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Original article

Relationship between Morphologic, Phenotypic and Pathogenic Characteristics in *Macrophomina phaselina* Isolates from Cucumber Plants

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Abstract

During 2018 summer season, surveys were carried out in cucumber growing areas of Hatay province of Turkey. Roots and crowns of cucumber plants showing disease symptoms such as yellowing, wilting, root rot, damping-off and gumming were collected. A total of 25 *Macrophomina phaseolina* isolates were determined by morphologic characteristics on PDA medium. Colony sizes were measured after incubation for 3 days on PDA and colony diameters ranged from 45 to 81mm. A strong positive correlation was present between mycelial growth and disease severity (R=0,801). PDA medium amended with 120 mM potassium chlorate was used for phenotyping. Eight isolates were dense, 12 isolates feathery and 5 isolates were restricted. A high correlation was present between mycelial growth and disease severity (R=0.920). Sclerotia size of M. phaseolina isolates ranged from 19.1 to 29.9. In the pathogenicity test, cucumber seedlings were transplanted to plastic pots containing potting mixture of soil, perlite, peat (1:1:1) amended with 50g of M. *phaseolina* inoculum grown in cornmeal-sand mixture. Disease severity was measured with a 0-4 scale according to the symptoms on roots. Disease severity index was varied from 2 to 4 and virulence was significantly different (P<0.05) among isolates. Dense isolates were most virulent with the 3.75 mean disease scale followed by Feathery and Restricted phenotyped isolates with 3.17 and 2.27 respectively. According to the results of this study, a high correlation (R=0.92) was determined between chlorate phenotype and virulence in M. *phaseolina* isolates from cucumber plants in Turkey.

Keywords: Macrophomina, Phenotype, Pathogenicity, Virulence, Soil-borne.

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INTRODUCTION

The cucumber plant is native to North America. Currently, different varieties of cucumber are cultivated in temperate climates and greenhouse conditions that allow the plant to thrive throughout the year. So, the people can find it fresh always in the market. On a global scale, the annual fresh cucumber production is approximately 150 million tonnes (Fao, 2017). According to cucumber production amounts in 2017, China ranks 1st in the world with 54,315,900 tonnes followed by Turkey with 1,754,613 tonnes, Iran with 1,570,078 tonnes, Russian Federation with 1,068,000 and Ukraine with 1,044,300 (Table 1) tonnes annually (Fao, 2017). In 2018, greenhouse cucumber production was 1,134,182 and field production was 714,091 tonnes in Turkey (Tuik, 2018).

Cucumber plants have many pest and disease problems (Caldwell et al., 2005; Retig at al., 1973; Rowe et al., 1995; Sherf et al., 1986; Young et al, 1940) but the one of them, *Macrophomina phaseolina*, has a potential damage to plants in both seedling and matured plant stages and cause huge yield losses worldwide (Jones et al., 1991). *Macrophomina phaseolina* is a soilborne fungus which can infect plants from over 500 different species and over 75 different families. This fungus was reported first time in India (Mitra, 1931), then reported in many countries such as Iran, Lebanon, Mexico, Syria, Turkey, United States of America, Australia, Ethiopia, Pakistan (Westerlund et al. 1974; Nene and Reddy, 1987). *M. phaseolina* generally infects plants in early stages, and it causes damping-off on seedlings and root and crown rots on plants (Karcılıoğlu et al., 1985). The fungus is polifag and can infect most of agricultural plants such as peanut, cotton, sunflower, chickpea, clover, potato, sweet potato, sugar beet, tomato, cabbage, pepper, cucurbits, soybean, strawberry, citrus and Rosaceae family members (Purkayastha et al., 2006). Damping off and root rot caused by *M. phaseolina* was reported on sunflower, melon, soybean, bean, tobacco, tomato and cucumber in Turkey by previous studies (Karcılıoğlu et al., 1985; Arca and Yıldız, 1990; Yıldız, 1989; Onan et al., 1992; Sağır, 1990; Maden, 1987).

Fungus cause more severe disease on plants especially in dry stress. It can infect plants in wide range of temperature (20-35ºC) (Olaya and Abawi, 1973; Diourte et al., 1995). The causel agent of charcoal rot is known to infect mostly matured plants but it can also infect younger plants. However, the diseases symptoms is not visible in all plant growing stages. In some cases, after infection in the seedling stage, symptoms are not visible untill blossoming stage then plants suddunly die. On younger plants, first symptoms appear on the cotyledon leaves and these plants mostly die soon. On matured plants, besides lesions on stem and roots plants show yellowing and wilting symptoms, and vascular tissues seems brown to black (Mihail and Taylor, 1995). *M. plaseolina* is a soil-borne and seed-borne plant pathogen so that it can spread by both soil and seed from field to field. Fungus can survive for years on the plant debrise in the soil as mycrosclerotia. Soil solarization and fumigation are being used to control of the fungus but these methods are not effective.

M. phaseolina an extremely cosmopolitan fungal plant pathogen which has a broad morphological (Mihail and Taylor, 1995; Mayek-Pérez, 1999), physiological (Manici et al., 1995; Mihail and Taylor, 1995), pathogenic (Manici et al., 1995; Mihail and Taylor, 1995; Miklas et al., 1998; Mayek-Pérez et al., 2001; Su et al., 2001), and genetic (Cloud and Rupe, 1991; Mihail and Taylor, 1995; Jones et al., 1998; Mayek-Pérez et al., 2001; Su et al., 2001) variability. Thus, the fungus can adapt to any extrem environmental conditions and its control is relatively harder than others.

The objectives of this study were to determine of growh rate, sclerotia size, chlorate phenotype and virulence of *M. phaseolina* isolates obtained from cucumber plants.

Materials and Methods

Isolate Collection

During 2018 summer season, surveys were carried out in cucumber growing areas of Hatay province of Turkey. Roots and crowns of cucumber plants showing disease symptoms such as yellowing, wilting, root rot, damping-off and gumming were collected and kept in an icebox until they get transported to the laboratory. Samples were washed in a running tap water for 10 mins and then dried for 1 hour in the laboratory. Vascular tissues of diseased plants were extracted with a sterile lanced and then cut into 3-4mm small pieces. Plant tissues were surface disinfested in 1% NaOCl, washed in sterilized distilled water and dried for 30 mins. in a laminar flow cabinet. Tissues then transferred to Petri dishes containing potato dextrose agar (PDA) medium amended with tetracycline (10 mg L^{-1}). Five days after incubation *M. phaseolina* isolates diagnosed with the macroscopic and microscopic criteria suggested by Sutton (1980). Isolates were subcultured and kept at +4°C until use.

Mycelial Growth

Edges of fresh growing *M. phaseolina* cultures were used in the mycelial growth study. Mycelial discs (5mm-diameter) from each isolates were transferred to 9cm-diameter Petri dishes containing 15ml of Potato Dextrose Agar medium. Then Petri dishes were incubated at 25ºC for 3 days. After incubation period, colony size was measured from 2 diffrent perspectives and mean diameter of colony size was calculated for each isolate. The experiment was conducted in randomized complete design and the test was repeated twice.

Sclerotial Size

Seven days old fresh and pure cultures of *M. phaseolina* on PDA were used for measuring the size of sclerotia. Culture slides were prepared with 25% glycerol solution and sclerotia were measured under a digital camera monted light mycroscop. Totally 20 randomly selected sclerotia size measured for each isolate and the mean sclerotia sizes were then comparised by Duncan's multiple range test using SPSS statical software (IBM SPSS Statistics V21.0).

Chlorate Phenotype

Minimal medium suggested by Pearson et al. (1986) was used for determining of chrorate phenotype of *M. phaseolina* isolates. Seven days-old fresh cultures of *M. phaseolina* were used in the phenotype tests. Mycelial disks (5mm) from fast growing edges of the colonies were transferred to 90mm Petri dishes containing 15ml minimal medium amended with 120mM potassium chlorate. Cultures grown on minimal medium without potassium chlorate were kept as control. Petri dishes containing mycelial disks were incubated at 25ºC for 7 days in the dark conditions. Five Petri dishes were used for each isolates and the phenotyping test was repeated twice. A completely randomized design was used in the test. The chlorate phenotype of each isolate was determined according to the colony morphology and the density of the microsclerotia characteristics described by Das et al. (2008).

Pathogenicity Test

Cornmeal sand culture described by Baird et al. (1996) was used in the pathogenicity tests. All the *M. phaseolina* isolates collected from cucumber plants were grown in 9mm Petri dishes containing PDA medium in dark conditions at 25ºC for 5 days. Six milimeter sized agar disks containing *M. phaseolina* mycelia were transferred to 500ml bottles containing 100g maize flour and 300g sand mixture. The bottles were kept in the dark growing cabinet at 25ºC for 6 days. Inoculum was shaked two times in a day for homogening the *M. phaseolina* culture. Cucumber seeds were sown in 48-cell seedling trays and kept in a greenhouse untill emenging. Seedlings in hypocotyl stage were then transferred to plastic pots containing sand-soil-peat (1:1:1, v:v:v) mixture amended with 5% *M. phaseolina* inoculum grown in cornmeal sand mixture. Seedlings were transplanted to pots including inoculum and then kept in an plant growing room at 25ºC and 70% humidity with 10/14 hours dark/light period. Each isolates had 3 replicates and 10 plants were used for each treatment. Experiment was conducted in a randomized complete design and pathogenicity test was repeated twice. Twenty days after inoculation, disease severity index was measured with a 0-4 scale described by Pekgöz and Tok (2018). The scale was; 0= no disease symtoms, 1= chlorosis on leaves, 2= slightly wilting and/or slightly crown necrosis, 3= crown rot and/or sever wilting, 4= dead plants. Mean disease index values were then comparised by Duncan's multiple range test using SPSS statical software (IBM SPSS Statistics V21.0).

Results and Discussion

Isolate Collection

During 2018 summer season, surveys were carried out in cucumber growing areas of Hatay province of Turkey. Totally 50 cucumber growing fields were examined. Wilting, yellowing and gumming symptoms were present in 30 fields and 157 samples collected from diseased plants. Isolation from roots and crowns of cucumber plants showing disease symptoms such as yellowing, wilting, root rot, damping-off and gumming was made on PDA medium amended with antibiotics. After isolation

colonies were determined with macroscopic and microscopic characteristics, and 80 F. oxysporum, 20 V. dahiae, 18 R. solani, 16 S. sclerotiorum and 25 M. phaseolina isolates were diagnosed with the macroscopic and microscopic criteria suggested by Sutton (1980) (Table 1).

Mycelial Growth

Colony diameters of *M. phaseolina* isolates were not homogenic and there was a wide range of variation. According to the measurement after incubation period for three days, colony diameters ranged from 45 to 81mm and replications for all the isolates were similar for the same isolate. The fastest growing isolate was CucMp01 with 81mm colony diameter and followed by CucMp02 and CucMp12 with 79, CucMp14 with 78, CucMp13, CucMp15 and CucMp23 with 75mm. The lowest growing isolate was CucMp08 and CucMp16 with 45mm colony diameter and followed by CucMp25 with 50, CucMp04 with 55, CucMp24 with 60 and CucMp07 with 62mm. According to Duncan's multiple range test, isolates separated into 12 different statistical group and the differences between colony diameter of isolates were significant ($P<0.01$) statistically (Table 1). Correlation analysis shows that mycelial growth is correaleted with sclerotia size (R=-0,499) negatively, that means, fast growing isolates produces smaller sized sclerotia. On the other hand, a strong positive correlation is present between mycelial growth and phenotype (R=0,817) and disease severity index (R=0,801) (Table 2).

Sclerotia Size

Sclerotia size of *M. phaseolina* isolates ranged from 19.1 to 29.9. The isolate having the biggest sclerotia size was CucMp08 with 29.9µm and followed by CucMp25 with 26.6, CucMp19 with 25.8, CucMp20 with 25.7, CucMp12 with 25,6 and CucMp09 with 25.2. On the other hand, CucMp10 had the smallest sclerotia size with 19.1 and followed by CucMp15 with 20.1, CucMp14 and CucMp02 with 20.1, CucMp03 with 20.3 and CucMp07 with 20.5mm. According to Duncan's multiple range test, isolates separated into 8 different statistical group and the differences between sclerotia size of isolates were significant ($P<0.01$) statistically (Table 1). According to our results in this study, sclerotia size of *M. phaseolina* isolates is related with mycelial growth, phenotype and disease severity. Data analysis shows that there is a negative correalation between sclerotia size and mycelial growth (R=-0.499). Correlation between sclerotia size and disease severity index (R=-0.318) and phenotype (R=-0.311) were not significant statistically (Table 2).

Table 1. *M. phaseolina* isolates and their morphologic, phenotypic and pathogenic characteristics.

Isolate Code	City	Mycelial Growth (mm)	Sclerotia Size (µm)	Phenotype	DSI[1]
CucMp01	Samandağ	81 a[2]	22.3 cdefg	Dense	3.8 a
CucMp02	Samandağ	79 abc	20.1 efgh	Fathery	3.2 abcd
CucMp03	Samandağ	63 fghi	20.3 gh	Fathery	3.5 ab
CucMp04	Samandağ	55 ij	23.6 bcdef	Restricted	2.5 bcde
CucMp05	Samandağ	66 fgh	24.6 abcd	Fathery	2.8 abcde
CucMp06	Samandağ	72 abcdefg	21.0 fgh	Dense	3.5 ab
CucMp07	Antakya	62 ghi	20.5 efgh	Fathery	3.2 abc
CucMp08	Antakya	45 k	27.9 a	Restricted	2.0 e
CucMp09	Antakya	68 bcdefg	25.2 abcd	Fathery	3.0 abcde
CucMp10	Antakya	74 abcde	19.1 h	Dense	3.5 ab
CucMp11	Antakya	70 bcdefg	22.5 bcdef	Fathery	3.0 abcde
CucMp12	Arsuz	79 ab	25.6 ab	Dense	3.5 ab
CucMp13	Arsuz	75 abcd	21.4 defg	Fathery	3.0 abcde
CucMp14	Arsuz	78 ab	20.1 gh	Dense	4.0 a
CucMp15	Arsuz	75 abcdefg	20.0 gh	Fathery	3.5 ab
CucMp16	Kırıkhan	45 kl	25.2 abc	Restricted	2.2 de
CucMp17	Kırıkhan	69 cdefg	24.6 abcd	Dense	3.8 ab
CucMp18	Kırıkhan	70 bcdefg	23.9 bcde	Fathery	3.0 abcde
CucMp19	Kırıkhan	72 abcdefg	25.8 abcd	Dense	3.8 a
CucMp20	Antakya	65 fgh	25.7 abcd	Fathery	3.5 ab
CucMp21	Antakya	66 efgh	23.2 bcdef	Fathery	3.5 ab
CucMp22	Antakya	70 defg	24.8 abcd	Fathery	3.0 abcde
CucMp23	Antakya	75 abcdef	24.8 abcd	Dense	3.5 ab
CucMp24	Antakya	60 hij	23.3 bcdef	Restricted	2.5 de
CucMp25	Antakya	50 jk	26.6 abc	Restricted	2.5 cde

[1]DSI means disease severity index.

[2]The numbers with diffrent letters are significantly different (P≤0.01) according to Duncan's multiple range test.

Chlorate Phenotype

Isolates were phenotyped according to their apperance on minimal medium amended with potassium chlorate. According to the results all isolates were eighter sensitive or resistant to potassium chlorate. Sensitive isolates were named restricted and resistant isolates were named fathery or dense. After growing phenotype selective medium, 8 isolates were dense, 12 were fathery and 5 isolates were determined as restricted (Table 1). Two tailed correation analysis shows that phenotype is highly correlated with mycelial growth (R=0.817) and disease severity (R=0.920) (Table 2).

Table 2. Correlation between morphologic, pathogenic and phenotipic characteristics.

Pearson Correlation Sig. (2-tailed)	Isolate	Mycelial Growth	Sclerotia Size	Phenotype	DSI[1]
Isolate	1	-0.149	0,423*	-0.109	-0.041
Mycelial Growth	-0,149	1	-0,499*	0.817**	0,801**
Sclerotia Size	0.423*	-0.499*	1	-0.311	-0.318
Phenotype	-0.109	0.817**	-0.311	1	0.920**
DSI	-0.041	0.801**	-0.318	0.920**	1

[1]DSI means disease severity index.

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Pathogenicity Test

In the pathogenicity test, all *M. phaseolina* isolates were pathogenic and caused charcoal root rot disease symptoms on cucumber plants. Disease symptoms varied from yellowing to dead plants. The most virulent isolate was CucMp14 with 4.0 and followed by CucMp19, CucMp17, CucMp01 and CucMp01 with 3.8, CucMp20, CucMp12, CucMp23, CucMp21, CucMp06, CucMp03, CucMp15 and CucMp10 with 3.5 disease severity index. CucMp08 was the least virulent isolate with 2.0 and followed by CucMp16 with 2.2, CucMp24, CucMp04 and CucMp25 with 2.5 disease severity index (Table 1). According to the statistical analysis, there is a high correlation between virulence and mycelial growth (R=0.801) and phenotype (R=0.920) (Table 2). Mean disease severity varied among phenotypes. Dense isolates gave more disease severity index with 3.7, fathery isolates 3.2 and restricted isolates gave 2.3 mean disease severity on cucumber plants. According to Duncan's multiple range test defferences among mean disease severities for phenotypes were significant statistically (Figure 1).

Iqbal and Mukhtar (2014), collected 65 *M. pheseolina* isolates from different districts of Pakistan and characterized them morphologically. They reported that *M. phaseolina* isolates had a wide range diversity in colony size and the colony sizes were significant statistically. The avagare radial growth of 65 *M. phaseolina* isolates ranged from 32.00 to 87.17mm in the study. On the other hand, they did not report any correlation between colony size and pathogenicity.

Morphologic characteristics such as growth rate, chlorate sensitivity, sclerotia size, colony color among *M. phaseolina* isolates from diffrent plant were studied by many researchers (Mayek-Pérez et al., 1997; Mayek-Pérez, 1999; Dhingra and Sinclair, 1978; Riaz et al., 2007). On the other hand, a close relationship between virulence and growth rate was reported by Rayner (1991) and a relationship between morphological variations and pathogenicity was reported by Purkayastha et al., (2004). However, Dhingra and Sinclair (1978) and Beas-Fernandez et al. (2006) reported no relationship between sclerotia size and sclerotia weight among *M. phaseolina* isolates. On the other hand, a high

relationship between virulence and growth rate was reported by Rayner (1991) and a relationship between morphologic characteristics and pathogenicity by Purkayastha et al. (2004).

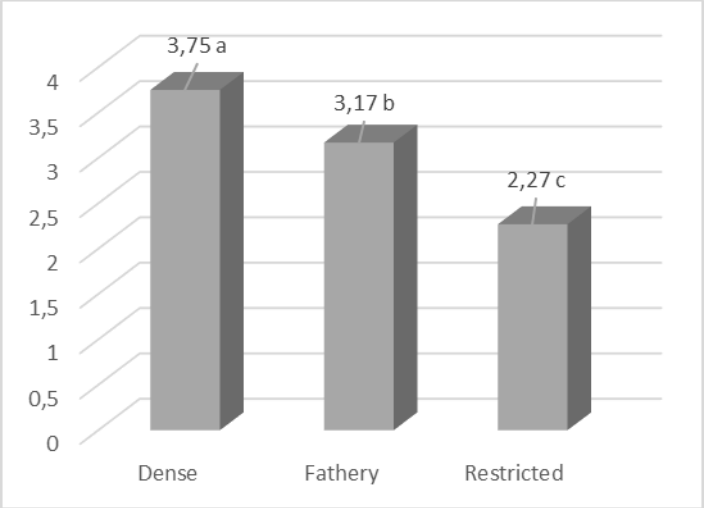

Figure 1. Mean disease severity index of different phenotypes.

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