

## RESEARCH NOTE

# Studies on variability in the growth of twenty isolates of *Fusarium oxysporum* f.sp. *ciceri* causing vascular wilt of chickpea in different liquid media

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## ARTICLE INFO

Received : 11.03.2013

Accepted : 06.06.2013

## Key Words :

*Fusarium oxysporum*, Vascular wilt, Chickpea, Liquid media

## ABSTRACT

Field survey was undertaken and Seventy one samples of chickpea wilted plants were collected from twenty three locations in different districts namely Bhopal, Raisen, Rajgarh, Sagar, Sehore and Vidisha of Vindhyan Plateau Zone of Madhya Pradesh. Out of Seventy one isolates, only twenty were found pathogenic to chickpea. These isolates were categorized into six different groups on the basis of mycelia dry weight, growth pattern and the number of micro and macro conidia. The physiological studies of the representative isolates of these six groups were made on six liquid media. All the media differed significantly from each other. The minimum mean mycelia dry weight (66.19 mg) was recorded in groups five of isolates Ri4, Ri5 and V2 and maximum (73.16 mg) in group one consisting of B2, B3, Se6 and Se8. The maximum mean mycelia dry weight (79.00 mg) was obtained on Potato dextrose broth (PDB) and minimum (60.70 mg) on Richard solution. These isolates were exhibited three types of growth pattern namely fluffy, partially submerged and submerged. The maximum numbers of micro conidia were produced on PDB (6.64 million/ml) and minimum (3.42 million/ml) on Elliot's Solution. Similarly, the maximum numbers of macro conidia were produced on PDB (2.48 million/ml) and minimum (0.73 million/ml) on Houston's solution. Chickpea (*Cicer arietinum* L) is an important pulse crop of India and suffers with various diseases caused by fungi, bacteria and virus of which vascular wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is much dangerous than other diseases Singh *et al.* (1973). The incidence of the disease varies from 10-100 per cent depending on the locality.

**How to view point the article :** Shrivastava, Ashish and Agrawal, Vijay (2013). Studies on variability in the growth of twenty isolates of *Fusarium oxysporum* f.sp. *ciceri* causing vascular wilt of chickpea in different liquid media. *Internat. J. Plant Protec.*, 6(2) : 480-483.

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In Madhya Pradesh, its incidence has been reported from 0-60 per cent Gupta *et al.* (1983). In order to find suitable lines resistant to this disease, it is necessary to study the variability of the pathogen. The isolates of the chickpea wilt pathogen obtained from various locations in Vindhyan Plateau Zone of Madhya Pradesh, were grouped into six groups on the basis of morphological as cultural characters Gupta *et al.* (1986) and physiological basis Kushwaha *et al.* (1974). The variability

in the growth of twenty isolates on six liquid media is reported in this paper.

All the pathogenic isolates were grown on six liquid media namely Potato dextrose broth, Armstrong fusarium solution, Czapeck's solution, Elliot's solution, Houston's solution and Richard's solution. Erlenmeyer flasks of 150 ml capacity were used and 30 ml medium was poured in each flask. The pH of medium was adjusted at 6.0 and autoclaved

three replications were maintained for each medium and isolates. Five mm of disc was cut from the margin of seven day old culture of *Fusarium oxysporum* f.sp. *ciceri* and transferred to each flask. The flasks were incubated at room temperature for 15 days. The type of growth pattern was noted as fluffy, partially submerged and submerged. A loopful suspension from each of the flask was added in 2.0 ml of sterilized water shaken well and was examined under the low power of the micro-scope on haemocytometer. The number of macro and micro-conidia/ml of suspension were calculated using the following formula:

$$\text{Conidia /ml} = X \times 250000$$

where X = Number of conidia/square of haemocytometer of 1/25 n<sup>2</sup>

Observation were recorded the mycelial dry weight /mg, growth pattern as fluffy, partially submerged and submerged and the number of micro and macro conidia/ml. It is evident from the data in Table 1, that all the groups of isolates of *Fusarium oxysporum* f.sp. *ciceri* differed significantly from each other in their mycelia dry weight/mg on six liquid media. The minimum mean mycelial dry weight (73.16 mg) was obtained in case of group 5 consisting of Ri4, Ri5 and V2, while maximum

(66.19mg) in group 1 of B2, B3, S6 and Se8. The maximum mean mycelia dry weight (79.85mg) was observed on Potato dextrose broth and minimum in Elliot's solution (59.73 mg). The interaction effect between isolates and media was also significant statistically. As regards the growth pattern only group 1 of B2, B3, S6 and Se8 isolates was found to produce the same growth pattern (fluffy). Other groups varied in this respect. On the basis of growth pattern, isolates could be placed into six groups as shown in Table 2.

The number of micro conidia of *Fusarium oxysporum* f.sp. *ciceri* on six liquid media are recorded in Table 3. The maximum sporulation (6.64 million/ml) was recorded on Potato dextrose broth. The sporulation was reduced significantly in all the other media. It was minimum in Elliot's solution (3.42 million/ml) followed by Houston's solution, Czapeck's solution, Richard solution and Armstrong fusarium solution.

The maximum micro conidia were produced by group 5 of (4.77million/ml) Ri4, Ri5 and V2 while group 6 of Se2, Se5 and V6 had minimum micro conidia (4.38 million/ml). The interaction effect between isolates and media with regard to micro conidia production was significant statistically indicating the difference among different groups.

**Table 1 : Mycelia dry weight (Mg) of 20 isolates of *Fusarium oxysporum* f.sp. *ciceri* on different liquid media**

Group No.	Medium						Mean	Isolate included
	Potato dextrose broth	Armstrong fusarium solution	Czapeck's solution	Elliot's solution	Houston's solution	Richard's solution		
1.	90.88	76.27	65.77	60.44	79.66	65.99	73.16	B <sub>2</sub> , B <sub>3</sub> , Se <sub>6</sub> and Se <sub>8</sub>
2.	80.84	66.99	64.99	59.99	76.33	62.99	68.68	Ri <sub>1</sub> and Sa <sub>2</sub>
3.	78.99	65.66	63.97	58.16	76.33	62.58	67.16	B <sub>5</sub> , Ri <sub>8</sub> Sa <sub>4</sub> and Se <sub>3</sub>
4.	77.99	68.49	61.87	58.53	73.91	61.16	66.99	B <sub>8</sub> , Rj <sub>4</sub> V <sub>4</sub> and V <sub>5</sub>
5.	75.44	67.77	62.83	60.99	72.55	57.56	66.19	Ri <sub>4</sub> , Ri <sub>5</sub> and V <sub>2</sub>
6.	74.99	64.99	61.70	60.45	70.33	58.16	65.10	Se <sub>2</sub> , Se <sub>5</sub> and V <sub>6</sub>
Mean	79.85	68.36	63.42	59.73	74.85	61.34		

CD at 5 % for Isolates 1.43 & for Media 0.78

B- Bhopal, Ri-Raisen, Sa- Sagar, Se- Sehore and V- Vidisha

**Table 2 : Growth pattern of 20 isolates of *Fusarium oxysporum* f.sp. *ciceri* on different liquid media**

Group No.	Medium						Isolate included
	Potato dextrose broth	Armstrong fusarium solution	Czapeck's solution	Elliot's solution	Houston's solution	Richard's solution	
1.	fluffy	fluffy	fluffy	fluffy	fluffy	fluffy	B <sub>2</sub> , B <sub>3</sub> , Se <sub>6</sub> and Se <sub>8</sub>
2.	fluffy	fluffy	Partially Submerged	Partially Submerged	Partially Submerged	fluffy	Ri <sub>1</sub> and Sa <sub>2</sub>
3.	Fluffy	Fluffy	Submerged	Partially Submerged	Submerged	Fluffy	B <sub>5</sub> , Ri <sub>8</sub> Sa <sub>4</sub> and Se <sub>3</sub>
4.	Partially Submerged	Partially Submerged	Submerged	Submerged	Partially Submerged	Partially Submerged	B <sub>8</sub> , Rj <sub>4</sub> V <sub>4</sub> and V <sub>5</sub>
5.	Fluffy	Fluffy	Fluffy	Submerged	Submerged	Fluffy	Ri <sub>4</sub> , Ri <sub>5</sub> and V <sub>2</sub>
6.	Submerged	Submerged	Partially Submerged	Submerged	Partially Submerged	Submerged	Se <sub>2</sub> , Se <sub>5</sub> and V <sub>6</sub>

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The number of macro conidia of *Fusarium oxysporum* f.sp. *ciceri* on six liquid media are recorded in Table 4. It is evident from data that the maximum macro conidia were produced on potato dextrose broth (PDB) (2.48 million/ml) while on other media, the macro conidia production was reduced significantly. The minimum macro conidia were observed on Elliot's solution (0.73 million/ml) followed by Houston's, Czapeck's solution, Richard solution and Armstrong fusarium solution.

As regard the groups, maximum macro conidia were formed by group No. 4 (1.61 million/ml) of B<sub>8</sub>, R<sub>j4</sub>, V<sub>4</sub> and V<sub>5</sub> isolates. The minimum macro conidia were produced by group No. 3 (1.18 million/ml) of B<sub>5</sub>, R<sub>i8</sub>, S<sub>a4</sub> and S<sub>e3</sub> isolates. The statistically significant effect of interaction between media and isolates with regard to formation of macro-conidia indicated that the isolates differed from each other. In general, it is clear that much less macro conidia were produced as compared to micro-conidia irrespective of isolates of the fungus and different media.

The mycelia dry weight of isolates in general reduced on all 5 media to a variable extent when compared with PDB. On the other hand, the mean mycelia dry weight did not differ much from each other. Although, the data was statistically significant with regard to isolates, the significant with regard

to isolates, the significant interaction effect however, showed the variability amongst the isolates. Probably the qualitative characters can only be the best criterion for such type of grouping of isolates.

The 20 isolates exhibited three types of growth pattern that is fluffy, partially submerged and submerged. As regards the growth pattern only group 1 of B<sub>2</sub>, B<sub>3</sub>, S<sub>e6</sub> and S<sub>e8</sub> isolates had same growth pattern (Fluffy) on all the media. Group 2 of R<sub>i1</sub> and S<sub>a2</sub> isolates had fluffy mycelium on PDB, Armstrong fusarium solution, and Richard solution but it changed partially submerged on Czapeck's solution, Elliot's solution and Houston's solution. Similarly, group 5 of R<sub>i4</sub>, S<sub>i5</sub> and V<sub>2</sub> isolates were fluffy on PDB and other media except on Elliot's and Houston's solution on which their growth was submerged.

As observed the sporulation was also influenced by different liquid media. The higher number of micro as well as macro conidia were recorded on PDB. While, the numbers were reduced drastically on other media. The number of macro conidia in all the cases was much less as compared to micro conidia. The data on sporulation were statistically significant so as the isolates on the basis of these characters Khare *et al.* (1975).

**Table 3 : Micro-conidia million/ml of 20 isolates of *Fusarium oxysporum* f.sp. *ciceri* on different liquid media**

Group No.	Medium						Mean	Isolate included
	Potato dextrose broth	Armstrong fusarium solution	Czapeck's solution	Elliot's solution	Houston's solution	Richard's solution		
1.	6.75	5.83	3.74	3.47	3.72	4.48	4.66	B <sub>2</sub> , B <sub>3</sub> , S <sub>e6</sub> and S <sub>e8</sub>
2.	6.66	5.74	3.75	3.33	3.49	4.79	4.62	R <sub>i1</sub> and S <sub>a2</sub>
3.	6.70	5.74	3.89	3.51	3.70	4.79	4.72	B <sub>5</sub> , R <sub>i8</sub> , S <sub>a4</sub> and S <sub>e3</sub>
4.	6.61	5.66	3.64	3.11	3.27	4.47	4.46	B <sub>8</sub> , R <sub>j4</sub> , V <sub>4</sub> and V <sub>5</sub>
5.	6.69	5.78	3.80	3.64	3.66	5.05	4.77	R <sub>i4</sub> , R <sub>i5</sub> and V <sub>2</sub>
6.	6.39	4.49	3.68	3.49	3.48	4.76	4.38	S <sub>e2</sub> , S <sub>e5</sub> and V <sub>6</sub>
Mean	6.64	5.54	3.75	3.42	3.55	4.73		

CD at 5 % for Isolates 0.18 & for Media 0.10

**Table 4: Macro-conidia million/ml of 20 isolates of *Fusarium oxysporum* f.sp. *ciceri* on different liquid media**

Group No.	Medium						Mean	Isolate included
	Potato dextrose broth	Armstrong fusarium solution	Czapeck's solution	Elliot's solution	Houston's solution	Richard's solution		
1.	2.66	1.72	1.26	0.73	0.81	1.16	1.39	B <sub>2</sub> , B <sub>3</sub> , S <sub>e6</sub> and S <sub>e8</sub>
2.	2.49	1.62	1.12	0.79	0.95	1.16	1.35	R <sub>i1</sub> and S <sub>a2</sub>
3.	2.19	1.58	1.08	0.63	0.61	1.0	1.18	B <sub>5</sub> , R <sub>i8</sub> , S <sub>a4</sub> and S <sub>e3</sub>
4.	2.64	1.89	2.41	0.72	0.85	1.16	1.61	B <sub>8</sub> , R <sub>j4</sub> , V <sub>4</sub> and V <sub>5</sub>
5.	2.35	1.76	1.22	0.75	0.78	1.28	1.35	R <sub>i4</sub> , R <sub>i5</sub> and V <sub>2</sub>
6.	2.58	1.94	1.42	0.75	0.97	1.33	1.49	S <sub>e2</sub> , S <sub>e5</sub> and V <sub>6</sub>
Mean	2.48	1.70	1.41	0.73	0.83	1.18		

CD at 5 % for Isolates 0.08 & for Media 0.04

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