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Study on growth variability of *Fusarium oxysporum* f.sp. *udum* causing wilt of Pigeon pea on different growing media

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Abstract
The present investigation was carried out in the research farm as well as laboratory of Birsa Agricultural University, Kanke, Ranchi on variability in *Fusarium oxysporum* f.sp. *udum* causing wilt of Pigeonpea and its management. The systematic investigation revealed that wilt disease in Pigeonpea was found to be infected with *Fusarium oxysporum* f.sp. *udum* at all ten locations surveyed in 2015-16 and 2016-17. Maximum wilt percentage was found at Bukru (32.14%) followed by Kanadu (29.40%) and Ranchi BAU (28.63%). After conducting elaborate survey at 10 locations, forty isolates were collected for studying variations among isolates characters. To identify *Fusarium* pathogen as it's actual casual agent for the disease pathogenicity test was conducted by using different effective isolates (Isolate-1, 14, 35). It clearly revealed that *Fusarium oxysporum* f.sp. *udum* is the cause for wilt disease in Pigeonpea. Morphological studies of *Fusarium oxysporum* f.sp. *udum* revealed that the dimensions of macro condia, micro conidia and chlamydospores shows larger variations among different isolates. Among four liquid media, maximum and fastest mycelial growth was obtained in PDA medium followed by Richards medium, Czapeks medium, & Martins medium. In PDA liquid media isolate Fou-Ran-1 given maximum mycelial dry weight that is 470 mg. After in detailed study of all forty isolates regarding their cultural, morphological studies these isolates are grouped into different categories based on criteria like size of macro and micro conidia, septation of macro condia, their growing speed on different semi solid mediums and number of spores under 10x microscopic field.

Keywords: Media, fusarium, inoculation, pigeon pea

Introduction
Pigeonpea [*Cajanus cajan* (L.) Millsp.] is widely grown by small farmers in the semi-arid tropics as a backyard subsistence crop. It is produced commercially in India, Myanmar, Kenya, Malawi, Uganda and a few countries of Central America. Pigeonpea commonly known as arhar or tur, Redgram, Cangopea and Gandul is the second most important pulse crop after chickpea in India. It is one of the extensively used pulses in India as an important source of protein in human diet. Generally, it is grown all over the country, but it is cultivated extensively in Jharkhand, Bihar, Uttar Pradesh, Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka, West Bengal and Gujarat.

Pigeonpea is a profitable and popular crop in the Jharkhand after Rice. It fetches good price in the market. It is a hardy plant, when intercropped with cereals, and ensures a measure of income stability. People use the dry grain as dhal, the green pod or seed as vegetable, feed, fodder and the sticks as fuel wood. In addition, it can be cut for forage and poor soil through its deep strong rooting systems, leaf drop at maturity and addition of nitrogen by symbiotic activities during crop growth. Considering importance of pulses in human nutrition, Government of India is giving much emphasis on increasing production of pulses in the country.

Pigeonpea (*Cajanus cajan* (L) Millsp.) is an important food legume grown in semi-arid tropical and sub-tropical farming systems under varied agro-ecological environments. It provides high quality vegetable protein to human beings and is one of the sources of animal feed and fire wood. Its cultivation is confined to developing countries, mostly in Asia and Africa. (Pande *et al*. 2013) [4].

The pathogen is mainly soil and seed borne. The genus *Fusarium* have wide host range and survives for long time in the field in the absence of host plant, and attacks the plants at any stage of their growth and life cycle. It causes complete failure of the crop especially in a warm spring and dry hot summer.

This disease is worldwide in distribution and causes considerable loss to the crop.

Methods and Materials

Present investigations were carried out during kharif and rabi seasons, 2015 and 2016 in the Department of Plant Pathology and also at the Research Farm of Ranchi Agricultural College, Birsa Agricultural University, Kanke, Ranchi. Geographically B. A. U. is situated at 23^0 17' North latitude and 85^0 19' East longitudes with an altitude of 625 meter above mean sea level in the Ranchi region of Jharkhand state. The plot had a fairly uniform topography and the soil was deep and well drained. The details of the methodology adopted in experimentation are described here under. Climatic condition is sub–tropical humid with moderate summer, heavy rain fall and cold winter. The average annual rainfall is about 1476 mm of which 80-85 percent is received during June to September. Generally, the monsoon breaks by the second week of June. Diseased samples of Pigeonpea plants carrying the characteristic symptoms of wilt were collected from farmer's fields of different places like of Nagri, Kanadu, Badhu, Kokdoro, Sukurhuttu, Hochar, Boreya, Bukru and Pithoria and Ranchi Agricultural College Research Farm, Ranchi.

Diseased specimens were brought to the laboratory and thoroughly washed in tap water. After washing, infected stems were cut into pieces measuring about 5 mm using sterilized blade. Glasswares to be used during experiment as test tube, Petri-plates, pipettes, Erlen Meyer's flasks, thistle funnels and all types of glassware's were washed first with vim powder in the running tap water and then cleaning solution prepared by dissolving 80 g of potassium dichromate ($K_2Cr_2O_7$) in 500 ml of warm water, cooled and added 500 ml concentrated sulphuric acid (H_2SO_4) very slowly and stirred with a rod for proper mixing. It was diluted 5 times by adding 4 litres of distilled water. Cleaned inoculating needle and forceps were dipped in spirit and heated over the flame until hot red. It was repeated 2-3 times. Inoculating needle was used in transferring inoculums from freshly prepared culture tube and Petri-plates to another culture tube and Petri-plates. 3 mm cork-borer was exclusively used for transferring measured quantity of inoculum. The inoculum transfer was carried out within laminar air flow. The inoculation chamber was sterilized with cotton soaked in spirit. The laminar air flow was subjected to ultraviolet light for thirty minutes by closing the door of the chamber.

To study the morphological and cultural characteristics, all the forty isolates of *F. oxysporum* f. sp. *udum* were grown on PDA medium, in petriplates at 28 ± 1^0C.Morphological characters like shape and size of microconidia, macroconidia and chlamydospores were examined with temporary slides prepared in lactophenol and cotton blue under calibrated compound microscope. Mean of 100 observations for each character was recorded.

Characters of radial growth, pigmentation and sporulation of all isolates were also observed from cultures grown on PDA, Richard's, Czepek's, Martin's rose agar media in Petridishes both in solid and liquid mediums.

Morphological, cultural and physiological characteristics

To study the morphological and cultural characteristics, all the forty isolates of *F. oxysporum* f. sp. *udum* were grown on PDA medium, in petriplates at 28 ± 1 ^{0}C. Morphological characters like shape and size of microconidia, macroconidia and chlamydospores were examined with temporary slides prepared in lactophenol and cotton blue under calibrated compound microscope. Mean of 100 observations for each character was recorded.

Characters of radial growth, pigmentation and sporulation of all isolates were also observed from cultures grown on PDA, Richard's, Czepek's, Martin's rose agar media in Petridishes both in solid and liquid mediums.

a) Solid medium

The ingredients of different solid media were dispensed in Erlenmeyer flasks and dissolved in 500ml distilled water. On the other hand the required amount of agar was added in 500ml distilled water in a separate flask and heated till it dissolved. The constituents were filtered through a muslin cloth and desired volume was made up by adding distilled water. These were then thoroughly mixed up and autoclaved at 15 lbs (15 pounds) pressure for 20 minutes cooled to 45 ºC and was poured into sterilized petriplates of 90mm diameter.

b) Liquid medium

Liquid medium were prepared by dissolving the constituents of each media separately. The required volume was made up with distilled water and 25ml of the medium was pipetted in to 50 ml flasks. The flasks were then pluggued with non-absorbent cotton plug and finally autoclaved at 20 lbs pressure for 20 minutes.

c) Inoculation

Twenty ml sterilized PDA medium was poured aseptically to each sterilized Petridish and allowed to solidify. Mycelial disc of 7 days old culture of the each isolate of the pathogen (5 mm diameter) were placed invertedly in the centre of Petridishes aseptically. While in case of culture flask, the inoculums was dropped in the liquid medium and shaken well in order to mix inoculums thoroughly. During inoculation, aseptic condition was maintained.

d) Incubation of culture

After inoculation, the petridishes and the flasks were incubated for 3 and 10 days, respectively at 28 ± 1^0C for growth of *Fusarium oxysporum.* Three replicates were kept for each isolate and treatment.

e) Measurement of radial growth

The observations for radial growth were recorded at 24 hr after incubation. The radial growth was measured by drawing two lines at right angles to each other on the back side of each plate and the average of the two was expressed as diameter of the colony it was expressed in mm (Lilly and Barnett, 1951). Thus, linear growth of the colony was measured into two directions.In the case of wavy, irregular growth, the colony average of the largest and shortest diameter was taken as the colony diameter (Brown, 1923) [1].

Result and Discussion

Among the isolated isolates it was clearly observed that there are lot of variations in between each other mostly in morphological, and cultural characters. Among 40 isolates variations like mycelial colour in white and pinkish at the center with dense to fluffy growth on PDA medium and some isolates showed fastest growth while others showed medium to very slow growth on different media.

Four semi solid media *viz*., PDA medium, Richard's medium, Czapek's medium, and Martin's medium were used for the

cultural studies. The different growth patterns of different isolates were presented in Table 1.

Results revealed that there are distinct variations in colony diameters of different isolates of *F. udum*. The data revealed that maximum mycelial growth was obtained in PDA medium and the range is between 72.90 mm for Fou-Ran-1 and 30.20 mm for Fou-Buk-3. In Richard's medium the growth range is in between 70.90 mm for Fou-Ran-1 and 32.60 mm for Fou-Buk-3. In the case of Czapek's medium it's range is between 70.10 mm for Fou-Ran-1 and 32.30 mm for Fou-Buk-3. At last in Martin's medium pathogen growth range is between 42.10 mm for Fou-Ran-1 and 22.60 mm for Fou-Buk-3. The fastest growth of mycelium observed on solid PDA media followed by Richard's, Czapek's media, it was clearly indicated from the table pathogen shows slowest growth on Martin's medium compared to other media. It was also revealed from Table 1 that isolates 1 (Fou-Ran-1) showed fastest growth on all mediums, isolate 14 (Fou-Hoc-2) showed medium growth on all mediums & finally isolate 35 (Fou-Buk-3) showed slowest growth on all mediums. So invariably all these 3 effective isolates were further studied in the research programme.

These isolates also depicted considerable variations while studying cultural characters mostly in growth of mycelial mat on different solid and liquid medias, and in their dry weights of mycelial mats, sporulation on different medias. On the basis of size of macro conidia, *Fusarium oxysporum* f.sp. *udum* isolates were grouped into five categories while in the grouping of isolates, out of forty isolates 37 and 3 number of *F.udum* isolates formed medium and large sized conidia respectively. In the case of size of micro conidia all isolates are classified into 3 groups, out of which studies revealed that 33 and 7 number of isolates were grouped in to medium and large categories respectively. Based on the number of septa observed in macro conidia, *Fusarium* isolates were again classified into three categories in those 13 isolates of *Fusarium udum* fall in first category (having 1-3 septa), 27 in second category (3-5 septa). The fastest radial growth of mycelial mat (72.90 mm) observed on solid PDA media followed by Richards, Czapeks media, it was clearly indicated from the result tables pathogen shows slowest growth on Martin's medium compared to other media. *Fusarium udum* isolates produced different number of macro conidia, micro conidia and chlamydospores on different media utilized in the experiment, then those are grouped into different categories based on total number of conidia. From experimental results identified that among the liquid media tested, maximum and fastest mycelial growth, highest dry mycelial weight (470 mg) was obtained on PDA medium followed by Richard's medium, Czapek's medium, & Martin's medium. Similar observations were also mentioned by Prasad and Chaudhary (1977), Upadhyay (2008) [5], Mahesh *et al*. (2010), Ram and Pandey (2011) and Islam (2015) [3].

Table 1: Mycelial growth diameters of *F. udum* isolates on different semi solid media

Sl. No	Isolates no	Medium (mm/144 hr's)			
		PDA	Richard's	Czapek's	Martin's
1	Fou-Ran-1	72.9	70.9	70.1	42.1
2	Fou-Ran-2	51.1	45.6	40.1	39.6
3	Fou-Ran-3	60.2	52.6	48.3	40.3
4	Fou-Ran-4	57.3	53.6	39.6	32.6
5	Fou-Pit-1	42.3	52.1	52.1	36.2
6	Fou-Pit-2	64	51.3	49.3	39.2
7	Fou-Pit-3	62.4	53.9	41.3	36.9
8	Fou-Pit-4	45.6	43.6	46	36.2
9	Fou-Nag-1	46.9	39.6	36	35.6
10	Fou-Nag-2	44	50.6	51.1	40.2
11	Fou-Nag-3	54	50.2	56.3	35
12	Fou-Nag-4	53.5	53.3	53.2	34.2
13	Fou-Hoc-1	60.2	53.2	46.9	32.8
14	Fou-Hoc-2	59.4	57.3	51.1	40.6
15	Fou-Hoc-3	51	52.3	45.6	36.2
16	Fou-Hoc-4	55.8	51.3	39.3	36.2
17	Fou-Bor-1	59.4	45.6	54	35.6
18	Fou-Bor-2	57.5	51.2	49.2	36.7
19	Fou-Bor-3	50.7	49.3	45.9	30.2
20	Fou-Bor-4	58.1	56.1	46	34.2
21	Fou-Kan-1	42.6	41.3	49.1	37.2
22	Fou-Kan-2	42.3	45.3	36.3	29.2
23	Fou-Kan-3	42.8	40	44.2	36.6
24	Fou-Kan-4	56.2	45.3	49.3	39.2
25	Fou-Bad-1	45.3	50.2	51.2	33
26	Fou-Bad-2	59.4	53.2	52.3	32.6
27	Fou-Bad-3	52.6	43	50.1	33.5
28	Fou-Bad-4	44.5	52.6	43.9	37.8
29	Fou-Kok-1	51.2	42	49.6	38
30	Fou-Kok-2	56.3	51.3	51.8	36.2
31	Fou-Kok-3	52.3	52	44.6	38
32	Fou-Kok-4	49.6	50.3	55.6	38
33	Fou-Buk-1	42.5	56.6	52.3	38.2
34	Fou-Buk-2	55.1	39.2	53.6	30.2
35	Fou-Buk-3	30.2	32.6	32.3	22.6

36	Fou-Buk-4	50.2	56.6	49.5	36
37	Fou-Suk-1	57.9	53.2	51.9	38
38	Fou-Suk-2	58.3	56.2	53.6	36.2
39	Fou-Suk-3	56.9	51.2	52.6	36.2
40	Fou-Suk-4	53.6	51.9	55.3	38.2
	SE(m)±	0.80	0.73	0.64	0.61
	CD(P = 0.05)	2.27	2.05	1.81	1.72
	CV (%)	2.64	2.54	2.30	2.97
*Average of three replications.					

Grouping of isolates of *F. udum* based on their radial growth on different solid media

Isolates of *Fusarium udum* were categorized on the basis of their radial growth on PDA media, Richard's media, Czapek's media, Martin's media as very slow (0-30 mm), Slow (31-45 mm), Moderate (46-60 mm), Fast (61-75 mm), Very fast (76-90 mm). Different isolates from different places of Ranchi district were placed in different categories based on their growth on above different mediums.

On PDA media

From the below table it was clearly indicative that 8 isolates comes under slow growth category, while another 29 isolates are comes in moderate category and 3 isolates were comes under fast growing category. It was clearly observed that there is no isolate comes under very slow & very fast category on solid PDA medium.

On Richard's media

From the above table it was clearly notified that 9 isolates of *Fusarium* pathogen came under slow growing category on Richard's medium, while 30 isolates comes under moderate growth category and 1 isolate comes under fast growing category. There is no isolate was classified under very slow and very fast growing category on Richard's medium.

On Czapek's media

From the above table we can observe that 9 isolates comes under slow growing category on Czapek's medium, while 30 isolates comes under moderate growth category and 1 isolate comes under fast growing category. There was no isolate classified under very slow and very fast growing category based on their growth speed on Czapek's medium.

On Martin's media

In the above table 40 isolates of *F. udum* classified into two categories those are, 2 isolates under very slow growth and 38 isolates under slow growth category on the basis of isolates mycelial growing speed in Martin's media. While there was no isolate was classified under moderate, fast, very fast categories.

The fastest radial growth of mycelial mat (72.90 mm) observed on solid PDA media followed by Richards, Czapeks media, it was clearly indicated from the result tables pathogen shows slowest growth on Martin's medium compared to other media.

Table 2: Grouping of isolates of *F. udum* based on their radial growth speed on different solid media

Sl. No	Growth dia (mm)	No. of isolates	Name of isolates (PDA media)	No. of isolates	Name of isolates (Richard's media)	No. of isolates	Name of isolates (Czapek's media)	No. of isolates	Name of isolates (Martin's media)
1	Very slow (0-30 mm)	0	-	0	-	0	-	2	Fou-Kan-2, Fou-Buk-3.
2	Slow (31-45 mm)	8	Fou-Pit-1, Fou-Nag-2, Fou-Kan-1, 2, 3, Fou-Bad-4, Fou-Buk-1, 3,	9	Fou-Pit-4, Fou-Nag-1, Fou-Kan-1, 3, 4, Fou-Bad-3, Fou-Kok-1, Fou-Buk- 2,3.	9	Fou-Ran-2,4, Fou-Pit-3, Fou-Nag-1, Fou-Hoc-4, Fou-Kan-2, Fou-Bad- 4, Fou-Kok- 3, Fou-Buk-3.	38	Fou-Ran-1,2,3,4, Fou-Pit-1,2,3,4, Fou-Nag-1, 2, 3,4, Fou-Hoc-1, 2, 3,4, Fou-Bor-1, 2, 3, 4, Fou-Kan-1, 3, 4, Fou-Bad-1, 2, 3, 4, Fou-Kok-1, 2, 3, 4, Fou-Buk-1, 2, 4, Fou-Suk-1,2,3,4.
3	Moderate (46-60 mm)	29	Fou-Ran-2,3,4, Fou-Pit-4, Fou-Nag-1, 3,4, Fou-Hoc-1, 2, 3,4, Fou-Bor-1, 2, 3, 4, Fou-Kan-4, Fou-Bad-1, 2, 3, Fou-Kok-1, 2, 3, 4, Fou-Buk- 2, 4, Fou-Suk-1,2,3,4.	30	Fou-Ran-2,3,4, Fou-Pit-1,2,3, Fou-Nag- 2, 3,4, Fou-Hoc-1, 2, 3,4, Fou-Bor-1, 2, 3, 4, Fou-Kan-2, Fou-Bad-1, 2, 4, Fou-Kok- 2, 3,	30	Fou-Ran-3, Fou-Pit-1,2, 4, Fou-Nag- 2, 3,4, Fou-Hoc-1, 2, 3, Fou-Bor-1, 2, 3, 4, Fou-Kan-1, 3, 4, Fou-Bad-1, 2, 3, Fou-Kok-1, 2, 4, Fou-	0	-

					4, Fou-Buk-1, 4, Fou-Suk-1,2,3,4.		Buk-1, 2, 4, Fou-Suk-1,2,3,4.		
4	Fast (61-75 mm)	3	Fou-Ran-1, Fou-Pit-2, 3	1	Fou-Ran-1	1	Fou-Ran-1	0	-
5	Very fast (76-90 mm)	0	-	0	-	0	-	0	-
	Total	40		40		40		40	

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