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## CULTURAL AND MORPHOLOGICAL VARIABILITY AMONG DIFFERENT ISOLATES OF *FUSARIUM UDUM* CAUSING WILT DISEASE IN PIGEONPEA [*CAJANUS CAJAN* (L.) MILLSP.] COLLECTED FROM HADOTI REGION OF RAJASTHAN

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### ABSTRACT

Pigeonpea (*Cajanus cajan* (L.), one of the major pulses cultivated and consumed in India, is also known as Arhar. Pigeonpea is a major and cheap source of protein (about 20-22%). *Fusarium* wilt of pigeonpea caused by *Fusarium oxysporum* f.sp. *udum* is the most serious disease of pigeonpea. Eight isolates of *Fusarium oxysporum* f.sp. *udum* were studied for its cultural and morphological variability. The Radial mycelial growth ranged from 81.92 to 90 mm at six days after incubation on PDA medium. Isolate RSBU1 showed the maximum and significantly superior mean radial growth of 90.00 mm after 144 hours of incubation, followed by RSBU2 (87.16 mm) and RSJH1 (86.61 mm). Pigmentation is found light yellow in colour in all eight isolates. Fluffy morphology was found in seven out of eight isolates except RSBR2 isolate. Colony colour is varied among the isolates i.e., light pinkish in RSKO1 and RSJH2, white colour in RSBU1 and RSBU2, pinkish in RSJH1, yellowish in RSBR1, light yellowish in RSBR2, and creamy in RSKO2. The micro conidia were 0-1 septate, hyaline and usually oval in shape. The size of micro conidia varied from 3.92 – 4.37 x 1.17 – 1.54 µm. The macro conidia were straight but curved at the tips and predominantly 3-4 septate. The size of macro conidia ranged from 9.93 – 13.30 x 1.46 – 1.73 µm.

**Keywords:** *Fusarium udum*, pigeonpea, isolates, cultural, morphological, variability

### Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a major grain legume crop, which belongs to the Leguminosae family. Pigeonpea has many common names like *arhar* and *tur* in India and is predominantly cultivated in tropical and subtropical regions under various agro-ecological environments. Pigeonpea is the second most important pulse crop of India, after chickpea. India ranks first in its production and contributes to 90 per cent of global pigeonpea (Mesapogu *et al.*, 2012). In India, Maharashtra, Karnataka, Madhya Pradesh, Uttar Pradesh, Bihar, Telangana, Andhra Pradesh and

Rajasthan are the major pigeonpea growing states. In India, it is grown in an area of 4.74 million hectares with the production of 4.22 million tonnes and productivity of 880 kg/ha (Anonymous, 2020). In Rajasthan, pigeonpea occupies an area of 6020 hectares with a production of 5680 tonnes and productivity of 899 kg/ha (Anonymous, 2022).

India is the leading producer of pigeon pea; however, the average yield of this crop falls short of its potential due to biotic and abiotic stresses as well as insufficient management practices. Among biotic stresses only a few diseases cause economic losses.

*Fusarium* wilt in pigeonpea, caused by the pathogen *Fusarium udum*, is widespread and has become a significant biotic threat to pigeonpea cultivation across all growing regions (Choudhary *et al.*, 2023). Crop yield losses vary based on the stage of infection. It causes upto 100 per cent losses in grain yield under extreme conditions (Pande *et al.*, 2011). The pathogen can survive on infected plant debris in the soil for several years as chlamydospores in dormant state. As a soil-borne pathogen, the fungus penetrates the host's vascular system through wounds on root tips, causing progressive leaf chlorosis, wilting, and root system collapse. Even though the infection starts in the early seedling stage, symptoms typically do not become apparent until the later stages of crop development. The early visible signs include a reduction in leaf turgidity and the appearance of interveinal chlorosis. The leaves exhibit mild chlorosis that can progress to bright yellow before eventually wilting (Hillocks *et al.*, 2000). Its tendency to inhabit deep inside host tissue and its ability to live and with stand for prolonged periods without host plants make the management of this disease a typically difficult task.

### Materials and Methods

To know the variability among the *Fusarium* isolates, diseased tissue of pigeonpea plants showing typical symptoms were collected from districts of Zone – V (Hadoti region) in *Kharif* season in 2023 of the Rajasthan. Isolates were named as RSKO1, RSKO2, RSB1, RSB2, RSBU1, RSBU2, RSJH1 and RSJH2.

#### Pathogenicity test

In order to ensure and confirm the identity of the isolated fungus, pot culture experiment was conducted in glass house. The required quantity of soil was sterilized to make soil free from microorganisms. The pots (12 inch) were surface sterilized by using 0.1% mercuric chloride and filled with 2 kg sterilized soil. The test fungus was inoculated @ 20g in soil seven days before sowing of pigeonpea seeds. Proper control was maintained where the test fungus was not inoculated. Ten seeds of pigeonpea were sown in each pot. Reisolation of fungus from artificially inoculated plants was following the process as mentioned in isolation and compared with the original isolate of the fungus.

#### Cultural and Morphological studies

This experiment was conducted under *in vitro* condition in completely randomized design (CRD) with three replications. Single spore cultures of all the isolates were cultured separately on PDA and master cultures of these isolates were maintained on PDA slants in test tubes at  $4\pm 1^\circ\text{C}$ . Cultural and

morphological variation among the isolates was determined by analyzing radial growth, colony colour, substrate colour, mycelial growth pattern and spore morphology.

The observations for radial growth were recorded at 24 hours after incubation. The radial growth was measured by drawing two lines perpendicular to each other on the back side of each plate as mentioned in 3.3.3 and the average of the two was expressed as diameter of the colony. Thus, linear growth of the colony was measured into two directions. In the case of wavy, irregular growth, the average of the largest and shortest diameter was taken as the colony diameter (Brown, 1923).

Colony colour was recorded by visual observation after 15 days of incubation at  $28 \pm 1^\circ\text{C}$  in BOD incubator.

For estimation of sporulation, equal mycelial contents along with medium was taken and homogenized with the help of pestle and mortar. It was transferred to 100 ml measuring flask and volume was made up to 100 ml and mixed thoroughly. The samples were taken from this mixture and number of conidia was counted directly under compound microscope with the help of haemocytometer.

Upadhyay (2008) had classified the *Fusarium* isolates on the basis of macro conidia, micro conidia and sporulation number into different groups as given below:

#### Based on size of macro conidia

Very small	:	< 10 $\mu\text{m}$
Small	:	10 -15 $\mu\text{m}$
Medium	:	15 -25 $\mu\text{m}$
Large	:	25 -35 $\mu\text{m}$
Very large	:	35 -45 $\mu\text{m}$

#### Based on size of micro conidia

Small	:	2 -5 $\mu\text{m}$
Medium	:	5 -10 $\mu\text{m}$
Large	:	> 10 $\mu\text{m}$

#### Based on septation of macro conidia

1-3 septa	:	First category
3-5 septa	:	Second category
5-8 septa	:	Third category

#### Categories based on the criteria of number of spores under 10x

-	:	no sporulation
+	:	1-10 conidia / microscopic field

- ++ : 11-25 conidia / microscopic field  
 +++ : 26-40 conidia / microscopic field  
 ++++ : >40 conidia / microscopic field

### Result and Discussions

The variability among the isolates of *Fusarium udum* was examined on the basis of some cultural and morphological characteristics. Eight isolates of the pathogen were collected from various locations of *Hadoti* region as mentioned in the material and methods. All of the isolates collected were purified. All the isolates showed a high level of variability in characteristics under study *viz.*, radial growth, mycelium morphology, colony colour and size and septation of macro and micro conidia. The isolates showed variation in cultural and morphological parameters have been shown in Table 1, Plate 1.

#### Mycelial growth and morphology

The data obtained revealed variation in mycelial growth pattern of various isolates of *Fusarium udum* collected from different locations. Out of eight isolates, RSBU1 showed the maximum and significantly superior mean radial growth of 90.00 mm after 144 hours of incubation, followed by RSBU2 (87.16 mm), RSJH1 (86.61 mm), RSJH2 (83.54 mm), RSKO2 (83.27 mm), RSKO1 (81.92 mm), RSBR1 (80.17 mm) and RSBR2 (79.91 mm).

The fluffy growth pattern was observed in seven isolates *viz.*, RSKO1, RSKO2, RSBR1, RSBU1, RSBU2, RSJH1 and RSJH2. However, appressed mycelial growth pattern was observed in RSBR2 isolates.

#### Colony colour

Out of eight isolates, two isolates (RSBU1 and RSBU2) showed white coloured colony, two isolates (RSKO1 and RSJH2) were light pinkish, one isolate (RSKO2) was creamy, one isolate (RSBR1) yellowish, one isolate (RSBR2) light yellowish and one isolate (RSJH1) found to be pinkish (Table 1 and Plate 1).

#### Spore morphology

Variation of micro and macro conidia was observed in all isolates. The micro conidia were 0-1 septate, hyaline, round to oval in shape. The size of micro conidia varied from 3.92–4.37 x 1.17–1.54  $\mu\text{m}$ . The macro conidia were either straight, spindle or sickle shaped. They were predominantly 3-4 septate. The size of macro conidia ranged from 9.93–13.30 x 1.46–1.73  $\mu\text{m}$  (Table 1).

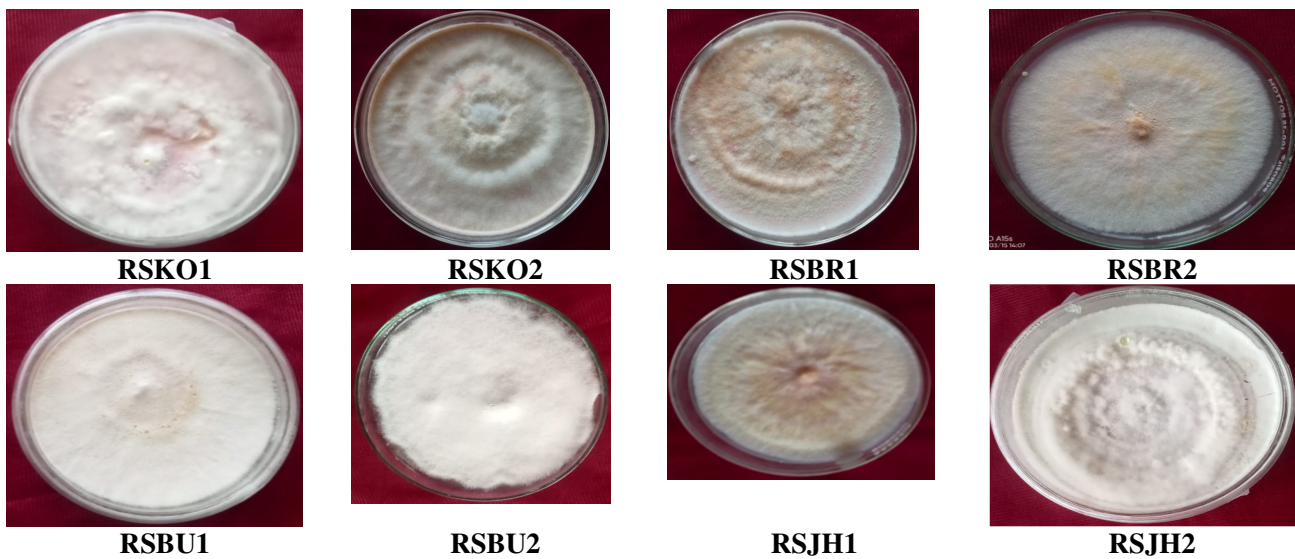
These findings are in close agreement with the finding of Groenewald *et al.*, 2006; Honnareddy and Dubey, 2007; Prasad *et al.*, 2008 and Mahesh *et al.*, 2010, who reported that *Fusarium* sp. vary in colour on PDA growth medium. The aerial mycelium was white and changed to a variety of colour from violet to dark purple depending on isolates. Reddy and Saifulla, 2006; Honnareddy and Dubey, 2007 and Mahesh *et al.*, 2009, observed that all the isolates showed the significant variations with respect to morphological characters *viz.*, the size of macro conidia and micro conidia varied from 10.51-18.70 x 1.27-3.10  $\mu\text{m}$  and 3.62-8.12 x 0.96-1.80  $\mu\text{m}$  respectively. Number of septa of macro conidia and micro conidia varied from 2.12-2.93 and 0-0.61 respectively. Colour of both the macro conidia and micro conidia was hyaline. Shape of macro conidia was sickle shaped with blunt ends to elongated sickle shaped with pointed at both ends while shape of micro conidia was oval to round.

**Table 1:** Cultural and morphological variability among *Fusarium udum* isolates on PDA medium

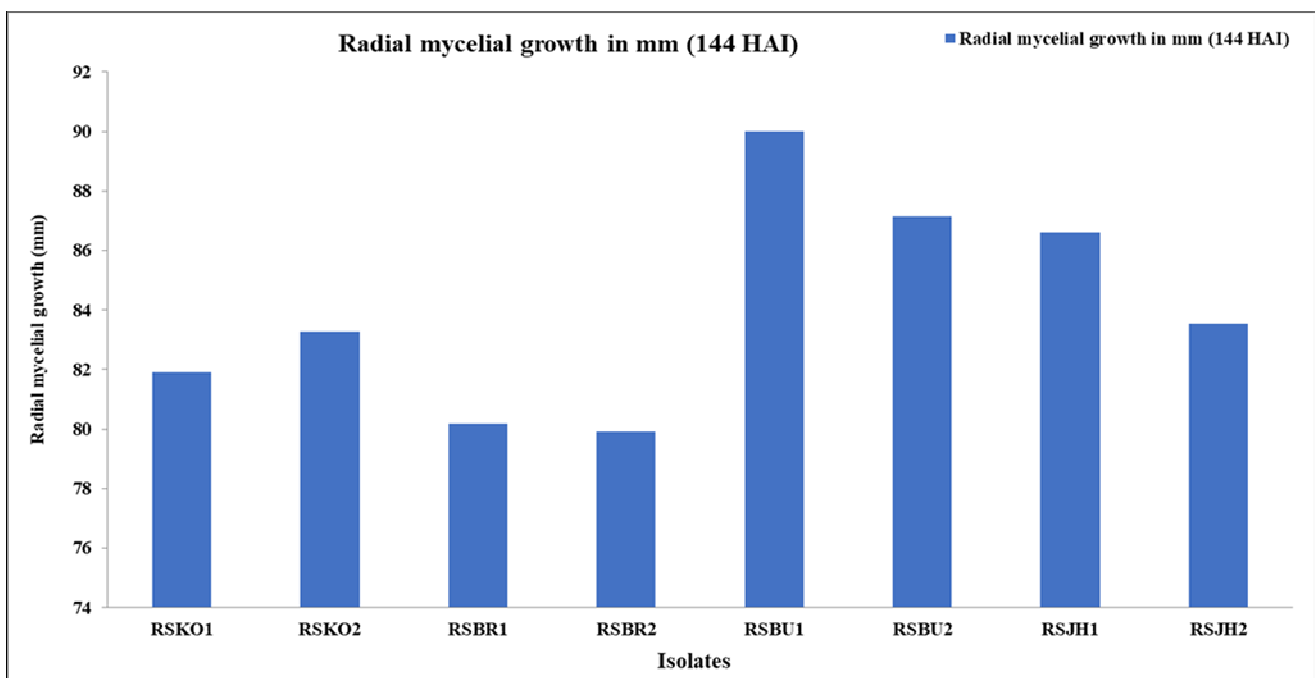
S. No.	Isolate	Radial mycelial growth in mm (144 HAI)	Substrate colour	Morphology	Colony colour	Micro conidia		Macro conidia	
						Size ( $\mu\text{m}$ )* Length and width	Septation	Size ( $\mu\text{m}$ )* Length and width	Septation
1.	RSKO1	81.92	Light yellow	Fluffy	Light pinkish	3.92x1.53	0-1	12.72x1.70	3-4
2.	RSKO2	83.27	Light yellow	Fluffy	Creamy	3.95x1.39	0-1	12.61x1.73	3-4
3.	RSBR1	80.17	Light yellow	Fluffy	Yellowish	4.08x1.49	0-1	12.30x1.65	3-4
4.	RSBR2	79.91	Light yellow	Appressed	Light Yellowish	4.12x1.53	0-1	11.78x1.71	3-4
5.	RSBU1	90.00	Light yellow	Fluffy	White	4.37x1.53	0-1	13.30x1.48	3-4
6.	RSBU2	87.16	Light yellow	Fluffy	White	4.18x1.54	0-1	13.22x1.46	3-4
7.	RSJH1	86.61	Light yellow	Fluffy	Pinkish	4.36x1.17	0-1	10.61x1.51	3-4
8.	RSJH2	83.54	Light yellow	Fluffy	Light pinkish	4.05x1.17	0-1	09.93x1.47	3-4
S Em $\pm$		0.43							
CD at 0.05%		1.32							

Here, RSKO1= ARS Field, Kota, RSKO2= Kaithoon, Kota, RSBR1= Siswali, Baran, RSBR2= Chhabra, Baran, RSBU1= Hindoli, Bundi, RSBU2= Keshoraipatan, Bundi, RSJH1= Khanpur, Jhalawar, RSJH2= Ummedpura, Jhalawar

\*Mean of ten spores randomly



**Plate 1:** Variability in isolates of *Fusarium udum*. Here, RSKO1= ARS Field, Kota, RSKO2= Kaithoon, Kota, RSBR1= Siswali, Baran, RSBR2= Chhabra, Baran, RSBU1= Hindoli, Bundi, RSBU2= Keshoraipatan, Bundi, RSJH1= Khanpur, Jhalawar, RSJH2= Ummedpura, Jhalawar



**Fig. 1:** Variability in colony diameter of different isolates of *Fusarium udum*

### Conclusion

The variability among the isolates of *Fusarium udum* was examined on the basis of some cultural and morphological characteristics. RSBU1 showed highest mycelial growth (90 mm) after 144 hours of inoculation and seven isolates (RSKO1, RSKO2, RSBR1, RSBU1, RSBU2, RSJH1 and RSJH2) showed fluffy growth pattern, however, appressed mycelial growth pattern was observed in RSBR2 isolate. Isolates showed variation with respect to colony colour

(White: RSBU1 and RSBU2; light pinkish: RSKO1 and RSJH2; Creamy: RSKO2; yellowish: RSBR1; light yellowish: RSBR2 and pinkish: RSJH1). The micro conidia were 0-1 septate, hyaline and usually oval in shape. The size of micro conidia varied from 3.92 – 4.37 × 1.17 – 1.54 μm. The macro conidia were straight but curved at the tips and predominantly 3-4 septate. The size of macro conidia ranged from 9.93 – 13.30 × 1.46 – 1.73 μm.

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## Conflict of interest

No conflict of interest.

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