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STUDIES ON CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY AMONG THE ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* CAUSING WILT OF CHICKPEA

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ABSTRACT: Chickpea (*Cicer arietinum L.*), one of the major pulses cultivated and consumed in India, is also known as Bengal gram. Chickpea is a major and cheap source of protein (about 17-20%) compared to animal protein. *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* is the most serious disease of chickpea. Eleven isolates of *F. oxysporum* f.sp. *ciceri* were studied for its cultural, morphological and pathogenic variability. The radial growth of isolates ranged from 72 mm to 87 mm at seven days after inoculation on PDA medium. The isolates, BRFOC-1, BRFOC-4, BRFOC-5, BFOC-3, PFOC-2, and PFOC-3 grow more than 85 mm after seven days of inoculation. Pigmentation is varied among the isolates. Pinkish found in isolates of BRFOC-1, BRFOC-5 and PFOC-2 while pale yellow found in BRFOC-2, BRFOC-4 and BFOC-1 isolates. Sporulation of isolates was profuse to moderate. The size of macro-conidia was ranged from 13-15 x 2-3 µm to 15-19 x 3-4 µm, in micro-conidia was from 3-4 x 1-2 µm to 5-6 x 2-3 µm. The number of septa in macro-conidia was mostly 2-3 and micro-conidia are mostly no septum and some are 0-1. Conidia are hyaline. Shape of most macro-conidia is sickle shape and micro-conidia are round to oval. Pathogenic variability revealed that most of the isolates were highly pathogenic.
Key words: Cultural, Morphological, Pathogenic variability, *Fusarium*, Chickpea

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INTRODUCTION

Chickpea (*Cicer arietinum L.*), one of the major pulse cultivated and consumed in India, is also known as Bengal gram. Chickpea is a major and cheap source of protein (about 17-20%) compared to animal protein. This pulse crop is frequently attacked by *Fusarium* wilt. The disease manifests as mortality of young seedlings (within 25 to 30 days after sowing) to wilting or death of adult plants. Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants [1]. The roots of the wilting plants don't show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen [2]. Chickpea wilt causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop [1,3,4,5]. Annual chickpea yield losses due to *Fusarium* wilt were estimated at 10% in India [6,7]. *Fusarium* wilt caused by fungal pathogen, *Fusarium oxysporum* f.sp. *ciceri*, is the most serious disease of chickpea. The fungus is a soil borne root pathogen, colonizing xylem vessels, blocking them and causing wilting [8]. The *F. oxysporum* f.sp. *ciceri* is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds [9].

The pathogen is facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years [10]. The fungus produced macro-conidia and micro-conidia and also chlamydospores. Fungal chlamydospores can survive in soil up to six years in the absence of the host plants [11]. The pathogen is mainly soil borne therefore needs to know the nature of pathogen for better management practice of *Fusarium* wilt disease of chickpea. However the present study was investigated to cultural, morphological and pathogenic variability of *Fusarium* isolates present in the red and lateritic zone of West Bengal.

MATERIALS AND METHODS

Survey was conducted at the red and lateritic zone of West Bengal i.e. different place of Birbhum, Bankura and Purulia district during the year of 2014-15, where pulses are cultivated extensively by the farmers and experimented at Department of Plant Protection, Visva-Bharati, Sriniketan.

Isolation of *Fusarium oxysporum* f.sp. *ciceri* isolates

Wilted plants of chickpea were collected from different farmers' field of red & lateritic zone of West Bengal and surface sterilized was done by 70 % ethyl alcohol. The samples are cut into pieces of disease part along with healthy tissue. These pieces are place aseptically on sterilized Potato Dextrose Agar (PDA) medium in Petri plates. Pure culture was done by transfer of a pinch of mycelium on sterilized Potato Dextrose Agar medium in Petri plates and incubated in BOD. The fungus was identified by colony growth, pigmentation and microscopic charactertics of *Fusarium oxysporum*.

Cultural and Morphological variability

In order to study cultural charactertics, 5 mm mycelia bits of each isolates were taken from the actively growing cultures and centrally placed on 90 mm Petri plates containing sterilized PDA medium. After inoculation Petri plates were incubated at 26 ± 1^0C for 7 days. Each plate is replicated three times. After seven days radial growth of pathogen was recorded. Others charactertics viz; pigmentation, sporulation of different isolates was recorded by observing culture plate after complete growth of the mycelium which showed slight pinches of colour.

In morphological studies were carried out by taking small amount of mycelium from fourteen days old pure culture plates using a sterile needle and transferred onto a cleaned glass slide. The culture was taken from five different positions of the culture plate, four from adjacent side and one from middle. The mycelium was stained with 0.1 % lactophenol cotton blue and observed under compound microscope. Spores size was measured by software Y W Camera.

Pathogenicity test

In pathogenicity test these collected isolates were mass multiplied on sand maize meal medium and inoculated in pot at the rate of 30 gm per pot and cover with transparent polythene sheet then incubated for seven days on natural condition. After incubation, chickpea cultivars are sown in pots and three replications were maintained. Control pot was maintained by pots without inoculums. Disease was recorded after emergence of seedlings and expressed as percentage wilting. On the basis of wilt symptom observed of plant, isolates graded different category. Ranked is as minus (-) for no symptom and plus (+) for wilt symptom on inoculated pot. Appearance of symptoms was again divided into four groups viz., up to 25 % wilt ranked as single plus (+), 25.1 to 50% ranked as double plus (++), 50.1 to 75% were ranked triple plus (+++) and more than 75% were ranked tetra plus sign (++++) and reacted as slow pathogenic, moderate pathogenic, pathogenic and highly pathogenic isolates respectively.

RESULTS AND DISCUSSION

Cultural variability in isolates

Among eleven isolates, colony of three isolates was observed fluffy growth; while four are compact, two is sparse growth and two is cottony mycelium growth. Variation in the mycelium colour was observed in the isolates on PDA medium. Initially the colour of all isolates was white, which changed gradually with different pigments like pink appears in isolates of BRFOC-1, BRFOC-5 and PFOC-2, pale yellow was isolates of BRFOC-2, BRFOC- 4, and BFOC-1, light yellow was BRFOC-3, BFOC-3 and PFOC-3 isolates. Straw yellow colour was BFOC-2 isolates and dark yellow was PFOC-1 isolates. Sporulation of isolates showed moderate to profuse. Profuse spores produce isolates was of BRFOC-1,

Table 1. Cultural charactertics of *Fusarium oxysporum* f.sp. *ciceri* isolates in PDA medium

S. No.	Isolates	Location	Colony Diameter (mm) (7 DAI)	Colony character	Pigmentation	Sporulation
1	BRFOC-1	Raipur, Birbhum	87	White compact mycelium	Pink	Profuse
2	BRFOC-2	Sian, Birbhum	80	White cottony mycelium	Pale yellow	Moderate
3	BRFOC-3	Kirnahar, Birbhum	78	White fluffy mycelium	Light yellow	Moderate
4	BRFOC-4	Illambazar, Birbhum	83	White fluffy mycelium	Pale yellow	Profuse
5	BRFOC-5	Dubrajpur, Birbhum	81	White compact mycelium	Pink	Profuse
6	BFOC-1	Chhatna, Bankura	78	White sparse growth mycelium	Pale yellow	Profuse
7	BFOC-2	Simlapal, Bankura	72	White cottony mycelium	Straw yellow	Moderate
8	BFOC-3	Indpur, Bankura	85	White compact mycelium	Light yellow	Profuse
9	PFOC-1	Balarampur, Purulia	74	White fluffy mycelium	Dark yellow	Moderate
10	PFOC-2	Hurah, Purulia	82	White sparse growth mycelium	Pink	Moderate
11	PFOC-3	Santuri, Purulia	87	White compact mycelium	Light yellow	Profuse

BR: Birbhum, B: Bankura, P: Purulia FOC: *Fusarium oxysporum ciceri*

BRFOC-4, BRFOC-5, BFOC-1, BFOC-3 and PFOC-3 while moderate produce was BRFOC-2, BRFOC-3, BFOC-2, PFOC-1 and PFOC-2 (Table 1). The radial growth of colony diameter was different of different isolates. The colony diameter ranged from 72 mm to 87 mm at seven days after inoculation at 26 ± 1^0C in 90 mm Petri plates. Radial growth are maximum (87 mm) found in isolates of BRFOC-1 and PFOC-3 followed by BFOC-3 isolates (85 mm). Least growth found in BFOC-2 isolates with diameter was 72 mm.

Morphological variability in isolates

Morphological character such as size, shape, septation and colour of conidia were studied using of Potato Dextrose Agar medium. Conidiophores were elongated and sparsely branched, each branch usually terminated with a bearing spore. The pathogen bearing two types of asexual spores i.e. macro-conidia and micro-conidia. The size of macro-conidia was ranged from 13-15 x 2-3 µm to 15-19 x 3-4 µm; size of micro-conidia was ranged from 3-4 x 1-2 to 5-6 x 2-3 µm (Table 2). The number of septation is varying with isolates. Three to four septa are found in isolates of BRFOC-5 and PFOC-3, left all isolates septa was two to three in macro-conidia. In micro-conidia, most of isolates was no septum, some isolates viz., BRFOC-1, BRFOC-5, BFOC-2 and PFOC-1 were found zero to one septum. Shape of most macro-conidia was sickle shape while isolates of BRFOC-5 and PFOC-3 are elongated with blunt end. In micro-conidia most of isolates are round to oval shape while some isolates viz, BRFOC-2, BRFOC-4, BFOC-3, PFOC-2 and PFOC-3 are round shape. All isolate are hyaline.

Pathogenic variability in isolates

In pathogenicity test, pot culture experiment was done and on the basis of percentage of wilt isolates are graded different way. Isolates of BRFOC-3, BRFOC-5, BFOC-2, PFOC-1 and PFOC-3 are showing more than 75% wilt, graded at highly pathogenic isolates. BRFOC-1, BRFOC-2, BRFOC-4, BFOC-1, BFOC-3 and PFOC-2 isolates is show 50.1 to 75 % wilt, then grade in pathogenic isolates. Therefore all isolates show causing wilt disease of chickpea.

Table 2. Morphological charactertics of *Fusarium oxysporum* f.sp. *ciceri* isolates

S No.	Isolates	Location	Macroconidia			Microconiadia			Colour
			Size (µm) L x B	Septation	Shape	Size (µm) L x B	Septation	Shape	
1	BRFOC-1	Raipur, Birbhum	15-19 x 3-4	2-3	Elongated sickle shape	5-6 x 2-3	0-1	Round to oval	Hyaline
2	BRFOC-2	Sian, Birbhum	13-15 x 2-3	2-3	Sickle shape	3-4 x 1-2	0	Round	Hyaline
3	BRFOC-3	Kirnahar, Birbhum	13-15 x 2-3	2-3	Sickle shape	4-5 x 1-2	0	Round to oval	Hyaline
4	BRFOC-4	Illambazar, Birbhum	14-16 x 2-3	2-3	Sickle shape with blunt ends	3-4 x 1-2	0	Round	Hyaline
5	BRFOC-5	Dubrajpur, Birbhum	16-18 x 3-4	3-4	Elongated with blunt ends	5-6 x 1-3	0-1	Round to oval	Hyaline
6	BFOC-1	Chhatna, Bankura	13-16 x 2-3	2-3	Elongated sickle shape	4-5 x 1-2	0	Round to oval	Hyaline
7	BFOC-2	Simlapal, Bankura	14-16 x 3-4	2-3	Sickle shape	3-4 x 1-2	0-1	Round to oval	Hyaline
8	BFOC-3	Indpur, Bankura	13-15 x 2-3	2-3	Sickle shape with blunt ends	3-4 x 1-2	0	Round	Hyaline
9	PFOC-1	Balarampur, Purulia	15-18 x 2-3	2-3	Elongated sickle shape	4-5 x 1-3	0-1	Round to oval	Hyaline
10	PFOC-2	Hurah, Purulia	13-15 x 2-3	2-3	Sickle shape	3-4 x 1-2	0	Round	Hyaline
11	PFOC-3	Santuri, Purulia	15-18 x 3-4	3-4	Elongated with blunt ends	3-4 x 1-2	0	Round	Hyaline

BR: Birbhum, B: Bankura, P: Purulia FOC: *Fusarium oxysporum ciceri*

Table 3. Pathogenic variability of *Fusarium oxysporum* f.sp. *ciceri* isolates

Sl No.	Isolates	Location	Pathogenicity	Reaction
1	BRFOC-1	Raipur, Birbhum	+ + +	Pathogenic
2	BRFOC-2	Sian, Birbhum	+ + +	Pathogenic
3	BRFOC-3	Kirnahar, Birbhum	+ + + +	Highly Pathogenic
4	BRFOC-4	Illambazar, Birbhum	+ + +	Pathogenic
5	BRFOC-5	Dubrajpur, Birbhum	+ + + +	Highly pathogenic
6	BFOC-1	Chhatna, Bankura	+ + +	Pathogenic
7	BFOC-2	Simlapal, Bankura	+ + + +	Highly pathogenic
8	BFOC-3	Indpur, Bankura	+ + +	Pathogenic
9	PFOC-1	Balarampur, Purulia	+ + + +	Highly pathogenic
10	PFOC-2	Hurah, Purulia	+ + +	Pathogenic
11	PFOC-3	Santuri, Purulia	+ + + +	Highly pathogenic

Up to 25 % wilt for + (single plus), 25.1 to 50% for + + (double plus), 50.1 to 75% for + + + (triple plus) and more than 75% for + + + + (tetra plus).

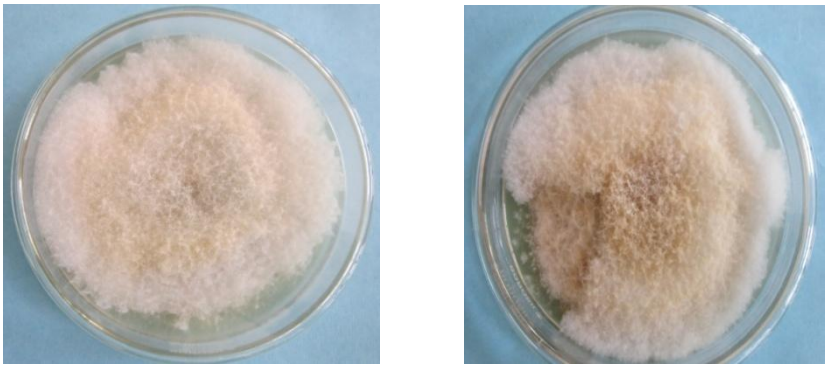

Plate 1: Cultural plate of *F. oxysporum* f.sp. *ciceri*

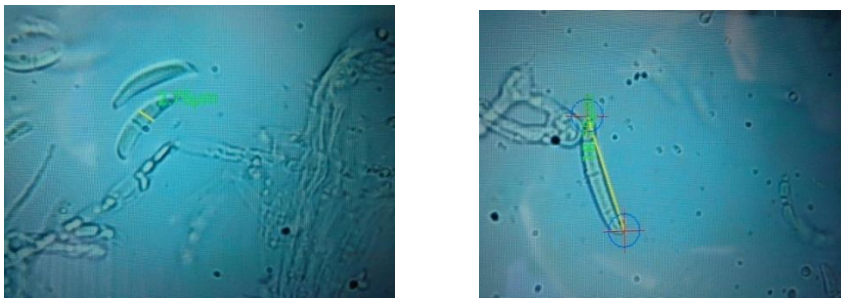

Plate 2: Microscopic view of *F. oxysporum* f.sp. *ciceri*

CONCLUSION

In the present study cultural, morphological and pathogenic variability of some isolates causing wilt of chickpea in the red and lateritic zone of West Bengal showed that the radial growth of colony diameter was different of different isolates. The colony diameter was ranged from 72 mm to 87 mm at seven days after inoculation at 26±1^{0}C in 90 mm Petri plates. Initially the colour of all isolates was white which changed gradually with different pigments like pink, pale yellow, light yellow etc. Growth of the isolates, some are cottony like, some are compact and some are fluffy growth. The size of macro-conidia was ranged from 13-15 x 2-3 µm to 15-19 x 3-4 µm and micro-conidia 3-4 x 1-2 to 5-6 x 2-3 µm. The shape of macro-conidia is sickle and micro-conidia are round to oval. All isolates show pathogenic causing wilt disease of chickpea. The present investigation has generated information in spite of cultural, morphological and pathogenicity variability among *F. oxysporum* f.sp. *ciceri* isolated from various region of red and lateritic belt of West Bengal for epidemiology and evolutionary potential of this pathogen and could development of improved practice for managing *Fusarium* wilt disease of chickpea.

REFERENCES

[1] Haware, M. P. and Nene, Y. L. 1980. Influence of wilt at different stages on the yield loss in chickpea. Trop. Grain Legume Bullet. 19: 38-40.

[2] Nene, Y. L., Reddy, M. V., Haware, M. P., Ghanekar, A. M. and Amin, K. S. 1991. Field diagnosis of chickpea disease and their control. In: *Information Bulletin no. 28*. ed. by International Crops Research Institute for the Semi Arid Tropics, Patancheru, India.

[3] Haware, M. P., Jimenez-Diaz, R. M., Amin, K. S., Phillips, J. C. and Halila, H. 1990. Integrated management of wilt and root rot of chickpea In: Chickpea in the Nineties: Procedings of the second international work shop on chickpea improvement, Patancheru, India. pp. 129-137.

[4] Halila, M. H. and Strange, R.N. 1996. Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f.sp. *ciceri* race 0. Phytopathol. Mediterr. 35: 67-74.

[5] Navas-Cortes, J.A., Hau, B. and Jimenez-Diaz, R. M. 2000. Yield loss in chickpea in relation to development to *Fusarium* wilt epidemics. Phytopathology 90: 1269-1278.

[6] Singh, K. B. and Dahiya, B. S. 1973. Breeding for wilt resistance in chickpea. Pages 13-14. In: Symposium on Wilt Problem and Breeding for Wilt Resistance in Bengal Gram. Indian Research Institute, New Delhi, India.

[7] Trapero-Casas, A. and Jimenez-Diaz, R. M. 1985. Fungal wilt and root rot diseases of chickpea in southern Spain. Phytopathology, 75: 1146-1151.

[8] Bateman, G. L., Kwasna, H. and Ward, E. 1996. Relationship among *Fusarium* spp. estimated by comparing restriction fragment length polymorphism in polymerase chain reaction amplified nuclear DNA. Canadian Journal of Microbiology 42: 1232-1240.

[9] Haware, M. P., Nene, Y. L. and Rajeswari, R. 1978. Eradication of *Fusarium oxysporum* f. sp. *ciceris* transmitted in chickpea seed. Phytopathology 68: 1364-1368.

[10] Haware, M. P., Nene, Y. L. and Mathur, S. B. 1986. Seed borne disease of chickpea. Technical Bulletin 1. Danish Government Institute of seed Technology for developing countries. Copenh., 1:1-32.

[11] Haware, M. P., Nene, Y.L. and Natarajan, M. 1996. Survival of *Fusarium oxysporum* f. sp. *ciceri*. Plant Disease, 66: 809-810.

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