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Study on variations in morphological and cultural characters of *Fusarium oxysporum* f.sp. *Udum*

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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 study 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. 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. The cultural characters observed on PDA medium and the mycelium colour was in white and pinkish at the center with dense to fluffy growth. The size of mycelial width varied from 3 µm (Fou-Bor -4) to 6.32 µm (Fou – Ran - 4). Where as microconidia were small, oval in shape and hyaline in colour, unicellular or with one or two septa, and measures in the range of size between 6.00 × 2.10 µm (Fou-Ran- 4) to 10.80 × 3.00 µm (Fou- Kok-1). The macroconidia were long, curved, sickle shaped, pointed at the tip, hyaline in colour and knotched at the base, septated (2-4 septa) and measured between 19.05 × 3.25 µm (Fou- Hoc-4) to 28.70 × 2.50 µm (Fou- Kok-1). Chlamydospores with spherical in shape and hyaline in colour and it's dimensions also varied in all 40 isolates of the pathogen. Chlamydospores dimension varied from 8.20 × 7.92 µm (Fou- Buk – 2) to 11.35 × 8.20 µm (Fou-Nag-3). Spore density also varied between conidia per ml of culture under 10 x microscopic field, it is varied for macro conidia in the range of 6 to 13, micro conidia it is between 20 to 42 and chlamydospores it is between 2 to 8 number per ml.

Keywords: Bioagents, isolates, trichoderma, *in vitro*

Introduction
Pigeonpea, a kharif season crop, is also commonly known as Red gram, Arhar or Tur. It is the IInd important pulse crop after the gram and a major kharif crop in the country. India ranks I st in area and production in the world with 80% and 67% of world's acreage and production respectively.

Pigeonpea represents about 5% of world legume production (Hillocks *et al.* 2000) and more than 70% is being produced in India. However, despite its immense importance in sustainable agriculture its global production per hectare remained static over last three decades. The yield gap observed between the potential yield and on-farm yield is mainly due to biotic and abiotic stresses and the lack of efficient management practices.

Among biotic stresses diseases such as Fusarium wilt, sterility mosaic, Phytophthora blight, cercospora leaf spot, collar rot, dry root rot, alternaria leaf spot, powdery mildew and phyllody are well known diseases of Pigeonpea. Among them, *Fusarium* wilt caused by *Fusarium udum* is the most important soil borne disease of pigeonpea capable of causing 30-100% loss in grain yield (Nene *et al.*1980, Upadhyay and Rai, 1989, 1992, Kannaiyan and Nene 1981, Reddy *et al.*1990) [5, 6].

The disease was first reported from Bihar state in India (Butler, 1906). Pigeonpea wilt is widely prevalent throughout the world and more important in India (Kannaiyan and Nene, 1981) [6] and in eastern Africa (Okiror, 2002).

The annual loss in pigeonpea due to wilt alone in India has been estimated to US $ 71 million (Reddy *et al.* 1993). The crop suffers heavily due to *Fusarium* wilt in the major growing areas resulting into huge production losses (Vishwa**d**har *et al.*2005).

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. Several places of Kanke block in Ranchi district were surveyed during the Rabi season of 2015 and 2016 to record the incidence of *Fusarium* wilt of Pigeonpea. 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. The diseased specimens were collected in paper bags and labeled properly and brought to the laboratory for further examination. Diseased samples were then preserved under dried conditions for further studies.

Isolation was made on PDA medium from different parts of the diseased plant showing characteristic symptoms of wilt complex. The specimens were first washed by passing them through running tap water to remove dust or soil particles, if any. Diseased parts just touching the healthy portion were chosen and separated with the help of sterilized blade and were cut into smaller pieces of 1-2mm length. The pieces were washed thoroughly in sterilized water in order to remove surface contamination and then surface sterilized with 1.0% NaOCl (Sodium hypochloride) solution for 30 seconds.

These pieces were washed thoroughly in three consecutive changes of sterilized distilled water to remove the residue of mercuric chloride completely. Excess moisture was removed by putting the pieces pressed in between two folds of sterilized blotting paper. Then it was transferred to PDA slants aseptically in laminar flow. The inoculated PDA slants were incubated at 28±1 °C to allow the pathogen to grow. After 72 hours, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants to make culture pure.

One week old cultures grown on PDA were used for inoculation. A 5 mm fungal disc was cut with the help of sterilized cork- borer. The discs were transferred at the center of petridish in an inverted position in fungicidal bioassay experiments under laminar flow so that it could come in direct contact with the surface amended medium. In dual culture 5 mm discs were cut by sterilized cork borer from actively growing colony of bio control agents and placed at one end of petridish over PDA medium. At the same time 5 mm diameter discs were also cut from the actively growing colony of the pathogen and kept opposite to the bio control agent. A control i.e. without inoculation of the antagonist was also maintained.

Result and Discussion

Variation in morphological & cultural characters of *Fusarium oxysporum* f.sp. *udum*

During above studies, the semi-permanent slides were prepared as per general procedure and measurement of fungi and it's spores was done with the help of microscope under oil immersion objective. Observations thus recorded are presented in Table 5. It is evident from the following data there were marked difference between different isolates in morphological & cultural characters. The colour of colony in the culture of all the 40 isolates varied from white, pinkish white, to pink. Some isolates showed central pink colour and white peripheral colour with dense to fluffy growth on PDA like isolate 1 (Fou-Ran -1) showed fastest growth while others showed medium (Isolate 14) to very slow (Isolate 35) growth. The size of mycelial width varied from 3 µm (Fou-Bor - 4) to 6.32 µm (Fou – Ran - 4). Where as microconidia were small, oval in shape and hyaline in colour, unicellular or with one or two septa, and measures in the range of size between 6.00 × 2.10 µm (Fou-Ran- 4) to 10.80 × 3.00 µm (Fou- Kok-1). The macroconidia were long, curved, sickle shaped, pointed at the tip, Hyaline in colour and knotched at the base, septated (2-4 septa) and measured between 19.05 × 3.25 µm (Fou- Hoc-4) to 28.70 × 2.50 µm (Fou- Kok-1). Chlamydospores were also formed on the host and in culture media with spherical in shape and hyaline in colour when the later are old. Chlamydospores dimensions also varied in all 40 isolates of the pathogen. Chlamydospores dimensions varied from 8.20 × 7.92 µm (Fou- Buk – 2) to 11.35 × 8.20 µm (Fou-Nag-3). Spore density also varied between conidia per ml of culture under 10 x microscopic field, it is varied for macro conidia in the range of 6 to 13, micro conidia it is between 20 to 42 and for chlamydospores it is between 2 to 8 number per ml.

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. The size of mycelial width varied from 3 µm to 6.32 µm. Where as Microconidia were small, oval in shape and hyaline in colour, unicellular or with one or two septa, and measured in the range of size between 6.00 × 2.10 µm to 10.80 × 3.00 µm. The macroconidia were long, curved, sickle shaped, pointed at the tip, hyaline in colour and knotched at the base, septated (2-4 septa) and measured between 19.05 × 3.25 µm to 28.70 × 2.50 µm. Chlamydospores were also formed with spherical in shape and hyaline in colour on the host and in culture media. Chlamydospores dimensions varied from 8.20 × 7.92 µm to 11.35 × 8.20 µm. Spore density also varied in both macro-and micro conidia. Variation was for macro conidia in the range of 6 to 13, micro conidia 20 to 42 and chlamydospores 2 to 8 per ml. Similar results were also reported by Kiprop *et al.* (2002) based on studies of differential reactions of seven pigeonpea varieties to 17 different isolates in Kenya, in India similar results reported by Sukumar *et al.* (2012). They found that these isolates differ in their mycelial colour, substrate colour, mycelial growth and virulence. The same type of results were also reported by Booth (1977) [1], Madhukeshwara and Seshadri (2001) [4], Pande *et al.*(2013) [7], Jalander and Gachande (2015) [2].

Table 1: Variation in morphological and cultural characters of *Fusarium oxysporum* f.sp. *udum*

Sl. No	Isolate name	Cultural characters	Mycelium width (µm)	Conidia size (µm)			Septation		Conidia (Macro)		Conidia (Micro)		Conidia (Chlamydo spores)		Spore density(per ml) under 10 X		
				Macro	Micro	Chlamydo spores	Macro	Micro	Shape	Colour	Shape	Colour	Shape	Colour	Macro	Micro	Chlamydo
1	Fou-Ran-1	Mycelium white dense, slightly pinkish at the center, uniformly raised with fast growth	5.21	21.65 × 4.10	7.50 × 3.50	9.70 × 9.10	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	40	6
2	Fou-Ran-2	Mycelium white dense, fluffy, slightly pinkish at the center	3.21	19.30 × 3.05	6.50 × 2.50	8.20 × 8.10	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	35	3
3	Fou-Ran-3	Mycelium white dense, slightly fluffy, uniform dense	4.56	20.40 × 3.65	7.00 × 3.10	9.75 × 8.10	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	8	24	4
4	Fou-Ran-4	Mycelium white dense, slightly pinkish at the center	6.32	20.45 × 3.70	6.00 × 2.10	9.05 × 7.08	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	30	7
5	Fou-Pit-1	Mycelium white appressed, slightly pinkish at the center	6	22.60 × 4.00	8.25 × 3.00	10.30 × 8.65	2-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	7	32	3
6	Fou-Pit-2	Mycelium white appressed, slightly pinkish at the center	5.23	22.25 × 3.05	8.35 × 3.20	10.15 × 8.25	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	8	41	5
7	Fou-Pit-3	Mycelium white in color, slightly pinkish at the center and uniform	3.15	21.15 × 4.60	7.65 × 2.55	9.20 × 7.65	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	21	4
8	Fou-Pit-4	Mycelium white dense, slightly violet color at the center	3.46	19.30 × 3.70	7.85 × 3.00	8.70 × 8.05	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	7	36	7
9	Fou-Nag-1	Mycelium white dense, slightly pinkish at the center	5.23	22.05 × 4.55	8.30 × 2.50	10.15 × 8.10	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	9	29	8
10	Fou-Nag-2	Mycelium white dense, slightly pinkish at the center	5.55	24.31 × 3.60	9.65 × 2.50	10.25 × 7.50	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	24	7
11	Fou-Nag-3	Mycelium white dense, slightly pinkish at the center	6.21	20.60 × 4.10	10.15 × 3.00	11.35 × 8.20	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	8	35	5
12	Fou-Nag-4	Mycelium white dense, slightly red color at the center	4.39	23.35 × 2.60	9.60 × 2.65	9.50 × 8.15	2-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	28	6
13	Fou-Hoc-1	Mycelium white dense, slightly pinkish at the center	3.15	20.20 × 5.15	7.20 × 3.60	10.55 × 8.85	3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	10	20	3
14	Fou-Hoc-2	Mycelium white dense, slightly pinkish at the center,with inermediate growth	6.2	21.65 × 3.25	7.65 × 3.00	10.05 × 8.20	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	26	5
15	Fou-Hoc-3	Mycelium white dense, slightly pinkish tinge at the center and in the colony	4.3	26.00 × 3.25	9.75 × 3.00	9.60 ×7.65	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	13	42	6
16	Fou-Hoc-4	Mycelium white dense, slightly pinkish at the center	5.23	19.05 × 3.25	9.85 × 2.50	8.40 × 8.22	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	40	5
17	Fou-Bor-1	Mycelium white dense, slightly pinkish at the center	6.12	22.70 × 4.50	9.60 × 3.05	8.60 × 8.20	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	7	29	5
18	Fou-Bor-2	Mycelium white dense, slightly pinkish at the center	4.52	23.75 × 4.35	8.60 × 2.65	9.15 × 8.85	3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	31	4
19	Fou-Bor-3	Mycelium white dense, slightly pinkish at the center uniformly raised	3.56	20.85 × 3.65	8.50 × 2.50	9.75 × 8.40	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	24	7
20	Fou-Bor-4	Mycelium white dense, slightly pinkish at the center	3	21.00 × 3.70	8.35 × 2.90	9.60 × 8.65	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	39	6
21	Fou-Kan-1	Mycelium white dense, slightly pinkish at the center	5.1	20.35 × 3.35	10.50 × 3.00	9.95 × 9.10	2-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	7	32	2
22	Fou-Kan-2	Mycelium white dense, slightly pinkish at the center	5.6	22.25 ×3.85	7.50 × 2.50	8.85 × 8.70	2-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	35	4
23	Fou-Kan-3	Mycelium white dense, slightly pinkish at the center	5.32	20.30 × 4.50	7.20 × 2.60	10.05 × 8.30	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	38	5
24	Fou-Kan-4	Mycelium white dense, slightly pinkish at the center	4.52	22.60 × 3.60	8.30 × 3.1	9.35 × 9.25	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	39	2
25	Fou-Bad-1	Mycelium white dense, slightly pinkish at the center	4.21	19.90 × 3.10	10.60 × 2.95	9.90 × 8.85	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	30	6
26	Fou-Bad-2	Mycelium white dense, slightly pinkish at the center	5.36	21.00 × 5.15	9.80 × 2.50	9.20 × 8.75	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	26	4
27	Fou-Bad-3	Mycelium white dense, slightly pinkish at the center	5.29	21.50 × 3.00	8.35 × 2.50	9.65 × 8.30	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	25	5
28	Fou-Bad-4	Mycelium white dense, slightly pinkish at the center	5.23	22.60 × 4.00	8.45 × 2.70	9.75 × 8.70	2	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	10	36	6
29	Fou-Kok-1	Mycelium white dense, slightly pinkish at the center	6.2	28.70 × 2.50	10.80 × 3.00	9.00 × 7.23	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	39	4
30	Fou-Kok-2	Mycelium white dense, slightly pinkish at the center	6.15	21.95 × 3.10	8.75 × 2.50	9.75 × 8.10	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	40	3
31	Fou-Kok-3	Mycelium white dense, slightly pinkish at the center	3.21	19.45 × 3.75	7.60 × 3.00	9.90 × 8.70	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	12	32	4
32	Fou-Kok-4	Mycelium white dense, slightly pinkish at the center	4.26	22.85 × 3.65	8.60 × 3.00	9.60 × 7.60	2-3	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	11	21	6
33	Fou-Buk-1	Mycelium white dense, slightly pinkish at the center	4.98	27.40 × 4.05	10.50 × 3.00	9.65 × 9.20	3-4	0-1	Sickle	Hyaline	Oval	Hyaline	spherical	Hyaline	6	25	5

Conclusion

While studying variability among isolates observations clearly indicated variation among different isolates of pathogen. The colony colour of 40 isolates ranged from white to pinkish white with pink center, with dense to fluffy growth on PDA medium and some isolates showed fastest growth while others showed medium to very slow growth. Mycelial width varied from 3 µm to 6.32 µm. Microconidia were small, oval in shape and hyaline in colour, unicellular or with one or two septa, and measured in the size range between 6.00 × 2.10 µm to 10.80 × 3.00 µm. The macroconidia were long, curved, sickle shaped, pointed at the tip, hyaline in colour and knotched at the base, septated (2-4 septa) and measured between 19.05 × 3.25 µm to 28.70 × 2.50 µm. Chlamydospores were spherical in shape and hyaline in colour. Chlamydospores dimensions varied from 8.20 × 7.92 µm to 11.35 × 8.20 µm. Spore density also varied for macro conidia in the range of 6 to 13, for micro conidia it was between 20 to 42 and for chlamydospores it was observed between 2 to 8 number per ml.

Reference

1. Booth C. *Fusarium*, laboratory guide to the identification of the major species. *CAB publication*, England, 1977, 1-37.
2. Jalander V, Gachande BD. Effect of Culture Media on Growth and Pigmentation of *Fusarium oxysporum* f. sp. *udum* Butler Isolated from Different Varieties of Pigeonpea [*Cajanus cajan* (L.) Millsp.]. International Journal of Current Research in Biosciences and Plant Biology 2015;2:50-53.
3. Kannaiyan J, Nene YL, Reddy MV, Ryan TN, Raju TN. Prevalence of Pigeonpea diseases and associated crop losses in Asia, Africa, and the Americas. Tropical Pest Management 1984;30:62-712.
4. Madhukeshwara SS, Seshadri VS. Variation and Management of *Fusarium* Wilt of Pigeonpea *[Cajanus cajan* (L.) Millsp.] *Tropical Agricultural Research*. 2001;13:380-394.
5. Nene YL, Kannaiyan J, Haware MP, Reddy MV. Review of the work done at ICRISAT on soil borne disease of Pigeonpea and chickpea. In Proceedings of the Consultants Group Discussion on the Resistance to Soil borne Diseases of Legumes. 8-11 January, 1979, ICRISAT Centre, Patancheru, A.P. India. 1980, 3-39.
6. Nene YL, Kannaiyan J, Haware MP, Reddy MV. Pigeonpea Diseases: Resistance-Screening Techniques. Information Bulletin no. 9. Patancheru, A.P, India: ICRISAT. 1981, 14.
7. Pande S, Sharma M, Gopika G. An updated review of biology, pathogenicity, epidemiology and management of wilt disease of Pigeonpea (*Cajanus cajan* (L.) Millsp.). *Journal of Food Legumes.* 2013;26(1, 2):1-14.
8. Upadhyay RS, Rai B. A new disease cycle of wilt of Pigeonpea. Current Science 1983;52:978-981.
9. Vishwa Dhar, Reddy MV, Chaudhary RG. Major Diseases of Pigeonpea and their management In: *Advances in Pigeonpea Research* (Eds. Ali, Masood and Shiv Kumar). Indian Institute of Pulses, Kanpur 2005, 229-261.