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Effects of inoculum density of *Fusarium oxysporum* f. sp. *ciceri* race 1 and 2 on chickpea wilt (*)

K.K. Zote, M.P. Haware *, S. Jayanthi * and J. Narayana Rao *

Agricultural Research Station, Badnapur, Maharashtra, India
* Crop Protection Division, ICRISAT, Patancheru, Andhra Pradesh, India

Summary. Studies on inoculum densities of the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* race 1 and 2 with thre chickpea cultivars representing early wilter (highly susceptible), late wilter (moderately susceptible) and resistant were conducted in glasshouse at 25°C. Highly susceptible cv. JG 62 showed 100% mortality at 15 days for race 1 and 17 days for race 2 at higher inoculum density. At the low inoculum density, the wilting was delayed and at this inoculum level two other cultivars showed no mortality. The wilt resistant cultivar cv. WR 315 and moderately susceptible cultivar cv. K 850 remained completely free from wilt symptoms up to 60 days while cv. JG 62 wilted in 27 days with *F.o.* f. sp. *ciceri* population ranging 4233-4667 propagules/g soil. However, xylem browning in the root and collar region was recorded in cv. K 850. The *F.o.* f. sp. *ciceri* population of race 1 and 2 increased with age of the plant in soils with wilt susceptible, moderately susceptible, and resistant chickpea cultivars. Moderately susceptible and resistant cultivars supported the multiplication of *F.o.* f. sp. *ciceri* propagules in the soil though the plants were free of wilt symptoms up to 60 days after planting. The multiplication ratio was higher at low inoculum density of the propagules compared to the higher inoculum density for both the races of *F.o.* f. sp. *ciceri*. As the inoculum density increased the multiplication ratio decreased.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops grown in India. Amongst the diseases reported on chickpea, wilt caused by *Fusarium oxysporum* Schl. emnd Snyd. *et* Hans. f. sp. *ciceri* [Padwick] Snyd. *et* Hans. *F. o.* f. sp. *ciceri* is one of the major diseases in North Africa, South Asia and Southern Europe (Haware, 1990) and causes up to 10% losses in yield. The disease is more prevalent in the lower latitudes (0-30°N), where the chickpea-growing season is relatively dry and warmer than in the higher latitudes (30-40°N). The pathogen is both seed and soilborne, survives in the soil for more than six years in the absence of susceptible host plants (Haware *et al.*, 1986). Haware and Nene (1982a) reported that the pathogen colonizes the roots of lentil, pea, pigeonpea without producing wilt symptoms.

Considerable progress has been made in the identification of wilt resistant sources and development of wilt resistant and high-yielding cultivars. Studies on the pathogenic variability revealed that the wilt pathogen has distinct physiological races (Haware and Nene, 1982; Jimenez-Diaz *et al.*, 1989). The severity of chickpea wilt in farmers' fields is directly related to inoculum density. The inoculum level may increase by cultivating chickpea every year thus increasing wilt severity. However, there is no information on the reaction of susceptible, moderately susceptible, and resistant chickpea cultivars to varying densities of *F.o.* f. sp. *ciceri* population and their influence on the population of the fungus in the soil. Such information would

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TABLE I. - Relationship between initial *Fusarium oxysporum* f. sp. *ciceri* (race 1) population levels and wilt incidence in susceptible, moderately resistant, and resistant chickpea cultivars at ICRISAT Asia Center, Patancheru.

Treatments (proportion of inoculum)	opulation	% Wilt incidence			Days to complete wilt in	*Fusarium* propagules g⁻¹ 60 days after sowing				Rate of multiplication in soil (a)			
		JG 62	WR 315	K 850	JG 62	JG 62	K 850	WR 315	Unsown	JG 62	K 850	WR 315	Unsown
T1 (1 g kg⁻¹ soil)	100	100	NW	NW	21	5933	3733	2500	2200	59.33	37.33	25.15	22.0
T2 (2 g kg⁻¹ soil)	133	100	NW	NW	19	3617	2483	1200	1967	27.19	18.66	9.02	14.78
T3 (5 g kg⁻¹ soil)	200	100	NW	NW	17	2950	2883	2233	2117	14.75	18.83	11.5	10.87
T4 (10 g kg⁻¹ soil)	217	100	NW	NW	16	3850	3033	2633	2367	17.74	13.97	12.13	10.94
T5 (15 g kg⁻¹ soil)	350	100	NW	NW	15	3150	2767	3200	2433	9.00	7.90	9.14	6.71
T6 (20 g kg⁻¹ soil)	383	100	NW	NW	15	3583	2533	4500	1883	9.35	6.61	11.76	4.87
T7 (25 g kg⁻¹ soil)	417	100	NW	NW	15	2183	2017	2900	2233	5.27	4.78	6.95	5.31
T8 (50 g kg⁻¹ soil)	1400	100	NW	NW	15	3250	1950	2250	2250	1.67	1.39	1.60	1.60
T9 (75 g kg⁻¹ soil)	2567	100	NW	NW	15	3267	1983	3467	2783	1.26	0.76	1.34	1.08
T10 (100 g kg⁻¹ soil)	3217	100	NW	NW	15	3800	3150	3383	3200	1.18	10.98	1.05	0.99
T11 (sterilized soil)	0	0	NW	NW									
SE	±85.0				±0.370	±136.2							
CV (%)	16.4				3.8	8.3							

(a) Rate of multiplication = Final population/initial population.
NW = No wilt.

be useful in the exploitation of host-plant resistance for management of the disease. This paper reports the results of pot culture experiments on the interaction of three chickpea cultivars with different degrees of susceptibility to wilt with a range of inoculum levels of race 1 and 2 of *F.o.* f. sp. *ciceri*.

Materials and methods

The experiments were conducted at ICRISAT Asia Center, Patancheru, in glasshouse at 25°±1°C. The *F.o.* f. sp. *ciceri* was isolated from roots of chickpea plants collected from Hyderabad (race 1) and Kanpur (race 2). The isolates were single-spored and maintained in autoclaved dry fine sand at 5°C.

Inoculum potential of *Fusarium oxysporum* f. sp. *ciceri*. The isolates of *F.o.* f. sp. *ciceri* race 1 and 2 maintained in autoclaved dry fine sand stored at 5°C were used. Fifteen-day old inoculum, multiplied on 100 g of 9:1 sand chickpea meal medium in 250 ml conical flask for 15 days at 25°C was thoroughly mixed with autoclaved soil (Vertisol) with different proportions of the inoculum, 1, 2, 5, 10, 15, 20, 25, 50, 75, and 100 g inoculum/kg soil. Physical characteristics of the soil used in experiment were: pH 8.1, electrical conductivity (d Sm^{-1}) 0.20 S, available P (mg kg^{-1}) 3.9, NH_4-N (mg kg^{-1}) 20.4, NO_3-N (mg kg^{-1}) 12.2. Inoculum levels were adjusted by adding non-infested soil. The plastic pots (15 cm) were washed in running water and disinfected with alcohol. The inoculum was mixed by shaking the soil in the closed container for 2-3 min. Two kg of infected soil were added in each pot. The initial *F.o.* f. sp. *ciceri* population in the different treatments were estimated (Singh and Chaube, 1970) by taking soil samples for each treatment, sub-sampling, and sprinkling on modified Czapek dox agar medium in Petri plates (ingredients:

$NaNO_3$ 2 g, K_2PO_4 1 g, $MgSO_4$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g, sucrose 30 g, agar 20 g PCNB 0.667 g, yeast extract 2 g, dissolved in 1 l distilled water autoclaved 6.8 kg sq in^{-1} or 15 lbs sq in^{-1} pressure for 20 min, before pouring into the plate 0.750 g of dicrysticin and 0.025 g of malachite green is added). Each treatment had 3 replicates. The plates were incubated at 25°C and initial *F.o.* f. sp. *ciceri* population was counted after 5 days.

The fungus was allowed to establish itself in the infested soil for 2 days before the seeds were sown. The experiment was laid out in a randomized block design with three cultivars (JG 62, K-850, and WR 315) and 10 inoculum treatments in three replications. For all tests, seed was surface-sterilized for 5 min with a 0.5% sodium hypochlorite solution before sowing. Six seeds were sown in each pot. In addition to the 10 treatments, one treatment was unsown (fallow). The experiment was set up in glasshouse with a temperature of 25±1°C. After germination, thinning was carried out in each pot to maintain 5 seedlings/pot. The pots were watered on alternate days with 100 ml sterilized water/pot.

Inoculum potential: Wilt sick soil (WSS) + sterilized soil (SS). Soil from a chickpea wilt sick plot (Vertisol, pH 8.2, EC 0.20 d Sm^{-1}), at ICRISAT Asia Center, Patancheru was collected, and air dried. The wilt sick soil was sieved and mixed with sterilized soil in various proportions i.e., 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 along with one treatment of wilt sick soil. The initial *F.o.* f. sp. *ciceri* population in the original wilt sick soil and in different treatments were estimated on modified Czapek dox agar medium. Seven treated seeds of cvs. JG 62, K 850 and WR 315 were sown in each pot. Later the seedlings were thinned out to 5 seedlings/pot.

In all 3 experiments wilt incidence was recovered at 3 days interval from the day of first appearance of wilt symptom for a period of 60

TABLE II. - Relationship between initial *Fusarium oxysporum* f. sp. *ciceri* (race 2) population levels and wilt incidence in susceptible, moderately resistant, and resistant chickpea cultivars at ICRISAT Asia Center, Patancheru.

Treatments (inoculum)	Initial population (propagules g^{-1} soil)	% Wilt incidence			Days to complete wilt in JG 62	*Fusarium* propagules g^{-1} 60 days after sowing				Rate of multiplication in soil (a)			
		JG 62	WR 315	K 850		JG 62	K 850	WR 315	Unsown	JG 62	K 850	WR 315	Unsown
T1 (1 g kg^{-1} soil)	83	73	NW	NW	26	2500	2389	1356	955	30.12	28.78	16.34	11.50
T2 (2 g kg^{-1} soil)	150	80	NW	NW	29	1855	4355	1533	955	12.36	29.03	10.22	6.36
T3 (5 g kg^{-1} soil)	217	53	NW	NW	27	2155	7800	811	478	7.77	28.15	5.85	3.44
T4 (10 g kg^{-1} soil)	217	100	NW	NW	18	2783	4172	1878	2328	12.82	19.22	8.65	10.72
T5 (15 g kg^{-1} soil)	350	100	NW	NW	19	2956	5383	4111	1811	8.44	15.40	11.74	5.17
T6 (20 g kg^{-1} soil)	400	100	NW	NW	18	2689	5205	2072	2944	6.72	13.01	5.18	7.76
T7 (25 g kg^{-1} soil)	450	100	NW	NW	25	3232	4511	3616	1922	7.18	10.02	8.03	4.27
T8 (50 g kg^{-1} soil)	1483	73	NW	NW	17	3567	4372	3495	3694	2.4	2.94	2.35	2.49
T9 (75 g kg^{-1} soil)	2417	100	NW	NW	17	4189	4244	3944	4284	1.62	1.64	1.52	1.65
T10 (100 g kg^{-1} soil)	3283	100	NW	NW	17	1872	3850	3100	3105	0.57	1.19	0.95	0.96
T11 (sterilized soil)	0	NW	NW	NW	NW								
SE		±78.4	±7.57		±0.291	±404.6							
CV (%)		15.0	14.7		2.4	22.9							

(a) Rate of multiplication = Final population/initial population.
NW = No wilt.

TABLE III. - Relationship between initial *Fusarium oxysporum* f. sp. *ciceri* from wilt sick plot on wilt incidence in susceptible, moderately resistant, and resistant chickpea cultivars at ICRISAT Asia Center, Patancheru (*a*).

Treatment soil mixture ratio WSS:SSS	*Fusarium* propagules g⁻¹ soil before sowing	% Wilt incidence			*Fusarium* propagules g⁻¹ 60 days after sowing				Days to complete wilt in	Rate of multiplication			
		JG 62	K 850	WR 315	JG 62	K 850	WR 315	Unsown	JG 62	JG 62	K 850	WR 315	Unsown
W.S.S.	4233	100	0.0	0.0	4667	3883	3617	3400	27	0.73	1.15	0.69	0.79
1:1	2083	100	0.0	0.0	3083	2500	2267	2500	27	0.96	1.21	0.98	1.19
1:2	1867	100	0.0	0.0	2783	2533	1833	2067	32	0.98	0.09	0.71	1.10
1:4	833	100	0.0	0.0	2417	1783	1583	1217	37	1.47	2.11	1.08	1.45
1:8	483	100	0.0	0.0	1800	1633	1200	800	44	4.70	5.61	3.19	1.62
1:16	350	80	0.0	0.0	1400	1667	1117	850	49	5.28	7.52	3.65	2.38
1:32	183	40	0.0	0.0	1517	1833	1950	850	60	13.32	11.10	12.36	4.64
1:64	117	13	0.0	0.0	1450	1167	1900	517	60	20.76	25.75	15.79	4.43
1:128	83	6	0.0	0.0	1300	1717	1100	400	60	268.93	408.78	201.96	60.60
SE	±129.6	±9.49				±249.1			±2.997				
CV (%)	19.7	23.4				22.7			11.5				

(*a*) Experiment was kept up to 60 days only; Xylem browning in cv. K 850 and slight discolouring xylem in cv. WR 315 at 60 days recorded. Ratio of multiplication = $\frac{\text{Final population}}{\text{Initial population}}$; W.S.S. = Wilt sick soil; S.S.S. = Sterilized soil sand mixture.

days. At 60th day, final *F.o.* f. sp. *ciceri* population in soil was estimated in all the treatments after removing the plants along with the roots from the pots. The rate of multiplication of the pathogen was calculated by dividing the final population by the initial population.

Results and discussion

Fusarium oxysporum is a monocyclic pathogen. The increase in population of *F.o.* f. sp. *ciceri* in the soil is because of longevity of reproductive units (Haware *et al.*, 1986). There were significant differences in the initial propagules for all the inoculum levels in all 3 experiments. Irrespective of the differences in the inoculum levels, cv. JG 62 showed 100% wilting 15-21 days after sowing in the case of *F.o.* f. sp. *ciceri* race 1, 17-25 days for *F.o.* f. sp. *ciceri* race 2, 27-44 days in the case of wilt sick soil + sterilized soil (Tables I, II, and III). Under field conditions, cv. JG 62 normally takes 30 days for 100% wilting but under controlled environmental condition 100% wilting was noticed in 27 days (Table III). With increase in the ratio between WSS:SSS the number of days required for 100% wilting was higher (32-60 days). Among the cultivars tested, only cv. JG 62 showed wilting in all the experiments, and cvs. K 850 and WR 315 did not show any wilting at all. However, after 60 days xylem browning was observed in cv. K 850 by splitting the roots at collar region whereas cv. WR 315 showed high level of resistance in spite of the increase in the number of propagules for *F.o.* f. sp. *ciceri* (4233-4667 *F.o.* f. sp. *ciceri* propagules/g soil). Bhatti and Kraft (1992) reported that wilt or root rot of chickpea increased with increased level of inoculum in the soil.

The susceptible, moderately susceptible, and resistant cultivar supported multiplication of *F.o.* f. sp. *ciceri*, but showed significant differences in wilt incidence in relation to initial *F.o.* f. sp. *ciceri* population in the soil. Initial inoculum in the soil determines severity of soilborne pathogen, and it can be seen from the results in all three experiments that with high level of initial *F.o.* f.

sp. *ciceri* propagules 100% wilting was noted much earlier than those treatments with low initial *F.o.* f. sp. *ciceri* propagules, hence the technique of assessing the disease potential of soils ought to be applicable for early forecast of diseases induced by soilborne pathogens (Fry, 1982). The results confirm that it is possible to forecast the severity of chickpea disease from the knowledge of the initial pathogen population. In all the experiments with *F.o.* f. sp. *ciceri* race 2 and wilt sick soil, cv. K 850 supported higher *F.o.* f. sp. *ciceri* multiplication followed by resistant (cv. WR 315) over the highly susceptible (cv. JG 62). In moderately susceptible and resistant cultivars, the fungus infects the roots and enters the xylem and takes longer to colonize than the susceptible cultivar which dies within a month after sowing. Hence the amount of diseased tissues contributed to the soil by moderately susceptible and resistant cultivar will be higher than the susceptible cultivar.

This study highlighted the level of genetic resistance to wilt available in cultivated chickpea. The cultivar WR-315 was resistant to the pathogen at approximately 100 times the population needed to kill the susceptible cultivar cv. JG 62, but in spite of this high level of resistance, it contributed to *F.o.* f. sp. *ciceri* multiplication in the soil. Therefore, it is clear that irrespective of its disease reaction, the resistant cultivar supports *F.o.* f. sp. *ciceri* multiplication in the soil. It may not be possible to eliminate wilt pathogen from infested soil by growing resistant cultivars alone. The above results confirm that there is a need for other management practices such as crop rotation to reduce the inoculum density in the soil. This study has also provided information on the *F.o.* f. sp. *ciceri* threshold levels for chickpea cultivars with different levels of susceptibility to wilt disease caused by *F.o.* f. sp. *ciceri*.

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