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Research Article

Comparative study of virulence of *Ophiognomonia leptostyla*

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Abstract: Anthracnose disease caused by *Ophiognomonia leptostyla*, is the most important and widespread fungal disease on *Juglans regia*. Walnut disease symptomatic samples were collected from different provinces of Iran, during 2015–2016. Fungal isolates were identified based on ITS-rDNA sequence data. Variance analysis of colony growth rate (mm/day) and acervulus density on medium, was significant. Acervulus density on medium was strongly correlated with colony growth rate. The Max acervulus density was 60% and > 80% for Hamedan and Mazandaran isolates respectively. The virulence of six selected isolates was examined on cv. Chandler. Virulence indices including spot diameter, disease severity, spot area average and logistic infection rate except spot number index, could successfully detect significant differences among isolates. SA-SE1 isolate from Mazandaran showed significantly the most virulence indices: disease severity (%), spot area and logistic infection rate. For the other five isolates, four significant levels in all virulence indices were observed. In summary after this isolate, other isolates including TA-ZY21, LA-SY21, U94-SR1, HA-GH22 and MA-K1 were placed in the next steps of virulence ranking. There was insignificant correlation between colony growth rate and disease severity. However, the acervulus density and disease severity were significantly correlated implying the importance of acervular conidial inoculum in secondary disease cycle progress. Disease severity was strongly correlated with number of spots, spot diameter and logistic infection rate. Disease severity was also negatively correlated with Mid-time (time to progress 50%). Moreover, there was positive relationship between logistic infection rate and three traits: number of spots, spot diameter and spot area average. This study was the first of the disease virulence components on cv. Chandler in Iran.

Keywords: cv. Chandler, disease severity, virulence, walnut anthracnose

Introduction

Persian walnut *Juglans regia* is an economically important tree that is widely cultivated for its nutritional kernels and valuable lumbers (Luppold and Bowe 2013; Bernard *et al*., 2018). According to FAOSTAT, Iran stood third in walnut production (394192 tones) in 2017 after China (1925403 tones) and US (571526 tones). Walnut trees get infected by several economically important diseases and amongst them walnut anthracnose, caused by the ascomycete fungal pathogen, *Ophiognomonia*

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leptostyla (Fr.) Sogonov, could be a severe threat for black walnut and Persian walnut production in the eastern half of the United States, South America, Europe, and Asia (Neely and Black 1976; Berry 1981; Juhasova *et al*., 2006; Belisario *et al*., 2008). By having tight host-fungus evolution (Walker *et al*., 2013), the pathogen could cause economic damage to the most important *Juglans* species including *J. nigra*, *J. hindsii* and *J. regia* (Belisario *et al*., 2008). Disease symptoms appear mainly on leaves, husks and twigs and rarely on shoots as necrotic lesions, circular or irregular in shape, and small black dots, the acervuli, patterned mostly on concentric circles on leaf lesions. Acervular macroconidia are two-celled spindle- or embowed -shaped (Berry, 1981; Belisario *et al*., 2001; Woeste and Beineke, 2001; Teviotdale *et al*., 2002). In severe epidemics, premature defoliation results in poorly-filled and low-quality kernels or even fruit dropping (Woeste and Beineke, 2001; Belisario *et al*., 2001). Successive and premature defoliation through years, may reduce annual growth and weaken or even destroy the infected tree (Berry, 1977; Belisario *et al*., 2001; Van Sambeek, 2003). *O. leptostyla* overwinters usually as perithecia in the infected leaf debris on the ground or rarely as mycelium in the twigs and fruit lesions (Belisario *et al*., 2001). With rising temperature to 15-21 °C and start of spring rainfalls, ascospores are released and splash to the leaf surface and begin the disease cycle upon germination and penetration. Free water film is vital for ascospore germination so walnut anthracnose occurs at high relative humidity (more than 95 %) at least for the beginning of infection. Although the disease severity is not affected by 10-32 °C temperatures, it is significantly reduced at low (below 10 °C) temperatures (Black and Neely, 1976). Disease secondary infection cycles, caused by asexual spores, are more successful and progressive in rainy cool climate and high relative humidity (Rosnev and Naidenov 1986). In Iran, walnut anthracnose was reported for the first time in 1952 and later reported from the north, west, northwest, and northeast of the country by a number of authors (Eskandari 1964; Behdad, 1991; Saremi *et al.,* 2003; Salahi *et al*., 2009; Najafi *et al*., 2014).

Despite little or no polymorphisms in population genetics of the pathogen (Salahi *et al*., 2009; Jamshidi and Zare, 2012), *O. leptostyla* isolates were marked to be different in terms of pathogenicity and virulence (Belisario *et al*., 2008; Dastjerdi *et al*., 2009; Jamshidi and Salahi 2010). Belisario *et al*. (2008) found a positive correlation between colony growth rate and virulence. Dastjerdi *et al*. (2009) reported significant differences between the leaf spot numbers and spot diameter caused by different isolates. According to their results, 15 isolates of *O. leptostyla* were divided into low and high virulence groups. Jamshidi and Salahi (2010), however recognized five virulence groups in studied isolates according to the disease index and date of acervulus formation. Jamshidi and Zare (2012) found out significant correlation between disease index and some RAPD and ISSR markers. In this study firstly, colony growth rate and acervulus density of different *O. leptostyla* isolates were compared and a short list of isolates from different provinces were selected. Then disease virulence of selected isolates on walnut cv. Chandler was surveyed.

Materials and Methods

Sample collection

More than 157 symptomatic walnut samples including leaf, petiole and fruit were collected from different provinces of Iran (Alborz, Fars, Hamadan, Mazandaran, Khorasan Razavi, Tehran, West Azerbaijan, Qazvin and Zanjan) during June to October in 2015-2016. Sampling was done randomly from Persian walnut trees in walnut orchards proper, margin of other orchards or fields and from trees growing in cities. Symptomatic samples were kept in dry condition and transferred to lab.

Fungal isolation and identification

Ophiognomonia leptostyla was isolated from leaf lesions using two methods: (A) leaf segments (5-10 mm in diameter) with lesions were surface-

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sterilized using 1% sodium hypochlorite solution for 2 minutes, then washed four times with sterile distilled water and cultured on Oat-Meal Agar medium (OMA: 30 gr oat meal, 20 agar, 1000 ml distilled water). The Petri dishes were incubated in 22 ± 2 °C and 18 h light/6 h darkness) for 4 weeks (Cline and Neely 1984). (B) Acervular conidia were directly streaked out on 2% water agar from dried leaf lesions or after their coming out by 3-4 days incubation in humid condition. Then, single germinated spore on water agar medium was transferred to OMA containing plates. Pure fungal cultures were incubated at 22 ± 2 °C and 18 h light /6 h dark photoperiod to stimulate spore production.

Growth rate and acervulus density

Sixty isolates were examined in a completely randomized design with three replications. The growth rate of colonies was measured over two perpendicular axes after 10 days. Acervulus density on the medium was scored 1 to 5 by comparative visual inspection of cultures (Belisario *et al*., 2008). Variance analysis of data was carried out by SAS software and six *O. leptostyla* isolates which had max growth rate and acervulus density on OMA medium were selected one from each province.

Pathogenicity analysis

Inoculum preparation

Selected isolates including HA-GH22, LA-SY21, MAK1, U94-SAR1, SA-SE1 and TA-ZY21 were used for pathogenicity analysis. Single-spore culture of each isolate, was used separately for inoculum preparation. The 25-30 days old cultures of isolates on OMA medium, incubated in suitable condition (22 ± 0.5 °C and 18/6 h photoperiod) were gently scratched by sterile scalpel and flooded with 10 mm distilled water. Spore suspension was filtered through several fold cheesecloths to remove mycelial fragments and its concentration was adjusted to 1×10^7 spores per ml (Cline and Neely, 1984).

Inoculation and virulence related traits

Pathogenicity test was conducted in a randomized complete block design experiment with four replications on two years old walnut cv. Chandler trees. Walnut trees were provided by RANA Company produced by tissue culture. Trees were grown in plastic bags containing a mixture of peat and sand (3: 1, V/V) and maintained by regular irrigation and supplying micronutrients. The plants were then transplanted individually into sterile plastic pots containing sterile sand and field soil mixture (1: 1, V/V). The conidial suspension was sprayed on fully expanded leaves, then inoculated leaves were covered with plastic bags and kept for 72 h at 22 °C. After removal of the plastic bags, trees were maintained in greenhouse at temperature ranging from 22 to 25 °C and 75% relative humidity (Cline and Neely, 1983). The control plants were sprayed with sterile distilled water. The experiment was carried out from April to June in greenhouse. Three days after inoculation, leaves were randomly sampled to confirm infection occurrence. Leaves were cleared by mounting them in ethanol-acetic acid solution (1: 1 V) for 16-20 h then colorless samples were transferred to lactophenol (Cline and Neely, 1983) and stained with cotton blue. Stained leaf segments were observed under microscope to detect fungal pathogen hyphae. Twenty-seven days after inoculation four traits including number of spots on a leaf, average diameter of spot, infected area on leaves (mm^2) and total leaves area (mm^2) were measured and recorded Cline and Neely, 1983; Dastjerdi *et al*., 2009). Logistic infection rate was calculated according to logistic model for disease progress (Madden, 2006).

Results

Growth rate and acervulus density

Sixty isolates (Table 1) were identified based on sequence of ITS-rDNA. The isolates had 98% or more similarity with *O. leptostyla* sequences in NCBI. Variance analysis of colony growth rate (mm/day) and acervulus density on medium, showed that block had no significant effect on colony growth rate and acervulus density, but isolates were significantly different in colony growth rate and acervulus density on medium (Table 2). In most of the studied provinces, there

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was at least one significantly different isolate in terms of colony growth rate and acervulus density. Colony growth rate was almost the same among West Azerbaijan isolates (1.6-1.8 mm/day) and only U71-SED1 had the most colony growth rate (2.2 mm/day) among the other provinces isolates (Fig. 1-B). While observing the same trend in Mazandaran isolates, most of the Mazandaran isolates grew faster (1.8 mm/day or more) compared to West Azerbaijan isolates, and SA-SE1 isolate from Mazandaran province had the highest colony growth rate (Fig. 1-J). None of Tehran-Alborz isolates or Khorasan-e-Razavi isolates grew 2mm/day or more. Two Tehran-Alborz isolates (SH-OSH2 and TAZY21) grew significantly slower (1.4 -1.6 mm/day) than the isolates from the other provinces (Fig. 1-D). Three significantly different levels of colony growth rate were observed in Hamedan isolates. Most of the Hamedan isolates had a colony growth rate 1.9-2.2 mm/day. (Fig. 1-F). Two isolates of Khorasan -e- Razavi including MA-K1 and MA-KH2 showed significant differences in colony growth rate. They had the most and the least colony growth rate among Khorasan-e-Razavi isolates respectively (Fig. 1-H). Acervulus density on culture medium was also significantly different among isolates. There was only one isolate in each of the three provinces, Khorasan-e-Razavi, Hamedan and Mazandaran i.e. MA-K1, HA-GH22 and SA-SE1, that showed the most acervulus density (Fig. 1-G, H and I).

Table 1 List of *Ophiognomonia leptostyla* isolates collected from different provinces of Iran in 2015-2016.

Isolate	Sampling location	Sampling province	Local climate	Sampling date
U11-UK1	Urmia- Anzal-Kahriz	Western Azerbaijan	semi cold -arid	2015
U12-UK2	Urmia- Anzal-Kahriz	Western Azerbaijan	semi cold and arid	2015
U21-UGH1	Urmia- Ghulengy	Western Azerbaijan	semi cold and arid	2015
U22-UGH2	Urmia- Ghulengy	Western Azerbaijan	semi cold and arid	2015
U31-UCHO1	Choghtary-e-pol	Western Azerbaijan	semi cold and arid	2015
U32-UCHO2	Choghtary-e-pol	Western Azerbaijan	semi cold and arid	2015
U71-SED1	Urmia- Sedaghe	Western Azerbaijan	semi cold and arid	2015
U72-SED2	Urmia- Sedaghe	Western Azerbaijan	semi cold and arid	2015
U94-SAR1	Saribeyglou	Western Azerbaijan	semi cold and arid	2015
U94-SAR2	Saribeyglou	Western Azerbaijan	semi cold and arid	2015
U-MO1	Urmia- Moshkabad-e- sofla	Western Azerbaijan	semi cold and arid	2015
U-MO2	Urmia- Moshkabad-e- sofla	Western Azerbaijan	semi cold and arid	2015
BN1	Behshr-Neka	Mazandaran	moderate and humid	2015
BN2	Behshr-Neka	Mazandaran	moderate and humid	2015
SHB1	Babol-Shirgah	Mazandaran	moderate and humid	2015
SHB2	Babol-Shirgah	Mazandaran	moderate and humid	2015
GA-SK1	Ghaemshar-Sarucola	Mazandaran	moderate and humid	2015
GA-SK2	Ghaemshar-Sarucola	Mazandaran	moderate and humid	2015
SA-SE1	Sari serah-e- Eslamabad	Mazandaran	moderate and humid	2015
SA-SE2	Sari serah-e- Eslamabad	Mazandaran	moderate and humid	2015
SA-JU1	Sari-be-Juybar	Mazandaran	moderate and humid	2015
SA-JU2	Sari-be-Juybar	Mazandaran	moderate and humid	2015
GHA1	Ghaemshar	Mazandaran	moderate and humid	2015
GH2	Ghaemshar	Mazandaran	moderate and humid	2015
HA-TUC1	Tuyserkan-center	Hamedan	semi cold and arid	2015, 2016
HA-TUC2	Tuyserkan-center	Hamedan	semi cold and arid	2015, 2016
HA-T44	Tuyserkan	Hamedan	semi cold and arid	2015, 2016
HA-T34	Tuyserkan	Hamedan	semi cold and arid	2015, 2016
HA-GH22	Serkan	Hamedan	semi cold and arid	2015, 2016
HA-SE13	Serkan	Hamedan	semi cold and arid	2015, 2016
HA-G11	Ganjeh	Hamedan	semi cold and arid	2015, 2016
HA-G121	Ganjeh	Hamedan	semi cold and arid	2015, 2016
HA-TUB1	Tuyserkan-piraliBaba	Hamedan	semi cold and arid	2015, 2016
HA-TUB3	Tuyserkan-piraliBaba	Hamedan	semi cold and arid	2015, 2016
HA-TUMO31	Tuyserkan-Mobarakabad	Hamedan	semi cold and arid	2015, 2016
HA-TUMO23	Tuyserkan-Mobarakabad	Hamedan	semi cold and arid	2015, 2016
MA-ZO21	Mashad-Zoshk	Khorasan-e-Rzavi	semi cold and arid	2015

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Table 1 continued.

MA-ZO22	Mashad-Zoshk	Khorasan-e-Rzavi	Cold	2015
MA-SH1	Mashad-Shandiz	Khorasan-e-Rzavi	Cold	2015
MA-SH2	Mashad-Shandiz	Khorasan-e-Rzavi	Cold	2015
MA-TO1	Mashad-Torghabeh	Khorasan-e-Rzavi	Cold	2015
MA-TO2	Mashad-Torghabeh	Khorasan-e-Rzavi	cold	2015
MA-JA1	Mashad-Jaghargh	Khorasan-e-Rzavi	cold	2015
MA-JA2	Mashad-Jaghargh	Khorasan-e-Rzavi	cold	2015
MA-K1	Mashad-Kalat	Khorasan-e-Rzavi	cold	2015
MA-K2	Mashad-Kalat	Khorasan-e-Rzavi	cold	2015
MA-KH1	Mashad-khalilabad	Khorasan-e-Rzavi	cold	2015
MA-KH2	Mashad-khalilabad	Khorasan-e-Rzavi	cold	2015
SH-VAR1	Shemiran-Varjin	Tehran	cold	2016
SH-VAR2	Shemiran-Varjin	Tehran	cold	2016
SH-OSH1	Shemiran-Oshan	Tehran	cold	2016
SH-OSH2	Shemiran-Oshan	Tehran	cold	2016
TA-ZY11	Taleghan-Zydasht	Alborz	cold	2016
TA-ZY21	Taleghan-Zydasht	Alborz	cold	2016
TA-GLY1	Taleghan-Glynak	Alborz	cold	2016
TA-GLY2	Taleghan-Glynak	Alborz	cold	2016
TA-Ce1	Taleghan-center	Alborz	cold	2016
TA-Ce2	Taleghan-center	Alborz	cold	2016
LA-SY1	Lavasan-Synak	Tehran	cold	2016
LASY2	Lavasan-Synak	Tehran	cold	2016

Table 2 Variance analysis of acervulus density and colony growth rate.

Source of variation	Degree of freedom	Acervulus density	Colony growth rate
Block	2	282.2^{ns}	0.012^{ns}
Isolate	59	616.8^{**}	0.166^{**}
Error	118	103.7	0.015

** significant difference at $p < 0.01$.

Isolates of Tehran and Alborz divided into two significantly different groups based on acervulus density. The first one including SH-VAR2 and TA-GLY isolates, whose acervulus density ranged from 50-60%, and in the second it ranged from 20-30%. The acervulus density of all West Azerbaijan isolates except U71-SED1 isolate was insignificant. High acervulus density of U71-SED1 isolate (48%) was significantly different from low density group (20-30%).

Pathological data

Variance analysis of virulence components including no. of spots, spot diameter (mm), disease severity (%), spot area average (mm^2), logistic infection rate and mid time (day) showed significant differences between isolates (Table 3). Two significantly different groups were observed based on the no. of spots on leaves. Mazandaran isolate, SA-SE1, produced significantly more spots i.e. 22, about double that of the other isolates (Fig. 2-A). Different isolates produced two significantly different ranges of spot diameter (Fig. 2-B), one was higher than 10 mm, caused by Mazandaran isolate, SA-SE1, and the other, ranging from 4-8 mm which were produced by the other isolates. TA-ZY21 and MAK1 isolates, caused the Max and Min of spot diameter in this range. Isolates of *O. leptostyla* were significantly different in disease severity. They showed four levels of disease severity on cv. Chandler (Fig. 2-C). SA-SE1 isolate ranked as the most severe isolate by about 10% of disease severity. Disease severity of TA-ZY21 isolate (4%) was significantly more compared to disease severity of LA-SY21, U94-SAR1 and HA-GH22 isolates but they themselves were not significantly different in terms of disease severity. MA-K1 isolate showed the least disease severity (1%) in our experiment. Three significant levels of spot area (mm^2) were identified in cv. Chandler's reaction to the pathogen isolates. The Max and Min spot area (3.8 and 1 and mm^2) were caused by SA-SE1 and MA-K1 isolates respectively. Other isolates including HA-GH22, LA-SY21, U94-SAR1 and TA-ZY21 produced necrotic spots of medium size (2.5-3.mm^2) (Fig.2-D).

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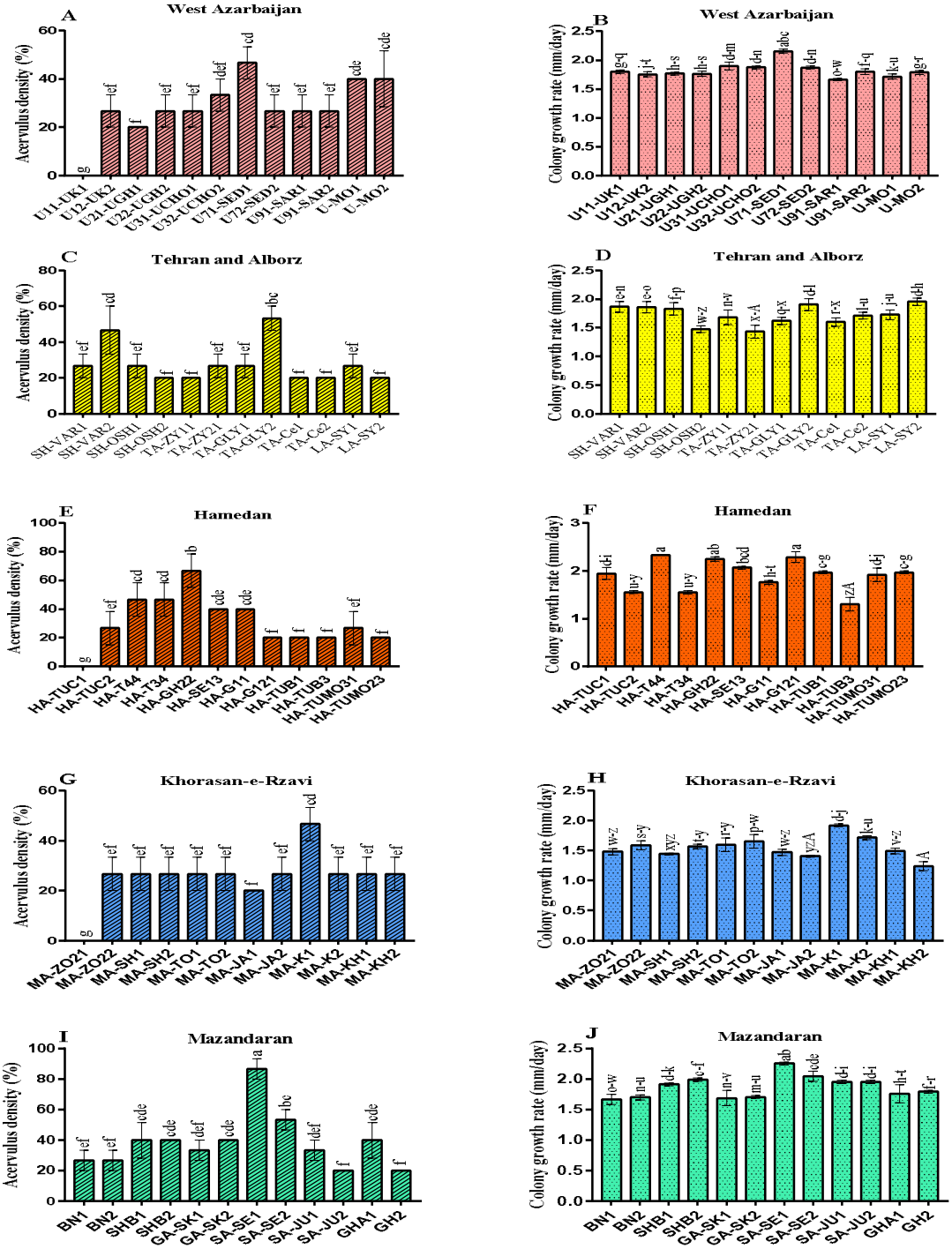

Figure 1 Acervulus density and colony growth rate in West Azerbaijan (A and B), Tehran-Alborz (C and D), Hamedan (E and F), Khorasan-e-Razavi (G and H) and Mazandaran (I and J) respectively.

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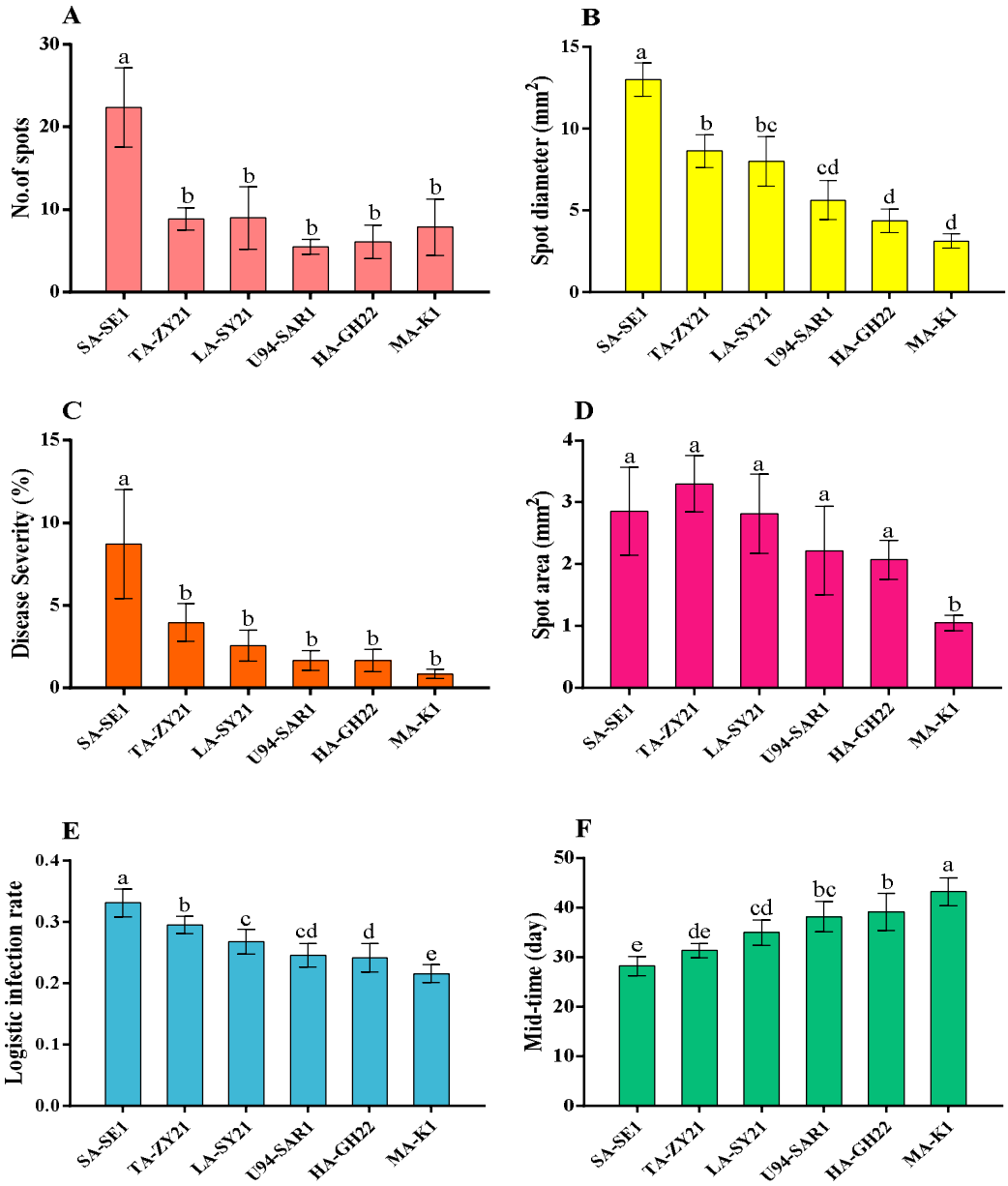

Figure 2 Means comparison (A) No. of spots, (B)Spot diameter, (C) Disease severity (%), (D) Spot area average), (E) Logistic infection rate and (F) Mid time (day) for six selected isolates from West Azerbaijan, Tehran-Alborz, Hamedan, Khorasan-e-Razavi and Mazandaran respectively.

Infection rate per day or logistic infection rate ranged 0.25-0.35 and its graph showed a more or less similar trend as disease severity (Fig. 2-E). SA-SE1 and MA-K1 isolates showed the most and the least logistic infection rates (0.88 and 0.28) respectively. TA-ZY21 and LA-SY21 isolates showed 2.4 -2.2 infection rate per day respectively. The two isolates U94-SAR1 and HA-GH22 were not significantly different.

Theoretically, mid time means days a disease needs to progress 50%. According to the results, SA-SE1 isolate, showed significant difference in

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mid time, compared to all other isolates except TA-ZY21 isolate. While it took 38 days for disease SA-SE1 isolate to progress 50%, it took 10 days longer for disease caused by MA-K1 to reach 50%. In fact, in this experiment, two isolates SA-SE1 and MA-K1 caused anthracnose disease with the fastest and the slowest infection rate, respectively. Mid time for the other isolates including TA-ZY21, LA-SY21, U94-SR1 and HA-GH22 increased sequentially in which it was significantly different in two pairwise comparisons (Fig. 2-F).

Correlation analysis

Based on correlation analysis results (Table 4), Disease severity was strongly ($p < 0.01$) correlated with both no. of spots and spot diameter by 95 correlation coefficient. It had also strong correlation ($p < 0.01$) with logistic infection rate by correlation coefficient equivalent to 99. But disease severity was negatively correlated ($p < 0.05$) with mid-time by correlation coefficient equivalent to -0.90. Mid time was also correlated ($p < 0.01$) with most of the virulence indices including spot diameter, spot area average and logistic infection rate. Moreover, there was positive relationship between logistic infection rate and the three virulence components: no. of spots, spot diameter and spot area average.

Correlation analysis showed no or weak correlation between colony growth rate and virulence but stronger correlation was observed between acervulus density and virulence components like disease severity (Table 5). Acervulus density was strongly ($p < 0.01$) correlated with colony growth rate.

Table 3 Variance analysis of virulence indices; No. of spots, Spot diameter, Disease Severity, Spot area average, Logistic infection rate and Mid-time.

Source of variation	Degree of freedom	Mean of square					
		No. of spots	Spot diameter	Disease Severity	Spot area average	Logistic infection rate	Mid-time
Block	3	108.7^{ns}	10.7^{*}	28.5^{*}	1.72^{ns}	0.0076^{**}	143.1^{**}
Isolate	5	156.1^{**}	50.7^{**}	33.3^{**}	3.14^{*}	0.0069^{**}	120.4^{**}
Error	15	23.1	3.0	5.5	0.97	0.0003	6.7

* and ** indicate significant difference $p < 0.05$ and significant difference $p < 0.01$, respectively.

Table 4 Correlation variation between virulence indices of different *Ophiognomonia leptostyla* isolates on walnut cv. Chandler.

Virulence indices	No. of spots	Spot diameter	Disease Severity	Spot area average	Logistic infection rate	Mid-time
No. of spots	1	0.86^{*}	0.95^{**}	0.58	0.82^{*}	-0.74
Spot diameter		1	0.95^{**}	0.90^{*}	0.98^{**}	-0.97^{**}
Disease Severity			1	0.78	0.94^{**}	-0.90^{*}
Spot area average				1	0.94^{**}	-0.97^{**}
Logistic infection rate					1	-0.99^{**}
Mid-time						1

Table 5 Correlation variation between anthracnose virulence indices and morphological traits.

Traits	No. of spots	Spot diameter	Disease Severity	Spot area average	Logistic infection rate	Mid-time	Colony growth rate	Acervulus density
No. of spots	1	0.86*	0.96**	0.51	0.84*	-0.78	0.46	0.70
Spot diameter		1	0.94**	0.84*	0.98**	-0.98**	0.09	0.36
Disease Severity			1	0.70	0.94**	-0.89*	0.35	0.63
Spot area average				1	0.84*	-0.87*	-0.15	0.08
Logistic infection rate					1	-0.99**	0.10	0.40
Mid-time						1	0.005	-0.29
Colony growth rate							1	0.92**
Acervulus density								1

* and ** indicate significant difference $p < 0.05$ and significant difference $p < 0.01$, respectively.

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Discussion

Among 157 symptomatic samples, 140 were infected by *O. leptostyla* and almost all samples from Zanjan and Qazvin were misdiagnosed in visual inspection and were not infected at all. Our sampling results showed that in spite of previous reports for walnut anthracnose prevalence in Zanjan and Qazvin, the disease was not found even in well-known walnut cultivation areas such as Qadimabad, Farsijin and Ziaabad in Qazvin and Khoramdare in Zanjan and the disease was found only in Alamut, a high latitude area in Qazvin. It could probably be due to the acute precipitation reduction in the recent years. So, we would expect that climate change could strongly change the disease distribution pattern in the country. We couldn't find walnut anthracnose in Fars although it is one of the most important provinces in walnut production. In a survey for walnut anthracnose, Jamshidi and Salahi (2010) also reported Fars as a pathogen free province.

The colony growth rate in isolates from different provinces was significantly different. In general, isolates from Tehran and Alborz also from Khorasan-e-Razavi didn't grow 2 mm/day or more. Among West Azerbaijan isolates, only U71-SED1 grew slightly more than 2 mm/day and growth rates for Hamedan isolates were more or less in fluctuation between 1.4 -2 mm/day. While most of the Mazandaran isolates grew 2 mm/day or more. Acervulus density percentage in Azerbaijan and Tehran isolates were mostly between 20-40% and few isolates slightly exceeded 40% acervulus density. Only one isolate of Khorasan-e-Razavi, MA-K1 could produce more than 40% acervulus density. About half of Hamedan and Mazandaran isolates exceeded 40% in acervulus density while the Max acervulus density percentage for Hamedan isolates was 60%, it was beyond 80% for Mazandaran isolates.

Ten days after inoculation, tiny spots of anthracnose emerged on leaves. Dastjerdi *et al*., (2009) reported that disease symptom appeared 16 days after inoculation. This difference in symptom appearance may be due to the different host susceptibility in the two experiments. Number of spots and spot diameter were significantly lower on upper leaflets. This is mainly due to the higher content of juglone in immature juvenile leaves (Cline and Neely, 1983).

Two significantly different levels of spots no. categorized isolates into two groups: SA-SE1 and the five other isolates. Thus, the number of spots is not indicative enough. Spot diameter, however, could successfully detect significant differences between isolates; SA-SE1 isolate gave the largest spots diameter. For the five other isolates, four significant levels, compared to SA-SE1 isolate were observed. A similar trend as spot diameter was observed for the other virulence components: disease severity, spot area average, logistic infection rate and Mid-time. In sum, the SA-SE1 isolate took the top place, in isolate virulence ranking and other isolates: TA-ZY21, LA-SY21, U94-SR1, HA-GH22 and MA-K1 were placed in the next steps, respectively.

Belisario *et al*., (2008) reported significant negative correlation between colony growth rate in culture and the altitude of sampling site. Our results also showed isolates of West Azerbaijan, Khorasan-e-Razavi, Tehran and Alborz from higher altitude had less growth rate than Mazandaran and Hamedan isolates from lower altitude. Belisario *et al*., (2008) reported that disease variability was correlated with colony growth rate. They did not calculate disease severity. We did not find out any positive significant correlation between colony growth rate and disease severity. However, acervulus density and disease severity were significantly correlated. Acervulus density was strongly ($p < 0.01$) correlated with colony growth rate.

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مطالعه پرآزاری جدایه‌های ایرانی *Ophiognomonia leptostyla*

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چکیده: بیماری آنتراکنوز گردو با عامل *Ophiognomonia leptostyla* یکی از مهم‌ترین بیماری‌های قارچی گردو است که انتشار جهانی دارد. نمونه‌های آلوده به علائم آنتراکنوز گردو، درطول سال‌های ۹۳-۹۴ از مناطق مهم گردوکاری کشور جمع‌آوری شد. قارچ بیمارگر پس از جداسازی، بر مبنای توالی ITS-rDNA شناسایی شد. شصت جدایه از *O. leptostyla* از استان‌های البرز، تهران، آذربایجان غربی، خراسان رضوی، مازندران و همدان از نظر سرعت رشد پرگنه و تراکم آسروول در محیط کشت در قالب طرح آزمایشی بلوک‌های کامل تصادفی، مقایسه شدند. آنالیز واریانس نشان داد که بین جدایه‌ها از نظر سرعت رشد پرگنه و تراکم آسروول، اختلاف معنی‌دار وجود دارد. سرعت رشد از ۱/۴ mm/day برای جدایه‌های SH-OSH2، TA-ZY21 و MA-JA2 به‌ترتیب از تهران، البرز و خراسان رضوی، تا ۲/۴ mm/day در جدایه SA-SE1 از مازندران، متفاوت بود. تراکم آسروول هم‌بستگی بالایی با سرعت رشد پرگنه نشان داد. تراکم آسروول در جدایه‌های آذربایجان غربی، تهران و خراسان رضوی اغلب بین ۲۰-، -۴۰٪ و تنها در تعداد کمی از جدایه‌های این استان‌ها، تراکم آسروول کمی بیش‌تر از ۴۰٪ بود. تراکم آسروول در نیمی از جدایه‌های همدان و مازندران ۴۰٪ یا بیش‌تر بود. بیش‌ترین تراکم آسروول (۸۰ <) در جدایه SA-SE1 از استان مازندران مشاهده شد. پر آزاری شش جدایه انتخابی از مناطق مختلف روی رقم چندلر مورد آزمون قرار گرفت. آنالیز واریانس داده‌های بیماری‌زایی جدایه‌ها نشان داد که بین جدایه‌ها از نظر شاخص‌های پرآزاری شامل متوسط قطر لکه‌ها (mm)، شدت بیماری (٪)، متوسط سطح لکه‌ها (mm^2) و نرخ آلودگی لوجستیک، اختلافات معنی‌دار وجود دارد. جدایه SA-SE1 از مازندران به‌طور معنی‌داری از نظر متوسط سطح لکه، شدت بیماری و نرخ آلودگی لوجستیک از دیگر جدایه‌ها پرآزارتر است. جدایه‌های دیگر شامل TA-ZY21، LA-SY21، U94-SR1، HA-GH22 و MA-K1 از نظر شاخص‌های پرآزاری به‌ترتیب در جایگاه‌های بعدی قرار گرفتند. بین سرعت رشد جدایه‌ها و پرآزاری هم‌بستگی معنی‌داری مشاهده نشد. اما بین تراکم تولید آسروول و پرآزاری هم‌بستگی مثبت وجود داشت. وجود این هم‌بستگی مثبت نشان‌دهنده اهمیت اسپورهای غیرجنسی در توسعه چرخه‌های ثانویه بیماری است. شدت بیماری با تعداد لکه، قطر لکه و نرخ آلودگی لوجستیک، هم‌بستگی قوی دارد. شدت بیماری با "مدت زمان متوسط" با ضریب ۹۰٪ ، هم‌بستگی دارد. هم‌چنین بین نرخ آلودگی لوجستیک و تعداد لکه، قطر لکه و متوسط سطح لکه، هم‌بستگی مثبت مشاهده شد. در این مطالعه، شاخص‌های پرآزاری جدایه‌های *O. leptostyla* روی رقم چندلر، برای نخستین بار به تفصیل مورد آزمون و بررسی قرار گرفت.

واژگان کلیدی: آنتراکنوز گردو، پرآزاری، شدت بیماری و کولتیوار چندلر

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