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Morphological and cultural variability in different isolates of *Fusarium udum* in Uttar Pradesh

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Abstract
India contributes nearly 90% of the total world production of pigeon pea but various diseases and pests are affecting this crop and reduce its productivity. Hence the present studies on the variability in the five isolates *of F. udum* were collected from different districts of Uttar Pradesh Morphological and cultural variability in comparative wet and dry mycelium weight were taken and range were observed from (0.707 to 3.713 and 0.167 to 0.707 gm) on potato dextrose broth medium after 10 days at 27±2 °C. Comparative radial growth rate ranged from 18.00 to 48.33 mm after 96 hrs after incubation at 27±2 °C on potato dextrose agar medium and antagonistic effect in dual culture techniques after 96 hrs of incubation, the inhibition percent of *F. udum* ranged between 16.00 to 27.34 mm, reduction percent of five isolates of *F. udum* ranged between 31.3 to 53.6 against *Trichoderma viride* (local strain). The size of micro and macro conidia also observed ranged from 2.5 x .50 to 3.5 x .75 um and 15.28 x 2.2 to 28.0 x 2.9 um.

Keywords: Pigeonpea, *Fusarium udum*, *Trichoderma viride*, wilt

Introduction
Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a perennial legume from the family Fabaceae. It is the fifth prominent pulse crop in the world and second most important pulse crop after chickpea in India (Patel and Patel, 2012) [14]. Annually 2.77 million tons of pigeonpea produced in India from 3.47 million hectare of land with average productivity of 7.99 q/ha. Pigeonpea represents about 5% of world legume production (Hillocks *et al*., 2000) [7] and more than 70% is being production in India. In india, pigeonpea is grown in an area of about 3.73 mha with annual production of 2.31mt and productivity of 678 kg/ha (Anonymous, 2010) [1]. The yield loss depends on the stage at which the plant wilts; it can approach 100% when wilt occurs at the pre - post stage, about 67% wilt occurs at maturity and 30% when it occurs at the pre - harvest stage (Kannaiyan and Nene, 1981) [8]. Pigeonpea crop suffers from over 210 pathogens (83 fungi, 4 bacteria, 19 viruses and mycoplasma and 104 nematodes) reported from 58 countries (Reddy *et al.*, 1996; Nene *et al*., 1996) [15, 13]. *Fusarium* wilt is the most important soil borne disease of pigeonpea and was first described in 1906 from Bihar state, India (Butler, 1906) [4]. The pigeonpea wilt pathogen was first described as *F. udum* by (Butler, 1910) [5]. The pathogen can survive on infected plant debris in the soil for about three years and caused serious disease yield losses, some time up to 100% in susceptible cultivars (Kiprop *et al*., 2005; Kumar and Upadhyay 2014). Use of bioagent along with combination with compatible fungicides gave effective control of the disease (Humka Ram and Pandey, 2011). The present investigation was therefore, conducted to study the morphological and cultural variability of *F. udum* isolates collected from different district of Uttar Pradesh.

Materials and Methods
Survey, collection, isolation, purification and identification of *F. udum* isolates
Survey was undertaken in different pigeonpea growing districts of Uttar Pradesh. These samples were collected during November 2013 and December 2013. The fungus was isolated from the stem tissue after peeling the bark on 2% Potato dextrose agar (PDA) medium in Petri plates. Stem tissues were cut into small pieces (4-6 mm) then these tissues were surface sterilized for 1 minute in 0.1% $Hgcl_2$. After three washings in sterilized water were dried on sterile blotter paper. These infected stem part was placed on PDA Petri plate and covered with parafilm after that incubated for 7 days at 27±2 °C in incubator. Later, the cultures were transferred on PDA slants and again incubated under same condition for growth and sporulation.

Pure culture of each isolate was obtained using spore dilution plate method and maintained on PDA slant for morphological and cultural variability studies. The cultures were examined microscopically and identified as *F. udum* Butler on the basis of description given by Booth (1971) [2].

Isolation and identification of *T. viride*

Soil samples collected from various rhizosphere site of infected pigeonpea field. *Trichoderma* spp. were isolated from collected soil-samples on PDA medium following serial dilution plate technique (Johnson and Crul, 1972) [16]. Ten gram soil sample from well pulverized air dried soil was added into 90 ml sterile water in a flask to make 1:10 dilution (10^{-1}). One ml of suspension from flask was transferred into a test tube containing 9 ml sterile of water under aseptic condition to make 1:100 (10^{-2}) dilution. Further dilution of 10^{-3} was made by pipetting 1ml suspension into additional water as prepared above. One ml of each liquid of 10^{-3} dilution was transferred into 10 sterile Petri plates, which was previously poured with 15 ml sterile PDA medium and spread uniformly. The Petri plates were incubated at 27±2 °C for 7 days in an incubator. As soon as the mycelial growth was visible in the PDA culture medium, the hyphal tips from the advancing mycelium were cut and transferred into the slants containing PDA medium. The pure culture of *Trichoderma* sp. was obtained by adopting single spore technique.

Morphological and cultural variability

All isolates of *F. udum* maintained on PDA medium, were studied for their morphological and cultural variability by growing them on PDA and PDB medium. Wet and dry weight of mycelium was observed in liquid medium (PDB) after 15 days incubation. The mycelium was filtered through filter paper with the help of funnel and wet and dry weight of mycelium was observed by electronic balance. All isolates ware grown on PDA poured petri plates and mycelium discs of 5 mm size cut with sterilized cork borer from 7 days old cultures were placed in the center of the Petri plate in the three replications and incubated at 25±2 °C and recorded the radial growth rate of mycelium after 24, 48, 72 and 96 hrs of the incubation. Size and septation in conidia were recorded. The size of conidia was measured by help with ocular micrometer. All isolates of *F. udum* using dual culture technique on PDA medium. 20 ml of medium after sterilization was poured in 90 mm Petri plate. The 5 mm disc was cut from 7 days old culture of *F. udum* with the help of sterilized cork borer. One disc of each isolate was inoculated on one side of Petri plate separately. Similarly, 5 mm disc of the *T. viride* (local strain) was cut from 7 days old culture and one disc was placed on opposite side of the pathogen inoculated Petri plates so as to maintain 80 mm distance between the pathogen and *T. viride* disc. Each treatment was replicated three times with control and the inoculated plates were incubated at 27±2 °C in the incubator. Observations on the radial growth of *F. udum* and *T. viride* were recorded at 24, 48, 72 and 96 hrs after incubation.

Results and Discussion

Identification of pathogen

Isolation of pathogen from all collected wilted pigeonpea plants which was purified and identified separately as *F. udum* an cultural and morphological characters as described-

I. Micro-conidia: They are small, elliptical or curved, unicellular or with 1-2 septa and measure 5-15 x 2-4 microns.

II. Macro-conidia: They are produced in small cushions of stromatic mycelium. The stromatic bases called sporodochia are tubercular in culture media. The macroconidia are long, fusariod, pointed at tip and noched at based, septate (3-4 septa), and measure 15-50 x 3-5 microns.

III. Chlamydospores: They often develop from the cells of macroconidia. The cells round of and become thick walled. These spores are oval or spherical, single or in chains, terminal or intercalary and persist in soil for long. (Booth 1978) [3].

Morphological and cultural variability

F. udum isolates were different in their cultural and morphological characteristics on PDA. Maximum colony diameter was showed in F_5 isolate (48.33 m) followed by F_4 (45.66 m) after 96 hrs incubation while minimum colony diameter was found in F_3 (18.00 m) isolate followed by F_1 (18.66 m). after 96 hrs. On the basis of above data it can be inferred that among the five isolates, F_5 (48.33 m) was the fastest growing isolate whereas as F_3 was slow growing isolate of the fungus. (fig. 1).

Maximum wet mycelium weight of *F. udum* was found in isolates F_4 (3.713 g) followed by F_5 (3.251). While minimum wet mycelium weight was found in F_3 (0.707 g) followed by F_2 (0.782 g). Maximum dry mycelium weight was found in isolate F_4 (2.546 g) followed by F_5 (2.461), and minimum dry mycelium weight was found in the F_3 (0.167) followed by F_2 (0.266). Similarl result was recorded by (Mishra, 2004) [11]. (Table – 1)

Variation of micro and macro conidia was observed in all isolates of *F. udum* the micro conidia were 0-1, septate, hyaline, round to oval in shape. The size of micro conidia varied from 2.5 × 0.50 to 3.5 × 0.75 µm. The macro conidia were produced in masses on pinots sporodochia and were hyaline, almost straight with a distinct foot cell and with strongly hooked or curved apices. They were predominantaly 3-5 septate less frequently 6-8 septate and rarely more than 8 septate. The size of macro conidia ranged from 15.28 × 2.2 to 28.0 × 2.9 µm. (Table - 1)

Antagonistic effect against *Trichoderma viride* (local strain)

Antagonistic effect in dual culture technique was observed all isolates in the presence of *T. viride* recorded after 96 hrs of incubation, Table 4. Colony growth of all the five isolates were ranged between 23.66 to 35.00 mm.. The pathogen's growth on control without (*T. viride*) showed colony growth of 51.00 mm. Maximum radial growth showed in *F. udum* against *T. viride* F_5 (35.00) followed by F_3 (27.66) while minimum radial growth was observed in F_1 (23.66) followed by F_2 (26.33). The maximum reduction percent was found F_1 (53.6) and minimum reduction percent (31.3) was found F_5 isolate. Percent reduction of five isolates of *F. were* ranged between 31.3 to 53.6 mm against *T. viride* (local strain) similar result was recorded by (Dhar *et al*., 2006) [6].

Table 1: Variability of *F. udum* isolates on the basis of morphological characteristics

Isolates	Districts	Micro conidia (µm)	Septation rang	Macro conidia (µm)	Septation rang	Wet mycelial weight (mg)	Dry mycelial weight (mg)
F_1	Kanpur	2.5×.50	0-1	28×2.9	3-5	0.853	0.390
F_2	Shahabad	3.0×.75	0-1	26.32x2.7	3-7	0.782	0.266
F_3	Pratapgarh	3.0x.75	0-1	15.28x2.2	3-5	0.707	0.167
F_4	Ajamgarh	3.5x.75	0-1	17.9x2.3	3-6	3.713	2.546
F_5	Varanasi	3.0x.50	0-1	18.8x2.4	3-7	3.251	2.461
C.D.(P=0.05)						0.746	0.286

Table 2: Radial growth of five isolates of *F. udum* after 24, 48, 72 and 96 hrs of inoculation on PDA medium

Isolates of *F. udum*	Radial growth of *F. udum* mycelium (mm)			
	24 hrs	48 hrs	72 hrs	96 hrs
F_1	4.66	7.66	15.66	18.66
F_2	4.66	7.66	15.66	19.66
F_3	3.33	6.66	11.66	18.00
F_4	8.66	21.00	35.00	45.66
F_5	8.66	22.00	38.00	48.33
S.Ed (±)	0.0014	0.0339	0.0122	0.045
C D (P=0.05)	0.100	0.167	0.293	0.574

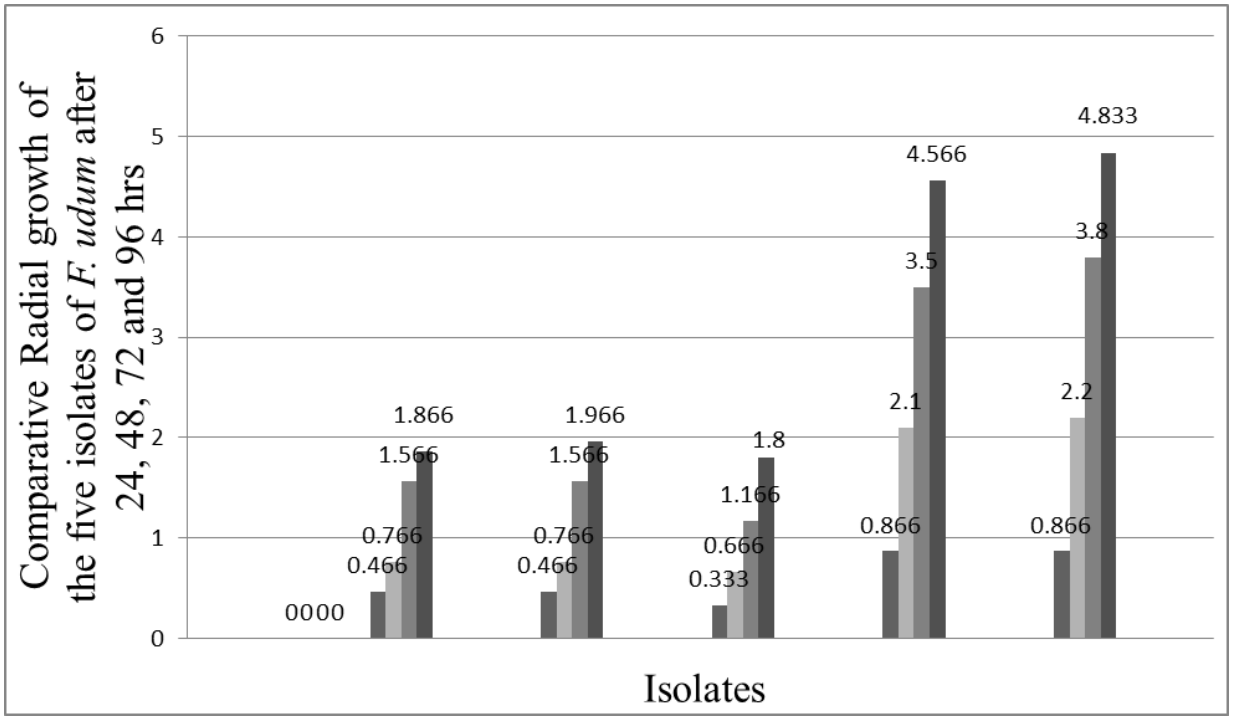

Fig 1: Comparative Radial growth of the five isolates of *F. udum* after 24, 48, 72 and 96 hrs

Table 3: Colony characters of different isolates of *F. udum* on PDA medium

Isolates of *F. udum*	Type of colony	Colour of colony	Pigmentation
F_1	Fluffy	White	Creamy
F_2	Fluffy	White	Creamy
F_3	Fluffy	White	Creamy
F_4	Appressed	White	Light yellow
F_5	Appressed	Off white	Light yellow

Table 4: Inhibition percentage of *T. viride* with *F. udum* on PDA medium

Isolates of *F. udum*	Antagonistic effect of *T. viride* with *F.* udum (cm)											
	24 hours			48 hours			72 hours			96 hours		
	F. udum	*T. viride*	Inhibition %	*F. udum*	*T. viride*	Inhibition %	*F. udum*	*T. viride*	Inhibition %	*F. udum*	*T. viride*	Inhibition %
F_1	0.966	1.933	0.356	1.366	2.533	1.572	1.800	4.866	2.010	2.366	5.866	2.734
F_2	0.833	1.733	0.456	1.300	2.266	1.638	1.700	4.300	2.110	2.633	4.833	2.467
F_3	1.233	2.466	0.089	2.366	3.133	0.572	2.733	4.400	1.077	2.766	5.333	2.334
F_4	1.300	2.366	0.022	2.100	3.066	0.838	2.266	4.600	1.544	2.700	5.600	2.400
F_5	1.233	2.033	0.089	2.433	2.766	0.505	2.600	5.233	1.210	3.500	6.000	1.600
Control	1.322			2.938			3.810			5.100		
S.Ed (±)	0.002	0.010		0.035	0.010		0.024	0.020		0.039	0.065	
C D (P=0.05)	0.163	0.278		0.505	0.315		0.411	0.383		0.522	0.686	

Conclusion

All the five isolate of *F. udum* were shown very good variation in their Morphological and cultural character, Radial growth rate was observed on Potato Dextrose Agar after 24, 48, 72 and 96 hrs of inocubation. Weight of mycelium mat and with bio-agent *T. viride* local were also taken. Comparative Wet and Dry weight of *F. udum* mycelium were recorded on Potato Dextrose Broth. Maximum mycelial weight was recorded in the isolate F_4 and dry weight showed the mass, where as F_3 wet weight and dry weight was having the minimum mass. Comparative radial growth was studied on PDA of the five isolates in which isolate F_5 showed the maximum radial growth where as isolate F_3 showed the minimum radial growth rate. Inhibition% is minimum with *T. viride* local strain has shown is a F_5 which was maximum growing fungus. Inhibition% is minimum of isolate F_1 which was slow growing fungus. Isolate F_5 is fast growing fungus and F_3 is slow growing fungus but inhibition% with local strain of *T. viride* is same approximately. On the result both a new pathotype may be exist in this area and further studied may be needed.

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