

## Influence of nutritional media, pH and temperature on mycelial growth and sporulation of *Fusarium udum*

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### ABSTRACT

Effect of different culture media, pH and temperature on mycelial growth and sporulation of three culturally and pathogenically different isolates of *Fusarium udum* (F<sub>3</sub>, F<sub>8</sub> and F<sub>17</sub>) was studied. Four liquid media viz., two synthetic media (Czapek's dox and Martin's media) and two semi synthetic media (potato dextrose and pigeonpea seed extract) were used. Mycelial growth and sporulation were studied at eight different temperatures ranging from 10-45°C and seven pH ranging from 5.0-8.0. Dry mycelial weight was maximum on pigeonpea seed extract medium by all the three isolates while in respect of sporulation (both micro and macro conidia production), potato dextrose broth was found the best. Isolate F<sub>8</sub> produced maximum mycelial growth while isolates F<sub>3</sub> and F<sub>17</sub> produced comparatively less mycelial growth. Temperature range of 25 to 30°C was, in general, most suitable for all the three isolates. These isolates showed reasonable growth at pH regimes ranging from 5.0-8.0, optimum being 7.0-8.0. No differential response of the isolates to temperature and pH regime was recorded. It is indicated that slow growing isolates with appressed mycelium are more pathogenic than faster growing isolates with fluffy mycelium.

Key words: *Fusarium udum*, Nutritional media, pH, Temperature

Vascular wilt caused by *Fusarium udum* Butler is one of the most serious and damaging diseases of pigeonpea in India. The fungus is a soil borne facultative parasite that enters through roots and becomes systemic. Survival of the fungus in the soil is influenced by many abiotic factors. Though the effect of different culture media, pH, temperature, etc., on *F. udum* have been studied by Mundkur (1935), Yogeshwari (1948), Singh (1968), Prasad and Chaudhary (1977), Reddy and Basuchaudhary (1985), Rai and Upadhyay (1983) and Chakraborty and Gupta (1995), only Subramanian (1961) and Laha (1978) made cultural and pathological studies on different isolates of *Fusarium udum* and indicated that isolates differ in their pH and temperature requirements. Since then, no such differential requirement of culture media, temperature, hydrogen ion concentration, etc., by *F. udum* isolates for their optimum growth and sporulation was studied. In view of this, experiments were conducted to find out the optimum media, temperature and pH for growth and sporulation of *F. udum* isolates differing in cultural, morphological and pathogenic characters.

### MATERIALS AND METHODS

**Selection of basal medium:** Three isolates of *Fusarium udum* (F<sub>3</sub>, F<sub>8</sub> and F<sub>17</sub>) collected from experimental farm of Indian Institute of Pulses Research, Kanpur differing in cultural and pathogenic characters (Table 1) were grown on four liquid media viz., two semi-synthetic media (potato dextrose and pigeonpea seed extract broth) and two synthetic media (Czapek's dox and Martin's medium broth) for selecting the best basal medium. Standard composition of the media was taken. For pigeonpea seed extract broth, aqueous extracts from 200 g pigeonpea seed and 20 g sucrose made to 1.0 litre was used. Five replications of each medium inoculated with each of the three isolates incubated at 25±1°C were maintained.

Table 1. Cultural characteristics and relative pathogenicity of *F. udum* isolates

Isolate	Cultural characters on PDA			Radial growth rate	Relative pathogenicity
	Colony type	Colony colour	Pigmentation		
F <sub>3</sub>	Appressed	Off white	Purple	Medium	> 90% wilting
F <sub>8</sub>	Fluffy	White	Yellow	Fast	51-70% wilting
F <sub>17</sub>	Moderately appressed	Off white	Cream	Slow	71-90% wilting

Chromic acid washed coming glassware, 'Analar' grade chemicals and double distilled water were used for all studies. The media were sterilized by autoclaving at 1.2 kg/cm<sup>2</sup> pressure for 25 minutes. Fifty ml of medium was taken into 250 ml conical flasks in three replicates for each treatment. Inoculations were made by placing a 5 mm disc containing mycelium and conidia from 7 days actively growing culture. The inoculated flasks were incubated at 25±1°C for 15 days. The pH of medium was adjusted to 6.0 in each case before autoclaving. After incubation, the contents of each flask were filtered through pre-weighed and dried Whatman filter paper No.42 and washed with distilled water to remove the adhering salts, if any. The mycelial mat was subsequently dried at 70°C in an electric oven for 48 hours and weighed on a single pan micro balance for determination of dry weight. The dry weight data of the fungal mats were statistically analyzed. For recording the extent of conidia production, a uniform amount of fungal growth was taken at random from three different places and observed under low magnification (40 X 10x) of research binocular. The degree of macroconidia production was calculated by averaging the number of macroconidia per microscopic field.

**Effect of temperature on mycelial growth and sporulation:** Potato dextrose broth medium was used as a basal medium for this experiment on the basis of results of studies on basal medium. After autoclaving, the flasks were kept at different temperatures for at least 24 hours before inoculation to remove the lag effect. The temperatures tried were 10, 15, 20, 25, 30, 35, 40 and 45°C. The flasks were inoculated with 5 mm disc of each isolate replicated thrice and incubated for 15 days at the respective temperatures. Dry weight of mycelial growth and conidia production by different isolates were recorded.

**Effect of pH on mycelial growth and sporulation:** Same isolates were grown on potato dextrose broth at seven different pH values viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. The pH was maintained with NaOH (Sodium hydroxide) for increasing the pH and H<sub>3</sub>PO<sub>4</sub> (Phosphoric acid) for decreasing the pH value and calibrated in a pH meter. An extra flask was maintained to measure the pH after autoclaving in each treatment. Inoculated flasks with 5 mm disc of each isolate replicated thrice were incubated for 15 days at 25±1°C. Later, the contents of the flasks were filtered and dried for recording the dry weight. The pH of the culture filtrate was measured to find out the change in pH value.

## RESULTS AND DISCUSSION

**Growth and sporulation of isolates of *F. udum* on different media:** Observations on dry mycelial weight of the isolates and sporulation are presented in Table 2. Among the different media, dry mycelial weight was maximum on pigeonpea seed extract broth by all the three isolates with 0.734 mg of isolate F<sub>3</sub>, 1.050 mg of F<sub>8</sub>, and 0.239 mg of F<sub>17</sub>. The next better medium was potato dextrose broth with 0.176, 1.031 and 0.210 mg mycelial dry weight of isolates F<sub>3</sub>, F<sub>8</sub> and F<sub>17</sub>, respectively. Among the isolates, F<sub>8</sub> yielded maximum mycelial dry weight on all the culture media studied. Isolate F<sub>17</sub> was next in production of mycelial mass while isolate F<sub>3</sub> produced least mycelial growth on all the media except pigeonpea seed extract broth.

In respect of sporulation, potato dextrose broth was found best for all the three isolates. Production of

chlamydo spores, however, was low in this medium. Pigeonpea seed extract medium yielding very high dry mycelial weight by all isolates, showed moderate microconidia production but no macroconidia were observed in this medium. Isolates F<sub>3</sub>, and F<sub>8</sub> did not produce chlamydo spore on this medium while moderate production of chlamydo spore was recorded by isolate F<sub>17</sub>. Micro and macroconidia production was high and low in Czapek's dox broth whereas chlamydo spores were observed in low to moderate numbers. In Martin's medium, the sporulation was very scanty with no production of chlamydo spores. Almost no variation in the extent of sporulation was observed among the isolates. Good sporulation on potato dextrose has also been recorded by Mundkur (1935), Yogeshwari (1948), Singh (1968) and Prasad and Chaudhary (1977).

### Effect of temperature Mycelial growth and sporulation

were studied at eight different temperatures ranging from 10 to 45°C (Table 3). The maximum dry weight of mycelium for isolates F<sub>3</sub> and F<sub>8</sub> was recorded at 25°C (0.412 mg in isolate F<sub>3</sub> and 1.008 mg in F<sub>8</sub>). For isolate F<sub>17</sub>, the maximum dry weight (0.712 mg) was, however, found at 30°C. There was a drastic reduction in mycelial dry weight below 20°C. Thus, all the isolates could grow well in the temperature range of 20-35°C with optimum temperature ranging between 25 and 30°C. A temperature range of 20-30°C has been reported optimum for growth and sporulation of *F. udum* (Yogeshwar 1948, Singh 1968). Our results are in conformity with these findings. Isolate F<sub>8</sub> invariably showed very high mycelial weight at all the temperatures tested as compared to isolates F<sub>17</sub> and F<sub>3</sub>. Isolate F<sub>17</sub> yielded moderate mycelial growth while F<sub>3</sub> showed least.

Isolate F<sub>3</sub> produced maximum microconidia and macroconidia at 25 and 30°C. However, the chlamydo spores formation in this isolate was more at 35 and 40°C. More or less similar situation was recorded in isolate F<sub>8</sub> and F<sub>17</sub>. In F<sub>8</sub> maximum microconidia, macroconidia and chlamydo spores were produced at 30°C while at 25°C chlamydo spores production was low. In F<sub>17</sub>, maximum microconidia and macroconidia were formed at 25°C while more chlamydo spores were produced at 30°C and above. Macroconidia production was very much reduced at 20°C in all three isolates showing no formation at temperature 15°C and 10°C. No macroconidia

Table 2. Growth and sporulation of three variable isolates of *F. udum* on different media

Nutritional media	Mycelial dry weight (mg)				Extent of conidia (sporulation)								
	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	Mean	Microconidia			Macroconidia			Chlamydo spore		
					F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>
Potato dextrose broth	0.176	1.031	0.210	0.472	++	++	++	++	++	++	+	+	+
Pigeonpea seed extract broth	0.734	1.050	0.239	0.674	++	+	++	-	-	-	-	-	++
Czapek's dox broth	0.184	0.545	0.197	0.308	++	++	++	+	+	+	++	+	+
Martins broth	0.174	0.563	0.192	0.309	+	-	-	-	+	-	-	-	-
Mean	0.317	0.797	0.209										
CD (5%) for Mycelial dry weight	Isolate	Nut. Media		Isolate x Nut. Med									
	0.020	0.031		0.012									

- Nil, + Scanty, ++ Moderate, +++ High

Table 3. Effect of temperature on growth and sporulation of three variable isolates of *F. udum*

Temp (0°C)	Mycelial dry weight (mg)				Sporulation								
	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	Mean	F <sub>3</sub>			F <sub>8</sub>			F <sub>17</sub>		
					Mic.	Mac	Chl.	Mic.	Mac	Chl.	Mic	Mac	Chl
10	0.103	0.372	0.148	0.207	-	-	-	-	-	-	-	-	-
15	0.188	0.504	0.178	0.290	+	-	+	+	-	+	-	-	+
20	0.356	0.881	0.543	0.593	++	+	+	++	+	+	++	+	+
25	0.412	1.008	0.708	0.709	+++	+++	+	+++	+++	+	+++	+++	+
30	0.402	0.924	0.712	0.679	+++	+++	+	+++	+++	++	++	+++	++
35	0.357	0.803	0.489	0.549	+	++	++	+	++	++	+	++	++
40	0.174	0.603	0.198	0.325	+	-	++	+	-	++	+	-	+
45	0.089	0.103	0.079	0.090	-	-	+	-	-	+	-	-	+
Mean	0.260	0.649	0.381										
CD (5%) for mycelial dry weight	Isolate = 0.003				Temperature = 0.006				Isolate x Temp. = 0.002				

- NIL. - Scanty. ++ Moderate. +++ High Mic. = Microconidia, Mac. = Macroconidia, Chl. = Chlamydo-spore

was recorded at 40 and 45°C. In fact, at temperature 10 and 45°C there was no sporulation (both micro and macroconidia) except scanty chlamydo-spore formation at 45°C. A temperature range of 20-30°C has been reported optimum for growth and sporulation of *F. udum* on potato dextrose medium by earlier workers (Yogeshwari 1948, Singh 1968). Rai and Upadhyay (1983) reported maximum growth at 22°C.

**Effect of pH:** The data on mycelial dry weight are presented in Table 4. All the isolates showed reasonable growth at pH regimes ranging from 5.0-8.0. However, the isolates differed in respect of total mycelial dry weight obtained at different pH. Isolate F<sub>3</sub> recorded maximum growth (0.400-0.428 mg) at pH 7.0-8.0 while optimum growth in isolate F<sub>17</sub> was obtained at 7.0-7.5 pH (0.327-0.334 mg). Isolate F<sub>8</sub> showed maximum mycelial dry weight of 0.422-0.461 mg at pH 7.5-8.0. Data on the pH of culture filtrate presented in Table 3 showed that isolate F<sub>3</sub> tended to bring the pH levels to near neutral (6.9 to 7.7) under all the pH regimes. Comparatively, the isolates F<sub>8</sub> and F<sub>17</sub> turned medium towards alkaline (pH 7.7 to 8.5). All the three isolates under present study could grow at the pH ranging from 5.0-8.0. However, the optimum pH for the isolates differed slightly. Isolate F<sub>3</sub> had pH optima at 7.0-8.0 while F<sub>17</sub> at 7.0-7.5 and F<sub>8</sub> at 7.5-8.0. Prasad and Chaudhary (1977) reported pH optima of 6.0 for best growth and sporulation in *F. udum*

isolates. The present results, however, are in conformity to Rai and Upadhyay (1983) who recorded pH range of 7-9 as more suitable for *F. udum*. All the isolates in present study exhibited differential ability in changing the reaction (pH) of culture medium. There is no such earlier report of this kind.

The three isolates F<sub>3</sub>, F<sub>8</sub> and F<sub>17</sub> of *F. udum* showed differential growth and sporulation on potato dextrose and other media. Isolate F<sub>8</sub> with fluffy cottony mycelium and fastest growth rate produced maximum mycelial dry weight followed by isolate F<sub>17</sub> with moderately appressed mycelium and comparatively slower growth rate. Isolate F<sub>3</sub> with appressed mycelium and moderate growth rate produced least mycelial dry weight. Reddy and Basuchaudhary (1985) recorded fluffy, cottony and sticky mycelial growth in 6 isolates of *F. udum* and categorized them into three distinct groups based on mycelial growth pattern and radial growth rate. Differential colony growth pattern and growth rate have been also reported by Gohil and Vala (1996), Okirar and Kimani (1997) and several other workers but none of them observed any correlation of these characters with the relative pathogenicity of isolates. This study indicated that slow growing isolates with appressed mycelium are more pathogenic than fast growing isolates having moderate to highly fluffy mycelium.

Table 4. Effect of pH on growth of three variable isolates of *F. udum*

pH	Mycelial dry weight (mg)				pH of culture filtrate after 15 days			
	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	Mean	F <sub>3</sub>	F <sub>8</sub>	F <sub>17</sub>	Mean
5.0	0.337	0.305	0.330	0.324	7.30	7.50	7.73	7.51
5.5	0.358	0.343	0.235	0.312	7.14	8.27	8.01	7.80
6.0	0.340	0.299	0.305	0.314	7.76	7.80	8.27	7.94
6.5	0.363	0.355	0.312	0.343	7.37	8.12	8.09	7.86
7.0	0.400	0.407	0.327	0.378	6.92	8.12	8.21	7.75
7.5	0.408	0.422	0.334	0.388	7.14	8.14	8.32	7.86
8.0	0.428	0.461	0.307	0.398	7.24	8.49	8.20	7.97
Mean	0.376	0.370	0.307		7.26	8.06	8.11	
CD(5%) for mycelial dry weight	Isolate			pH	Isolate x pH			
	0.068			0.025	0.044			
CD (5%) for pH change	1.02			NS	NS			

## REFERENCES

- Chakraborty A and Sen Gupta PK. 1995. Factors affecting cross protection of *Fusarium* wilt of pigeonpea by soilborne nonpathogenic fungi. *Phytoparasitica* 23(4): 323-33
- Gohil VP and Vala DG. 1996. Pathogenic and cultural variability among the fungal isolates associated with sugarcane wilt. *Indian Journal of Mycology and Plant Pathology* 26(1): 69-74.
- Laha SK. 1978. Cultural and pathological studies on *Fusarium udum* Butler M Sc thesis, IARI, New Delhi.
- Mundkur BB. 1935. Influence of temperature and maturity on the incidence of sun hemp and pigeonpea wilt at Pusa. *Indian Journal of Agricultural Sciences* 5: 609-619.
- Okirar MA and Kimani PM. 1997. Pathogenic variation of *Fusarium udum* of pigeonpea. *Indian Journal Genetics and Plant Breeding* 57 (2): 186-192
- Prasad M and Chaudhary SK. 1977. Relation of pH levels and varied nutrient media to growth and sporulation of *Fusarium oxysporum* f.sp. *udum* Butler. *Research Journal of Ranchi University* 13: 214-222.
- Rai B and Upadhyay RS. 1983. Competitive saprophytic colonization of pigeonpea substrate of *Fusarium udum* in relation to environmental factors, chemical treatments and microbial antagonism. *Soil Biology and Biochemistry* 15: 187
- Reddy NPE and Chaudhary KCB. 1985. Variation in *Fusarium udum*. *Indian Phytopathology* 38(1): 172-173.
- Singh GP. 1968. Extracellular hydrolytic enzymes of *Fusarium lateritium* f.sp. *udum cajan*. *Indian Journal of Microbiology* 8: 95-100.
- Subramanian S. 1961. Studies on wilt of pigeonpea. Ph D thesis University of Madras Tamil Nadu, India
- Yogeshwari L. 1948. The element nutrition of fungi I. The effect of Boron, Zinc, and Manganese on *Fusarium* spp. *Proceeding of Academic Sciences (Sec. B)* 28: 177-201.