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Cultural and morphological variability in single spore isolates of *Fusarium oxysporum* f. sp. *Corianderii* on different media

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Abstract
Among eight isolates from different districts surveyed, representing different groups of *Fusarium oxysporum* f.sp. *corianderii* incited wilt of coriander. Variability was observed in terms of cultural and morphological characters on different agar and broth media. PDA medium was found to be significantly superior and best suited among all media in the present investigations for growth and sporulation of the pathogen. All the isolates produced maximum mycelial growth on potato dextrose agar media followed by Czapek Dox agar media. Both these media were also found better for production of macro and micro-conidia. The *Fusarium oxysporum* f.sp. *Corianderii* isolate I_2 showed maximum (256.13 mg) mean dry fungal biomass and isolate I_6 showed minimum (179.39 mg) mean dry fungal biomass on broth media.

Keywords: *Coriander*, variability, *Fusarium oxysporum*, Media.

Introduction
Fusarium oxysporum f. sp. *Corianderii*, incitant of wilt disease in coriander (*Coriandrum sativum* L.) is a destructive disease in major coriander growing districts of Rajasthan resulting into considerable economic losses. Annual yield losses due to wilt have been estimated 10%–60% (Singh and Reddy, 1991) [9]. Persistence of the pathogen in soil and its capacity to survive there for years even in the absence of host (Haware *et al*., 1996) [3] renders its management difficuly. The isolated pathogenic strains may vary morphologically, physiologically and in pathogenicity, thus studies were undertaken to find out variability in *Fusarium oxysporum* f.sp. *Corianderii* and their effect on disease development.

Materials and Methods
Pure culture of different isolates of *Fusarium oxysporum* f.sp. *corianderii* collected from different coriander growing area of Rajasthan. A total of eight isolates obtained from the surveyed districts and a local isolate from Agronomy Farm of SKNCoA, Jobner were used in present study. Eight isolates of *Fusarium oxysporum* f. sp. *coriandrii* were transferred on 2 per cent PDA in Petri dishes to study their cultural characters such as the colour of aerial mycelium, colony growth and pigmentation of substratum and sporulation.
The isolates were coded as:

Code	Place of collection of isolates
I_1	Kota
I_2	Bundi
I_3	Baran
I_4	Jhalawar
I_5	Jodhpur
I_6	Jaipur
I_7	Sawai Madhopur
I_8	Chittorgarh

1. Cultural variability
a) Variability on agar media
All the eights isolates were grown on different solid and broth media for their growth and sporulation. The composition of each medium used is given as under:

1) Modified Czapek Dox Agar Medium

Sodium nitrate	2.0 g
Dipotassium monohydrogen phosphate	1.0 g
Mangnesium sulphate	0.5 g
Potassium chloride	0.5 g
Ferrous sulphate	0.01 g
Sucrose	30.0 g
Pentachloro nitrobenzene (PCNB)	500.0 mg
Yeast extract	2.0 g
Agar	20.0 g
Distilled water	1000.0 ml

2) Potato dextrose Agar (PDA)

Peeled potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000

3) Richard's medium

Potassium nitrate	10 g
Potassium dihydrogen phosphate	5 g
Magnesium sulphate	2.5 g
Ferric chloride	0.02 g
Sucrose	50 g
Agar	20 g
Distilled water	1000 ml

4) Broun's medium

Glucose	2.0 g
Asparagine	2.0 g
Potassium dihydrogen phosphate	1.25 g
Magnesium	0.72 g
Agar	20 g
Distilled water	1000

5) Malt extract agar

Malt extract	20.0 g
Dextrose	20.0 g
Peptone	1.0 g
Agar	25.0 g
Distilled water	1000 ml

6) Corn medal agar

Corn meal	30 g
Agar	20 g
Distilled water	1000 ml

7) Oat meal agar

Oat meal	30 g
Agar	20 g
Distilled water	1000 ml

8) Martin's agar

Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Rose Bengal (1%)	3.3 ml
Agar	20.0 g
Distilled water	1000 ml

9) Mung bean agar

Mung bean (crushed)	30 g
Agar	20 g
Distilled water	1000 ml

Required quantity of the above mentioned solid medium was prepared and sterilized at 1.05 kg per cm^2 pressure for 20 minutes. Sterilization of Petri dishes was done at 180°C for 2 h in a hot air oven. In each Petri dish, 25 ml of respective medium was poured. Each treatment was replicated four times. Each Petri dishes/flask was inoculated with bit of 2 mm having a single germinating conidium maintained on plain agar. The inoculated Petridishes was incubated at 25±1°C temperature and observation on mycelial colour, mycelial growth characters, substrates colour and production of macro or micro conidia were recorded.

b) Variability on broth media

All the nine solid media prepared using agar-agar were also tested as broth media by excluding agar-agar. After sterilization, 25 ml of each medium was poured into 100 ml conical flask, keeping quadruplicate for each treatment. The flasks were inoculated with 2 mm bit of the pathogen and were incubated in BOD incubator at 25±1 °C. After seven days of incubation, mycelial mat was harvested on oven-dried and weighed whatman No.1 filter paper. Mycelial mat along with filter paper was dried at 60 °C for over night in hot air oven and weighted. For conidial production, 3 separate flasks were maintained. The each of the eight isolates was cultured on each broth medium separately in Erlenmeyer flasks for 10 days at 25±1 °C. The mycelial mat was fragmented in warring blender at low speed intermittently for a total time of 5 min. The homogenous suspension so obtained was strained through the muslin cloth and supernatants were received in a beaker. A drop of the suspension was taken on the slide and examined for conidia count under low power magnification. In each of 3 drops, 5 microscopic fields were observed and mean number of conidia in each isolate was calculated.

2. Morphological variability

To detect morphological variability in conidia formed by different isolates, purified culture of each isolate was grown in PDA plate. From 7-day old culture of each isolate, 3 mm diameter bits were cut from periphery of the growth with the help of a sterilized cork borer. One such bit was transferred aseptically with the help of sterilized inoculation needle to inoculate one PDA plate. Four Petridishes each inoculated with 1 bit of the same isolate constituted 4 replications for that isolate. The inoculated Petridishes were incubated at 25±1 °C temperature. On 7th day of inoculation, spores were collected from culture in each Petridish according to the following procedure. From each Petridish, constituting one replication, 3 bits (each of 3 mm diameter) were cut with the help of sterilized cork borer from random points on peripheral growth. Three bits were shaken in 5 ml water for 10 minutes on a horizontal shaker. The harvested spores were stained with dilute solution of cotton blue and examined microscopically. For each isolate,10 macro- and 10 micro-conidia from the total number of spores separated out from 3 bits per replication were separately examined for their size, shape and septation. Thus, 60 macro- and micro-conidia from each isolates were examined for their size, shape and septation.

Isolates were transferred separately on PDA in Petri dishes to study in detail for their discernible characters on the basis of cultural characters such as the amount and colour of aerial mycelium, colony growth and pigmentation of substratum.

Result and discussion

Cultural variability

a) Variability on Agar media

To know the cultural variability in pathogen isolates they were grown on different nutritional media. Mycelial

pigmentation in cultures of the isolates varied with the type of medium, however, isolates I_2, I_5, I_7 and I_8 showed white mycelial pigmentation on all the media (Table 1). The colony diameters of all the isolates were 8.0 mm to 9.0 mm range equal on PDA and Czapek Dox medium. The colony diameters of all the isolates were smaller on Richard's and Broun's medium than on the other media. (Table 1)

Table 1: Cultural variability among eight isolates of *Fusarium oxysporum* f.sp. *corianderii* on different media at 10 days growth

Isolates/ Media	Colony diameter (cm)*	Mycelium colour	Mycelial growth characters	Substrate colour	Production of conidia	
					Macro conidia	Micro conidia
1. PDA						
I_1	8.5	White	SC, SM	Light yellow	36	31
I_2	9.0	White	CO, RM	Creamy	45	41
I_3	8.7	White	WO, SM	Dirty white	43	20
I_4	8.6	Pinkish	PD, SM	Pink	30	38
I_5	8.2	White	CO, SM	Creamy	33	24
I_6	8.0	Pinkish white	WO, SM	Voilet	39	16
I_7	9.0	White	WO, RM	Light yellow	42	18
I_8	8.3	white	CO, SM	Yellowish	37	20
2. Czapek Dox						
I_1	8.3	White	SC, SM	Light yellow	40	20
I_2	9.0	White	SC, RM	Light dark	37	23
I_3	8.5	Violet	SC, RM	Dirty white	40	30
I_4	8.0	White	PD, SM	Voilet	33	35
I_5	8.5	White	CO, SM	Dark pink	35	32
I_6	9.0	Pinkish yellow	PF, RM	Dirty white	30	27
I_7	8.5	White	PS, RM	Light yellow	28	30
I_8	8.1	White	PF, SM	Dirty white	32	24
3. Richard's						
I_1	6.0	Pinkish White	SC, SM	Light yellow	25	21
I_2	7.6	White	CO, SM	Pink	34	30
I_3	6.8	White to cream	WO, SM	Dirty white	36	20
I_4	7.4	Pinkish	PD, SM	Pink	28	22
I_5	5.0	White	CO, SM	Creamy	30	24
I_6	6.3	Pinkish white	PF, SM	Dark pink	20	22
I_7	7.5	White	WO, SM	Light yellow	25	21
I_8	6.2	White	PF, SM	Yellowish	22	20
4. Broun's						
I_1	5.1	White	PS, SM	Light yellow	21	19
I_2	6.0	White	CO, RM	Creamy	26	17
I_3	4.9	white	PF, RM	Dirty white	35	19
I_4	4.5	Pinkish	WO, RM	Pink	25	16
I_5	3.3	White	CO, SM	Creamy	30	18
I_6	4.0	Pinkish white	PF, RM	Voilet	17	13
I_7	4.7	White	PS, RM	Light yellow	19	15
I_8	3.9	White	SC, SM	Pink	22	18

SC= Slightly cottony growth, CO= Cottony growth, WO= Wooly growth, PS= Poor suppressed growth, PD= Poor growth slightly, PF= Poor felt light, SM= Smooth margin, RM= Roof margin

Six types of mycelial growth characters and two types of margins were recorded in cultures of eight isolates on four different media. Mycelial growth characters varied with the type of medium, while majority of the isolates displayed either cottony or slightly cottony growth of aerial mycelium. Isolates I_4 and I_7 did not display these types of growth on any medium. None of the isolates displayed woolly growth on modified Czapek Dox agar medium. Poor felt-like growth was displayed only by isolate I_6 and that too not on PDA. Margins of the colonies of most of the isolates were smooth on PDA but reverse was observed on modified Czapek Dox and Broun's agar medium, where as margins of the colonies of the isolates were smooth on Richard's medium. Margins of the colonies of the isolates I_1, I_5 and I_8 remained smooth irrespective of the type of medium. Different colours of substrate pigmentation were observed as a result of growth of different isolates on different media. One isolate producing one type of substrate pigmentation on one type of medium, produced different type of substrate pigmentation on different media, however, isolate I_1 and I_7 produced light yellow coloured substrate pigmentation on all the four media. Isolate I_3 produced dirty white coloured substrate pigmentation on all the four media. The formation of macro-conidia by different isolates was affected by the type of medium. The isolates differed amongst themselves in production of macro-conidia. On PDA, maximum number of macro-conidia were formed by isolate I_2, I_3 and I_7. On Czapek Dox, maximum number of macro-conidia were formed by isolate I_1 and I_3. Isolate I_6 and I_7 produced minimum number of macro-conidia modified Broun's agar medium. Most of the isolates could not sporulate as efficiently on modified Broun's agar medium as on the other three media. The formation of micro-conidia by most of the isolates was again not as efficient on modified Broun's agar medium as on other three media. Isolate I_2 produced maximum number of micro-conidia on PDA. Taking both macro-conidia and macro-conidia formation into account, it can be seen that I_2 produced maximum number of both micro-conidia and macro-conidia on PDA. Variability in colour of mycelium and sporulation *Fusarium oxysporum* f.sp. *ciceri* were also reported by Tetarwal *et al.* (2016) [10], Mathur and Parsad (1967) [7].

b) Variability on broth media

The data on dry fungal biomass of different isolates on broth media revealed that mean dry fungal biomass of all the eight isolates was maximum (244.72 mg) on potato dextrose medium followed by Czapek Dox medium (238.98 mg). Almost all the isolates produced highest dry fungal biomass on potato dextrose medium except isolate I_6 which showed highest fungal dry mass on Czapek Dox medium (236.50 mg). The isolate I_2 produced maximum dry fungal biomass (256.13 mg) on potato dextrose medium and isolate I_7 showed minimum dry fungal biomass (139.30 mg) on Broun's medium whereas, isolate I_4 showed minimum dry fungal biomass (140.10 mg) on Martin's medium.((Table 2) In general, the potato dextrose, Czapek Dox and moong bean meal broth media were found excellent for macro- and micro-conidia formation. The potato dextrose showed highest number of macro-conidia of isolate I_2, (46) and I_3, (46) whereas, corn meal and Martin's media showed lowest macro-conidia of isolates I_1 and I_3. (Table 3) Variation among wilt fungi is well known. In *Fusarium*, the more common variations are reduction in aerial mycelium or growth rate, changes in production of chalamydospores variation in production of macro and micro conidia and colony pigmentation. Teterwal *et. al* (2016) [10], reported that variability of *Fusarium oxysporum* f.sp. *ciceri* the color of the mycelium and sporulation in different nine isolates. Mathur and Prasad (1967) [7] also observed variability in pigmentation, topography, sporulation and relative production and size of macro and micro-conidia among isolates of coriander wilt *Fusarium oxysporum*. The morphological studies conducted by Reddy and Chaudhary (1985) [8] also revealed that six isolates of *Fusarium oxysporum* f.sp. *ciceri* had variation in number and size of macro and micro-conidia, cultural characters, growth pattern, pigmentation and sporulation. Six types of mycelial growth characters and two types of margins in cultures of 9 isolates of *Fusarium oxysporum* f.sp. *cumini* on three different media were also observed by Champawat (1986) [2]. He further reported that isolates varied in pathogenicity and produced sickle shaped macro and micro conidia.

Table 2: Effect of different broth media on Fungal biomass of *Fusarium oxysporum* f.sp. *corianderii* isolates

Broth media	Dry fungal biomass (mg)*								
	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	Mean
Czapek Dox	246.72	229.40	231.37	227.41	231.25	236.50	240.52	241.97	238.98
Potato dextrose	253.47	256.13	249.18	243.21	240.16	224.20	243.84	247.63	244.72
Richard's	171.40	178.31	153.07	156.30	152.40	163.27	153.44	164.56	171.32
Broun's	146.10	147.52	162.10	151.87	154.10	156.86	139.30	145.95	164.05
Malt extract	242.51	185.21	181.51	163.75	169.32	165.42	167.13	191.50	192.15
Corn meal	187.23	184.60	194.65	186.39	163.05	179.29	164.37	176.40	188.43
Oat meal	236.54	205.12	181.20	191.60	207.29	191.41	198.03	187.67	206.23
Martins	161.43	166.73	163.81	140.10	164.21	155.57	159.31	160.05	170.07
Moong bean	197.69	187.33	191.14	165.23	151.36	142.05	154.51	161.42	177.44
Mean	198.70	256.13	189.78	180.65	181.46	179.39	180.05	186.35	

* Mean of four replications

Table 3: Effect of different broth media on conidia formation in *Fusarium oxysporum* f.sp. *corianderii* isolates

Agar media	Number of conidia per ml*																	
	Macro-conidia**									Micro-conidia**								
	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	Mean	I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	Mean
Czapek Dox	44	42	42	37	38	35	38	39	39.38	23	25	33	37	41	36	28	30	31.63
Potato dextrose	41	46	46	36	38	43	41	40	41.38	35	40	28	40	36	29	23	26	32.13
Richard's	26	37	39	32	29	24	26	26	29.88	24	15	19	16	26	23	20	21	20.50
Broun's	19	24	38	28	32	23	24	22	26.25	19	29	25	20	25	24	16	17	21.87
Malt extract	35	29	37	22	39	21	22	21	28.25	20	31	27	37	20	18	26	28	25.88
Corn meal	19	36	34	28	35	38	35	33	32.25	28	36	39	27	18	16	31	21	27.00
Oat meal	37	21	32	29	24	19	20	21	25.38	22	21	37	26	35	23	19	31	26.75
Martins	24	29	16	38	36	24	24	25	27	35	24	30	23	31	13	29	29	26.76
Moong bean	31	41	37	39	43	30	32	33	35.75	34	40	38	18	34	21	37	24	30.75
Mean	30.67	33.89	35.67	32.11	34.89	28.56	29.11	28.89		26.67	29.00	30.67	27.11	29.56	22.56	25.44	25.22	

* Mean of four replications

** Conidia per microscopic field

Morphological variability

All the isolates produced sickle shaped macro-conidia. Amongs different isolates, isolate I_3 was produced conidia of minimum length (23.44 µm) and isolate I_2 (31.15 µm) produced conidia of maximum length as compared to other isolates. (Table 4) The different isolates produced macro-conidia of varying width and the isolate I_6 showed significantly maximum width of conidia (5.19 µm) than all other isolates. The isolate I_7 showed minimum width of conidia (3.47 µm) that was at par with isolate I_5 and significantly lower than all other isolates. All the isolates showed wide variability in number of septa present (Table 4.6). The isolate I_7 produced significantly lowest number of septa (2.43) that at par with isolate I_3 and while isolate I_2 produced significantly highest (3.75) number of septa than all other isolates.

Table 4: Size of macro and micro conidia and number of conidial septa in different isolates of *Fusarium oxysporum* f.sp. *corianderii* on potato dextrose agar medium

Isolate	Macro-conidia			Micro-conidia		
	Length (µm)	Width (µm)	No. of range septa	Length (µm)	Width (µm)	No. of range septa
I_1	27.30	4.21	3.50	10.31	3.61	0.93
I_2	31.15	5.03	3.75	10.71	3.59	0.97
I_3	23.44	4.13	2.47	9.42	3.50	0.68
I_4	27.54	4.56	3.64	8.21	3.63	0.79
I_5	26.13	3.63	2.97	9.56	3.56	0.84
I_6	25.68	5.19	3.29	7.92	3.53	0.89
I_7	24.63	3.47	2.43	9.47	3.49	0.77
I_8	26.98	4.52	3.34	10.53	3.57	0.86
SEm±	0.69	0.09	0.07	0.21	0.07	0.02
CD at 5%	2.04	0.26	0.20	0.62	0.20	0.05
CD at 1%	2.78	0.35	0.27	0.85	0.27	0.07

All the isolates formed ovoid micro-conidia. The isolate I_6 gave minimum length (7.92 µm) whereas maximum length of micro-conidia was found in isolate I_2 (10.71 µm). The different isolates did not show variation with regard to width of micro-conidia (Table 4).The isolate I_5, I_6 and I_8 did not differ significantly so far as number of septa is concerned. However, the isolates I_3, I_7 and I_4 produced significantly lower number of septa than I_2 that produced highest number of septa amongst all isolates. Thus, isolates of the pathogen showed great variation in morphological characters as reported by Mathur and Prasad (1967) [7]. Some isolates ceased sporulation on continuous culturing but regained it on passing through the host. Mall (1969) [5] also observed two distinct type of macro and micro conidia. *Fusarium oxysporum* f. sp. *corianderii* isolates varied morphologically and in their pathogenic nature in the present investigations as earlier observed by Honnareddy and Dubey (2006) [4] while studying pathogenic and genetic variability of *Fusarium oxysporum* f. sp. c*iceri* isolates collected from different parts of India. Similarly, Mandharea *et al* (2011) [6] and Arvayo-Ortiz *et al.* (2011) also observed pathogenic and morphological variation in isolates of *Fusarium oxysporum* f. sp. c*iceri*, thereby, confirming the findings of present investigations.

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