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Prevalence of *Fusarium oxysporum* f. sp. *ciceris* Causing Wilt in Chickpea and Its Pathogenic, Cultural and Morphological Characterization

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A B S T R A C T

Chickpea (*Cicer arietinum* L.) contributes 18% of the global production of grain legume and provides as an important source of dietary protein for living things. The area and production of chickpea has been reduced due to several abiotic and biotic factors. Among them soil borne pathogen of *F. oxysporum* f. sp. *ciceris* causing severe yield loss now a days. In this study conducted for a prevalence of wilt incidence percentage varied from 34.00 to 57.33 per cent in chickpea due to *F. oxysporum* f. sp. *ciceris* in Tamil Nadu. Continuously the pathogenic ability, cultural and morphological characterization was carried out. Among the fifteen isolates Foc4 (Gomangalampudur) is highly pathogenic when compared to other and causing early wilt in JAKI-9218. Grouping of isolates based on their virulence potential isolates like, Foc4, Foc5, Foc6, Foc8, Foc10, Foc11, Foc12, Foc13 and Foc14 are highly pathogenic nature and other isolates were strongly pathogenic. The cultural variability of these isolates have pale yellowish to dark pinkish (Foc4) in pigmentation with aerial compact mycelial growth within 7 – 9 DAI. The morphological characterization all the isolates produced micro, macro-conidia and chlamydospores within 20 DAI and the size of the spores varied from (micro conidia) 5.6 x 2.5 µm (Foc2) to 12.7 x 3.1 µm (Foc14) and the isolate (Foc4) maximum size of macro conidia in 29.1x 4.9 µm and mycelial dry weight of 700 mg at 100 ml.

Introduction

Chickpea (*Cicer arietnum* L.) is a most important grain legume crop of *Cicer* genus and cultivated throughout the world for its easy available form of edible proteins and vitamins. In India it is cultivated at cool winter (Rabi) season in semi arid tropics by irrigated or rain-fed conditions (Nene *et al*., 1984). India is largest producer of chickpea in world sharing 65.25 per cent in area and 65.49 per cent in production and is grown on 10.23 million ha area with production 9.88 million tonnes and productivity 967 kg/ha (Thaware *et al*., 2017). Despite the production was reduced due to several biotic and abiotic factors. Chickpea is noticed to be more than 52 pathogens at cropping season (Harware and Nene, 1980; Nene *et al*., 1984). Among these pathogens *F. oxysporum* f. sp. *ciceris*

causing a potential yield loss for both in seed yield and seed weight by wilt about 10 to 15 per cent (Navas-Cortes *et al*., 2000b; Khilare *et al*., 2009).

Fusarium oxysporum f. sp. *ciceris* is ubiquitous soil borne pathogen and providing severe economic losses about 10-40% in worldwide (Kaiser *et al*., 1994). *Fusarium* genus was highly variable nature in survive, growth and infection in all seasons with crop or without also. Because absence of susceptible host it can survive in soil due to their production of resting spores like, micro, macro conidia and chlamydospores for distribution with diverse niches (Leslie and Summerell, 2006; Nelson *et al*., 1983). Hence traditionally following methods *viz*., crop rotation, using resistant cultivars, chemical managements is presence with some limitation factors like, location specific pathogen races and wide geographical distribution (Singh *et al*., 2006). So, the cultural and morphological variability is primary diagnosis for typical identification of different isolates of *F. oxysporum* f. sp. *ciceris* and classically determining by their virulence ability.

In the present study was carried out for disease prevalence and extensively discriminating the different isolates of *F. oxysporum* f. sp. *ciceris* by cultural, morphological characterization and virulence ability through grouping it.

Materials and Methods

Survey and occurrence

An extensive survey was conducted in major chickpea growing areas of Tamil Nadu during Rabi, 2015. In each districts locations were selected randomly and a total of fifteen locations were selected from four districts for assessment of wilt incidence by *F. oxysporum* f. sp. *ciceris*. The name of the villages surveyed along with districts given in Table 1. The wilt incidence was calculated by using the following formula and impact of disease incidence grouped into classes like, (0% - Nil, 0.1 - 1.0 % - low, 1.1 – 20.0% - moderately high, 20.1-50.0% - high and >50.0% - very high) (Traperos-Casas, 1983).

Per cent disease Incidence =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Fungal isolates

A large systematic collection of fungal isolates were used in this study, consisting of 15 isolates from infected plant samples. The infected root bits were surface sterilized with 1% sodium hypochlorite for 30 seconds, and subsequently three washings were given with sterile distilled water. Then, they were placed in sterilized Petri dishes containing potato dextrose agar (PDA) medium (Potato 200 g, dextrose 20 g, agar 15 g and water 1L) and incubated at the laboratory conditions at 25 ± 2°C for seven days. Pure culture of the pathogen was obtained by single hyphal tip method (Rangaswami, 2005).

Pathogenic characterization

Pathogenic ability was assessed by using local cultivar of JAKI-9218 by the method derived by Harware and Nene (1980). The mixtures of sand and chickpea meal (90 g sand + 10 g chickpea meal) in 250 ml conical flasks were inoculated with 5 plugs (8mm in diameter) from different isolates of *F. oxysporum* f. sp. *ciceris* well grown on PDA medium containing Petri plates at 23 ± 1°C for ten days. Fifteen days after incubation the multiplied fungal mass was well mixed with 2kg of autoclaved soil in 15cm diameter plastic pots. Five seeds were sown in each pot and kept under 25 ± 1°C with relative humidity of 30-50%. In each isolate three

replications were maintained and the control was maintained by uninoculated healthy. These were kept in glasshouse conditions for 40 days till flowering. Plants were observed for symptom development and the pathogenic variability given in to five groups *viz*., (0% wilting – Non pathogenic; 1-20% wilting – weakly pathogenic; 21-50% wilting- moderately pathogenic; 51-70% wilting- strongly pathogenic and >70% wilting- highly pathogenic) was described by Rakhonde *et al.,* (2015).

Cultural characterization

Fifteen isolates of *Fusarium oxysporum* f. sp. *ciceris* obtained aseptically and invidually inoculated in PDA contained Petri dishes and incubated at 28 $\pm$ 2°C for a week. Observations of cultural characteristics *viz*., Colony morphology, colony diameter, growth rate, growth habitat, pigmentation and sporulating potential were recorded at two weeks after incubation respectively. The grouping was done in the basis of mycelial growth for Slow (10 mm/day), medium (10-12 mm/day) and fast (>12 mm /day) denoted by Dubey *et al.,* (2010).

Morphological characterization

The morphological characteristics of conidial size (Micro and macro conidia) in respect of each test isolate were studied. The size of conidia was measured using ocular micrometer (calibrated using stage micrometer) under the compound microscope (Labomed Vision 2000) at 400X magnification.

Results and Discussion

Understanding the basic information of pathogen's *viz*., pathogenic diversity, cultural and morphological variability was most playing a important role in the development of durable resistance. *F*. *oxysporum* f. sp. *ciceris* is a highly variable nature on other soil borne pathogens. Because of their survival nature and virulence is known to be a vital role in disease incidence and resistance occurrence in chickpea plants.

Survey and occurrence

Survey was conducted to assess the incidence of wilt disease in major chickpea growing districts of Tamil Nadu *viz*., Coimbatore, Dindigul, Dharmapuri and Tiruppur during Rabi, 2015 (Table 1; Plate 1). The result revealed that the maximum disease incidence of 57.33 per cent was recorded at Gomangalampudur in Tiruppur district of cultivar JAKI-9218 and a minimum of 34.00 per cent incidence was recorded at Idigarai in Coimbatore district in CO4 cultivar (Table 1, Figure 1). These results revealed that Kumar *et al.,* (2012) he reported that the chickpea was attained highly incidence of *F*. *oxysporum* f. sp. *ciceris* at 38.79 to 59.23 in Ranchi of Jharkhand state. In 25 to 48 per cent of local cultivars of chickpea in field conditions were influenced by wilt at 9.7% to 13.8% in major central and southern parts of India (Ghosh *et al*., 2013).

Collection of fungal isolates

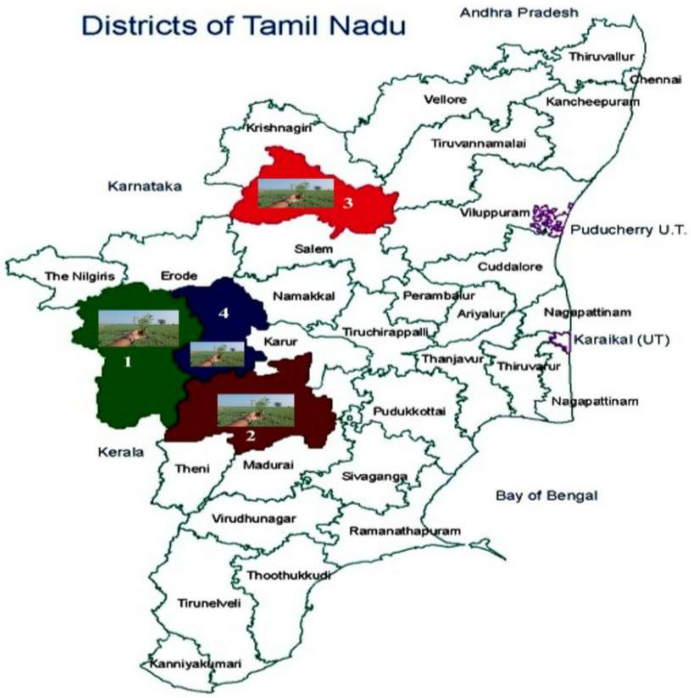
An extensive survey was conducted during Rabi, 2015 in major chickpea growing areas of Tamil Nadu. The prevalent incidence of wilt was recorded and the fifteen *Fusarium oxysporum* f. sp. *ciceris* isolates were isolated and confirmed through their production of spores and phenotypic appearance of cultural growth.

Pathogenic characterization

The pathogenic variability test indicates that all the fifteen isolates of *Fusarium oxysporum*

f. sp. *ciceris*, proved to be pathogenic to local cultivar JAKI-9218. The fifteen isolates were grouped under five forms like, (Non pathogenic, weakly pathogenic and moderately pathogenic have no represent isolates). Among the all isolates, there are six isolates *viz*., Foc1, Foc2, Foc3, Foc7, Foc9 and Foc15 were strongly pathogenic nature and causing wilt incidence ranged from 53.3 % to 66.7 %. Another nine isolates *viz*., Foc4, Foc5, Foc6, Foc8, Foc10, Foc11, Foc12, Foc13 and Foc14 were recorded incidence at 73.3 % to 93.3 % under highly pathogenic respectively (Table 2 and 5). These results revealed that Sharma *et al.*, (2009) reported forty eight *F. oxysporum* f. sp. *ciceris* isolates from India, among the forty one isolates have been identified as highly pathogenic and remaining seven isolates were non-pathogenic respectively. The existence of pathogenic variability in *F. oxysporum* f. sp. *ciceris* isolates was also reported by Gupta *et al.*, (1986), Paul *et al.*, (2001) and Mandhare *et al.*, (2011) from isolated from different regions of India.

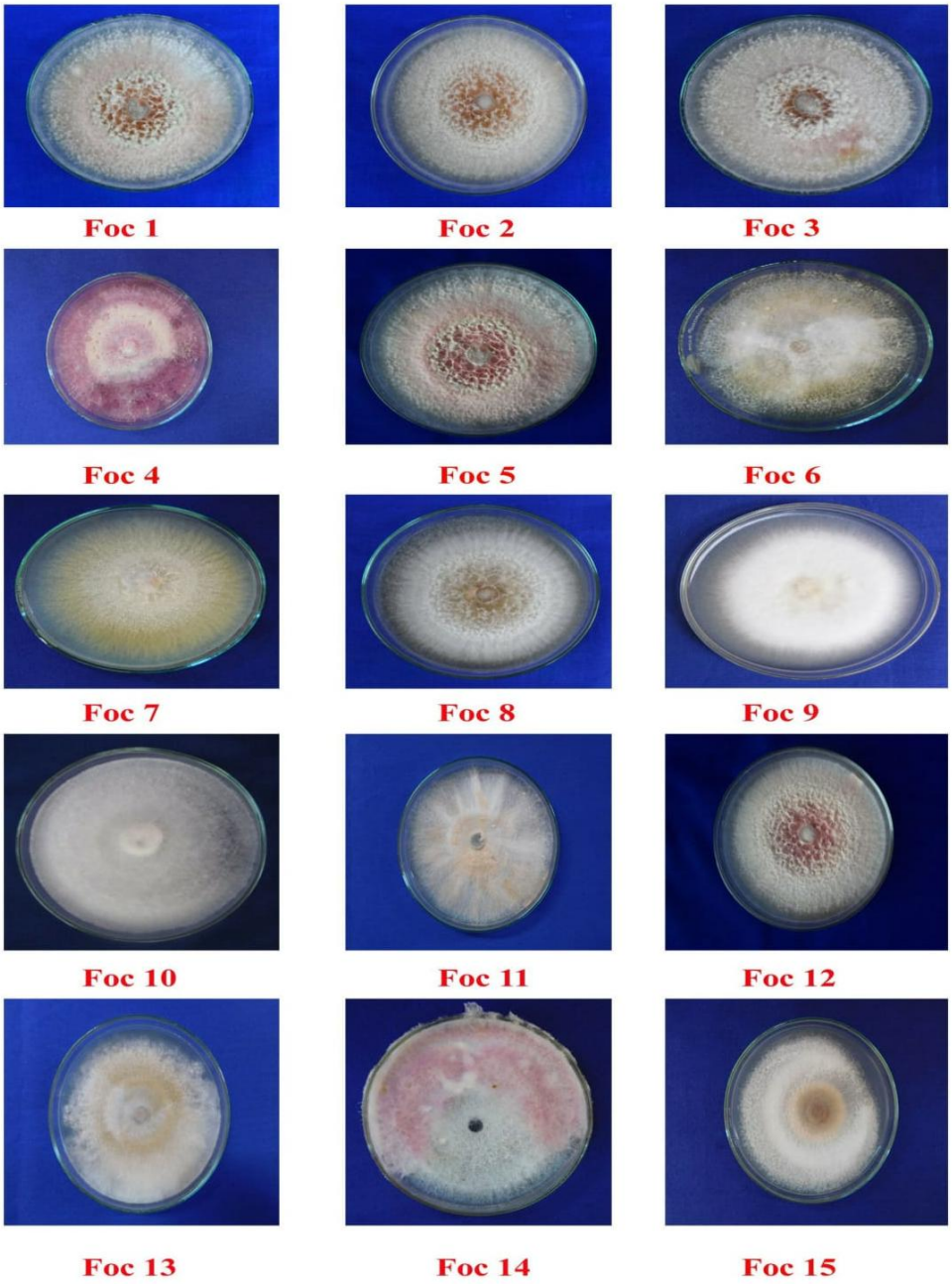
Figure 1. Survey for the incidence of wilt in major chickpea growing areas of Tamil Nadu



Survey conducted districts

1. **Coimbatore**
2. **Dindigul**
3. **Dharmapuri**
4. **Tiruppur**

Figure 2. Different isolates of *Fusarium oxysporum* f. sp. *ciceris* from major chickpea growing areas of Tamil Nadu



Foc1- Adivalli
Foc2- Anthiyur
Foc3- Athakkampapu
Foc4- Gomangalampudur
Foc5- Idigarai
Foc6- Konnur
Foc7- Modakkupatti
Foc8- Mukkonam
Foc9- Pannaikinaru
Foc10- Periyanayakanpalayam
Foc11-Poolankinaru
Foc12- Ragalpavi
Foc13- Ramachandrapuram
Foc14- Thippampatti
Foc15- Valzavadi

Figure 3. Microscopic view of *Fusarium oxysporum* f. sp. *ciceris* isolates

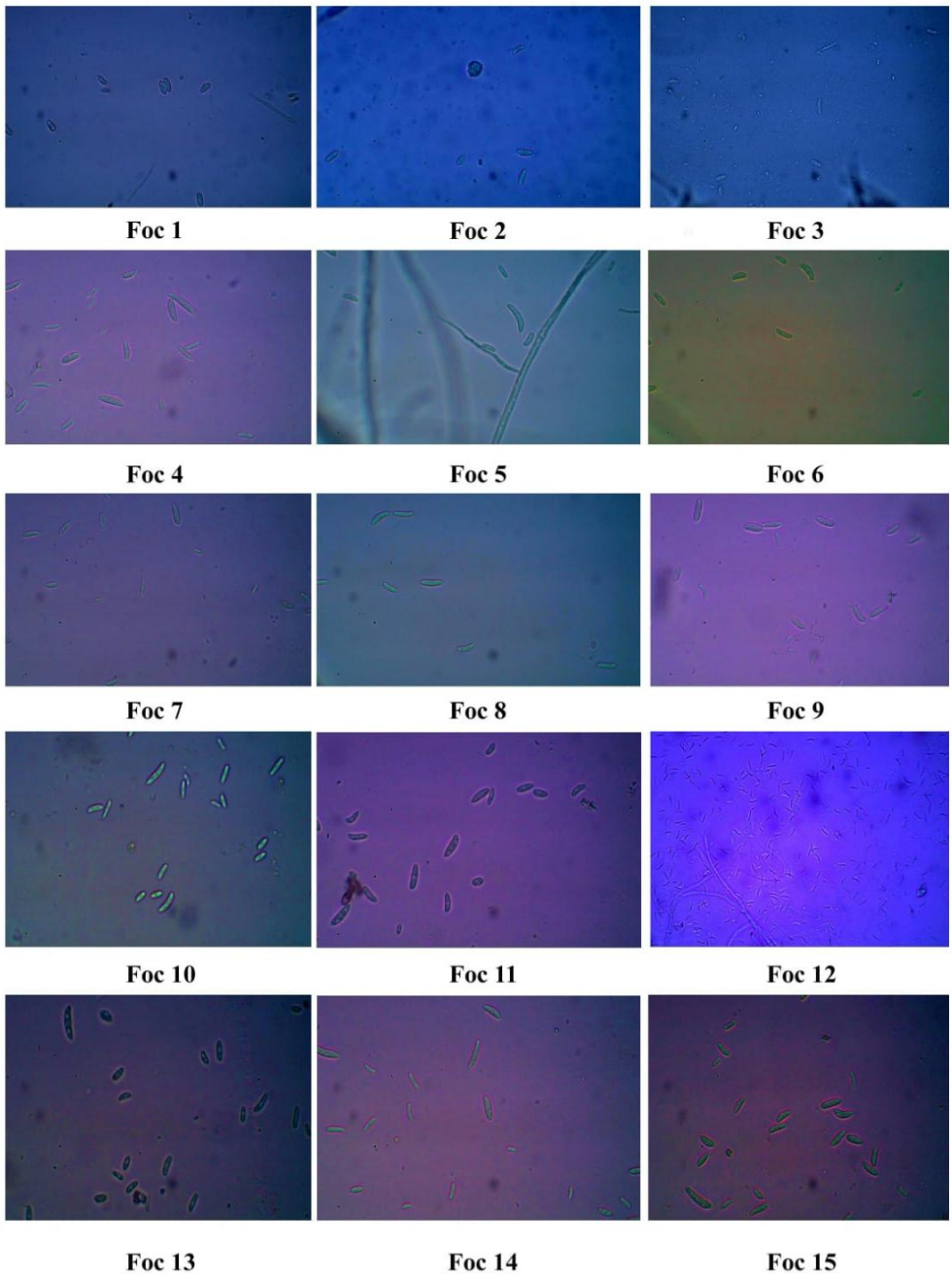


Table.1 Survey and occurrence of *Fusarium* wilt disease incidence of major chickpea (*C. arietinum* L.) growing areas of Tamil Nadu

S. No.	Districts	Location	Cultivar	Isolate No	Per cent disease incidence (PDI)*	Impact
1	Coimbatore	Idigarai	CO4	Foc5	34.67^{h}	High
		Periyanakanpalayam	CO4	Foc10	41.00^{def}	High
2	Dharmapuri	Athakampapu	JAKI -9218	Foc3	35.33^{h}	High
3	Dindigul	Konnur	JAKI-9218	Foc6	39.67^{defg}	High
4	Tiruppur	Adivalli	JAKI-9218	Foc1	37.00^{gh}	High
		Anthiyur	JAKI-9218	Foc2	38.33^{fg}	High
		Gomangalampudur	JAKI-9218	Foc4	57.33^{a}	Very high
		Modakkupatti	JAKI-9218	Foc7	42.33^{cd}	High
		Mukkonam	JAKI-9218	Foc8	44.33^{bc}	High
		Pannaikinaru	JAKI-9218	Foc9	39.33^{efg}	High
		Ragalpavi	JAKI-9218	Foc12	37.33^{gh}	High
		Ramachandrapuram	JAKI-9218	Foc13	41.33^{de}	High
		Poolankinaru	JAKI-9218	Foc11	40.67^{def}	High
		Thippampatti	JAKI-9218	Foc14	46.33^{b}	High
		Valzavadi	JAKI-9218	Foc15	42.33^{cd}	High

Means followed by a common letter are not significantly different at the 5% level by the DMRT.

Table.2 Pathogenic variability for different isolates of *Fusarium oxysporum* f. sp. *ciceris* against with local cultivar JAKI-9218 under *in vitro* conditions

S. No	Locations	Isolate Name	Germination (%)	(%) Wilting	Type of pathogenic
1	Adivalli	Foc1	80.0	53.3	SP
2	Anthiyur	Foc2	86.7	53.3	SP
3	Athakkampapu	Foc3	86.7	60.0	SP
4	Gomangalampudur	Foc4	100.0	93.3	HP
5	Idigarai	Foc5	86.7	73.3	HP
6	Konnur	Foc6	93.3	80.0	HP
7	Modakkupatti	Foc7	93.3	60.0	SP
8	Mukkonam	Foc8	100.0	73.3	HP
9	Pannaikinaru	Foc9	80.0	66.7	SP
10	Periyanakanpalayam	Foc10	93.3	73.3	HP
11	Poolankinaru	Foc11	100.0	73.3	HP
12	Ragalpavi	Foc12	100.0	80.0	HP
13	Ramachandrapuram	Foc13	100.0	80.0	HP
14	Thippampatti	Foc14	93.3	86.7	HP
15	Valzavadi	Foc15	93.3	66.7	SP
16	Control	Uninoculated	100.0	0.00	NIL

Table.3 Cultural characteristics for different isolates of *F. oxysporum* f. sp. *ciceris*

S. No	Isolate No	Colony morphology	Mean mycelial growth (mm) / 7DAI	Growth rate (mm)	Growth habitat	No. of. days taken to cover the plate	Pigmentation	Sporulation
1.	Foc 1	Circular compact aerial mycelia	80.00 (63.43)	11.42^{de}	Medium	8	Pale pinkish	1-2 celled sparsely dispersed microconidia
2.	Foc 2	Smooth circular compact aerial mycelia	77.00 (61.34)	11.00^{f}	Medium	9	Pale pinkish with white	Sparsely dispersed microconidia with minimum curvature
3.	Foc 3	Circular profuse compact aerial mycelia	74.00 (59.34)	10.57^{g}	Medium	9	Pale pinkish with white	Sparsely dispersed microconidia
4.	Foc 4	Circular compact 5.aerial mycelia	90.00 (71.56)	12.85^{a}	Fast	**7**	Deep pinkish	Abundantly dispersed micro and 3-5 septate macroconidia
5.	Foc 5	Circular smooth compact aerial mycelia	77.00 (61.34)	11.00^{f}	Medium	9	Pale pinkish	Sparsely dispersed microconidia and macroconidia
6.	Foc 6	Circular sparsely dense aerial mycelia	78.67 (62.48)	11.23^{e}	Medium	9	Pale yellowish	1-2 celled sparsely dispersed microconidia
7.	Foc 7	Circular sparsely flattened mycelia	80.67 (63.91)	11.52^{d}	Medium	8	Yellowish with centre white	1-2 celled Abundantly dispersed microconidia
8.	Foc 8	Circular smooth compact aerial	83.00 (65.65)	11.85^{c}	Medium	8	Pale white	Sparsely dispersed 1-2 celled

		mycelia						microconidia
9.	Foc 9	Circular smooth dense mycelia	75.33 (60.22)	10.76^{g}	Medium	9	Milky white	Abundantly dispersed microconidia
10.	Foc 10	Circular smooth compact mycelia	69.67 (56.58)	9.95^{hi}	Slow	10	Milky white	Abundantly dispersed microconidia and 3-5 celled macroconidia
11.	Foc 11	Circular sparsely dense flattened mycelia	70.33 (57.62)	10.04^{h}	Medium	10	Pale yellowish	Abundantly dispersed 1-2 celled microconidia with minimum curvature
12.	Foc 12	Smooth circular compact mycelia	71.33 (57.62)	10.19^{h}	Medium	10	Pale pinkish	Abundantly dispersed 3-5 celled macroconidia
13.	Foc 13	Circular smooth sparsely dense mycelia	70.33 (56.99)	10.04^{h}	Medium	10	Pale yellowish	Abundantly dispersed 1-2 celled microconidia
14.	Foc 14	Circular compact aerial mycelia	86.00 (68.05)	12.28^{b}	Fast	8	Deep pinkish	Abundantly dispersed 3 - celled macroconidia
15.	Foc 15	Circular smooth compact aerial mycelia	68.33 (55.62)	9.76^{i}	Slow	10	Milky white with pale yellowish	Abundantly dispersed microconidia

Table.4 Conidial characteristics for different isolates of *F. oxysporum* f. sp. *ciceris*

S. No.	Isolate No	Microconidia		Macroconia		No of conidia / µL	Mycelial dry weight (mg)/ 100ml*
		Length (µm)	Width (µm)	Length (µm)	Width (µm)		
1.	Foc 1	6.5	2.6	16.9	4.1	8	553^{hi}
2.	Foc 2	5.6	2.5	17.3	3.8	5	550^{fg}
3.	Foc 3	6.9	2.7	18.5	4.1	5	540^{gh}
4.	Foc 4	11.7	2.9	29.1	4.9	18	700^{b}
5.	Foc 5	10.5	2.7	20.2	3.8	7	653^{d}
6.	Foc 6	11.3	2.8	21.2	4.4	9	663^{d}
7.	Foc 7	8.1	2.6	20.5	4.2	15	535^{hi}
8.	Foc 8	8.8	2.8	22.9	4.1	7	678^{c}
9.	Foc 9	10.9	2.6	24.9	4.3	15	528^{ij}
10.	Foc 10	7.8	2.6	28.4	4.4	17	522^{j}
11.	Foc 11	8.1	2.7	20.6	3.8	18	597^{e}
12.	Foc 12	10.9	3.0	25.6	4.5	70	551^{f}
13.	Foc 13	10.1	2.6	22.0	4.4	18	496^{k}
14.	Foc 14	12.7	3.1	23.6	4.4	18	723^{a}
15.	Foc 15	11.0	2.7	26.3	4.1	22	492^{k}

* Mean of three replications

Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table.5 Grouping of *F. oxysporum* f. sp. *ciceris* isolates on the basis of pathogenic nature and growth habitat

S. No	Pathogenic Nature		Growth Habitat	
	Group ((% wilting)	Name of the isolates	Group (Colony diameter mm/day)	Name of the isolates
1.	Non-pathogenic (0%)	Nil	Slow (10mm /day)	Foc10 and Foc15
2.	Weakly pathogenic (1-20%)	Nil	Medium (>10-12mm / day)	Foc1, Fo2 Foc3, Foc5, Foc6, Foc7, Foc8, Foc9, Foc11, Foc12 and Foc13
3.	Moderately pathogenic (21-50%)	Nil	Fast (>12mm / day)	Foc4 and Foc14
4.	Strongly pathogenic (51-70%)	Foc1, Foc2, Foc3, Foc7, Foc9 and Foc15		
5.	Highly pathogenic (>70%)	Foc4, Foc5, Foc6, Foc8, Foc10, Foc11, Foc12, Foc13 and Foc14		

Cultural characterization

All the fifteen isolates of *Fusarium oxysporum* f. sp. *ciceris* exhibited a high variability in colony morphology, colony diameter, growth rate, growth habitat, pigmentation and sporulating potential respectively. The colony morphology varied from compact dense aerial mycelia to sparsely flattened mycelia of pale yellowish to deep pinkish coloured with slow to fast growth habitat. Among the fifteen isolates (Foc4) recorded a three types of growth like, slow (Foc10 and Foc15), medium (Foc1, Foc2, Foc3, Foc5, Foc6, Foc7, Foc8, Foc9, Foc11, Foc12 and Foc13), fast (Foc4 and Foc14) are recorded and abundantly production of micro and macro conidia in 7 DAI (Day after inoculation) (Table 3 and 5, Figure 2). These results are coincided with earlier workers like, Gupta *et al.,* (1986), Desai *et al.,* (1994). Burgess *et al.,* (1989) reported that the *Fusarium oxysporum* were extensively variable of cultural and morphological diversity and it's concluded for identification of genus not species.

Morphological characterization

All the isolates were highly variable in morphological *viz*., production of micro, macro conidia and chlamydospores. The mean size of micro conidia of the test isolates ranged from 5.6 x 2.5 µm (Foc2) to 12.7 x 3.1 µm (Foc14) and the isolate (Foc4) maximum size of macro conidia in 29.1 x 4.9 µm and mycelial dry weight of 700 mg in 100 ml and highly pathogenic also (Table 4 and Figure 3). Above the results were revealed that Dubey *et al.,* (2010) documented one hundred and twelve isolates by twelve categories, among the isolates were produced micro conidia size varied from 5.1-12.8 x 2.5-5.0 µm and macro conidia (16.5-37.9 x 4.0-5.9 µm) with 1-5 septations. Kaur *et al.,* (2015) reported twenty four isolates of *Fusarium oxysporum* f. sp. *ciceris* produced significant variation to size of micro (8.9-16.9 x 3.1-6.3 µm) and macro (21.7-64.9 x 2.7-10.0 µm) conidia was observed also.

In conclusion, collection of different isolates of *F. oxysporum* f. sp. *ciceris* was highly variable nature through their presence pathogenic nature and growth habitat and cultural characters also. Because the virulent isolate of *F. oxysporum* f. sp. *ciceris* (Foc4) growing fast and production of spores abundantly within seven days and their causing severe incidence in local cultivar of JAKI-9218 in Tamil Nadu.

Acknowledgements

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