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Searching about Resistance of Common Cultivated Varieties in Varamin to Separated Fungal *Phytophthora drechsleri* from the Same Place

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Abstract

Dieback plant disease caused by fungi species Phytophthora is one of the most important soil borne disease in Iran. During studies in Varamin, one of the most harmful factors on crops is *Phtophtora* species in that place. So that in almost all different villages of this city, limitation of cultivation of crops such as cantaloupe, that is one of the most important product in this region, has arisen. This study had been done for searching about reaction of common cultivars in some plants in Varamin to the separated *Phtophthora drechsleri* from the same place by measuring plant growth factors. Seeds of melon in Ivanaki cultivar, cantaloupe in Samsoori cultivar, tomato in Urbana cultivar, red bean in Mahalli cultivar, were planted in pots containing sterile soil, then mentioned fungal were infected by Zoospore suspension and kept in greenhouse condition. In addition, the reaction of safflower seedlings of species *Phytpophthora melonis* was used to differentiate species. Percentage of disease as well as growth factors such as stem fresh and dry weight, stem length and root length during the time of 1, 2 and 3 weeks after inoculation had been measured. Symptoms in different hosts were seen such as reducing growth, root, and crown rot, yellowing and wilting of aerial organ and ultimately these symptoms led to death in susceptible hosts and destroyed them. Due to the discussed factors, cantaloupe and, melons were very sensitive hosts, tomatoes were sensitive hosts and beans were relatively resistant hosts, the results indicated sensitivity of used cultivars to the phytophthora in this area. Also in checking the germination percentage of seeds, separated *Phtophthora drechsleri* could affect the germination of melon and cantaloupe seeds, their germination percentage is drastically reducing. In a parallel study, the same research had been done to measure the effect of biofertilizers Trichodermin B and Subtilin for *Phtophthora drechsleri* control in mentioned cultivars, the results indicated positive effects of these two biofertilizers in pathogenic phytophthora control.

Keywords: *Phtophthora drechsleri*, varamin, trichodermin B, subtilin

1. Introduction

Phytophthora has more than 60 species, they were known as the main factors for different diseases in lots of crops and vegetables (Shekari et al., 2006). *Phtophthora drechsleri*, is known as the most important factor for root and crown rot in different places in Iran, sometimes its damage had been reported about 80 percent in some farms in Fars province of Iran (Ghaderi et al., 2011).

Crown and root rot diseases of cucurbits caused by the Oomycete pathogen, *Phytophthora drechsleri* Tucker, have been reported from many cucurbits in Iran and other countries (Ershad, 1992).

in original description of *Phtophthora drechsleri* from Iran, separated the putative *Phtophthora drechsleri* isolates into two cucurbit and non-cucurbit groups (Mostowfizadeh-Ghalamfarsa et al., 2015). *Phytophthora drechsleri* rotting can damage other economically important plants like tomato and alfalfa as well as safflower (Mostowfizadeh-Ghalamfarsa et al., 2015). For the first time *Phtophthora drechisleri* was reported from sugar beet root (Tompkinst et al., 1936). Also this species was reported from cucumber and melon in Varamin, Karaj,

Hamedan, Ghazvin, Isfehan, Yazd, Mahhad, Saveh in 1969, from beet root in Ghazvin in 1971 and 1998 in Kermamshah.

In other searching about root and crown rot of beet in Khorasan in 1998 *Phtophthora drechsleri* were isolated and reported (Shekari et al., 2006). In other researches *Phtophthora drechsleri* Tucker was reported as one of the most important pathogens in Iran that causes the disease in cucurbits, sugar beet and sunflower. Also this pathogen is the cause of death in planted pine in Australia and root rot in safflower and fruit in United States (Mansoori & Banihashemi, 1982).

Mansoori and Banihashemi (1981) studied resistance of different varieties of squash to the *Phtophthora drechsleri*, then they announced that the most sensitive variety was Cucumis melon and Cucurbita pepo variety was the most resistant variety to the *Phytophthora*. El-Helaly et al. (1968) isolated this species of cucumber plants in Egypt with root rot signs in 1981.

Ho et al. (1984) tested on separated *Phytophthora* species of cucumber and they had been identified based on morphological characteristics called *Phtophthora drechsleri*.

In other research *Phtophthora drechsleri*, *Phtophthora cryptogea*, *Phtophthora nicotiana* and *Phtophthora capsici* as productive root rot of cucurbits have been mentioned in Khuzestan province (Shekari et al., 2006). During the study, Shekari et al. (2006) had isolated *Phtophthora drechsleri*, *Phtophthora cryptogea*, *Phtophthora nicotiana* and *Phtophthora capsici* species from a number of crops and vegetables in East Azerbaijan province.

Due to huge losses of this species to the farms and greenhouses, its controlling by chemical pesticides requires spending too much expense (Heidari Faroughi et al., 2005). The alternatives of biological control methods are appropriate, because the continued usage of these pesticides cause environmental pollution and pathogen resistance. Among the types of bio-control methods, the use of *Trichoderma* spp. has been attracted so many researchers due to control a wide variety of plant diseases and enhance the growth of some products (Subash et al., 2014).

Trichoderma spp. is an imperfect fungi in an order of *Hypocreales* that can do all three biological decomposition mechanisms of reproductive organ, survival, reproduction, expelling pathogens from chaff, and preventing of residue contamination source, as a result it can be used as a biological fungicide, plant growth promoter and activity of beneficial micro organisms (Behboudi et al., 2005). As an example, one of the bio-control agents is *T. harzianum* that is used to control lots of *Phytophthora* species diseases in commercial plants (Subash et al., 2014).

Determining the host range of pathogens often is essential to control and reduce the damages that caused by them. Damping off in cucurbit plant that caused by *Phtophthora drechsleri* is an important disease, it has extensive host range, and it causes massive damages on its host that many searching have not been done on the host range of that in Iran.

The aims of this study are determining the potential host of this fungus, comparing with sensitive and resistant host and studying about the effect of biological fertilizers Trichodermin B and Subtilin to control it.

2. Materials and Methods

2.1 Separation and Identification of Phytophtora Species

Studied Species were separated from Aliabad and Ghareh tape in Varamin by method of baiting (Grimm & Alexander, 1973) and it was identified in morphological method by valid keys.

2.2 Preparing of Phytophthora Zoospore

Some sterilized cannabis seed were put on the seven-day pure culture of phytophthora, after 24 hours, cannabis seeds were put in other sterile Petri dishes containing 20 ml of distilled water. Dishes were kept in 15 degree of centigrade under fluorescent light in 30 centimeter distance for 24 hours. Petri dishes were put in 20-22 degree of centigrade for hours to exit zoospore in a same time.

2.3 Reaction of Safflower Seedling

Reaction of safflower seedling method had been used to distinguish studied species of *Phytpophthora melonis* (Banihashemi & Mitchell, 1975).

2.4 Planting, Inoculation, and Reaction of Common Cultivars in Varamin

In this study, Urbana tomato cultivar, Samsoori cantaloupe cultivar, Ivanaki melon cultivar, Mahalli red been cultivar were used to determine host range of separated *Phtophthora drecsleri* fungi. Seeds of mentioned plants

were prepared and they were disinfected by one percent sodium hypochlorite. Then the seeds had been planted in mixture of sterilized soil of farm, sand and peat soil in ratio of 1:1:2 in pots.

After the tow leaves stages, three seedlings had been selected in each pot and others had been omitted. After that 50 ml of a zoospore suspension of separated *Phtophthora drechsleri* to concentration of 1×10^5 per ml, was added to the soil around the crown. Distilled water had been added to control pots and then adapted plants had been irrigated with water requirement. To determine the percentage of germination of the plants, potting soil contaminated with prepared suspension then 4 seeds were planted in each pot. All the pots were kept in a greenhouse at temperature of 28±1 in days and 22±1 at nights.

In the period of 1, 2 and 3 weeks after inoculation, the plants had been sampled and the samples to measure specific factors had been transported to the laboratory. In order to determine the authenticity of disease percentage, fragments of their root and crown had been cultured in specific mediums as well as the percentage of each plant infection was determined by the level meter device. Fresh and dry weight of aerial organ and root and their length were measured. After reviewing the degree of contamination in the samples, the degree had been awarded to them that was 0 to 4 based on the severity of the disease. Number 0 means no disease; one means1 to 25 percent of disease in host organ, 2 shows 25 to 50 percent of disease in host organ, 3 indicates 50 to 75 percent of disease in host organ and 4 shows 75 to 100 percent of disease in host organ.

For each treatment, 4 times of repetition were considered for both tests of determining host range and germination percentage. This test had been done completely random.

2.5 To Study Biological Control of Fertilizers, Subtilin and Trichodermin B

The effect of biological fungicide Trichodermin B and Subtilin had been evaluated with fungicidal properties against separated *Phytophthora drechsleri* in green house condition. The test was completely random in 4 treatments and 4 repetitions. Treatments had been done as said in below:

One: Phytophthora zoospore without any biological fertilizers (control);

Two: Phytophthora zoospore in a pot contains Subtilin;

Three: Phytophthora zoospore in a pot contains Trichodermin B;

Four: Phytophthora zoospore in a pot contains both fertilizers.

Seeds should be a little wet for mixing them with biological fertilizers, after adding Trichodermin B (powder in particles of 120 microns) and the required amount of Subtilin (nearly 10 to 15 kg for each kilogram of seed) mixed them so that fertilizer covered seeds then seeds were planted. Also by preparing suspension 2% and irrigation plants by it during the period of growth the effect of fertilizer in pathogenic control was studied after a week of inoculation by 50 ml of zoospore suspension of separated *Phytophthora drechsleri* with the concentration of 1×10^5 per ml. Infection of each treatment had been studied by using scoring system of 0-4. Evaluation of treatment effects with determining infection percentage and disease severity and performance calculation had been done.

Statistical analysis of data was done by Mini-Tab and JMP-9 software and comparison of data were performed with LSD test. Excel 2013 software was used for drawing diagrams of the results.

3. Results

3.1 Reaction of Safflower Seedling

The results of infection of safflower seedlings indicated that separated relevance was *Phtophthora drechsleri* because it had ability to infect seedlings and all seedlings showed dieback signs. Mirtalebi and Banihashemi (2006) announced the resistance of all safflower cultivars to the *Phytpophthora melonis* so that mentioned separated, *Phtophthora drechsleri* was confirmed.

3.2 Germination

In terms of effect on germination of seeds, *Phtophthora drechsleri* could reduce the amount of 31.25% in generation percentage compared to the control in Samsoori cultivar cantaloupe, the lowest amount of germination percentage reducing had been seen in Mahalli cultivar red bean, this ratio was 6.25%. Germination percentage reducing, in Ivanaki cultivar melon was 25% compared to the control that showed nearly extreme reducing of seeds germination in cucurbits compared to the other plants (Figure 1).

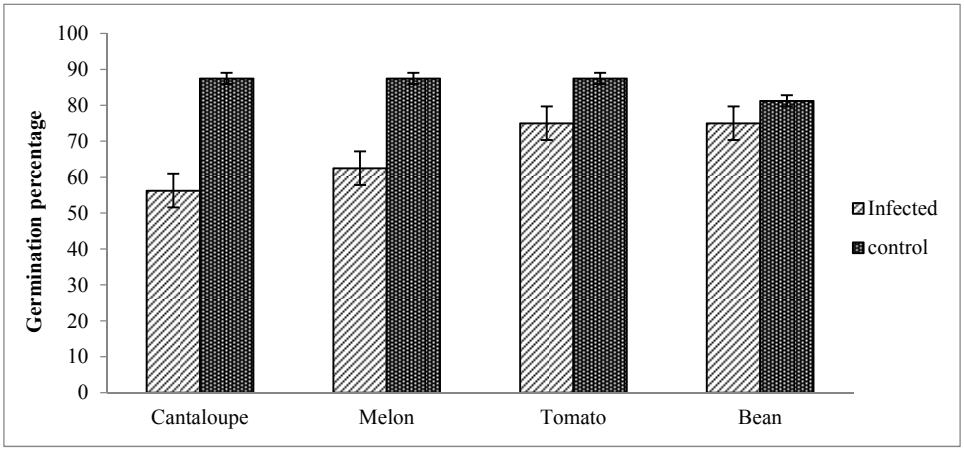

Figure 1. Comparing seeds germination percentage of different plants in control and infected soil to the *Phtophthora drechsleri*

3.3 Host Rang

In host range studied, plants showed many differences to each other and data had the significant difference in 1% level (Table 1). So that disease progressed so fast in Samsoori cultivar cantaloupe, disease percentage was 79.80% after passing 3 weeks of inoculation but results showed the low speed of disease progress in Urbana cultivar tomato and the lowest speed of disease progress was seen in Mahalli cultivar bean after 3 weeks.

For disease percentage, level meter device reported number 8.05% of disease compared with the control for been (Figure 2), in cantaloupe and melon plant growth was so poor and slow even in some samples no growth in root and stem. In some samples, roots were atrophied because of increasing disease so that they could not continue to their growth. Weight and percent of dry root and stem had reduced (Table 2) in plants with high disease percentage.

Table 1. Analysis of variance of measured mean square factors during the time

Sources changes	DF	Mean squares						
		Length stem	Length root	Stem fresh weight	Root fresh weight	Stem dry weight	Root dry weight	%Diseases
Plant	3	4171.667**	4328.031**	7312.071**	8596.564**	7147.294**	7366.099**	4455.115**
Error (main factor)	8	1.209	2.088	1.459	1.535	1.025	1.102	0.546
Time	2	249.357**	175.976**	305.775**	440.434**	232.098**	333.152**	2603.222**
Plant × Time	6	20.147**	6.477**	32.433**	49.148**	19.771**	36.812**	632.952**
Error (Sub)	16	0.487	0.498	0.57	0.46	0.68	0.46	0.63

Table 2. Comparison of the mean measured factors during the time

Plant	Time	Length stem (%)	Length root (%)	Stem fresh weight (%)	Root fresh weight (%)	Stem dry weight (%)	Root dry weight (%)	%Diseases
Bean	First week	9.69^{l}	9.84^{k}	11.42^{k}	8.20^{k}	7.97^{j}	11.96^{k}	6.27^{i}
	Second week	11.49^{k}	11.13^{j}	12.49j^{k}	8.89^{k}	9.95^{i}	13.37^{j}	9.05^{h}
	Third week	13.17^{j}	13.82^{i}	13.77^{j}	11.30^{j}	11.20^{i}	14.70^{i}	8.05^{h}
Melon	First week	33.19^{f}	46.16^{e}	47.51^{f}	53.42^{f}	50.92^{e}	51.98^{e}	20.70^{e}
	Second week	35.91^{e}	50.01^{d}	54.51^{e}	66.51^{d}	55.74^{d}	58.00^{d}	44.17^{d}
	Third week	47.06^{d}	54.60^{c}	64.87^{d}	70.16^{c}	63.68^{c}	64.20^{c}	70.08^{b}
Cantaloupe	First week	55.74^{c}	53.40^{c}	67.40^{c}	62.48^{e}	64.01^{c}	63.77^{c}	20.78^{e}
	Second week	59.21^{b}	58.98^{b}	73.31^{b}	77.38^{b}	72.65^{b}	78.56^{b}	53.17^{c}
	Third week	67.12^{a}	64.40^{a}	80.27^{a}	81.19^{a}	76.88^{a}	82.02^{a}	79.80^{a}
Tomato	First week	18.26^{i}	21.65^{h}	18.27^{i}	18.86^{i}	22.10^{h}	20.31^{h}	7.90^{h}
	Second week	21.73^{h}	24.79^{g}	22.79^{h}	23.86^{h}	25.69^{g}	24.73^{g}	13.08^{g}
	Third week	25.24^{g}	28.82^{f}	26.01^{g}	27.35^{g}	28.38^{f}	28.70^{f}	15.41^{f}

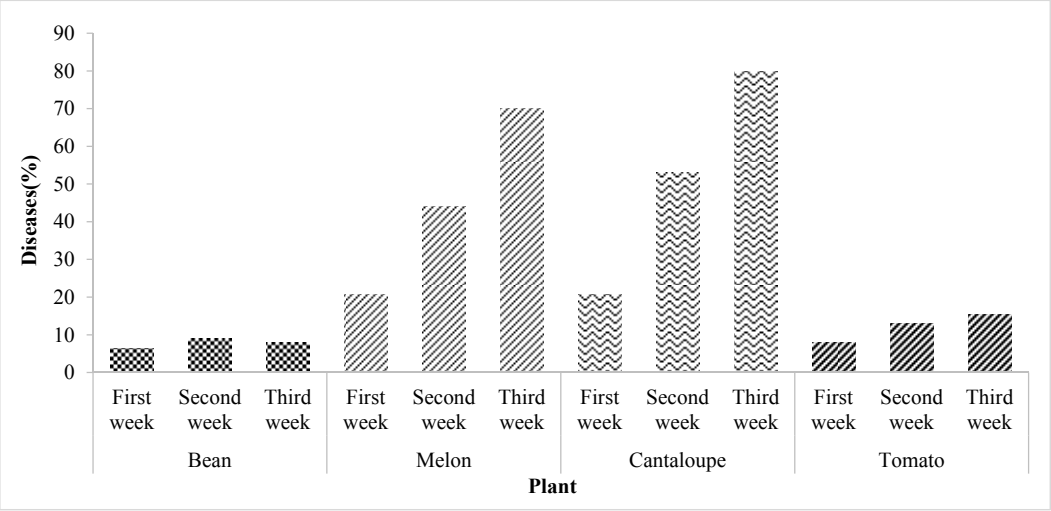

Figure 2. The percentage of disease changes in different plants during 3 weeks

3.4 The Effect of Fertilizers on Control of Separation

The study factors in the biological control test had shown significant difference at 1% level in all of the plants (Table 3). Collection and final evaluation of the results had done with determining of infection percent and evaluation of performance.

The results showed (Table 4) there was a meaningful difference in treatments in terms of infection amount and performance compared with the control and fertilizers was the reason for reducing infection and decreasing product performance compared with the control. The results indicated positive effect of biological fertilizers on disease control of *Phytophthora drechsleri* and the most effective one was treatment of using both fertilizers together.

Table 3. Analyzing variance of mean squares results of biological control by fertilizers

Sources changes	DF	Mean squares			
		Length stem	Stem fresh weight	Length root	Root fresh weight
Fertilizer treatment (safflower)	3	46.19000**	0.0284750*	8.110000**	0.2510570**
Error	8	0.0625	0.005025	0.08000	0.007408
Fertilizer treatment (melon)	3	136.1475**	1.471453**	20.22000**	0.7118750**
Error	8	0.092	0.00404	0.0600	0.002250
Fertilizer treatment (tomato)	3	7.347500**	0.6962000**	8.827500**	0.0789667**
Error	8	0.09750	0.005783	0.06500	0.008208
Fertilizer treatment (bean)	3	34.74750**	8.106600**	109.3875**	2.098608**
Error	8	0.1050	0.00543	0.088	0.01198
Fertilizer treatment (cantaloupe)	3	54.32750**	3.947800**	9.587500**	1.878608**
Error	8	0.0925	0.00930	0.09000	0.00702

Table 4. Comparing the mean results of biological control of the pathogen

plant	Fertilizer treatment	Length stem (cm)	Stem fresh weight (gr)	Length root (cm)	Root fresh weight (gr)	Stem disease degree*	Root and crown disease degree*
Safflower	control	18.2^{d}	2.22^{b}	6.4^{c}	0.92^{c}	3	3
	subtilin	19.6^{c}	2.34^{ab}	7.2^{b}	1.14^{b}	2	2
	Trichodermin	20.2^{b}	2.40^{a}	7.6^{b}	1.23^{b}	1	1
	Trichodermin+subtilin	27.0^{a}	2.44^{a}	10.2^{a}	1.61^{a}	1	1
Melon	control	12.5^{d}	1.14^{b}	3.7^{d}	0.16^{c}	4	4
	subtilin	13.4^{c}	1.16^{b}	5.2^{c}	$0.24b^{c}$	3	3
	Trichodermin	18.4^{b}	1.22^{b}	6.1^{b}	0.30^{b}	3	3
	Trichodermin+subtilin	27.2^{a}	2.57^{a}	9.8^{a}	1.20^{a}	1	1
Tomato	control	5.9^{c}	0.82^{c}	3.8^{d}	0.73^{b}	3	3
	subtilin	6.3^{c}	1.17^{b}	4.4^{c}	0.75^{b}	2	2
	Trichodermin	7.3^{b}	1.28^{b}	5.4^{b}	0.98^{a}	2	2
	Trichodermin+subtilin	9.4^{a}	1.97^{a}	7.7^{a}	1.05^{a}	1	2
Bean	control	23.5^{d}	2.44^{d}	15.7^{d}	1.40^{d}	2	2
	subtilin	26.9^{c}	3.11^{c}	19.6^{c}	1.91^{c}	2	1
	Trichodermin	28.4^{b}	4.09^{b}	21.4^{b}	2.86^{b}	1	1
	Trichodermin+subtilin	31.7^{a}	6.21^{a}	30.0^{a}	3.21^{a}	0	0
Cantaloupe	control	7.9^{d}	0.87^{d}	5.7^{d}	0.52^{c}	4	4
	subtilin	13.8^{c}	2.53^{c}	7.3^{c}	1.08^{b}	2	2
	Trichodermin	14.4^{b}	2.80^{b}	8.1^{b}	1.16^{b}	2	3
	Trichodermin+subtilin	18.2^{a}	3.60^{a}	10.0^{a}	2.40^{a}	2	2

Note. *Grading the severity of infection: 0 = no disease, 1 = 1 to 20 percent of disease in host organ, 2 = 25 to 50 percent of disease in host organ, 3 = 50 to 75 percent of disease in host organ and 4 = 75 to 100 percent of disease in host organ.

4. Discussion

Based on measured factors and the percentage of virulence in studied plants, it could be said that all cultivated cultivars in Varamin are sensitive to the separated *Phytophthora drechsleri* in the same place, that distribution area of the region is high. Mehrabi Koshki et al. (2007) had done evaluation of the effect of two biological products, Trichodermin B and Subtilin, on the wheat take-all disease control. The searches showed Terchodermin B had the most biocontrol effects on separated pathogen. Since in Mehrabi Koshki et al. (2007) research there was no combination of two products, therefore in this study have been seen the effect of both products on biological control of separated *Phytophthora drechsleri* had the most effects and it could be good option for usage, however using resistant cultivars seem the best way to control. For this reason, it is recommended that

studying about the amount of intended separated effects on different cultivars of each plants are planted in the area could be the next research.

5. Conclusion

Achievements of these study shows that not only searching about the resistance of different cultivated varieties of a same place and selection among them can be a good solution in building combat with pathogen, but also biological fertilizers could be safe and secure alternative to chemical fertilizers and pesticides. In addition, we can help to increase level of soil fertility by continuous and timely usage of these biological products.

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