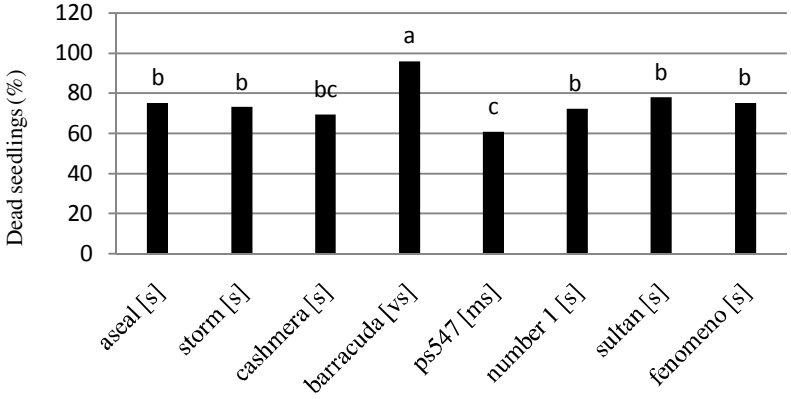
Cultivars Dark Red Norland, Russet Norkotah, Goldrush and Russet Burbank were susceptible to P. ultimum, while Snowden was resistant (Taylor *et al*., 2008).

Table 1: Analysis of variance of the percentage of dead cucumber seedlings due to *P. Drechsleri.*

Source	DF	Mean Square		
		2-3 leaf stage	5-6 leaf stage	Flowering
Treat	7	**1.34	**1.24	**6.97
Error	24	0.16	0.15	0.39
CV (%)		4.65	5.02	11.11

**: Significant at 1 percent.

With respect to the seedling damping-off at different levels of growth (Fig. 1), in the 2-3 leaf stage with 95.87 percent of death, Barracuda was recognized as very sensitive; cultivars of Sultan, Aseal, Fenomeno, Storm, Number one and Kashmir showed, 77.77, 74.99, 74.99, 73.05, 72.21, 69.43 death percentages, respectively, and were placed in susceptible group, hence PS 547 with 60.83 death percentage was classified as moderately susceptible.

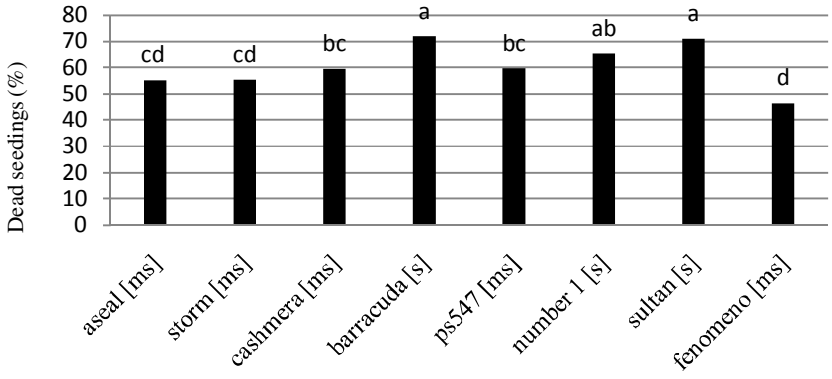


*Means with common letters in each column are not significantly different

**The responses have been determined based on: 0-20% death (r; resistant), 21-40% death(mr; moderately resistant), 41-60% death (ms; semi-sensitive), 61-80% death (s; sensitive), 81-100% death (vs; very sensitive)

Fig. 1. Percentage of dead cucumber seedlings infected with Ph. drechsleri at 2-3 leaf stage.

Ghaderi (2011), also performed an investigation to identify resistant varieties of cucumber to *Pythium aphanidermatum* and *P. melonis* and concluded that different varieties showed different reactions to these pathogens. Comparison of root and crown colonization and seedling mortality in response to Ph. melonis showed revealed that Close and Mehr cultivars showed the lowest sensitivity to the pathogen and Fadya and Bahman were the most susceptible cultivars. Besides, different varieties showed different responses in reaction to *P. aphanidermatum.* In 5-6 leaf stage, cucumber cultivars showed significant differences in terms of seedling mortality (Table 1). Results of the mean percentage of seedling mortality (Fig. 2) revealed that Barracuda, Sultan and Number one cultivars showed 71.66, 70.83 and 65.41 percent mortality and classified as susceptible; other cultivars including PS 547, Kashmir, Storm, Aseal and Fenomeno showed 59. 59, 59.41, 55.27, 55.08 and 49.25 percent mortality and were grouped as moderately susceptible. In this stage, cucumber cultivars were classified into two groups of sensitive and semi-sensitive.



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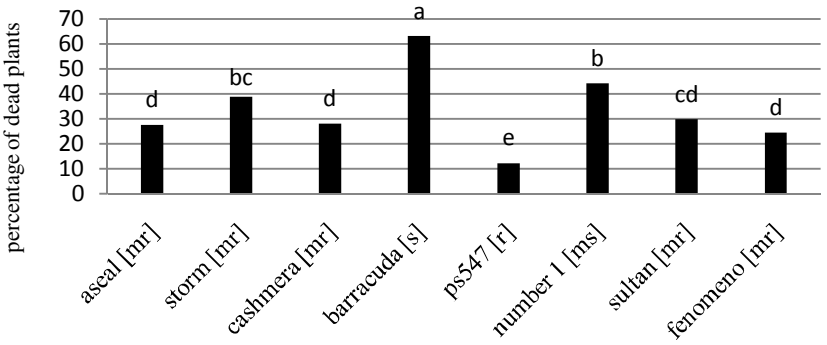
**The responses have been determined based on: 0-20% death (r; resistant), 21-40% death(mr; moderately resistant), 41-60% death (ms; semi-sensitive), 61-80% death (s; sensitive), 81-100% death (vs; very sensitive)

Fig. 2. The mean percentage of cucumber dead seedlings in reaction to Phy. drechsleri at 5-6 leaf stage.

Eikemo *et al* (2003), assessed 26 strawberry cultivars for their resistance to crown rot caused by *P. cactorum*. The results showed that different varieties had different resistance levels against pathogen, and cultivars such as Bogota, Glima, Melody and Sengana were introduced as resistant, whereas Evita, Inga and Tamella cultivars were among the most sensitive ones.

Krebs *et al* (2002), evaluated 57 varieties of peony to assess the resistance to root rot caused by *P. cinnamomi*. Their results indicated that the majority of the cultivars (about 77%) were sensitive to disease, six cultivars showed average resistance and seven cultivars were highly resistant to the pathogen. In their grouping method, classification, severity of root rot disease did not increase significantly with a threefold increase in inoculom concentration.

According to figure 3 and table 1, in this stage , same as former stages, significant differences was observed between different cultivars. In flowering stage, greenhouse cultivar Barakouda with 63.28 percent seedling death was placed in sensitive group and Number one cultivar with 44.22 percent of seedling death was classified as semi-sensitive; Storm, Sultan, Kashmir, Aseal and Phenonemo with 38.88, 29.72, 28.04, 27.77, 24.44 percent death, respectively, were categorized in semi-resistant group and PS 547 by 12.03 percent mortality was placed in resistant group.



Cucumber cultivars

*Means with common letters in each column are not significantly different

**The responses have been determined based on: 0-20% death (r; resistant), 21-40% death(mr; moderately resistant), 41-60% death (ms; semi-sensitive), 61-80% death (s; sensitive), 81-100% death (vs; very sensitive)

Fig. 3. The percentage of dead plants at the Flowering of *Phytophthora drechsleri*.

Mansoori and Banihashemi (1982) performed an experiment during which 116 variety of melon, cucumber, squash and watermelon were infected with *P. drechsleri* and concluded that different varieties showed different sensitivity to the pathogen. Melon cultivars were susceptible to the pathogen and squash cultivars were the most resistant ones. Cucumber variety Ohio MR17, local Iranian watermelon cultivar named Klondike NO. 7 and melon cultivar Gold & Silver showed the highest resistant towards pathogen.

Nasr-e-Esfahani *et al* (1391), studied the resistance of 14 cucumber and squash cultivars against *P. drechsleri* and concluded that the cultivars were classified in five groups of resistant, semi-resistant, semi-sensitive, sensitive and very sensitive and lower percentage of death happened in adult plants comparing to seedlings.

In this research, according to figure 4, it was concluded that Barakoda and PS 547 were the most sensitive and most resistant to the pathogen in all growing stages, respectively.

Sensitivity of plants in the seedling stage is due to the fact that their tissues have not still compeletely developed and they do not have the necessary strength. In the flowering stage, given that the plant tissues are now stronger and their resistance to the disease has been increased, fewer plants become infected and as a result lower death will be seen. According to various studies conducted in this field, it has been proven that plants in the seedling stage are the most susceptible to damping-off caused by *Phytophthora* (Howard *et al*., 1976; Kannaiyan *et al*., 1981).

Plants sensitiveness through plantlet is because their tissues are not complete and solid yet, means that they don't have enough consistency. In blooming level relying on the consistence tissues and their resistance which increased against pathogen factors, less plants were infected and at last less damping-off wilt would presented.

Kim *et al* (2001), Atrectylis figures in Korea in the field of resistance against *P. drechsleri* bioassay methods used and of the 638 varieties collected 18 varieties were identified as resistant varieties that the figures have been used as a rootstock resistant.

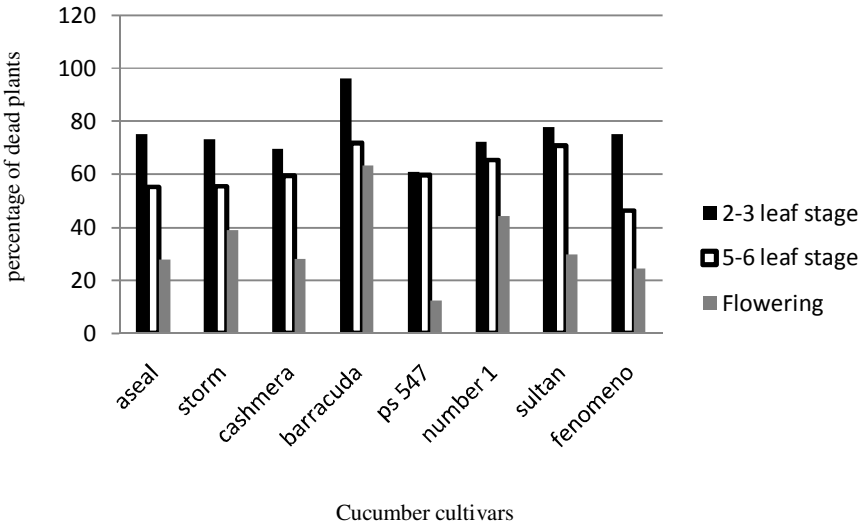
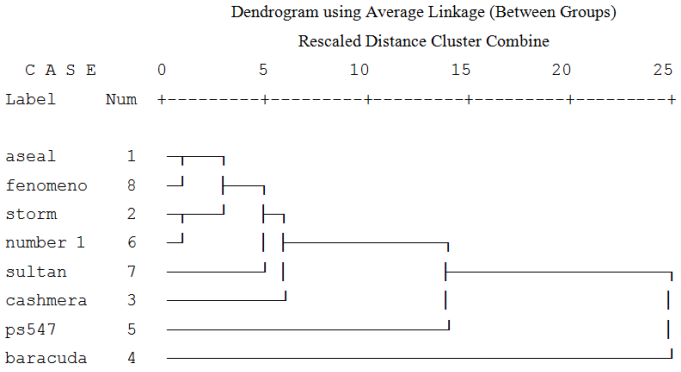


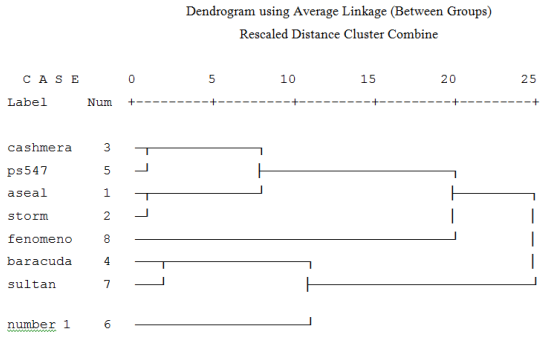
Fig. 4. Comparison of percentage of dead plants infected with Ph. drechsleri in three growth stages.

Rostami *et al* (2015), studied relative sensitivity of commercial cucumber hybrid varieties including Alpha, Caspian 340, Storm 5970, Delta scar, Janette 810, Royal, Negyn, Sultan and Fadya after hybridization on a pumpkin stem. The results showed Caspian 340 and Alfa with 15.7 and 100 percent seedling death had the highest and lowest tolerance to *P. drechsleri.* The Among cucurbits, squash is known as the most resistant to damping-off disease.

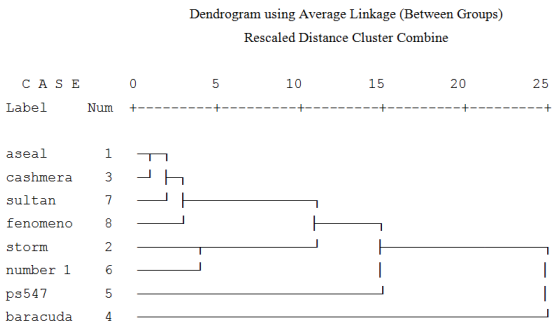
Different sorts in reaction with *P. drechsteri* and their analysis outcomes also depend on their similarity between groups and their consequences are classified.



Dendrogram 1: Cluster analysis 2-3 leaf stage



Dendrogram 2: Cluster analysis 5-6 leaf stage



Dendrogram 3: Cluster analysis flowering stage

Dunn *et al* (2014), performed a study on resistance to *Phytophthora* damping off and found that in pepper plant varieties Archimedes, Aristotle, Intruder and steel were resistant to disease, cultivars ACR285, PS09941819, and Vangrad showed moderate levels of resistance and Askalada, Karysma, Keyston Gyant, King of the North and the Red Knight were introduced as very sensitive.

According to medium intensity in the rate of homogeny and biotypes distance the product clusters are divided in specific groups. In this way of classification relying on the first number showed biotypes clustering through their reactions against pathogen' factors in the second or third level of foliar illustrate three various groups included very sensitive (Barracuda), sensitive (Sultan, Aseal, Fenonemo, Storm, Numberone, Cashmera) and semi-sensitive (PS547) which results seconder in the first figure. Also in the second Dendergaram related to care doing in the fifth to sixth level of foliar all sorts classified into the two groups of sensitive (Sultan, Storm, Numberone) and semi-sensitive (PS547, Aseal and Fenonemo)

In a study that was performed on cucumbers and squash cultivars it was revealed that squash cultivars including pumpkin stewed solid and short, and cucumber cultivars like Bosco and Dastgerdi with 100, 100, 99.30 and 92.34 percent seedling death, respectively, were recognized as very sensitive and squash hybrids of RS841, RS152, RS107 and pumpkin with 23.60, 20.12, 15.03 and 10.18 death, respectively, were classified in the resistant group (Nasr-e- Esfahani *et al*., 2012)

Additionally the reaction of sorts in the level of plantlet in Dendergram of number three marked that the sorts are classified in four groups of sensitive (Barracuda), semi-sensitive (Numberone), semi resistant (Storm, Sultan, Cashmera, Aseal, and Fenonemo) and resistance (PS547). In the specific aim of resistance towards wilting and the essence of breeding actions in the way of identifying resistant genes crossing, making resistant sorts, exploring adequate parents are very important. Relying on the results of the research concluded the ability which to be used in breeding studies then could be present Barracuda sorts (sensitive) and PS547(semi-sensitive) those used in breeding programs included genes identification, crossing, doing different roll call, transmittance, aggregation, and increasing useful genes in the population and commercial sort.

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