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PLANT PATHOLOGY

Wilt Incidence and Cultural Variability of *Fusarium oxysporum* f.sp. *udum* Collected from different Districts of Uttar Pradesh

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Abstract

Pigeonpea (*Cajanus cajan*) L. Millsp. is an important legume crop widely used as food grain as it is rich source of protein, carbohydrate, essential amino acids, minerals and fibres. India is reknowned as a major pigeonpea producer country all over the world. Pigeonpea is susceptible to a number of pathogens, among which *Fusarium oxysporum* f.sp. *udum* is considered as the most important fungal pathogen causing considerable economic loss in India and all over the world. Among different states in India, Uttar Pradesh is the major pigeonpea growing state having most of the wilt susceptible pigeonpea growing areas causing considerable yield losses. In the present study, collection of diseased samples from wilt affected areas of different districts, isolation of test fungi, test of pathogenicity in pots under wirenet house condition was conducted. Further test of wilt incidence of the selected strains of *Fusarum oxysporum* f.sp. *udum* through root dip method and soil inoculation method was undertaken in earthen pots under wirenet house to make a precise comparison between the two methods. Cultural variabilities like radial growth, growth rate and mycelia dry weight among the selected isolates were studied under laboratory conditions.

Highlights

- Isolation of *Fusarium udum* from the diseased samples collected from different districts of Uttar Pradesh.
- Test of pathogenicity of the isolated strains of *F. udum*.
- Study of disease incidence of selected isolates by root dip and soil-inoculation methods.

Keywords: Cultural variability, *Fusarium udum*, pathogenicity, pigeonpea, root dip, soil inoculation, wilt incidence

Pigeonpea [(*Cajanus cajan*) L. Millsp.] is an important legume crop extensively used as food grain due to its high value of protein, carbohydrate, essential aminoacids, minerals and fibres. It is also used as green manure crop in different areas to increase the soil fertility. The countries having major contributions in global pigeonpea production are India (63.74% of global production), Myanmar (18.98%), Malawi (6.07%), Tanzania (4.42%) and Uganda (1.98%). The global productivity of green pods vary from 1000-4000 kg/ha, while the dry seed productivity average was 714 kg/ha. (Duke, 1983, Saxena *et al.*, 2006). The global production area of pigeonpea is over 4.92 Mha according to Saxena (2009). In India, pigeonpea was cultivated on 4.65 Mha resulting a total production of 3.02 MT and productivity of 650kg/ha (Gowda *et al.*, 2015). In India, it had a low growth rate of 0.8% in production between 1949–1950 and 2004 because of various biotic and abiotic stresses (Singh *et al.*, 2005). The lower productivity of pigeonpea in India is due to different biotic and abiotic stress. Though pigeonpea is attacked by a number of pathogens including fungi, bacteria, viruses, nematodes, and mycoplasma like organisms, the diseases of considerable economic importance at present are *Fusarium* wilt (*Fusarium udum*), sterility mosaic virus, and phytophthora blight (*Phytophthora* *dreschleri* f.sp. *cajani*). Among these, wilt caused by *Fusarium udum* is considered as the most important soil born pathogen of pigeonpea (Kumar *et al.*, 2010). This disease was first reported by Butler (1906) in India. According to ICRISAT report 1987, in India, pigeonpea wilt is of serious concern in Maharastra, Bihar, Uttar Pradesh, Madhya Pradesh and Andhra Pradesh and less severe in other states. Singh *et al*. (2013) reported *Fusarium* wilt of pigeonpea from different districts of Uttar Pradesh and 22.33-95% disease incidence was recorded from different places on susceptible cultivar '*Bahar*'. Saxena *et al.* (2010) reported that *Fusarium* wilt disease in pigeonpea is so devastating that it can cause production loss up to 97000 tonnes per year in India alone. According to Datta *et al*. (2013), *Fusarium* wilt is a serious fungal disease in pigeonpea (*Cajanus cajan*) which causes severe yield loses up to 90%. In Malawi susceptible pigeonpea line ICP 2376 was inoculated by 60 isolates of *F. udum* but only 7 isolates were pathogenic (Soko *et al.*, 1995). Kiprop *et al*. (2002) tested 56 isolates from different pigeonpea growing areas in Kenya and observed that cultural characteristics of *F. udum* appeared to be independent of aggressiveness and no relationship between aggressiveness and geographical origin of the isolates was found. The isolates of *F. udum* from the same or diverse geographical origins have shown high degree of cultural variability (Reddy and Choudhary 1985, Gaur and Sharma, 1989) and variability in virulence or pathogenicity on pigeonpea genotypes (Soko *et al*.,1995, Kiprop *et al.*, 2002, Parmita *et al.*, 2005, Karimi *et al.*, 2012).

Very limited reviews have been available on the wilt causing ability of *F. udum* isolates from different pigeonpea growing locations of Uttar Pradesh and their cultural variations. Hence, the main objective of this study is to survey the different wilt susceptible districts of Uttar Pradesh, isolation of *F. udum* from diseased samples, test of pathogenicity and study of wilt incidence in susceptible pigeonpea variety '*Bahar*' though different methods (root dip and soil inoculation).

MATERIALS AND METHODS

Survey for wilt incidence at different districts of Uttar Pradesh

Random roving method of survey was carried out to record the severity of *Fusarium* wilt in pigeonpea. The survey was conducted during *Rabi* 2011 - 12 in different districts of Uttar Pradesh. The areas having maximum wilt incidence were selected and infected samples were collected from those areas. Observations on stage of the crop and disease incidence of the surveyed plots were recorded. The percent disease incidence was assessed by the formula:

$$PDI = \frac{\text{Number of plants showindg wilting symptoms}}{\text{Totalnumber of plants}} \times 100$$

Pigeonpea plants showing typical wilting symptoms were collected separately in paper bags and brought to the laboratory for isolation of pathogens and further investigations. Fifty samples were collected from different places of Uttar Pradesh and were named.

Isolation, purification, maintenance of fungal isolates and test for pathogenicity

After collection of diseased samples, the collar region of each samples were washed thoroughly in running tap water to remove the soil particles. Each sample was cut into small bits and at least 5 bits were surface sterilized by dipping in 0.1% mercuric chloride for 1 min. and rinsed three times in sterilized distilled water. The surface sterilized bits were then transferred to separate Petriplates containing PDA medium under aseptic condition. This procedure was repeated for each sample. The Petriplates were than incubated at 25±2^{o}C till visible fungal colonies appeared. From the colonies a small bit of actively growing mycelium were cut and transferred to separate sterilized Petriplates. Each isolate was purified by adopting single spore isolation technique (Kiprop *et al.*, 2002). The cultures of all isolates were maintained on PDA slants at 25 ±2^{o}C for further studies.

After isolation and purification of *F.udum* from the collected samples, they were subjected to pathogenicity tests on susceptible genotype of pigeonpea variety '*Bahar*' through 'root dip' inoculation method as described by Haware and Nene (1994).

Study of disease incidence of selected isolates of *F. udum*

Fifteen selected isolates of *F.udum* were tested for their ability to cause wilt on susceptible genotype '*Bahar*' by adopting 'root dip' method and 'soil inoculation' method. In root dip method, the data were recorded on 20, 30 and 40 days of inoculation. Data on wilt incidence were recorded at 15 days interval from 15^{th} to 135^{th} days after sowing in soil inoculation method. *F. udum* untreated plants were taken as control plant.

Wilt incidence in root dip and soil inoculation methods was studied and the data were recorded by calculating the proportion of wilt affected plants to initial plant stand by the formula:

$$PDI = \frac{\text{Number plants showing wilting symptoms}}{\text{Totalnumber of plants}} \times 100$$

The above experiments were performed in sterilized earthen pots in three replications in which one earthen pot containing 4 numbers of plants representing one replication.

Procedure for root dip inoculation method

In this procedure, different isolates of *F. udum* were grown on PDA medium separately. Mycelial discs of 5mm diameter from the periphery of 6 days old cultures were inoculated in 250 ml conical flasks containing 100 ml potato dextrose broth (PDB). Those flasks were inoculated for 7 days at 25±2°C. After incubation the broth from each conical flasks were decanted gently and the collected mycelial network were macerated separately by mortar and pestle. The macerated fungal growth was suspended in 100 ml distilled water and the spore concentration was approximately adjusted to 2×10^6 conidia/ml (Marley and Hillocks, 1993). Pegionpea seedlings were grown in pots containing mixture of sterilized sand and soil in the ratio of 3:7. Ten days old seedlings were removed carefully and dipped in spore suspension for 5 minutes (Haware and Nene, 1994).

Seedlings from the control pots were dipped in sterilized distilled water for 5 minutes. The inoculated seedlings and the seedlings of the control pots were transplanted back in the same pots. The pots were watered regularly to avoid moisture stress to the plants. Observations on disease appearance were recorded as percent disease incidence (PDI). The experiment was performed in three independent replications with each pot representing a single replication and four numbers of seeds were shown in each pot.

Procedure for soil inoculation method

Each isolate was grown on PDA plates. Two actively grown mycelia discs (5mm dia) from the periphery of 6 days old culture of each isolates were separately inoculated in 500 ml conical flasks containing 100g pigeon pea meal medium. The flasks were incubated at 25±2°C for 20 days. A fungus-soil mixture was prepared by mixing 200 g of inoculums with 2kg of autoclaved sand: soil mixture (3:7). Fifteen cm diameter earthen pots were sterilized by formalin (0.1%). These were than filled with fungus-soil mixture. Seeds sterilized with mercuric chloride (1%) were sown in each pot. Seeds sown in un-inoculated pots served as control (Haware and Nene, 1994). Three independent replications were taken, each pot representing a single replication and four numbers of seeds were shown in each pots.

Study of cultural variability of selected isolates of *F. udum*

Study of radial growth and radial growth rate of *Fusarium udum* isolates

Well sterilized Petridishes were poured with 20 ml of potato dextrose agar (PDA) medium under laminar flow. After this 5mm diameter of healthy mycelial bits of 6 days old cultures of *F.udum* isolates were transferred separately to the centre of the newly prepared Petriplates under laminar flow with the help of sterilized needle and forceps. The Petriplates were incubated at 25± 2°C. The radial growth of each isolate was measured in mm using vernier callipers at an interval of 24 hours till the mycelial growth covered the full surface of Petridish (Gaur and Sharma, 1989). Data were recorded up to 7^{th} day of inoculation. Three Average cumulative growth rate/day were calculated by using mathematical formula as follows:

$$\text{Growth rate} = \frac{(\text{Final growth} - \text{Initial growth})}{\text{Time}}$$

Study of mycelial dry weight of isolates

Selected isolates of *F.udum* were grown separately in sterilized glass test tubes containing potato dextrose broth (PDB) and incubated at 25±2°C. Mycelial mat grown on PDB was filtrated over the pre-weighed filter paper. The mycelium accompanied by pre-weighed filter paper was dried at 60°C for 48. The mycelium weight was determined by subtracting the weight of filter paper from the weight of mycelium accompanied dried filter paper.

Statistical analysis

Experiments were repeated once using one way ANOVA. The data are expressed as the mean of three independent replications ± standard deviations. Means were compared by DMRT ($P \leq 0.05$), using SPSS version 16. Different letters superscript in the column data indicate significant difference between the variants across the treatments.

RESULTS AND DISCUSSION

Collection of diseased samples, isolation and purification of test pathogens

The areas having maximum wilt incidence were selected and infected samples were collected from those areas. The observations on crop stages of pigeonpea showing the typical wilting symptoms, the disease incidence of the surveyed plots and the name of the isolated strains of *F. udum* were presented in Table 1. The disease incidence of the surveyed plots ranged from 10.7%-60.9% at different crop stages like flowering, pre-podding, podding and post-podding stages. Fifty samples were collected from different districts of Uttar Pradesh from which fifty isolates of *F. udum* were isolated in laboratory in aseptic condition on PDA plates. Further purification of the isolated strains was done by using PDA plates to avoid contaminations (Nikam *et al.,* 2011). These findings were corroborated with the reports of Nene *et al*. (1979) who reported thatthe wilt disease of pigeonpea appears in early stages of plant growth i.e. when plants are 6-8 weeks old and maximum incidence was recorded at flowering and podding stage. The incidence of disease has been reported 30-60% at crop maturity and flowering stages by Kannaiyan and Nene, 1981. Datta *et al*. (2013) reported maximum yield loss up to 90% caused due to *Fusarium udum* (*Fud*) in pigeonpea (*Cajanus cajan*). Okiror (2002) suggested that this disease depends upon the stage of the crop infection, which approach over 50% and even upto 100% when wilt occurs at the pre-pod stage.

Test for pathogenicity

After isolation and purification of *F.udum* from the collected samples, they were subjected to pathogenicity tests on susceptible genotype of pigeonpea variety '*Bahar*' through 'root dip' methods as described by Haware and Nene (1994). Out of 50 isolates of *Fusarium udum* tested for pathogenicity, 15 isolates showed the typical wilting symptoms and proved the Koch's postulate and results were shown in Table 2. The 15 isolates which proved the Koch's postulate were FU-2, FU-4, FU-6, FU-7, FU-9, FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-18, FU-19 and FU-20. These selected isolates were further studied for percent disease incidence (PDI) in both 'root dip' and 'soil inoculation' methods.

In the present study, out of 50 isolates of *F. udum*, 15 isolates were found to show typical wilting symptoms as cited by Reddy *et al*. (1990) and Jain *et al*. (1995) and 15 out of 50 isolates were able to prove Koch's postulate and proved to be truely pathogenic to pigeonpea cultivar '*Bahar*'. This result is corroborated by the findings of Soko *et al*. (1995) inoculated 60 isolates of *F. udum* in pigeonpea line ICP 2376 out of which only 7 isolates were found to be pathogenic. Okiror and Kimani (1997), who explored some true variants of *F. udum* during his experiment in Kenya.

Study of disease incidence of selected isolates of *F. udum*

Root dip inoculation method

The results of tests for PDI of 15isolates of *F.udum* at 20, 35 and 40 DAS by root dip method were represented in Table 3. At 60DAI, FU-6 showed maximum disease incidence of 100% followed by FU-7 and FU-17 which caused 91.66% and 66.66% PDI respectively. FU-11 caused minimum 25% of PDI at 60 DAI.

Soil inoculation method

As the results obtained from Table 3, no disease incidence were recorded up to 30 DAS. Maximum

Table 1: *Fusarium* wilt incidence in major pigeonpea growing districts of Uttar Pradesh

Sl. No.	Name of the District	Locations	Stages of the Crop	Isolates	PDI (%)
1	Varanasi	Rajatalab	Flowering	FU-1	12.5
2	Varanasi	Rajatalab	Flowering	FU-1a	10.7
3	Varanasi	Rajatalab	Flowering	FU-1b	13.9
4	Varanasi	Harauwa	Podding	FU-2	23.7
5	Varanasi	Harauwa	Podding	FU-2a	26.3
6	Varanasi	B.H.U. campus	Pre-podding	FU-3	17.5
7	Varanasi	Babusarai	Pre-podding	FU-4	27.9
8	Varanasi	B.H.U. campus	Pre-podding	FU-5	25.6
9	Varanasi	Harauwa	Podding	FU-6	60.9
10	Varanasi	B.H.U. campus	Podding	FU-6a	54.6
11	Barabanki	Dariyabad	Pre-podding	FU-7	39.3
12	Barabanki	Puredelai	Pre-podding	FU-7a	29.4
13	Barabanki	Bhitariya	Pre-podding	FU-7b	26.7
14	Gorakhpur	Badhalganj	Flowering	FU-8	16.7
15	Gorakhpur	Kaudiram	Flowering	FU-8a	19.6
16	Gorakhpur	Dohrighat	Pre-podding	FU-9	34.5
17	Mirzapur	Lalpur	Podding	FU-10	45.3
18	Mirzapur	Tedia	Podding	FU-10a	27.3
19	Mirzapur	Mirzamurad	Podding	FU-10b	22.5
20	Mirzapur	Persodha	Post-podding	FU-10c	21.9
21	Basti	Kaptanganj	Flowering	FU-11	36.8
22	Basti	Kaantae	Flowering	FU-11a	33.4
23	Basti	Harriya	Flowering	FU-11b	32.5
24	Faizabad	Bhelsar	Podding	FU-12	35.7
25	Faizabad	Mawai	Podding	FU-12a	38.9
26	Faizabad	Maholi	Pre-podding	FU-12b	39.7
27	Sultanpur	Dhanpatganj	Post-podding	FU-13	59.6
28	Sultanpur	Chhanda	Post-podding	FU-14	39
29	Sultanpur	Lamhua	podding	FU14a	36.5
30	Bhadohi	Amawa	Podding	FU-15	41.3
31	Ghazipur	Muhamadabad	Flowering	FU-16	49.7
32	Ghazipur	Kaithi2	Flowering	FU-16a	39.5
33	Ghazipur	Udiara	Flowering	FU-16b	36.3
34	Ambedkarnagar	Kathari	Pre-podding	FU-17	56.4
35	Ambedkarnagar	Gopalpur	Pre-podding	FU-17a	45
36	Ambedkarnagar	Malipur	Pre-podding	FU-17b	43.2
37	Pratapgarh	Gudwara	Flowering	FU-18	47.2
38	Pratapgarh	Biharganj	Flowering	FU-18a	44.3
39	Pratapgarh	Patti	Flowering	FU-18b	39.6
40	Jaunpur	Dulhanpur	Podding	FU-19	33.2
41	Jaunpur	Khanpur2	Podding	FU-19a	19.1
42	Mau	Ghosi	Post-podding	FU-20	53.5
43	Mau	Tajaepur	Podding	FU-20a	25.8
44	Raibareilly	Gangaganj	Podding	FU-21	19.6
45	Raibareilly	Latwa	Podding	FU-21a	18.1
46	Raibareilly	Inhauna	Podding	FU-22	13.6
47	Allahabad	Bikrampur	Podding	FU-22a	12.9
48	Allahabad	Bhiti	Podding	FU-22b	10.4
49	Faizabad	Darshnagar	Post-podding	FU-23	28.6
50	Faizabad	Khandasa	Post-podding	FU-23a	27

100% PDI was acquired by FU-6 followed by 83.33% by FU-7 at 135 DAS. Minimum PDI of 25% was recorded by FU-10 at 135 DAS.

Table 2: Pathogenicity test using different isolates of *Fusarium* under pot conditions

Sl. No.	Name of the Isolates	Wilting Symptoms	Koch's Postulate
1.	Control	-ve	-ve
2.	FU-1	-ve	-ve
3.	FU-1a	-ve	-ve
4.	FU-1b	-ve	-ve
5.	FU-2	+ve	+ve
6.	FU-2a	-ve	-ve
7.	FU-3	-ve	-ve
8.	FU-4	+ve	+ve
9.	FU-5	-ve	-ve
10.	FU-6	+ve	+ve
11.	FU-6a	-ve	-ve
12.	FU-7	+ve	+ve
13.	FU-7a	-ve	-ve
14.	FU-7b	-ve	-ve
15.	FU-8	-ve	-ve
16.	FU-8a	-ve	-ve
17.	FU-9	+ve	+ve
18.	FU-10	+ve	+ve
19.	FU-10a	-ve	-ve
20.	FU-10b	-ve	-ve
21.	FU-10c	-ve	-ve
22.	FU-11	+ve	+ve
23.	FU-11a	-ve	-ve
24.	FU-11b	-ve	-ve
25.	FU-12	+ve	+ve
26.	FU-12a	-ve	-ve
27.	FU-12b	-ve	-ve
28.	FU-13	+ve	+ve
29.	FU-14	-ve	-ve
30.	FU14a	-ve	-ve
31.	FU-15	+ve	+ve
32.	FU-16	+ve	+ve
33.	FU-16a	-ve	-ve
34.	FU-16b	-ve	-ve
35.	FU-17	+ve	+ve
36.	FU-17a	-ve	-ve
37.	FU-17b	-ve	-ve
38.	FU-18	+ve	+ve
39.	FU-18a	-ve	-ve
40.	FU-18b	-ve	-ve
41.	FU-19	+ve	+ve
42.	FU-19a	-ve	-ve
43.	FU-20	+ve	+ve
44.	FU-20a	-ve	-ve
45.	FU-21	-ve	-ve
46.	FU-21a	-ve	-ve
47.	FU-22	-ve	-ve
48.	FU-22a	-ve	-ve
49.	FU-22b	-ve	-ve
50.	FU-23	-ve	-ve
51.	FU-23a	-ve	-ve

In root dip method the disease has been appeared at early stages of plant growth and maximum disease incidence was found from flowering to podding stage in soil inoculation method, which was supported by the findings of Nene *et al*. (1979), who reported the incidence of wilt disease in pigeonpea in early stages of plant growth i.e. when the plants are 6-8 weeks old and maximum incidence was recorded at flowering and podding stage. Kannaiyan and Nene (1981) reported 30-60% incidence of disease at crop maturity and flowering stages. Also there may be differences in pathogenicity of *F. udum i*solates as the experimental reports of Shit and Sengupta (1980) bring up the similar results from India. They provided the reports by examining seven different isolates of *F. udum* collected from different parts of the country. Two of them were moderately to highly pathogenic against four cultivar *viz*., B7, EB-3, C-11 and Mukti, whereas one of them was found to be weakly pathogenic to EB-3 and isolate V to B7.

Study of cultural variability of selected isolates of *F. udum*

Radial growth of fungal mycelium was studied and observations were recorded up to 7th day of inoculation as 100% radial growth was achieved by FU-6 after 7th day of inoculation. The results have been shown in Table 5. FU-6 showed maximum radial growth consistently up to 7th day of inoculation followed by FU-7. The experiment was conducted by taking 15 treatments in three replications. Each treatment representing one selected isolate of *F.udum*.

Radial growth, radial growth rate and mycelia dry

Table 3: The effect of *Fusarium udum* isolates on percent disease incidence of pigeonpea through root dip method of inoculation method

Sl. No.	Name of the Isolates	PD I (%) Days after inoculation		
		20 DAI	30 DAI	40 DAI
1	Control	0±0^{a}	0±0^{a}	0±0^{a}
2	FU-2	8.33±14.43^{ab}	16.66±14.43^{abc}	33.33±8.33^{bc}
3	FU-4	25±25^{ab}	41.66±14.43^{cd}	50±14.43^{bcde}
4	FU-6	25±14.43^{b}	75±25^{e}	100±0^{g}
5	FU-7	25±0^{ab}	58.33±14.43^{de}	91.67±8.33^{fg}
6	FU-9	8.33±14.43^{ab}	33.33±14.43^{bcd}	41.67±8.33^{bcd}
7	FU-10	8.33±14.43^{ab}	25±0^{abc}	33.33±8.33^{bc}
8	FU-11	0±0^{a}	8.33±14.43^{ab}	25±14.43^{ab}
9	FU-12	8.33±14.43^{ab}	25±25^{abc}	33.33±8.33^{bc}
10	FU-13	25±0^{ab}	41.66±14.43^{cd}	58.33±8.33^{cde}
11	FU-15	8.33±14.43^{ab}	25±25^{cd}	50±14.43^{bcde}
12	FU-16	16.66±14.43^{ab}	33.33±14.43^{bcd}	58.33±8.33^{cde}
13	FU-17	25±25^{ab}	41.66±14.43^{cd}	66.67±8.33^{def}
14	FU-18	16.66±14.43^{ab}	33.33±14.43^{cd}	58.33±8.33^{cde}
15	FU-19	0±0^{a}	16.67±14.43^{abc}	41.67±8.33^{bcd}
16	FU-20	25±0^{ab}	58.33±14.43^{de}	75±14.43^{efg}

The data in the tables were analysed using one way ANOVA. The data are expressed as the mean of three independent replications ± standard deviations. Means were compared by DMRT (P ≤ 0.05), using SPSS version 16.

Table 4: The effect of *Fusarium udum* isolates on percent disease incidence of pigeonpea through soil inoculation method

Sl. No.	Name of the Isolates	PDI (%) Days after sowing								
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105DAS	120 DAS	135DAS
1	Control	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
2	FU-2	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	16±14.43^{abc}	25±0^{abc}	33.33±14.43^{bc}
3	FU-4	0±0	0±0	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	16.66±14.43^{ab}	25±0^{abc}	33.33±14.43^{bcd}	41.66±14.43^{bcd}
4	FU-6	0±0	0±0	8.33±14.43^{a}	25±0^{c}	33.33±14.43^{d}	41.66±14.43^{c}	58.33±14.43^{d}	75±25^{e}	100±0^{f}
5	FU-7	0±0	0±0	8.33±14.43^{a}	16.66±14.43^{bc}	25±0^{cd}	33.33±14.43^{bc}	41.66±14.43^{cd}	58.33±14.43^{de}	83.33±14.43^{ef}
6	FU-9	0±0	0±0	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	8.33±14.43^{ab}	16.66±14.43^{abc}	25±0^{abc}	33.33±8.33^{bc}
7	FU-10	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	16.66±14.43^{ab}	25±0^{b}
8	FU-11	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	16.66±14.43^{abc}	25±0^{abc}	33.33±14.43^{bc}
9	FU-12	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	25±0^{abc}	33.33±14.43^{bcd}	33.33±14.43^{bc}
10	FU-13	0±0	0±0	0±0^{a}	8.33±14.43^{ab}	16.66±14.43^{bc}	25±0^{abc}	33.33±14.43^{bcd}	41.66±14.43^{bcd}	50±25^{bcd}
11	FU-15	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	16.66±14.43^{abc}	25±0^{abc}	41.66±14.43^{bc}
12	FU-16	0±0	0±0	0±0^{a}	16.66±14.43^{bc}	25±0^{cd}	25±0^{abc}	33.33±14.43^{bcd}	50±25^{cde}	50±25^{bcd}
13	FU-17	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	16.66±14.43^{ab}	33.33±14.43^{bcd}	41.66±14.43^{bcd}	58.33±14.43^{cde}
14	FU-18	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	16.66±14.43^{abc}	25±0^{abc}	33.33±14.43^{bc}
15	FU-19	0±0	0±0	0±0^{a}	0±0^{a}	0±0^{a}	8.33±14.43^{ab}	25±25^{abc}	33.33±14.43^{bcd}	41.66±14.43^{bcd}
16	FU-20	0±0	0±0	0±0^{a}	0±0^{a}	16.66±14.43^{bc}	25±25^{abc}	33.33±14.43^{bcd}	41.66±28.86^{bcd}	66.66±14.43^{de}

The data in the tables were analysed using one way ANOVA. The data are expressed as the mean of three independent replications ± standard deviations. Means were compared by DMRT (P ≤ 0.05), using SPSS version 16.

Table 5: Study of the radial growth of selected isolates of *Fusarium udum*

Sl. No.	Name of the *Fusarium isolates*	Radial growth (DAI)						
		1DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI
1	FU-2	0.25±0	0.25±0	0.5±0.028	0.85±0.076^{ab}	1.9±0.104^{abc}	2.45±0.08^{cd}	3.41±0.058^{bcd}
2	FU-4	0.25±0^{a}	0.25±0^{a}	0.56±0.04^{ab}	0.95±0.057^{ab}	2.85±0.076^{f}	3.23±0.06^{g}	3.46±0.06^{cd}
3	FU-6	0.5±0.05	0.95±0.057^{f}	1.91±0.18^{f}	2.45±0.076	3.4±0.076^{h}	3.85±0.057^{i}	4.5±0.07
4	FU-7	0.48±0.01^{b}	0.8±0.028^{e}	1.8±0.06^{f}	2.25±0.08^{f}	3.2±0.076^{h}	3.65±0.076^{h}	4.25±0.057^{f}
5	FU-9	0.25±0^{a}	0.46±0.016^{b}	0.66±0.04	1.05±0.057^{bc}	2.11±0.06^{c}	2.55±0.057^{cd}	3.3±0.076^{abc}
6	FU-10	0.25±0^{a}	0.25±0^{a}	0.5±0.028^{a}	0.95±0.057^{ab}	1.8±0.076^{ab}	2.25±0.057^{ab}	3.25±0.057^{ab}
7	FU-11	0.25±0^{a}	0.45±0.028^{b}	0.75±0.07^{bcd}	1.35±0.076^{d}	2.65±0.076^{ef}	2.83±0.06^{ef}	3.66±0.04^{e}
8	FU-12	0.25±0^{a}	0.25±0^{a}	0.5±0.028^{a}	0.75±0.057	1.7±0.06	2.13±0.06	3.1±0.06
9	FU-13	0.25±0^{a}	0.25±0^{a}	0.55±0.028^{ab}	1.06±0.04^{bc}	2.05±0.057^{bc}	2.56±0.04^{cd}	3.13±0.06^{a}
10	FU-15	0.25±0^{a}	0.46±0.016^{b}	0.75±0.057^{bcd}	1.2±0.076^{cd}	2.4±0.028^{d}	2.65±0.057^{de}	3.25±0.04^{ab}
11	FU-16	0.25±0^{a}	0.48±0.016^{b}	0.6±0.028^{ab}	1.06±0.04^{bc}	2±0.076^{bc}	2.53±0.06^{cd}	3.38±0.02^{bcd}
12	FU-17	0.25±0^{a}	0.5±0^{bc}	0.8±0.06^{cd}	1.18±0.06^{cd}	2.1±0.12^{c}	2.58±0.04^{cd}	3.46±0.04^{cd}
13	FU-18	0.25±0^{a}	0.56±0.03^{cd}	0.6±0.06^{ab}	0.93±0.04^{ab}	1.95±0.08^{abc}	2.38±0.04^{bc}	3.28±0.058^{abc}
14	FU-19	0.25±0^{a}	0.5±0^{bc}	0.91±0.06^{d}	1.3±0.10^{d}	2.5±0.076^{de}	3±0.10^{f}	3.76±0.11^{e}
15	FU-20	0.46±0.016^{b}	0.58±0.04^{d}	1.4±0.028^{e}	1.88±0.04^{e}	2.9±0.076^{g}	2.95±0.076^{f}	3.5±0.06^{de}

The data in the tables were analysed using one way ANOVA. The data are expressed as the mean of three independent replications ± standard deviations. Means were compared by DMRT (P ≤ 0.05), using SPSS version 16.

Table 6: Study of the radial growth, radial growth rate and mycelial dry weight of different isolates *Fusarium udum* (7 Days after Inoculation)

Sl. No.	Name of the *Fusarium* strains	Radial growth(cm)	Radial growth rate (mm/day)	Mycelial dry weight(mg)
1	FU-2	3.41±0.10^{bcdef}	4.51±0.065^{bc}	28.66±1.52^{a}
2	FU-4	3.46±0.115^{cdef}	4.58±0.075^{c}	39±3.60^{b}
3	FU-6	4.5±0.132^{h}	5.71±0.247^{f}	169±6.55^{j}
4	FU-7	4.25±0.1^{g}	5.38±0.072^{e}	146.33±6.11^{i}
5	FU-9	3.3±0.13^{abcde}	4.35±0.132^{abc}	72.66±2.08^{e}
6	FU-10	3.25±0.1^{abcd}	4.28±0.075^{ab}	63.33±1.201^{d}
7	FU-11	3.66±0.076^{f}	4.87±0.043^{d}	86±3^{g}
8	FU-12	3.11±0.104^{a}	4.08±0.072^{a}	47.66±1.52^{c}
9	FU-13	3.13±0.104^{ab}	4.11±0.2^{a}	53.33±1.52^{c}
10	FU-15	3.25±0.076^{abc}	4.25±0.160^{ab}	74±2.64^{e}
11	FU-16	3.38±0.036^{cdef}	4.47±0.036^{bc}	77±2^{ef}
12	FU-17	3.46±0.076^{ef}	4.58±0.045^{bc}	84±2.64^{fg}
13	FU-18	3.76±0.14^{def}	4.32±0.167^{bc}	80±2.64^{efg}
14	FU-19	3.28±0.101^{bcdef}	4.41±0.085^{c}	81±3^{g}
15	FU-20	3.55±0.10^{ef}	4.9±0.055^{d}	97±2^{h}

The data in the tables were analysed using one way ANOVA. The data are expressed as the mean of three independent replications ± standard deviations. Means were compared by DMRT (P ≤ 0.05), using SPSS version 16.

weight were represented in Table 6. Data of the observations were taken after 7th day of inoculation. FU-6 showed the highest radial growth i.e.4.5cm followed by FU-7 and FU-18 i.e. 4.25cm and 3.76cm respectively. Least radial growth was observed in case of FU-12 i.e. 3.1cm after 7th day of inoculation. Also the highest radial growth rate was observed in case of FU-6 i.e. 5.71mm/day followed by FU-7 and FU-20 which showed 5.38mm/day and 4.9mm/day respectively.

Least radial growth rate was recorded in case of FU-12 i.e. 4.08mm/day. Mycelial dry weight was observed to be highest in case of FU-6 i.e.169mg followed by FU-7 and FU-20 i.e.146.33mg and 97mg respectively whereas least amount of mycelia dry weight was found in case of FU-4 i.e. 3.9mg after 7days of inoculation.

The experimental reports of Reddy and Choudhury (1985) as well as Gaur and Sharma (1989) point out the high degree of cultural variabilities among isolates of *F. udum* regardless of their geographical origins. Gwata *et al*. (2006) agreed with most of the available reports which relates different isolates to different races. Okiror and Kimani (1997), who explored some true variants of *F. udum* during his experiment in Kenya in which they tested 12 isolates of *F. udum* collected from different locations. Patel *et al*. (2011) revealed that owing to existence of physiologic races among isolates of *F. udum,* the dry mycelial weight of different isolates ranged from 221 to 494 mg. High degree of variations were observed in case of radial growth rate and mycelia dry weight after 7 days of inoculation which was corroborated by Singh *et al*. (2013) who observed the variation in mycelia dry weight from 27mg-236mg and radial growth rate from 4.80-11.93mm/day.

CONCLUSION

From the present study, it can be concluded that *F. udum* isolates isolated from different districts of Uttar Pradesh were varied in the appearance of wilting symptoms and degree of pathogenicity as only some isolates proved the Koch's postulate. Those isolates were varied in their ability cause wilt in pigeonpea which was measured as percent disease incidence. Disease incidence was measured by root dip method or soil inoculation method. In root dip method early appearance of disease symptoms and wilt were observed as compared to the soil inoculation method. These isolates were found to be highly variable in their cultural characteristics like radial growth and radial growth rate of fungal mycelium and mycelia dry weight.

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