RESEARCH ARTICLE Legume Research- An International Journal, Volume 43 Issue 5: 735-741 (October 2020)

Current Distribution and Virulence of *Fusarium oxysporum* f. sp. *ciceris* in Turkey

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10.18805/LR-520

ABSTRACT

This study was undertaken to define current data on chickpea Fusarium wilt severity, its relation with epidemiological parameters and virulence levels of isolates in Turkey. The disease was detected in 7 regions and 37 provinces of Turkey indicating that *Fusarium oxysporum* f. sp. *ciceris* is an emerging pathogen of chickpea. Yellowing and wilting types were detected in surveyed areas wherein yellowing was observed in 31 of 37 provinces. Epidemiological parameters were effective on wilting and yellowing syndrome development wherein negative correlations were observed among disease severity, relative humidity (%) ($r = -0.25$; $P < 0.01$) and the number of rainy days ($r = -0.23$; $P < 0.01$) for yellowing, however there was no statistically significant relationship for wilting phenotype ($P > 0.05$).

Key words: Chickpea, Disease severity, Epidemiological parameters, *F. oxysporum* f. sp. *ciceris*, Pathogenicity.

INTRODUCTION

Chickpea Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend. : Fr. f. sp. *ciceris* (Padwick) (*Foc*) Matuo and K. Sato (*Foc)* can severely limit the production in many chickpea growing areas of the world. Disease was first reported in India (Chand and Khirbat, 2009) and then, it was also notified from Turkey (Dolar, 1997), South and West Asia, East and North Africa, USA and the Soviet Union. Fusarium wilt can lead to total crop loss and annual chickpea yield diminish varies between 10-15% (Jiménez-Díaz *et al*., 2015). *Foc* is seed and soil-borne, can survive more than 6 years in crop residues and symptomless carrier legumes like lentil, pigeon-pea and pea hinders in the control. The use of resistant cultivars has been reported to be the best and economic way to control chickpea wilt (Singh *et al*., 2007). The diversity of pathogenic races reduces the efficiency of control methods thus characterization of pathogenic variability is very important in effective disease resistance breeding (Jiménez-Díaz *et al*., 1993).

Foc comprise rampant pathogenic diversity wherein eight races have been identified (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) worldwide via a set of 10 differential chickpea genotypes (Jiménez-Gasco and Jiménez-Díaz, 2003). The isolates from Spain were divided into two pathotypes inducing the symptoms of yellowing and wilting exhibiting different pathogenicity degrees on chickpea lines through induction of vascular infections. The occurrence of races 0, 2 and 3 in *Foc* were reported from 9 provinces of Turkey (Bayraktar and Dolar, 2012), however, studies on the disease prevalence, incidence and relation of disease severity with epidemiological parameters have not been reported. The objectives of this study were (i) to assess disease severity in chickpea growing provinces of Turkey through surveys; (ii) to explore relations of Fusarium disease severity with ecological parameters; (iii) to define the distribution of yellowing and wilting phenotypes in Turkey. Therefore, the present study was undertaken to determine current comprehensive investigation on the variability of *Foc* population in Turkey.

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How to cite this article: Kocalar, H., Kafadar, F.N., Ozkan, A., Talapov, T., Demirel, O., Anay, A., Mart, D. and Can, C. (2020). Current Distribution and Virulence of *Fusarium oxysporum* f. sp. *ciceris* in Turkey. Legume Research. 43(5): 735-741.

Submitted: 23-08-2019 **Accepted:** 05-12-2019 **Published:** 18-03-2020

MATERIALS AND METHODS

Disease surveys and *Foc* isolations

Systematic field surveys were performed on chickpea growing seasons between 2015 and 2016 in the main chickpea growing areas in Turkey (Fig 1, Table 1). Disease severity (%), location (province and district), field size (da), weed density (m^2), growth stage of chickpea plants, average nodule number of infected and healthy plants with plant height (cm) were recorded for each field. Latitude, longitude and altitude (m) were also recorded by using Global Positioning System (GPS).

Chickpea plant samples exhibiting wilting and yellowing symptoms (Fig 2a and b) were collected from different points of each field and fungal infections were assessed on a scale of 0-4 based on percentage of infected foliage accompanied

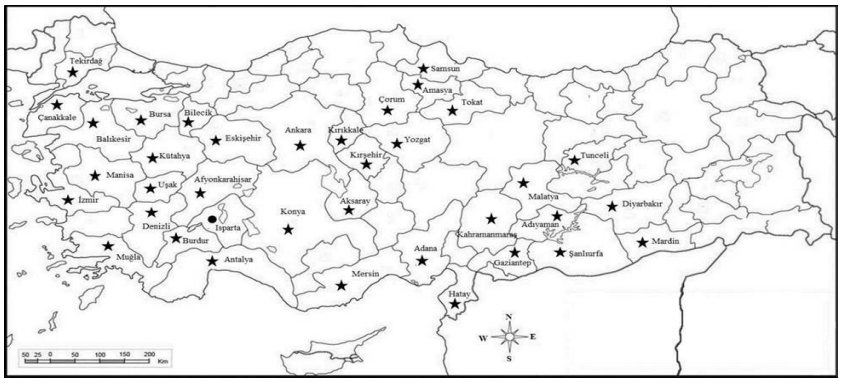

Fig 1: Geographic distribution of chickpea fields surveyed during 2015 and 2016 in Turkey. The provinces are indicated with stars.

Table 1: Percentage prevalence and incidence of Fusarium wilting and yellowing forms in 2015/2016 growing season in Turkey.

Regions	Provinces	#Fields inspected /infected	Inspected field area (da)	Prevalence (%)	Incidence (%)
Southeastern	Adıyaman	20/11	1423	55.00	2.75
Anatolia	Mardin	5/5	20	100.00	36.49
	Diyarbakır	6/6	25	100.00	30.13
	Şanlıurfa	6/1	222	16.67	2.47
	Gaziantep	37/5	1489	13.51	0.56
Eastern Anatolia	Malatya	7/2	214	28.57	8.46
	Tunceli	5/2	167	40.00	40.00
Mediterranean	Antalya	15/13	201	86.67	6.46
	Burdur	6/4	41	66.67	5.47
	Hatay	4/4	65	100.00	4.27
	Kahramanmaraş	10/2	555	20.00	0.58
	Adana	8/6	151	75.00	5.17
	Mersin	41/22	457	53.66	4.83
	Isparta	15/13	162	86.67	4.01
Aegean	Denizli	9/9	135	100.00	5.36
	Muğla	5/4	47	80.00	3.71
	Uşak	19/9	1265	47.37	2.07
	Manisa	12/8	80	66.67	1.83
	Kütahya	19/9	166	47.37	1.26
	Afyonkarahisar	7/6	85	85.71	3.29
	İzmir*	1/1			
Bosphorus	Balıkesir	7/5	93	71.43	16.53
	Bilecik	6/2	52	33.33	1.50
	Bursa	1/1	2		
	Çanakkale	12/7	98	58.33	1.69
	Tekirdağ	7/5	34	71.43	3.61
Central Anatolia	Aksaray	11/2	121	18.18	3.12
	Kırşehir	21/5	1018	23.80	0.16
	Yozgat	21/5	663	23,80	0,23
	Ankara	13/10	221	76.92	1.61
	Konya	22/14	732	63.64	1.07
	Eskişehir	10/10	350	100.00	4.80
	Kırıkkale	4/0	270	0.00	0.00
Black Sea	Amasya	8/6	26	75.00	5.63
	Samsun	4/0	13	0,00	0,00
	Çorum	13/11	147	84.62	1.70
	Tokat	6/3	27	50.00	1.91
Total/Mean	37	423/228	10837	53.90	4.04

*The isolates in İzmir were obtained from Aegean Agricultural Research Institute (Turkey).

by wilting or yellowing through acropetal progression (Jiménez-Díaz *et al*., 2015) and disease severity index (DSI) was calculated. Disease incidence (percentage of diseased plants) and disease prevalence (percentage of diseased fields) ratios were defined.

Pathogen isolation was performed from chickpea samples exhibiting wilting and yellowing symptoms (Fig 2). Colony color, macroconidia, microconidia, chlamydospores, conidiophores and phialide features were investigated to identify *F. oxysporum* isolates (Leslie and Summerell, 2006).

Pathogenicity tests

Isolates were recultured for 10-14 days and conidia suspensions were adjusted to 1×10^6 conidia/ml. ILC482 cultivar seeds were planted in a soil mixture containing soil:torf:vermiculite (1:1:3, v/v/v) in viols and were germinated in growth chambers adjusted to 12-hour photoperiod of cool florescent light at 11000 lux with 50±70% humidity at 25±2°C. Pathogenicity test was performed when plants had 4-5 side branches (twelve-days-old seedlings) according to the root dipping method (Altinok and Can, 2010). The disease reactions were assessed on a scale of 0-4 for each plant periodically at intervals of 7 days for 28 days from the day when the first symptoms were seen until the beginning of the death appearance in the plants (Jiménez-Díaz *et al*., 2015). The 0-4 scale was used to define disease severity (%) and the area under the disease progress curve (AUDPC) was calculated (Simko and Piepho, 2012). The experiments were repeated twice.

Statistical analyses

These data were analyzed using SPSS 16.0 statistical package programs. Results were evaluated as mean of ± Standard Error (Mean±SE). Results of Duncan Multiple Comparison (post-hoc) in ANOVA were defined as statistically significant ($P < 0.05$). Principal components analysis (PCA) was used to define the effect of ecological parameters on wilting and yellowing symptom development.

Results and Discussion

Field surveys

Fusarium wilting and yellowing symptoms were observed in 228 of 423 chickpea fields surveyed (Table 1). Totally 880 *F. oxysporum* isolates was obtained from chickpea plants exhibiting symptom and 442 isolates were included in the pathogenicity tests that identified 416 *Foc* isolates (Table 2). The pathogenic diversity of *Foc* has been reported by Bayraktar and Dolar (2012) in Turkey. However, there is no current information on disease severity/prevalence/ incidence, pathogenicity, epidemiological parameters and regional comparisons of *Foc* in Turkey. The present study

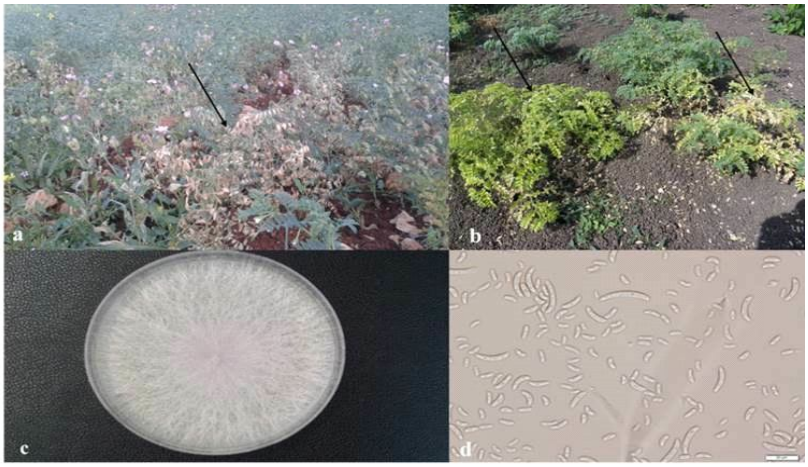

Fig 2: Wilting (a) and yellowing (b) phenotypes of chickpea plants in the field, *Fusarium oxysporum* f. sp. *ciceris* colony morphology on PDA medium (c), macro and micro conidia (d).

Table 2: AUDPC distribution of *Fusarium* wilt isolates among regions of Turkey.

Regions	Number of isolates		AUDPC (%)
	F. oxysporum used in pathogenicity tests	*F. oxysporum* f. sp. ciceris	
Southeastern Anatolia	36	32	30.92
Eastern Anatolia	8	5	30.83
Mediterranean	174	170	39.45
Aegean	96	92	29.96
Bosphorus	58	51	27.88
Central Anatolia	41	41	59.73
Black Sea	29	29	29.36
Total	442	416	

showed that *Foc* has a wide pathogenic diversity at the level of virulence in different geographic regions of Turkey and the disease is prevalent in majority of chickpea producing provinces. Chaithra *et al*. (2019) reported similar results indicating that the current *Foc* isolates of India vary for their pathogenicity and morphology.

During the surveys in Turkey, two distinct symptomatic types (wilting/yellowing) were detected in diseased plants affected by the *Foc*. Previously, chickpea yellowing and wilting with vascular discoloration were induced by different biotypes of *Foc* (Jiménez-Díaz *et al*., 2015). The wilting type, which is more virulent than the yellowing (Jiménez-Gasco and Jiménez-Díaz, 2003), was found in Mersin, Isparta, Aksaray and Eskişehir provinces in our study. Yellowing type was more common in chickpea growing areas in Turkey and was determined in 31 of the 37 provinces (Table 1). Wilted plants with severe chlorosis and flaccidity, complete collapse of the seedlings including death occurred earlier than the yellowed plants. Yellowing symptoms were observed in different vegetation states of the chickpea plant, while wilting frequently affected plants during pre-flowering and flowering stages (Fig 2). Studies conducted to determine race distribution in Turkey revealed that race 0 (yellowing type) existed in chickpea growing areas and is a less virulent race when compared to races 2 and 3 (Bayraktar and Dolar, 2012; Dolar, 1997). However, surveys conducted in this study determined widespread occurrence of yellowing type in Turkey and the diseased plants did not have flowers and capsules (Fig 2) that in turn may negatively effects chickpea yield.

The overall disease prevalence of *Foc* in Turkey was 53.9% (Table 1). The prevalence of wilting and yellowing types were 61.04% and 52.31%, respectively. Although wilting had less frequency distribution than yellowing in Spain (Jiménez-Díaz *et al*., 2015), the prevalence of wilting (61.04%) was higher than that of yellowing (52.31%) in Turkey (Table 1) and *Foc* isolations have been performed from 72.40% of the collected plants. The prevalence of the Mediterranean region was the highest (64.65%) and 65.97% of the collected samples were infected with *Foc*. In the Central Anatolia that is the major chickpea producing region of Turkey, variability in prevalence was very high. All fields in Kırıkkale province were free of the disease whereas the mean disease prevalence of the other provinces varied between 51.0-100% (Table 1). The Eastern Anatolia had the highest disease incidence (21.60%), followed by the Southeastern Anatolia (6.13%), Bosphorus (4.57%) and Mediterranean (4.57%) regions. Black Sea, Aegean and Central Anatolia regions exhibited the lowest disease incidences of 2.54%, 2.47% and 1.32%, respectively (Table 1).

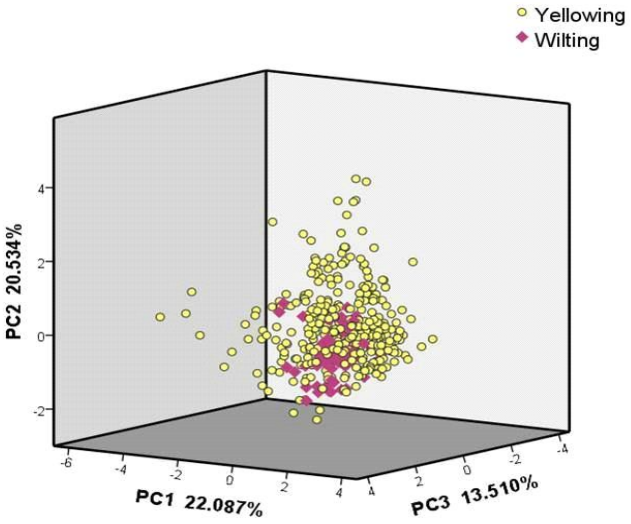

Fig 3: Effect of factors on distribution of yellowing and wilting types in chickpea plants.

Table 3: *Fusarium* wilt disease severity of different geographic regions of Turkey.

Geographic Regions	Disease Severity (%)*	Grouping
Central Anatolia	0.48±0.16^{c}	Group-I
Black Sea	1.03±0.31^{bc}	Group-II
Aegean	1.11±0.20^{bc}	
Mediterranean	2.17±0.43^{bc}	
Bosphorus	3.23±1.01^{bc}	
Southeastern Anatolia	3.61±1.02^{b}	Group-III
Eastern Anatolia	8.38±4.79^{a}	Group-IV

*Means±SE in the same column by the same letter is not significantly different to the test of Duncan (P ≤ 0.05).

Table 4: Pearson correlations among disease severity and geographic parameters of yellowing phenotype (N = 343).

	(1)	(2)	(3)	(4)	(5)
Disease severity (%)	1				
Relative humidity (%)	-,252**	1			
Temperature (°C)	,383**	-,275	1		
Wind speed (m/sec)	,004	,235**	-,008	1	
Rainfall (mm)	-,077	,429**	-,215**	-,063	1
Number of rainy days	-,229**	,383**	-,434**	,079	,371**

*P < 0.05, **P < 0.01.

Surveyed regions of Turkey were divided into four groups according to average values of disease severity. The highest disease severity (8.38±4.79) was detected in the Eastern Anatolia region that was placed statistically in Group IV. Intermediate disease severity was observed in Group-III and Group-II. Southeastern Anatolia region (3.61±1.02) in which of 74 fields, 28 fields were found to be infected and placed statistically in Group III (Table 3). Chickpea fields of Mardin, Diyarbakır and Gaziantep provinces exhibited yellowing and dwarfing and 29.48% of symptom exhibiting plants resulted *F. oxysporum* isolations. Because reduction of soil water potential causes symptoms of wilting and chlorosis (Passioura, 2002), drought, physical change of the soil, may bring along chickpea yellowing and dwarfing in this region. Although chickpea is a drought-resistant legume, it shows a reaction against water during the periods of insufficient rainfall (Ullah *et al*., 2002). Biotic and abiotic factors including fungal infections influence the formation of yellowing or chlorosis. Low level of *Foc* isolations in the Southeastern Anatolia region supports the conclusion that yellowing symptoms were not caused solely by *Foc* and could be due to environmental stress conditions (Jiménez-Díaz *et al*., 2015).

Disease severity (%) values were compared with climatic indices including relative humidity (%), temperature (°C), wind speed (m/sec), rainfall (mm) and number of rainy days for the years 2015 and 2016. Negative correlation among disease severity, relative humidity ($r = -0.25$; $P < 0.01$) and number of rainy days ($r = -0.23$; $P < 0.01$) and positive correlation between disease severity and temperature ($r = 0.38$; $P < 0.01$) showed that increasing temperature and decreasing humidity are critical factors affect chickpea yellowing in the field and increases the severity. Temperature affects several mechanisms in a disease cycle of pathogens including germination, dissemination, survival, penetration, progression and propagation rate (Sharma and Verma, 2019). Disease symptoms of *Foc* were more severe at higher temperatures (25°C and 30°C) than at lower temperatures (10°C, 15°C and 20°C). (Sugha *et al*., 1994). There was not significant interaction between disease severity and wind speed and rainfall ($P > 0.05$) (Table 4). It was concluded that the wilting form was not related to any climatic factors tested in this study ($P > 0.05$) (Table 5). According to the regression analysis, the climatic parameters affected the disease severity by 18% ($R^2 = 0.180$) in the yellowing and by 0.6% ($R^2 = 0.006$) in the wilting, indicating that climatic parameters had a supporting effect on yellowing disease (Tables 4 and 6).

Disease severity were also compared with values including altitude (m), average weed density (m^2), plant height (cm) and nodule number of infected and healthy plants. There was no statistically significant relationship between disease severity and plant parameters for in yellowing and wilting type ($P > 0.05$) (Tables 6 and 7). The plant parameters affected the disease severity by 8.6%, ($R^2 = 0.086$) in the yellowing and by 22.1% ($R^2 = 0.221$) in the wilting according to the regression analysis.

PCA was performed by using the variables of disease severity (wilting and yellowing groups), temperature, number of rainy days, relative humidity, rain quantity, altitude, healthy plant height, mean nodule and weed numbers (Table 8). Discrimination force of factor scores on yellowing and wilting groups through PCA is given in Fig 3. The agreeableness of data for PCA was verified by Kaiser-Meyer-Olkin value (KMO coefficient = 0,657 Bartlett test Chi-square test = 546,585, df = 36, $P < 0.001$). The variables were separated into three components. Varimax vertical rotation method was applied after factor loadings were calculated for components.

Table 5: Pearson correlations among disease severity and geographic parameters of wilting phenotype (N = 77).

	(1)	(2)	(3)	(4)	(5)
Disease severity (%)	1				
Relative humidity (%)	,077	1			
Temperature (°C)	-,049	-,625**	1		
Wind speed (m/sec)	-,076	-,986**	,619**	1	
Rainfall (mm) (5)	,075	,976**	-,760**	-,980**	1
Number of rainy days	,064	,823**	-,762**	-,733**	,818**

*P < 0.05, **P < 0.01.

Table 6: Pearson correlations among disease severity and plant parameters of yellowing phenotype (N = 343).

	(1)	(7)	(8)	(9)	(10)	(11)
Disease severity (%)						
Altitude (m)	-,037					
Average weed density (m^2)	-,044	,146**				
Average nodule number of healthy plants	-,080	-,051	-,069			
Average nodule number of infected plants	,102	-,114	,013	,327**		
Average height of healthy plants (cm)	-,042	-,303**	-,115*	,005	-,037	
Average height of infected plants (cm)	-,167	-,463**	-,046	,015	,115	,823**

*P < 0.05, **P < 0.01.

Eigenvalue had three factors greater than 1. The explained total variance rate was 56.131%. The first component explained 22.087% of total variance wherein temperature, disease severity, number of rainy days and relative humidity were defined as significant characters. The second component explained 20.534% of total variance in which altitude, rain quantity and healthy plant height was significant characters. The third component explained 13.510% of total variance that the mean weed and nodule numbers were defined as significant characters (Table 8). In the case of the emergence of disease epidemics, it is necessary to regard the effects of the interaction of environmental factors. The environmental conditions affect the incidence and development of wilt disease in chickpea (Merkuz and Getachew, 2012).

Virulence diversity of *Foc* isolates

Foc isolates were divided into five groups based on DSI: 0% nonpathogenic; 1-20% weakly pathogenic, 21-50% moderately pathogenic, 51-70% strongly pathogenic and >70% highly pathogenic. All regions except Central Anatolia included moderately pathogenic isolates and when the pathogenic variability of geographical regions was assessed, the DSI value ranged from 27.9%-39.5%. The isolates from

Table 7: Pearson correlations between disease severity and plant parameters of wilting phenotype (N = 77).

	(1)	(7)	(8)	(9)	(10)	(11)
Disease severity (%)	1					
Altitude (m)	-,177	1				
Average weed density (m^2)	,032	-,049	1			
Average nodule number of healthy plants	,122	,107	,137	1		
Average nodule number of infected plants	,371	,105	-,028	,424**	1	
Average height of healthy plants (cm)	,188	-,059	-,061	,189	-,031	1
Average height of infected plants (cm)	,155	-,068	-,076	,140	,061	,862**

*P < 0.05, **P < 0.01.

Table 8: The factor loadings of each component.

	Component*		
	PC1	PC2	PC3
Temperature (°C)	-,815		
Disease severity (%)	-,656		
Number of rainy days	,649		
Relative humidity (%)	,547		
Altitude (m)		-,784	
Rain quantity (mm)		,731	
Mean healthy plant height (cm)		,624	
Mean number of weeds (m^2)			,686
Mean number of nodules in healthy plants			-,651
Eigen value	1.988	1.848	1.216
Explained variance rate %	22.087	20.534	13.510

*Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization Rotation converged in 7 interations.

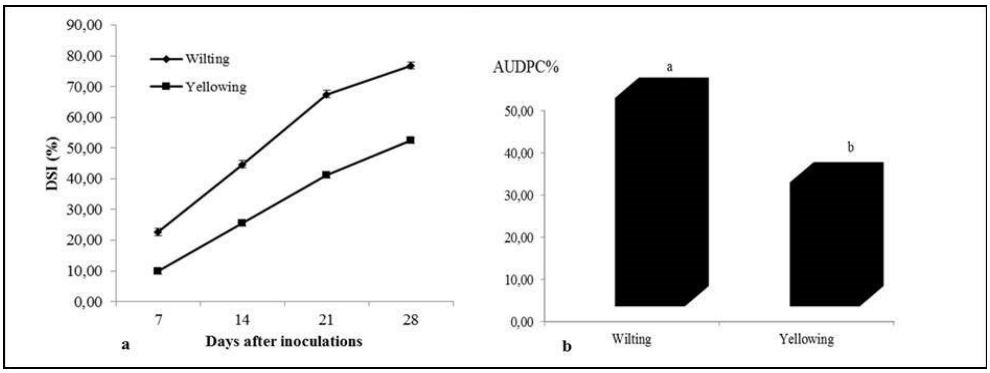

Fig 4: Disease progress in chickpea seedlings following inoculation with *Fusarium oxysporum* f. sp. *ciceris* isolates. DSI (%) (a) and AUDPC (%) values of yellowing and wilting types (b). Means±SE in the same column by the same letter are not significantly different to the test of Duncan (P ≤ 0.05).

the Central Anatolia Region was determined as strongly pathogenic with DSI value of 59.73%. DSI and AUDPC% values of wilting isolates were found to be higher than yellowing isolates (Fig 4a and b).

Environmental conditions impress in spreading phytopathogens by influencing host-pathogen interactions (Garrett *et al*., 2006) and make plants susceptible to disease that in turn affecting pathogen physiology, host susceptibility/ resistance or host-pathogen interactions. These condition, called predisposition (Bostock *et al*., 2014), facilitate diseases caused by facultative pathogens such as *Pythium ultimum*, *Fusarium* spp. and air-borne pathogens such as *Alternaria* spp. and *Botrytis cinerea* (Marek *et al*., 2013). Experiments conducted under controlled conditions in our study, the yellowing and wilting isolates exhibited differences in the level of virulence on ILC482 cultivar. Solely wilting type symptoms were observed in growth chamber experiments unlike two types of symptomatic development in the field. Similar results were reported by Jiménez-Díaz *et al.* (1989). Overall we claim that yellowing symptom development of chickpea plants in the field may be under the effect of predisposition.

Conclusion

High level of pathogenic diversity of *Foc* in both types, wilting/ yellowing, was determined by the statistical assessment of current survey studies and pathogenicity results. The study provided a general insight in understanding of chickpea Fusarium wilt-environment interactions based on correlation, regression and PCA analysis. Immediate need to breed resistant/tolerant chickpea varieties to *Foc* in Turkey is underlined considering widespread occurrence of yellowing type and high prevalence/severity of wilting type distribution of Fusarium wilt.

Conflict of Interest

The authors declare no conflict of interest in the publication.

Acknowledgement

Hatice Kocalar acknowledges the PhD grand provided by the Scientific and Technical Research Council of Turkey (TUBITAK). This study was supported partly by TUBITAK (project number 113O071) and University of Gaziantep Scientific Research Project Governing Unit (project number FEF.DT.17.04).

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