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Epidemiology of Cotton Verticillium wilt in Golestan Province, the North of Iran

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Authors' contributions

The authors performed the whole research work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The efficiency of *Verticillium dahliae* inoculum density (ID) on tolerant cotton Verticillium wilt in cotton growing of northern province of Iran (Golestan) and the influence of temperature (T) and relative humidity (RHA) on this relationship.
Place and Duration of Study: In several fields in Golestan province, northern Iran, between 1992-2009.
Methodology: The microscleotia per gram of soil samples and disease severity of cotton Verticillium wilt were determined in several fields. Physiological time was determined as the cumulative number of degree-days from the time of sowing.
Results: ID for overall fields and years varied between 2 and 47 propagules per gram of air-dried soil with average 18.961 ± 0.730. The pattern of diseased plants varied with fields and years. Linear regression analysis between ID (propagules per gram of air-dried soil) of *V. dahliae* at planting time and the disease severity for all years closely followed the linear curves. The straight line models described the increase in disease intensify index over the accumulated physiological time from sowing. The effect of temperature, number of days with above 28ºC and the area under RHA from sowing with respect of pathogen ID in soil (MS) on the final disease severity (Y) were significant and fitted a Y= 65.840- 0.0034 RHA + 0.57MS- 1.7T model with R^2 = 0.859 and significant F function ($p \leq 0.0001$).
Conclusion: this study revealed that Verticillium wilt severity of cotton related to *V. dahliae* ID in soil at planting and negative exponential models described the relationships. This work also demonstrated the influence of temperature (days with above 28°C) and relative

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humidity on the efficiency of Verticillium ID for disease severity. This simulation may be important to consider when selecting a cotton cultivar for planting or in breeding programs.

Keywords: Cotton; Verticillium wilt; inoculum density; epidemic; Iran.

1. INTRODUCTION

Verticillium dahliae Kleb., a soil borne fungus distributed worldwide, colonizes the vascular tissues of plants, causing wilt in many crops [1,2]. The Verticillium wilt as a major disease affecting cotton has been known since 1914 when it was noticed on greenhouse-growing plants in Virginia in USA [2]. In Iran, the disease originally was noticed in 1952 and 1959 in the eastern (Ajarbaijan) and northern (Golestan) provinces, respectively. However, nowadays the disease can be seen in almost all cotton fields [2,3]. Because the extensive cotton and intensive cultivation in Golestan province (northern of Iran), the Verticillium wilt is partly responsible for reducing yield of cotton [3,4]. The disease cause economic losses in this province between 4-18% and the losses are often greater than losses caused by other diseases [3]. The resistance to Verticillium wilt under field conditions is strongly influenced by a number of factors, including the environment, ID, pathogen virulence, and management practices [5,6,7,8,9]. These factors vary widely in the Guadalquivir Valley (southern Spain) as a result of highly variable soil types, T, and irrigation regimes. In addition, a number of major herbaceous hosts of the pathogen, such as potato, sugar beet, sunflower, and vegetables, typically are grown close to cotton fields [10]. In Golestan province, the disease has been managed by use of wilt tolerant cultivar 'Sahel' in the region, but the present of highly virulent of the pathogen is accomplished with increase the Verticillium wilt [4,6].

Although, two species of *Verticillium* Nees., *Verticillium dahliae* Kleb. and *Verticillium albo-atrum* Reinke and Berth. have been mentioned as causing the Verticillium wilt of cotton, the Iranian workers identified the *V. dahliae* as a primary pathogen of cotton wilt in Iran [2,6,11]. The pathogen survives by means of microsclerotia in soil for more than 14 years and causes monocyclic diseases [2]. ID of the pathogen in field soils at planting plays a critical role in the epidemiology of Verticillium wilt [1,12,13]. Therefore, the workers carried out to determine the relationship between the soil ID at time of sowing and the disease severity during plant growth and development [12,14,15]. Primary work on Verticillium wilt has emphasized the direct relationship of vascular discoloration and foliar symptoms to the ID of the pathogen in soil [4,15,16,17,18,19]. However, the relationship between ID of the pathogen and disease progression is more variable and depends on environmental fluctuations [7,12,20], a little is known about the epidemiology of cotton Verticillium wilt under different conditions especially the temperature (T) and relative humidity (RHA) as the main environmental conditions [21].

The objective of this work were to determine the efficiency *V. dahliae* ID on tolerant cotton Verticillium wilt in cotton growing of northern province of Iran (Golestan) and the influence of T and RHA on this relationship.

2. MATERIALS AND METHODS

2.1 Sampling Soil

The soil samples collected during the years 1992, 1993, 1994, 1995, 1996, 1999, 2004, 2005 and 2009 in Golestan province in Iran (Fig. 1), near Gorgan region from several cotton

fields in each year (Table 1). 30 sites in each field were selected randomly before planting by z pattern for soil sampling. The soil samples were collected from depth of 0-30 cm and composited into single sample. Each soil samples placed into individual bags.

2.2 Inoculum Density

The soil samples were air-dried for 4 weeks at room temperature (23-27ºC), pulverized, mixed well and passed through a 2 Cm screen [22]. Four 10 gram of soil from each samples was mixed with 2 ml DL-methionine (7.5 gr/l) to break the dormancy of microsclerotia [20,23]. Then the samples were incubated at 32ºC for 1 week. After incubation, the soil was air-dried for 7 days. ID of *V. dahliae* was estimated by wet-sieving technique [24] and ethanol-streptomycin agar medium [25,26]. Plates were incubated in the dark at 25ºC for 3 weeks. After incubation, plates were washed with tap water to remove particles and aerial mycelia from the agar surface and facilitate the identification of colonies of *V. dahliae* under the stereomicroscope. Colonies of *V. dahliae* were identified based on morphology of microsclerotia formed into the agar, their distribution within the medium, and the absence of dark hyphae or dark mycelium. The ID in a sample was estimated as number of microsclerotia per gram of dry soil, and it was recorded for each sample area studied.

2.3 Disease Data

Disease severity [sum of all numerical rating (foliar disease index on scale of 1 to 5: 1 = no symptoms; 2 = slight necrosis of lower leaves; 3 = chlorosis of all leaves, some defoliation; 4 =total defoliation; 5 =plant killed) * number of infected plant per total number of plant] [12] were evaluated in each field 5 intervals mid-May, mid-June, mid-July, mid-August and mid-September during the experimental years.

2.4 Environmental Data

To calculate the physiological time, maximum and minimum air temperature and relative humidity were recorded in a metrological station near the experimental fields (Hashem abad near Gorgan region, Fig. 1). Celsius degree-days accumulated were calculated from the area enclosed over 11.9ºC (threshold temperature for cotton growth) [23]. Physiological time was determined as the cumulative number of degree-days from the time of sowing. For determination of T and RHA effects on the efficiency of *V. dahliae* ID on foliar symptoms, data from area under the variables were assayed using SAS software version 9.1.

2.5 Data Analyses

The relationships between ID at planting and disease severity of different growing seasons were determined by linear regression analysis using SAS software version 9.1. Analyses of variance were performed on data obtained from fields studied during one and two consecutive growing seasons and the effect of RHA and T were compared by the least significant difference test ($P \leq 0.01$) using the same software.

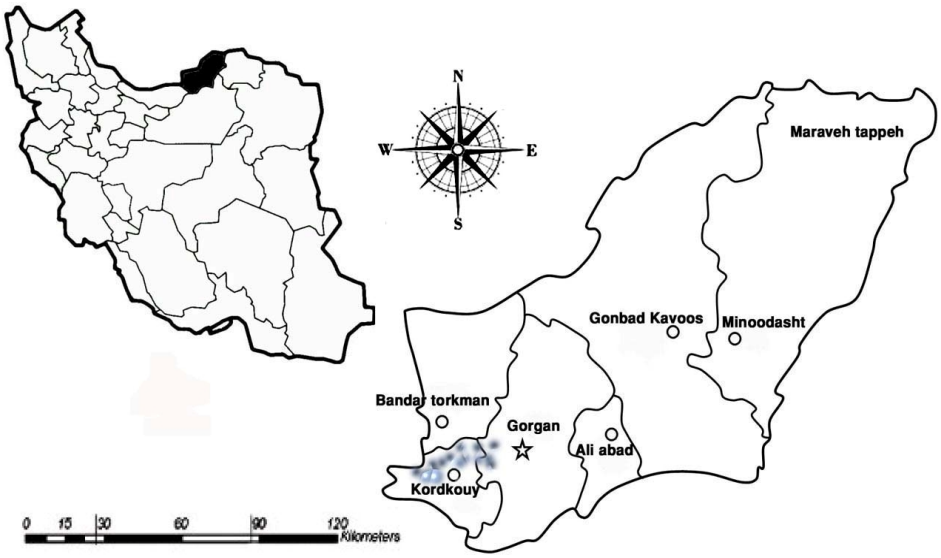

Fig. 1. Map of Iran for Golestan location (left) and regional distribution of randomly assigned cotton fields surveyed for Verticillium wilt in Golestan province (right)

Table 1. Linear regression analysis of Verticillium wilt severity (%) of plants with foliar symptoms over cumulative physiological time from sowing in cotton field with different IDs of *V. dahliae* propagules during the years studied

Year	Field number	Regression equation	r	p
1992	25	Y= -9.509 + 0.026X	0.910 **	≤0.001
1993	28	Y= -10.339 + 0.028X	0.941 **	≤0.001
1994	25	Y= -3.341 + 0.016X	0.801 **	≤0.001
1995	21	Y= -8.242 + 0.017X	0.796 **	≤0.001
1996	22	Y= -2.420 + 0.014X	0.757 **	≤0.001
1999	20	Y= -5.451 + 0.016X	0.811 **	≤0.001
2003	12	Y= -7.111 + 0.019X	0.799 **	≤0.001
2005	5	Y= -8.132 + 0.017X	0.697 **	≤0.001
2009	6	Y= -6.131 + 0.015X	0.799 **	≤0.001
All years		Y=-9.112 + 0.025X	0.812 **	≤0.001

Y= wilt severity index (%), X= cumulative physiological time (degree days)

3. RESULTS AND DISCUSSION

ID for all fields and years varied between 2 and 47 propagules per gram of air-dried soil with average 18.961±0.730. The pattern of diseased plants varied with fields and years. The mean wilt severity in fields ranged from 5 to 51% with average 18.68±0.831 for all years. In all years, disease increased showed a positive and significant linear relationship (r=0.812) with physiological time (Table 1). The predicted line model Y= -9.112 +0.025X (p≤0.001) obtained from regressional analysis (Y= wilt severity%, X= cumulative physiological time). The onset of infection was 500-610 degree days (Fig. 2).

Wilt severity was significantly different among fields with various level of *V. dahliae* ID. The Verticillium wilt severity (%) increased with the ID increased from 2 to 47 microsclerotia per gram of air-dried soil and the data closely followed the linear curves (Table 2). Coefficient of determination of predicted model for final wilt severity and ID of *V. dahliae* was 0.519 with $p \leq 0.001$ for all years. A simulation using the model showed that 5 microsclerotia per gram of air-dried soil cause 10.4% wilt severity (%). Also, the threshold of cotton plant infection under 47 microsclerotia per gram of air-dried soil was 34.65% wilt severity for the maximum ranges in this experiment.

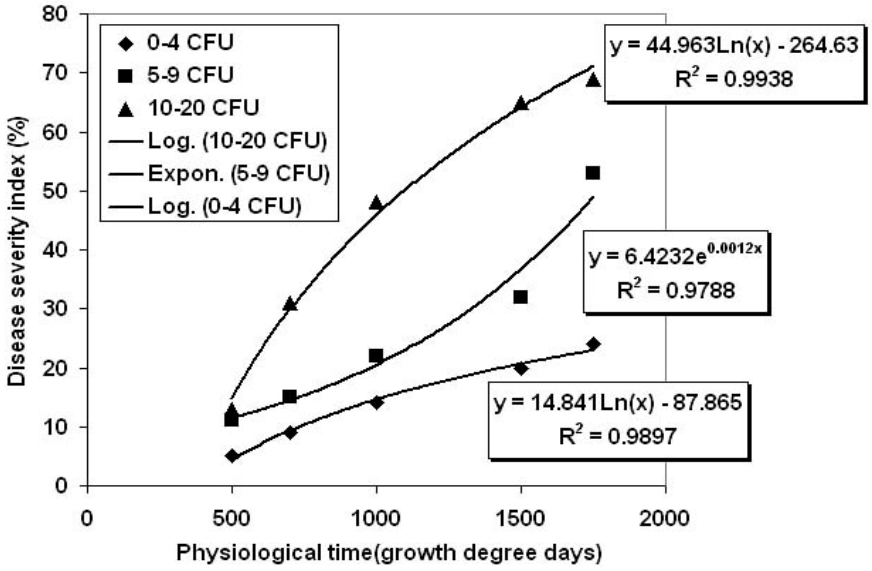

Fig. 2. Increase of Verticillium wilt severity (%) over cumulative physiological time from sowing in cotton field with different IDs during 9 years of experiment study

Table 2. Linear regression analysis of Verticillium wilt severity (%) of plants with foliar symptoms (Y) over cumulative physiological time from sowing in cotton field in defined IDs of *V. dahliae* propagules per gram of air-dried soil

ID*	Regression line slope (b)	r	p
0-5	0.007	0.743 **	≤0.001
5-10	0.005	0.652 **	≤0.001
10-15	0.008	0.641 **	≤0.001
15-20	0.009	0.654 **	≤0.001
20-25	0.015	0.612 **	≤0.001
25-30	0.012	0.618 **	0.003
30-35	0.002	0.728 **	0.002
35-40	0.010	0.493 **	0.001
>40	0.014	0.730 **	0.001

** Propagules of V. dahliae per gram of air-dried soil.*

For epidemic analysis, the relationship between foliar symptoms and cumulative physiological time were linearized to describe disease increase over time. When the wilt

severty of foliar symptoms was expressed as a linear function against physiological time, the parameters of disease curves increase related on initial density (Table 2). In general, a decrease in disease severity was observed in 1995 and 1996 especially for temperature above 28ºC. The relationship between number of days with 28°C and above (T1), degree accumulation above 28ºC (T2), relative humidity (RHA) and ID on disease progression fitted in a Y= 65.840 - 0.0034 RHA + 0.57MS - 1.7T1 with probability and correlation matrix 0.001, 0.657; -0.0004, 0.495 and 0.003, -.717 for all variables in the model, respectively. The F function was significant $p \leq 0.0001$ for the model. The degree accumulation above 28ºC has high correlation matrix (r=-0.703) but the stepwise regression analysis omitted the variable from the model ($p \leq 0.082$).

Because Verticillium wilt is a single cycle disease, inoculum levels of *V. dahliae* in the soil at planting time play a critical role in disease progression [5]. The results indicate the relatively low tolerance of cotton crop to *V. dahliae* microsclerotia in soil and revealed that high wilt severity associated with populations of *V. dahliae* microsclerotia occurred in the soil. The data documents that even five microsclerotia per gram of air-dried soil has planted to infect 10.4% of cotton plants. Therefore, low levels of the pathogen ID could potentially pose a significant risk to cotton production.

With respect of cotton wilt severity to affected productivity, the ID and wilt severity relationships have been characterized for cotton [1,4,12,14,27] and a number of crops in other reports [5,10,15,28,29,30,31]. By way of example, Bejarano-Alcázar *et al*. [12] found that the mean *V. dahliae* IDs in soil in cotton fields affected by Verticillium wilt in the upper, central, and lower areas of the Guadalquivir Valley (Southern Spain) were 3.4, 6.4, and 37.1 microsclerotia per gram, respectively, with an overall mean of 14.4 microsclerotia per gram and a range of 2 to 132 microsclerotia per gram. Similarly, ID values ranging from 100 to 200 microsclerotia per gram have been reported for cotton-growing areas in California [32]. In general, higher of ID increase the probability of contact cotton roots with microsclerotia of *V. dahliae* which followed with multiple infections of a root system and higher wilt severity [20, 34]. Also, the incubation period between adventitious root infection and the progressive of the pathogen to the main root vascular system expected considerably shorter for multiple infections compared with solitary infection [33]. Therefore, higher ID of the pathogen may be closely accompanied with sooner infection. This relation has also observed on wilt diseases of cotton and other crops [10,33,34,35]. However, in this experiment the relations vary from field to field and year to year.

The progression of symptom severity over time was characterized in the present study by using a simple straight linear regression of untransformed data. A comparison of regression coefficients revealed that the rate of increase in severity over time differed among ID treatments. The highest ID (47 microsclerotia per gram of soil) resulted in a continuous increase in disease incidence throughout the study period with higher line slope; whereas, the lower ID (2 microsclerotia per gram of soil) with lower line slope, there were long periods over which no disease incidence increase was recorded. The threshold for cotton plant infection under Californian field conditions was 0.03 microscleotia per gram of soil. At the end of the growing season, with 0.3-1.0 microscleotia per gram of soil, infection was between 20 and 50%. With 3.5 microscleotia per gram of soil or greater, infection was 100% [1]. A similar threshold level was reported previously in cotton by Bejarano- Alcázar *et al*. [12] for the Guadalquivir Valley; they found the ID of a defoliating pathotype of *V. dahliae* required to cause 100% disease incidence in the highly susceptible cv. Coker-310 to range from 6 to 10 microsclerotia per gram of soil. In other report [27], the disease intensity of

susceptible cotton increases progressively, with 100% infection achieved by 10 microscleotia per gram of soil.

The relationship between the amount of *V. dahliae* in soil and wilt disease progression has been studied in various herbaceous hosts of this pathogen [2]. Some studies have revealed positive relationships [14,15,29,30,36]; in others, the results were variably influenced by diverse factors including soil type, cultivar, and pathogen race or pathotype [35]. For example, Bejarano-Alcazar *et al.* [12] found no correlation between the *V. dahliae* ID determined at sowing time in Spanish cotton field soils with vascular discoloration, incidence of foliar symptoms or disease intensity index at harvest time. Although the authors refer the diverse factors, but there is no precise data about these factors, but in this report, the result shows the influence of T and RHA in this report.

As the cotton planting date occur in cold season (mid-April to early-May) in Golestan province, therefore, only the isolates of *V. dahliae* with lower optimal growth can attack the seedlings [4]. These results explain our finding for onset of infection which mostly related to mild and severe non-defoliate pathotypes with having lower optimal growth than defoliate pathotypes [3,35,13]. The coefficient of determination for regression was lowered between observed and predicted disease severity based on data from different years in some years. The lower correlation could have resulted from other factors that influence the wilt severity, environmental conditions, cultural practices, virulence variation of *V. dahliae* strains, host resistance and etc. [37,38]. In this experiment similar cultivar ('Sahel') and close of field omitted some factors, such as host resistance and some environmental conditions for each year. The variation of the field experiment may have been caused by irrigation practice (drip or sprinkler) and uneven distribution of soil moisture in fields or by other factors [20]. For all years, the effect of environmental factors, temperature and relative humidity show that these variables can influence the efficiency of ID on foliar symptoms which influence both on wilt severity.

This result can be explained by the influence of environmental factors especially temperature on internal population of *V. dahliae* in cotton which can decrease or increase disease progression [3]. The results in this experiment agreed with those of DeVay *et al.* [23] and Farsad *et al*. [21] who concluded that ID of the pathogen in soil is not only determinate of final foliar symptoms and consequently that the ID is not a unique parameter indicator of the potential development of Verticillium wilt in cotton field.

The data was to develop a model based on the ecological traits and the disease progression. The model suggest that for screening of cotton cultivar the tolerant of cotton to Verticillium wilt as well as the responses of cultivars to ID of *V. dahliae* in soil, are dependent on temperature and relative humidity changes during experiment. This simulation may be important to consider when selecting a cotton cultivar or line for planting or in breeding programs.

4. CONCLUSION

In summary, this study revealed that Verticillium wilt severity on cotton related to ID of *V. dahliae* in soil at planting and negative exponential models described the relationships. This work also demonstrated the influence of temperature (days with above 28ºC) and relative humidity on the efficiency of Verticillium ID for disease severity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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