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Sporulation and chlamydospore formation of different isolates of *Fusarium culmorum* under *in vitro* condition

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In vitro study of different isolates of *Fusarium culmorum* like CF_2, CF_5, CF_6, and CF_7 was done on different cultural media like potato dextrose agar, oat meal agar, papavizas and Potato sucrose agar in respect of their sporulation behaviour and chlamydospore formation. It was observed that the intensity of sporulation was significantly different among the isolates and highest sporulation was observed on PDA medium (46.43×10^5 / ml by CF_2) after 20 days. With increasing age of culture the sporulation intensity was reduced irrespective of isolates and media used. In case of intensity of chlamydospore formation it was observed that with increasing age of culture the production was reduced and maximum production was observed after 20 days by CF_6 (60×10^4 / ml) as compared to other cultures irrespective of media used. All the culture produced highest chlamydospore on PDA except CF_2 isolates. All the isolates produced chlamydospore on papavizas medium after 10 days and others media showed different in chlamydospore production by different isolates.

Key words : Carnation, basal rot, *Fusarium culmorum*, isolates, chlamydospore, sporulation behaviour.

INTRODUCTION

Basal rot of carnation by different fusarial species like *Fusarium culmorum* (Smith) Sacc., *F. equiseti* (Conda) Sacc, *F. roseum ; F. avenaceum* cause 70% plant mortality in different parts of world (White, 1936). In West Bengal, only one species *Fusarium culmorum* (Smith) Sacc. causes plant mortality (Das, 2004). The present experiment was carried out to find the different isolates of *Fusarium culmorum* collected from different infected plant parts and their survival behavious by producing chlamydospore and intensity of sporulation in different culture media under *in vitro* condition.

MATERIALS AND METHODS

The diseased specimens collected from different carnation fields in Nadia, Hooghly and 24 Parganas (North) were taken for isolating the causal organism *Fusarium* species associated with the symptoms.

Isolation was made within 24 hours of collecting the samples. A small bit of infected host tissue was washed thoroughly in distilled water, surface sterilized by immersing in 0.1% sodium hypochorite solution for about 1 minute, washed in sterile water and then transferred aseptically to sterilized PDA slants and incubated at 28°C for about 3-4 days. When mycelial growth from the host tissue was noticed, a small portion of mycelium was aseptically trnasferred to PDA slants. This culture was regarded as original culture and subcultures of all the isolates were made at 2 months intervals. Stem and root pieces at different levels above and below the ground level were used to isolate the organism. The isolated causal organism was inoculated to healthy plants to reproduce the disease symptoms. For pathogenicity tests, selected cuttings were carefully examined for freedom from disease and grown under conditions that precluded infection from outside sources. Also a large number of control plants were grown, thus

reducing the chances of error from incipient cutting infection.

The inoculations were made by adding the *Fusarium* culture grown in sand-maize meal media to the soil @ 1.0% on dry weight basis in which the cuttings were planted. The plants, if susceptible, developed a typical symptom, while the adjacent control plants remained healthy. From various parts of infected plants the *Fusarium* was reisolated and these reisolations, when compared with each other and with original isolate, were found to agree in culture and morphological characters. Also the re-isolations were tested by inoculating plants and proved pathogenic.

The isolates of *Fusarium* were identified on tbe basis of macroscopic and microscopic growth feature (Booth, 1971) followed by pathogenicity tests. For noting the intensity of sporulation 6 discs of 6 mm size were cut by means of a sterilized cork borer at random from ech petriplate of growing culture of 10, 20 and 30 days old on potato-dextrose agar (PDA), potato sucrose agar (PSA), oat-meal agar (OA), and Papavizas medium and 10 ml of distilled water was added to it in a culture tube. The tube was vigorously shaken in an electric shaker for 10 minutes and the suspension was passed through folded bandage cloth to eliminate mycelial and the substratum in which the fungus was grown. The number of conidia and chlamydospores per ml of the suspension were then calculated by means of a haemacytometer. Three petriplates were taken for each isolate. Sporulation of different isolates of *Fusarium culmorum* on different media at different time intervals (10, 20 and 30 days) was observed. The results of an average of three replications were analyzed statistically to find out the differentiation among the isolates and media used.

RESULTS AND DISCUSSION

The different isolates like CF_2, CF_5, CF_6, and CF_7 were taken into consideration for the study according to their pathogenicity. The isolates for *Fusarium culmorum* varied greatly among themselves in respect to intensity of sporulation and growth rate on different media.

The average sporulation from 10 days old culture was highest in isolates CF_2 (42.25 × 10^5/ml) irrespective of medium. In isolate CF_6 and CF_5 sporulation was also quite high. It was lowest in isolate CF_7 (16.30 × 10^5 / ml) Similarly, average sporulation was highest on PDA medium (45.07 × 10^5 /ml) and a lowest on Papavizas medium (23.7 × 10^5 /ml) irrespective of isolates. It also observed that sporulation was highest in isolate CF_2 grown in PDA medium which was significantly higher as compared to other interactions. The lowest sporulation ws observed by CF_7 isolate grown on PDA, and no sporulation on PSA medium (Table 1).

Table 1 : Intensity of sporulation of conidia of different isolates of *F. culmorum* on different medium at 28°C (10 days old).

Isolates	Intensity of sporulation of conidia (× 10^5 / ml) on different medium (10 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	82.98	35.83	41.25	8.93	42.25
CF_5	48.00	31.33	38.13	19.46	34.23
CF_6	48.26	52.00	18.93	30.93	37.53
CF_7	1.06	0.00	28.66	35.46	16.30
Mean	45.07	29.79	31.74	23.70	
	S. Em±	CD at 5%			
Isolates		1.76	5.09		
Medium		1.76	5.09		
Medium × Isolates		3.53	10.19		

Table 2 : Intensity of sporulation of conidia of different isolates of *F. culmorum* on different medium at 28°C (20 days old).

Isolates	Intensity of sporulation of conidia (× 10^5 / ml) on different medium (10 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	86.66	70.00	21.66	7.40	46.43
CF_5	37.20	41.06	24.26	13.33	28.96
CF_6	34.00	49.86	17.60	31.20	33.16
CF_7	1.86	2.26	15.73	20.40	10.06
Mean	39.93	40.80	19.81	18.08	
	S. Em±	CD at 5%			
Isolates		1.03	2.97		
Medium		1.03	2.97		
Medium × Isolates		2.06	5.94		

The average sporulation from 20 days old culture was highest in isolate CF_2 (46.43 × 10^5 /ml) and lowest significantly among themselves. The highest

sporulation was obtained on PSA medium (40.80 × 10^5 /ml) irrespective of isolates, followed by PDA medium and both were statistically at par, Lowest sporulation was recorded on Papavizas medium (18.08 × 10^5 /ml). The interaction between isolate and medium showed that sporulation was significantly highest in isolate CF_6 grown in PDA medium (71.6 × 10^5 / ml) and lowest in isolate CF_7 (1.06 × 10^5/ml) (Table 3). The rate of development of conidia was independent of the growth rate but the rate of production of conidia was greatest at high growth rate and also depends upon the pH of the medium (Larmour and Marchant, 1977).

Table 3 : Intensity of sporulation of conidia of different isolates of *F. culmorum* on different medium at 28°C (30 days old).

Isolates	Intensity of sporulation of conidia (× 10^5 / ml) on different medium (30 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	52.50	60.00	14.16	3.73	32.60
CF_5	42.40	58.53	32.26	4.53	34.43
CF_6	71.60	46.93	12.93	19.46	37.73
CF_7	1.06	3.33	6.13	28.53	9.76
Mean	41.89	42.20	16.37	14.06	
	S. Em±	CD at 5%			
Isolates		1.66	4.81		
Medium		1.66	4.81		
Medium × Isolates		3.33	9.62		

Table 4 : Intensity of chlamydospore production of different isolates of *F. culmorum* on different medium at 28°C (10 days old).

Isolates	Intensity of chlamydospore production (× 10^4 / ml) on different medium (10 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	0.00	0.00	0.00	5.33	1.33
CF_5	0.00	0.00	5.33	10.66	4.00
CF_6	0.00	66.66	10.66	25.33	25.66
CF_7	57.33	0.00	16.00	10.66	21.00
Mean	14.33	16.66	8.00	13.00	
	S. Em±	CD at 5%			
Isolates		3.02	8.71		
Medium		3.02	NS		
Medium × Isolates		6.04	17.42		

and medium showed that isolate CF_2 achieved significantly highest sporulation on PDA medium (86.66 × 10^5 / ml) and lowest in isolate CF_7 (1.86 × 10^5 / ml) (Table 2). Whereas from the 30 days old culture it was observed that sporulation was highest in isolate CF_6 (37.73 10^5 / ml) irrespective of medium followed by CF_6 (34.43 × 10^5 /ml). Similarly, highest sporulation achieved from PSA medium (42.20 × 10^5 / ml) followed by PDA medium irrespective of isolates and their differences were statistically at per and lowest sporulation was achieved from Papavizas medium (14.06 × 10^5 / ml). The interaction between isolates

Table 5 : Intensity of chlamydospore production of different isolates of *F. culmorum* on different medium at 28°C (20 days old).

Isolates	Intensity of sporulation of conidia (× 10^4 / ml) on different medium (20 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	1.41	18.75	25.00	9.33	13.62
CF_5	38.66	21.33	8.00	45.33	28.33
CF_6	145.33	40.00	22.66	32.00	60.00
CF_7	64.00	12.00	5.33	29.33	27.66
Mean	62.35	23.02	15.25	29.00	
	S. Em±	CD at 5%			
Isolates		4.05	11.67		
Medium		4.05	11.67		
Medium × Isolates		8.10	23.34		

Table 6 : Intensity of chlamydospore production of different isolates of *F. culmorum* on different medium at 28°C (30 days old).

Isolates	Intensity of chlamydospore production (× 10^4 / ml) on different medium (30 days)				
	PDA	PSA	OA	PAP	Mean
CF_2	37.50	25.00	16.66	18.66	24.45
CF_5	6.66	0.00	8.00	20.00	8.66
CF_6	37.33	37.33	34.66	53.33	40.66
CF_7	28.00	8.00	8.00	41.33	21.33
Mean	27.37	17.58	16.83	33.33	
	S. Em±	CD at 5%			
Isolates		3.24	9.36		
Medium		3.24	9.36		
Medium × Isolates		6.49	NS		

Intensity of chlamydospore production of by *F. culmorum* isolates was examined microscopically and observed that 10 days old culture produced maximum chlamydospores in isolate CF_6 (25.66 × 10^4 / ml) and minimum in isolate CF_2 (1.33 × 10^4 / ml) irrespective of medium. Isolate CF_6 and CF_7 were statistically at par in that respect. The chlamydospore production was highest in PSA medium (16.66 × 10^4 / ml) and lowest in OA medium (8.0 × 10^4 / ml) irrespective of isolates and

the difference among themselves were non-significant. The highest interaction was achieved by isolate CF_6 grown on PSA medium (66.66 × 10^4 / ml), although some isolates did not produce any chlamydospore (Table 4).

Whereas in 20 days old culture, maximum chlamydospore were produced in isolate CF_6 (60.0 × 10^4 / ml) and minimum in isolate CF_2 (13.62 × 10^4 / ml) irrespective of medium. Isolate CF_5 and isolate CF_7 were statistically at par. Similarly maximum chlamydospore were produced in PDA (62.35 × 10^4 / ml) which was significantly highest and lowest in OA medium (15.25 × 10^4 / ml) irrespective of isolates. It is also seen that isolates CF_6 produced maximum chlamydospore in PDA medium (145.33 × 10^4 / ml), which was significantly higher as compared to other interactions. Minimum chlamydospore produced by isolate CF_6 in Papavizas medium (53.33 × 10^4 / ml) but the difference among interactions between isolate and mediums were non-significant (Table 5). 30 days old culture showed that all the isolates produced lower number of chlamydospores except CF_5 as compared to 20 days old culture (Table 6). It means chlamydospore production gradually decreased statistically with increase in culture age. It was concluded from the above experiment that 20 days old culture produced maximum sporulation and chlamydospore formation in PDA medium and when isolates were considered CF_2 produced maximum sporulation and CF_6 produced maximum chlamydospore formation though CF_2 isolates produced minimum chlamydospore in PDA medium. All the isolates produce chlamydospore on Papavizas medium after 10 days and others media showed difference in chlamydospore production by different isolates.

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