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Pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates of Marathwada region (Maharashtra)

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Abstract
Fusarium oxysporum f. sp. *ciceri* causing wilt disease is an major loophole in the lesser productivity of chickpea in Marathwada region (Maharashtra). It is an highly variable pathogen. However the high pathogenic variability may limit the effectiveness of resistant cultivars. So the present study was conducted to determine the pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region (Maharashtra) using sick soil inoculation method and water culture method. In sick soil inoculation method, FOC-8 isolate (Parbhani) was proved most virulent on chickpea wilt susceptible cultivar JG-62 with mortality percent (89.33%), followed by FOC 5 (Nanded isolate) and FOC 1 (Hingoli isolate) with 86.66% and 84.48% respectively. In water culture method, all isolates of *Fusarium* were showed 100 percent mortality of chickpea seedlings, where isolate FOC-8 (Parbhani isolate) showed early symptoms on seedlings.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, pathogenic variability, Marathwada region, sick soil inoculation method, water culture method, mortality

1. Introduction
Chickpea, Bengal gram, or Garbanzo bean is an annual legume belonging to the Fabaceae family and sub family: Faboideae. It is said to be one of the world's oldest and third most important pulses, after beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) that are grown in Asia and Europe. Globally, Bengal gram is produced on an area of 137 lakh ha. with a production of 142.4 lakh tonnes and productivity of 1038 kg/ha (Anonymous, 2019) [1]. India supplies 70 percent of the world's bengal gram production of 116.2 lakh tonnes cultivated under 112 lakh ha. with productivity of 1036 kg/ha in 2020-21 (Anonymous, 2021) [3]. India is the largest producer of world gram production followed by Australia, Myanmar and Ethiopia (Anonymous, 2019) [1].

Maharashtra contributes the total area of 20.38 lakh ha. under chickpea cultivation with the production of 17.29 lakh tonnes and productivity was 848.55 kg/ha. (Anonymous, 2020) [2]. It is one of the significant *rabi* pulse crop grown in Marathwada region of Maharashtra state. In Marathwada region, chickpea was cultivated on an area of 10.59 lakh ha. with a production of 7.96 lakh tonnes and productivity of 707.56 kg/ha (Anonymous, 2020) [2].

Despite the high total production, yields of chickpea are low due to wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* causing devastating yield losses to the tune of 100 percent in severe condition (Jalali and Chand, 1992) [5]. In Marathwada region (Maharashtra), the disease incidence is widely distributed ranged from 21.50-33.06 percent (Sontakke *et al*., 2020) [11]. The typical symptoms of chickpea wilt disease characterized by drooping of petioles and rachis, yellowing and drying of leaves, browning in vascular bundles, and finally wilting of plants.

Fusarium oxysporum f. sp. *ciceri* is seed and soil borne, facultative saprophytic fungus belongs to Kingdom: Fungi, Phylum: Ascomycota, Class: Sordariomycetes, Order: Hypocreales and Family: Nectriaceae. The pathogen survives in the soil in the form of conidia (Microconidia and macroconidia), chlamydospores and mycelia (Jimenez-Diaz *et al*., 2010) [6]. Since the disease is soil borne, it is difficult to manage the disease either through crop rotation or application of chemicals. The pathogen has ability to survive for a longer time up to six years even in the absence of host (Haware *et al*., 1986) [4]. Due to its soil borne nature, exploitation of resistance sources is an feasible option for the management of disease. *Fusarium oxysporum* f. sp. *ciceri* is an highly variable pathogen, however the high pathogenic variability may limit the effectiveness of resistance.

So the present study was conducted to determine the pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region (Maharashtra).

2. Materials and Methods

Chickpea plants exhibiting typical symptoms of chickpea wilt disease were collected from three different agroclimatic zones of Marathwada region, Maharashtra during *Rabi* season of 2020-21. Such symptomatic plants showing visible symptoms such as yellowing, withering, drying of leaves, complete drying of plants, partial wilting and vascular discoloration etc. were collected in the paper bags, labelled with requisite information, brought to the laboratory and subjected to the tissue isolation on potato dextrose agar medium.

2.1 Isolation and purification of *Fusarium* wilt pathogen

Chickpea plants showing typical wilt symptoms were collected from Parbhani, Nanded, Aurangabad, Jalna, Beed, Hingoli, Latur and Osmanabad districts of Marathwada region. Tissue isolations (Tuite, 1969) [12] were made to isolate associated pathogen from wilted plants collected from different locations and purified using hyphal tip technique. The pure cultures of the isolates were maintained on PDA slants and stored at 4 ± 1 °C in laboratory for further studies.

2.2 Pathogenicity and pathogenic variability

Pathogenicity of *Fusarium oxysporum* f. sp. *ciceri* isolates will be proved by applying sick-soil inoculation technique and water agar technique using susceptible chickpea cv. JG-62 under controlled conditions.

In the sick soil inoculation technique, healthy seeds of susceptible chickpea cv. JG-62 will be sown (10 seeds / pot) were sown in the pots containing sick soil of test isolates of pathogen *Fusarium oxysporum* f. sp. *ciceri* @ 2% w/w inoculated separately in to earthen pots. Three replications were maintained for all treatments. The observations on seed germination, pre-emergence seedling mortality and post-emergence seedling mortality were recorded after 25 days of sowing. Based on these observations, pathogenic/non-pathogenic potential of the test isolates of pathogen were determined.

Pathogenicity of all test isolates of *Fusarium oxysporum* f. sp. *ciceri* was also be proved by applying water culture technique (Nene and Kanniyan, 1982) [8]. Water agar @ 1 percent will be prepared, dispensed in sterile glass test tubes, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure for 15 min. After cooling at room temperature, these tubes were inoculated with the pure culture discs of the test pathogens, separately and incubated at room temperature for 4-5 days. Then about 25 days old seedlings of chickpea cv. JG-62 with intact root system were transplanted into test tubes suspension and incubated at room temperature. The chickpea seedlings transplanted into the test tubes with sterile water were maintained as control. Three replications were maintained for each treatment. After a week of transplanting, observations were recorded for seedling mortality. Re-isolation was made from such affected portions of plant tissues and compared with that of original culture. Based on percentage of mortality and time taken for disease incidence, virulent isolate was selected for further studies.

3. Results and Discussion

3.1 Symptomatology

Symptoms observed during survey in the chickpea fields were yellowing and drooping of leaves. At severe stage, partial wilting or complete wilting of plant has been observed. When roots of the plant split open longitudinally, it showed internal brown vascular discoloration of the plant (Fig 1).

In case of artificially inoculated plants, symptoms appeared at 25 to 30 days after sowing in wilt susceptible cv. of chickpea JG-62. Initially diseased plants showed drooping of leaflets followed by chlorosis, stunted growth and finally complete drying of the plant. The prominent wilting starts from seedling to adult stage of the plants. When split open longitudinally, infected plant roots shown brownish discoloration of vascular tissues. These symptoms were developed due to the clogging of xylem vessels with the mycelia of pathogen, which obstructs the flow of water and nutrients within the plant and leads to the yellowing and wilting of the diseased plants. Further, it led to browning of vascular tissues. Initially lower stems begin to wither and finally complete chlorosis and withering occurred.

(Healthy field) (Diseased field)

Fig 1: Symptoms of chickpea *Fusarium* wilt under field conditions

3.2 Isolation, purification and designation of *Fusarium* wilt pathogen isolates

The *Fusarium* isolates collected from eight districts of Marathwada region were identified based on morphological and cultural characteristics. The identified *Fusarium* isolates were designated, based on location of collected samples and presented in Table 1 & Figure 2.

Table 1: Designation of *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region, Maharashtra

Sr. no.	Location	Isolate code	GPS location		
			Latitude	Longitude	Altitude (mt)
1	Hingoli	FOC 1	19.75151	75.71392	290.09
2	Osmanabad	FOC 2	18.55863	76.24682	578.01
3	Latur	FOC 3	17.88761	76.48266	595.70
4	Jalna	FOC 4	19.86864	75.71734	521.00
5	Nanded	FOC 5	19.13604	77.34759	294.38
6	Beed	FOC 6	18.99046	75.95262	435.73
7	Aurangabad	FOC 7	20.28367	75.37571	124.87
8	Parbhani	FOC 8	19.71867	76.52347	455.19

Fig 2: *Fusarium oxysporum* f. sp. *ciceri* isolates collected from Marathwada region (Maharashtra)

3.3. Pathogenicity test and pathogenic variability

Pathogenicity test of eight isolates of *Fusarium oxysporum* f. sp. *ciceri* was performed on susceptible cv. JG-62 by using sick soil inoculation method and water agar method under screen house and laboratory conditions.

In sick soil inoculation method, after 25-30 days of inoculation, all the isolates were proved pathogenic by showing typical wilting symptoms *i.e.* yellowing and drying of leaves, drooping of petioles, partial withering of plants and finally death of entire plants, whereas no symptoms were observed on control (Figure 3). Similarly in water agar method, after one week of transplanting in test tubes, all isolates showed mortality symptoms in chickpea seedlings. Re isolations were made and compared with the original cultures. All the eight isolates, satisfied the Koch's postulates, hence, proved the pathogenicity. (Figure 3a).

In sick soil inoculation method, among 8 *Fusarium* isolates, FOC-8 was proved most virulent on the basis of least seed germination percent (21.34%), highest pre-emergence mortality percent (78.66%), post emergence mortality percent (100%) and mean mortality percent (89.33%) (Plate 4.7). Followed by FOC 5 (Nanded isolate) and FOC 1 (Hingoli isolate) with seed germination percent (26.68%, 31.04%), pre-emergence mortality percent (73.32%, 68.96%), post emergence mortality percent (100%, 100%) and mean mortality percent (86.66%, 84.48%). While control showed 100 percent seed germination with zero percent mortality of chickpea seedings (Table 2 & Figure 3a).

In water agar method, all isolates of *Fusarium* were showed 100 percent mortality of chickpea seedlings, where isolate FOC-8 showed early symptoms on seedlings at 4th day. Thus, based on above results, isolate FOC-8 was proved most virulent which were used for further studies (Table 2 & Figure 3b).

(A)

(B)

Fig 3: Pathogenicity test and pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates a) Sick soil inoculation method b) Water culture method

Observations recorded were in the same line with the reports of Kohire *et al.* (2012) [7], who demonstrated the pathogenicity of the *Fusarium oxysporum* f. sp. *ciceri* by sick soil inoculation method using cv. JG-62 which exhibited wilting symptoms after 30 days of inoculation with 40 percent pre-emergence mortality and 72 percent of post-emergence mortality. Similarly, Qureshi *et al.* (2021) [10] proved the pathogenicity of wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* on variety RVKG-101 in sick soil pot. Further, Patil *et al.* (2017) [9] also confirmed the pathogenicity of the *F. oxysporum* f. sp. *ciceri* isolates using highly susceptible cv. JG-62 by using water culture technique, isolates 1-28, 1-13, and 1-1 took five days for the initiation of disease, on the other hand, isolates 1-19 and 1-20 took seven and eight days, respectively.

Table 2: Pathogenicity test and pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* isolates

Sr. no.	Isolate code	Sick soil inoculation method				Water agar method
		*Germination (%)	Mortality (%)			Mortality (%)
			*Pre emergence mortality (%)	Post emergence mortality (%)	*Mean mortality (%)	
1	FOC 1	31.04 (33.82)	68.96 (56.13)	100.00	84.48 (66.78)	100.00
2	FOC 2	43.62 (41.31)	56.38 (48.65)	100.00	78.19 (62.14)	100.00
3	FOC 3	49.41 (44.64)	50.59 (45.32)	100.00	75.30 (60.17)	100.00
4	FOC 4	54.49 (47.57)	45.51 (42.41)	100.00	72.76 (58.51)	100.00
5	FOC 5	26.68 (31.07)	73.32 (58.90)	100.00	86.66 (68.57)	100.00
6	FOC 6	33.21 (35.20)	66.79 (54.80)	100.00	83.40 (65.94)	100.00
7	FOC 7	36.62 (37.22)	63.38 (52.75)	100.00	81.69 (64.65)	100.00
8	FOC 8	21.34 (27.48)	78.66 (62.48)	100.00	89.33 (70.92)	100.00
9	Control	100.00 (90.00)	0.00 (0.00)	0.00	0.00 (0.00)	0.00
S. Em (±)		1.120	0.761	-	0.481	-
C.D. (P=0.01)		1.583	2.279	-	1.439	-

*Figures in the parenthesis are angular transformed values

4. Conclusion

The survey in the chickpea growing areas of eight districts of Marathwada region (Maharashtra) revealed the prevalence of *Fusarium* wilt in all the locations surveyed and occurrence of wide pathogenic variability of pathogen. Among eight isolates collected, *Fusarium oxysporum* f. sp. *ciceri* isolates of Parbhani, Nanded and Hingoli district proved to be more virulent in the virulent assays of sick soil pots and water culture assays.

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