



STUDIES ON CULTURAL VARIABILITY OF *FUSARIUM UDUM* ISOLATES IN INDIA

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ABSTRACT

Existence of variation among forty one *Fusarium udum* isolates collected from different parts of India were studied with special reference to cultural characters on PDA medium. All the 41 *F. udum* isolates showed wide variations with respect to mycelial colour, pigmentation, colony characters. Based on pigmentation, 41 isolates were categorised into five groups viz., Group I produced brown colour pigmentation and consisted of three isolates viz., KFu-9, KFu-10 and TNFu-1, Group II produced dark yellow pigmentation and consisted of eight isolates viz., APFu-1, APFu-2, APFu-3, APFu-4, APFu-7, KFu-11, KFu-13 and KFu-14, Group III produced light yellow pigmentation and consisted of 21 isolates viz., APFu-9, APFu-11, BFu-1, KFu-1, KFu-2, KFu-3, KFu-12, KFu-15, KFu-16, MPFu-1, MSFu-1, MSFu-2, MSFu-3, MSFu-4, NDFu-1, TNFu-2, TNFu-3, TNFu-4, TNFu-5, UPFu-1 and WBFu-1, Group IV produced light yellow to brown colour pigmentation and consisted of four isolates viz., APFu-5, APFu-6, APFu-8 and APFu-10 and group V produced pink colored pigmentation and consisted of five isolates viz., KFu-4, KFu-5, KFu-6, KFu-7 and KFu-8. Based on mycelial colour, the isolates were categorised into two groups viz., white and pink. Group I comprised of 36 isolates viz., APFu-1, APFu-2, APFu-3, APFu-4, APFu-5, APFu-6, APFu-7, APFu-8, APFu-9, APFu-10, APFu-11, BFu-1, KFu-1, KFu-2, KFu-3, KFu-9, KFu-10, KFu-11, KFu-12, KFu-13, KFu-14, KFu-15, KFu-16, MPFu-1, MSFu-1, MSFu-2, MSFu-3, MSFu-4, NDFu-1, TNFu-1, TNFu-2, TNFu-3, TNFu-4, TNFu-5, UPFu-1 and WBFu-1 which produced white colored mycelia. Whereas, five isolates viz., KFu-4, KFu-5, KFu-6, KFu-7 and KFu-8 produced pink colored mycelia which was considered as Group II. Based on colony character 41 isolates were categorised into three groups viz., fluffy, partially appressed and appressed growth. Group I produced fluffy growth and consisted of seventeen isolates viz., APFu-2, APFu-3, BFu-1, KFu-1, KFu-2, KFu-3, KFu-4, KFu-5, KFu-11, KFu-13, KFu-14, KFu-15, KFu-16, MPFu-1, NDFu-1, UPFu-1 and WBFu-1, Group II produced partially appressed growth and consisted fifteen isolates viz., APFu-1, APFu-4, APFu-5, APFu-6, APFu-8, APFu-10, APFu-11, KFu-6, KFu-7, KFu-8, KFu-9, KFu-12, TNFu-1, TNFu-3 and TNFu-4. Group III produced appressed growth and consisted of nine isolates viz., APFu-7, APFu-9, KFu-10, MSFu-1, MSFu-2, MSFu-3, MSFu-4, TNFu-2 and TNFu-5.

KEY WORDS: Variation, Mycelia, pigmentation and colony character.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the major grain legume (pulse) crops of tropics and sub tropics. It finds an important place in the farming systems adopted by small and marginal farmers in a large number of developing countries as it restores the soil fertility by fixing atmospheric nitrogen. In India, it is commonly known as red gram, tur or arhar in Hindi, togari in Kannada (Reddy *et al.*, 1990). The crop is grown in the world in 4.26 million hectares with the production of 3.05 million tonnes and average yield of about 716.5 kg/ha. In India, it accounts for 3.73 million hectares of area with a production of 2.90 million tonnes and yield of about 776 kg/ha (Anon., 2007b). Karnataka accounts for 16.76 per cent of the India's output with an area of 0.60 million hectares and a production of 0.44 million tonnes and yield potential of about 727 kg/ha (Anon., 2007a). The main constraints in boosting the yield of the crop are its susceptibility to diseases, insects and other physiological stresses. Pigeonpea is known to be affected by more than hundred pathogens (Nene *et al.*, 1989). Incidentally, only a few of them cause economic losses (Kannaiyan *et al.*,

1984) and the distribution of the most important diseases is geographically restricted.

The pathogen is primarily a soil inhabitant; hence controlling the disease is very difficult as no effective chemicals are available at present. The development of resistant varieties and combined application of bioagents and fungicides is considered as more practicable. However, developing resistant varieties is a tedious and time consuming procedure. Though several attempts have been made to develop resistant varieties, as ICRISAT developed wilt resistant variety "ICP 8863" and released by the name "Maruthi". Recently, a disease incidence upto 10 per cent in some locations is common. This may be due to the prevalence or development of new strains of *F. udum* in various geographical locations in India. Presently, the information on the detection or identification of *F. udum* races/strains in the world in general and more particularly in India is lacking. Hence, there is a need to collect information on the existence of variability in *F. udum* from among isolates collected from different geographical locations in India. Keeping this in view, present investigations were envisaged by using 41

Fusarium udum isolates collected from major pigeonpea growing areas of India.

MATERIALS AND METHODS

Collection of *Fusarium udum* isolates from major pigeonpea growing areas of India

Pigeonpea plants showing typical symptoms of wilt induced by *F. udum* were collected from different states of India viz., Andhra Pradesh (11 isolates), Bihar (1 isolate),

Karnataka (16 isolates), Madhya Pradesh (1 isolate), Maharashtra (4 isolates), New Delhi (1 isolate), Tamil Nadu (5 isolates), Uttar Pradesh (1 isolate) and West Bengal (1 isolate) during *Kharif* seasons of 2005-06 and 2006-07 (Table-1). The samples were brought immediately to the laboratory and washed thoroughly in running water. The stalk and roots of wilted plants were separated and dried in shade for 3-4 days and preserved for further studies.

Table-1. List of *Fusarium udum* isolates collected from different locations of India

Sl. no	State	District	Taluk	Village	Isolate designation
1	Andhra Pradesh	Guntur	Chilakalurpet	Appanpuram	APFu-1
2	Andhra Pradesh	Guntur	Inkallu	Inkallu	APFu-2
3	Andhra Pradesh	Guntur	Narasaraopet	Kesanapalli	APFu-3
4	Andhra Pradesh	Guntur	Narasaraopet	Merakapudi	APFu-4
5	Andhra Pradesh	Karnool	Karnool	Kichvala	APFu-5
6	Andhra Pradesh	Karnool	Karnool	Mahadevanpet	APFu-6
7	Andhra Pradesh	Khammam	Veira	Menavalu	APFu-7
8	Andhra Pradesh	Mahabub Nagar	Jetcherla	Mannemkonda	APFu-8
9	Andhra Pradesh	Sangareddi	Kodangal	Regadimylapuram	APFu-9
10	Andhra Pradesh	Sangareddi	Thandur	Bandimidipalli	APFu-10
11	Andhra Pradesh	Sangareddi	Thandur	Thandur	APFu-11
12	Bihar	Muzaffarpur	Muzaffarpur	Muzaffarpur	BFu-1
13	Karnataka	Bangalore	Bangalore	GKVK	KFU-1
14	Karnataka	Bangalore	Magadi	Byraganapur	KFU-2
15	Karnataka	Bangalore	Magadi	Dhannampalya	KFU-3
16	Karnataka	Bijapur	Hindi	Salotagi	KFU-4
17	Karnataka	Gulbarga	Aland	Aland	KFU-5
18	Karnataka	Gulbarga	Aland	Nimbala	KFU-6
19	Karnataka	Gulbarga	Gulbarga	Sirunuru	KFU-7
20	Karnataka	Gulbarga	Gulbarga	Uppalav	KFU-8
21	Karnataka	Gulbarga	Sedam	Kolluru	KFU-9
22	Karnataka	Gulbarga	Sedam	Konkanahalli	KFU-10
23	Karnataka	Hassan	Arasikere	Margundanahalli	KFU-11
24	Karnataka	Hassan	Channarayapa ttana	Kandali	KFU-12
25	Karnataka	Hassan	Hassan	Ankapur	KFU-13
26	Karnataka	Kolar	Malur	Chokkandahalli	KFU-14
27	Karnataka	Mysore	Mysore	Beerihundi	KFU-15
28	Karnataka	Raichur	Raichur	Hidapanur	KFU-16
29	Madhya Pradesh	Khargaon	Khargaon	JNKVV Farm	MPFu-1
30	Maharashtra	Akola	Akola	PKV Akola	MSFu-1
31	Maharashtra	Ahamad Nagar	Kopergoan	Chanai	MSFu-2
32	Maharashtra	Badnapur	Badnapur	ARS Badnapur	MSFu-3
33	Maharashtra	Latur	Latur	Oil seed research station, Latur	MSFu-4
34	New Delhi	Zaidapur	Zaidapur	Zaidapur	NDFu-1
35	Tamil Nadu	Coimbatore	Coimbatore	Coimbatore	TNFu-1
36	Tamil Nadu	Dharmapuri	Palkad	Kodavadi	TNFu-2
37	Tamil Nadu	Krishnagiri	Krishnagiri	Vamban Station	TNFu-3
38	Tamil Nadu	Thiruchi	Thiruchi	Thiruchi	TNFu-4
39	Tamil Nadu	Pudukotti	Alangudi	Thiruvarankulam	TNFu-5
40	Uttar Pradesh	Kanpur	Kanpur	Kanpur	UPFu-1
41	West Bengal	Murshidabad	Murshidabad	Murshidabad	WBFu-1

Isolation and identification of *Fusarium udum* isolates

The wilt pathogen was isolated using potato dextrose agar medium by following standard tissue isolation method. Pigeonpea plants showing vascular wilt symptoms collected from different places of the country (Table-1) were used for isolation. The infected stem of pigeonpea plants were split opened longitudinally with the help of sterilized scalpel. The plant parts showing brown

discoloration of vascular tissues were cut into small bits and washed well in running tap water. These bits were surface sterilized with 0.1 per cent sodium hypochlorite solution for fifteen seconds. These pieces were washed thoroughly in sterile distilled water so as to remove traces of sodium hypochlorite. These pieces were aseptically transferred on to each Petridish containing sterile potato dextrose agar (PDA) at equal distance, the inoculated

plates were incubated at $28^{\circ} \pm 1^{\circ}\text{C}$. The isolates of *F. udum* isolated from wilted plants collected from different locations were identified based on the characters described by Booth (1971). The respective isolates of *F. udum* were used subsequently for further studies.

Variability of *Fusarium udum* isolates on the basis of cultural characters on PDA medium

The variation in cultural characters among the 41 isolates of *F. udum* collected from different locations was studied

on PDA medium. The cultural characters viz., colony diameter, growth pattern, mycelial colour and pigmentation were recorded. Colony diameter was recorded by measuring the radial growth of the mycelium in mm after eight days of incubation at $28 \pm 1^{\circ}\text{C}$. Mycelial colour and pigmentation were recorded as per the Munsell colour chart.

Table 2. Variability of *Fusarium udum* isolates on the basis of cultural characteristics on PDA medium

Sl. No	Isolates	Colony diameter (mm) at 30°C	Colony characters	Pigmentation
1	APFu-1	55.00	White mycelial colour, serrated margin with partially appressed growth	Dark yellow
2	APFu-2	75.00	White mycelial colour, serrated margin with fluffy growth	Dark yellow
3	APFu-3	74.50	White mycelial colour, serrated margin with fluffy growth	Dark yellow
4	APFu-4	57.00	White mycelial colour, serrated margin with partially appressed growth	Dark yellow
5	APFu-5	70.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow to brown
6	APFu-6	59.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow to brown
7	APFu-7	38.00	White mycelial colour, serrated margin with appressed growth	Dark yellow
8	APFu-8	62.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow to brown
9	APFu-9	50.00	White mycelial colour, serrated margin with appressed growth	Light yellow
10	APFu-10	68.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow to brown
11	APFu-11	71.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow
12	BFu-1	88.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
13	KFu-1	90.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
14	KFu-2	85.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
15	KFu-3	89.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
16	KFu-4	82.00	Pink mycelial colour, serrated margin with fluffy appressed growth	Pink colour
17	KFu-5	73.75	Pink mycelial colour, serrated margin with fluffy growth	Pink colour
18	KFu-6	72.00	Pink mycelial colour, serrated margin with partially appressed growth	Pink colour
19	KFu-7	66.00	Pink mycelial colour, serrated margin with partially appressed growth	Pink colour
20	KFu-8	73.00	Pink mycelial colour, serrated margin with partially appressed growth	Pink colour
21	KFu-9	63.00	White mycelial colour, serrated margin with partially appressed growth	Brown
22	KFu-10	52.00	White mycelial colour, serrated margin with appressed growth	Brown
23	KFu-11	90.00	White mycelial colour, serrated margin with fluffy growth	Dark yellow
24	KFu-12	66.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow
25	KFu-13	88.00	White mycelial colour, serrated margin with fluffy growth	Dark yellow
26	KFu-14	86.00	White mycelial colour, serrated margin with fluffy growth	Dark yellow
27	KFu-15	87.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
28	KFu-16	75.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
29	MPFu-1	78.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
30	MSFu-1	45.00	White mycelial colour, serrated margin with appressed growth	Light yellow
31	MSFu-2	48.00	White mycelial colour, serrated margin with appressed growth	Light yellow
32	MSFu-3	52.00	White mycelial colour, serrated margin with appressed growth	Light yellow
33	MSFu-4	46.00	White mycelial colour, serrated margin with appressed growth	Light yellow
34	NDFu-1	86.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
35	TNFu-1	72.00	White mycelial colour, serrated margin with partially appressed growth	Brown
36	TNFu-2	45.00	White mycelial colour, serrated margin with appressed growth	Light yellow
37	TNFu-3	62.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow
38	TNFu-4	55.00	White mycelial colour, serrated margin with partially appressed growth	Light yellow
39	TNFu-5	42.00	White mycelial colour, serrated margin with appressed growth	Light yellow
40	UPFu-1	90.00	White mycelial colour, serrated margin with fluffy growth	Light yellow
41	WBFu-1	78.00	White mycelial colour, serrated margin with fluffy growth	Light yellow

Based on the mycelial colour, the forty one *F. udum* isolates were categorised into two groups viz., white and pink colour (Anon., 2006). Based on pigmentation produced, the forty one *F. udum* isolates were categorised into five groups viz., brown colour, dark yellow, light yellow, light yellow to brown and pink colour

pigmentation (Anon., 2006). Based on the colony growth pattern on solid medium, the forty one *F. udum* isolates were categorised into three groups viz., fluffy growth, partially appressed growth and appressed growth (Anon., 2006).

RESULTS AND DISCUSSION

Collection and isolation of *Fusarium udum* isolates from different locations of India during *Kharif*, seasons of 2005-06 and 2006-07.

Forty one *F. udum* infected samples were collected from major pigeonpea growing states of India viz., Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Tamil Nadu, Uttar Pradesh and West Bengal during *Kharif* seasons of 2005-06 and 2006-07 were isolated and presented in Table 1. In order to obtain the pure cultures of the pathogen, tissue isolations were made as described under material and methods.

Studies on Cultural Variability of *Fusarium udum* isolates

The cultural characters of 41 *F. udum* isolates were studied on potato dextrose agar medium as described in material and methods. The results of colony growth as measured by colony diameter in mm and colony characters viz., fluffy, appressed or partially appressed growth, mycelial colour, and pigmentation produced were recorded. All the isolates showed wide variations in

respect of mycelial colour and pigmentation. These characters were considered to assess the existence of variation in the pathogen (Table-2).

Based on pigmentation, 41 isolates were categorised into five groups viz., Group I produced brown colour pigmentation and consisted of three isolates viz., KFu-9, KFu-10 and TNFu-1, Group II produced dark yellow pigmentation and consisted of eight isolates viz., APFu-1, APFu-2, APFu-3, APFu-4, APFu-7, KFu-11, KFu-13 and KFu-14, Group III produced light yellow pigmentation and consisted of 21 isolates viz., APFu-9, APFu-11, BFu-1, KFu-1, KFu-2, KFu-3, KFu-12, KFu-15, KFu-16, MPFu-1, MSFu-1, MSFu-2, MSFu-3, MSFu-4, NDFu-1, TNFu-2, TNFu-3, TNFu-4, TNFu-5, UPFu-1 and WBFu-1, Group IV produced light yellow to brown colour pigmentation and consisted of four isolates viz., APFu-5, APFu-6, APFu-8 and APFu-10 and group V produced pink colored pigmentation and consisted of five isolates viz., KFu-4, KFu-5, KFu-6, KFu-7 and KFu-8 (Table-3 and Plate 1).

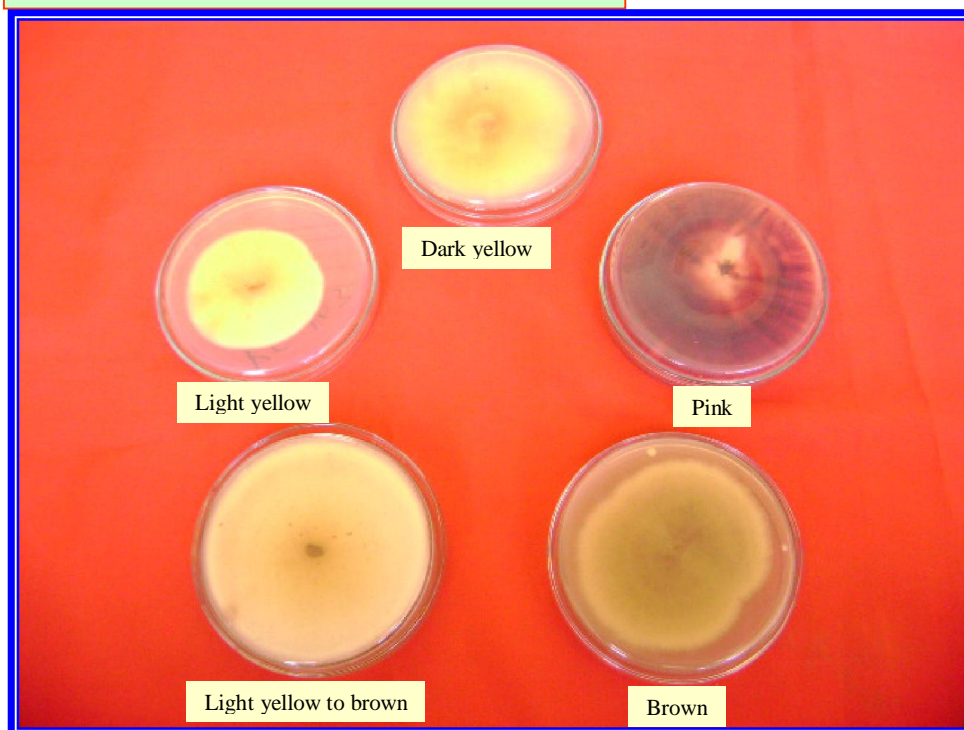
Table 3 Grouping of *Fusarium udum* isolates based on pigmentation

Sl. no.	Pigmentation	Name (no.) of the isolates									Total no. isolates
		Andhra Pradesh	Bihar	Karnataka	Madhya Pradesh	Maharashtra	New Delhi	Tamil Nadu	Uttar Pradesh	West Bengal	
1	Brown	-	-	KFU-9 & KFU-10 (2)	-	-	-	TNFu-1 (1)	-	-	3
2	Dark yellow	APFu-1, APFu-2, APFu-3, APFu-4 & APFu-7 (5)	-	KFU-11, KFU-13 & KFU-14 (3)	-	-	-	-	-	-	8
3	Light Yellow	APFu-9 & APFu-11 (2)	BFu-1 (1)	KFU-1, KFU-2, KFU-3, KFU-12, KFU-15 & KFU-16 (6)	MPFu-1 (1)	MSFu-1, MSFu-2, MSFu-3 & MSFu-4 (4)	NDFu-1 (1)	TNFu-2, TNFu-3, TNFu-4 & TNFu-5 (4)	UPFu-1 (1)	WBFu-1 (1)	21
4	Light yellow to brown	APFu-5, APFu-6, APFu-8 & APFu-10 (4)	-	-	-	-	-	-	-	-	4
5	Pink	-	-	KFU-4, KFU-5, KFU-6, KFU-7 & KFU-8 (5)	-	-	-	-	-	-	5
Total no. of isolates		11	1	16	1	4	1	5	1	1	41

Based on mycelial colour, the isolates were categorised into two groups viz., white and pink (Table-4). Group I comprised of 36 isolates viz., APFu-1, APFu-2, APFu-3, APFu-4, APFu-5, APFu-6, APFu-7, APFu-8, APFu-9, APFu-10, APFu-11, BFu-1, KFu-1, KFu-2, KFu-3, KFu-9, KFu-10, KFu-11, KFu-12, KFu-13, KFu-14, KFu-15,

KFu-16, MPFu-1, MSFu-1, MSFu-2, MSFu-3, MSFu-4, NDFu-1, TNFu-1, TNFu-2, TNFu-3, TNFu-4, TNFu-5, UPFu-1 and WBFu-1 which produced white colored mycelia. Whereas, five isolates viz., KFu-4, KFu-5, KFu-6, KFu-7 and KFu-8 produced pink colored mycelia which was considered as Group II.

Plate-1. Pigmentation of *Fusarium udum* isolates on PDA



Based on colony character 41 isolates were categorised into three groups viz., fluffy, partially appressed and appressed growth (Table-5 and Plate 2). Group I produced fluffy growth and consisted of seventeen isolates viz., APFu-2, APFu-3, BFu-1, KFu-1, KFu-2, KFu-3, KFu-4, KFu-5, KFu-11, KFu-13, KFu-14, KFu-15, KFu-16, MPFu-1, NDFu-1, UPFu-1 and WBFu-1, Group II

produced partially appressed growth and consisted fifteen isolates viz., APFu-1, APFu-4, APFu-5, APFu-6, APFu-8, APFu-10, APFu-11, KFu-6, KFu-7, KFu-8, KFu-9, KFu-12, TNFu-1, TNFu-3 and TNFu-4. Group III produced appressed growth and consisted of nine isolates viz., APFu-7, APFu-9, KFu-10, MSFu-1, MSFu-2, MSFu-3, MSFu-4, TNFu-2 and TNFu-5.

Table-4: Grouping of isolates of *Fusarium udum* based on mycelial colour

Sl. no.	Mycelial colour	Name (no.) of the isolates									Total no. isolates
		Andhra Pradesh	Bihar	Karnataka	Madhya Pradesh	Maharashtra	New Delhi	Tamil Nadu	Uttar Pradesh	West Bengal	
1	White	APFu-1, APFu-2, APFu-3, APFu-4, APFu-5, APFu-6, APFu-7, APFu-8, APFu-9, APFu-10 and APFu-11 (11)	BFu-1 (1)	KFu-1, KFu-2, KFu-3, KFu-9, KFu-10, KFu-11, KFu-12, KFu-13, KFu-14, KFu-15 and KFu-16 (11)	MPPu-1 (1)	MSFu-1, MSFu-2, MSFu-3 and MSFu-4 (4)	NDFu-1 (1)	TNFu-1, TNFu-2, TNFu-3, TNFu-4 and TNFu-5 (5)	UPFu-1 (1)	WBFu-1 (1)	36
2	Pink	-	-	KFu-4, KFu-5, KFu-6, KFu-7 and KFu-8 (5)	-	-	-	-	-	-	05
Total no. of isolates		11	1	16	1	4	1	5	1	1	41

Table-5 Grouping of *Fusarium udum* isolates based on growth pattern of the colonies on PDA medium

Sl. no.	Colony character	Name (no.) of the isolates								Total no. isolates	
		Andhra Pradesh	Bihar	Karnataka	Madhya Pradesh	Maharashtra	New Delhi	Tamil Nadu	Uttar Pradesh		West Bengal
1	Fluffy growth	APFu-2 and APFu-3 (2)	BFu-1 (1)	KFU-1, KFU-2, KFU-3, KFU-4, KFU-5, KFU-11, KFU-13, KFU-14, KFU-15 and KFU-16 (10)	MPFu-1 (1)	-	NDFu-1 (1)	-	UPFu-1 (1)	WBFu-1 (1)	17
2	Partially appressed growth	APFu-1, APFu-4, APFu-5, APFu-6, APFu-8, APFu-10 and APFu-11 (7)	-	KFu-6, KFu-7, KFu-8, KFu-9 and KFu-12 (5)	-	-	-	TNFu-1, TNFu-3 and TNFu-4 (3)	-	-	15
3	Appressed growth	APFu-7 and APFu-9 (2)	-	KFu-10 (1)	-	MSFu-1, MSFu-2, MSFu-3 and MSFu-4 (4)	-	TNFu-2 and TNFu-5 (2)	-	-	09
Total no. of isolates		11	1	16	1	4	1	5	1	1	41

Plate-2. Colony growth pattern of *Fusarium udum* isolates on PDA



Variation in cultural characters observed in first cultures nevertheless is important from the point of view of the biology of the fungus as it occurs in nature. Since it is closely linked with the question of physiologic races of pathogens. Eshwarareddy and Basu Choudhary (1985) grouped six isolates of *F. udum* into three distinct groups

based on radial growth and colony characters. These results are in agreement with Anjaneya Reddy (2002) and Mahesh (2004).

Similarly Sataraddi (1998) recorded that the distinct variability among forty *F. udum* isolates with respect to cultural and morphological characters viz., colony

diameter and pigmentation, size of spores, He categorised 41 isolates into six distinct groups based on cultural and morphological characters. But in the present findings forty one isolates of *F. udum* are categorised into four major groups based on cultural and morphological characters.

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