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Variation and Management of Fusarium Wilt of Pigeon Pea [*Cajanus cajan* (L.) Millsp.]

S.S. Madhukeshwara and V.S. Seshadri

Department of Plant Pathology
University of Agricultural Sciences
GKVK, Bangalore - 560 065, India

ABSTRACT. *A study was conducted during 1996-98 at University Farm, AICRP on Pulses, University of Agricultural Sciences, Bangalore, India, with the objectives of understanding the variation among the six isolates of Fusarium udum Butler causing wilt of pigeon pea [Cajanus cajan (L.) Millsp.] chosen from distinct agro climatic regions of Karnataka and Andhra Pradesh and management of them by various methods. The isolates were designated as I_1, I_2, I_3, I_4, I_5 and I_6 for wilted samples collected from Bangalore, Bijapur, Chitradurga, Dharwad, Gulbarga and Hyderabad respectively.*

Studies on existence of variability, with respect to morphological, cultural, nutritional, physiological, spore germination, isozyme and pathogenicity characters revealed considerable variation among six isolates. The size of macro and microconidia varied from 18-21 × 4-5 µm to 23-26 × 4-5 µm respectively. Chlamydospores measured from 10-17 µm in diameter and varied pigmentation was noticed from white to dusky red. The isozyme studies in six enzyme systems showed great variation with respect to matrix of similarity index. The dendrogram showed two broad group with isolates I_1 and I_3 in one and I_2, I_4, I_5 and I_6 in another. The latter were further divided in to three sub groups as I_2 and I_4 in one, I_5 and I_6 as separate groups. In all there was a difference of total genetic distance of 7.08. All the isolates of F. udum showed virulence on genotype ICP 2376 and C-11, where as none of the isolates except isolate I_5 showed virulence on ICP 8863.

The three soil antagonists isolated from the rhizosphere of the wilted plants in native sick soil viz., Trichoderma viride, Pseudomonas fluorescens and Bacillus subtilis showed significant results in suppression of F. udum both in-vitro and plot culture experiments. The studies on management of F. udum by various methods in sick plot imposing 20 treatments showed significant effect. Among the treatments, combined application of T. viride + T. flourescens + B. subtilis + Neem cake + mixed cropping had least percent wilt and highest mean yield.

There was drastic reduction in the pathogen population in the soil corresponding to the effective treatments.

INTRODUCTION

Pigeon pea, commonly known as redgram or tur or arhar [*Cajanus cajan* (L.) Millsp.], is one of the important legume crops of tropics and subtropics. Pigeonpea is grown in almost all states of India. It restores the soil fertility by fixing atmospheric nitrogen. The heavy shedding of leaves adds considerable organic matter to the soil. Pigeon pea has multiple uses such as tender green seeds used as vegetables, stem and roots

as fuel wood, besides its main use as dhal. It is affected by more than hundred pathogens (Nene *et al.*, 1989). Incidentally, only a few of them cause economic losses (Kannaiyan *et al.*, 1984) among which wilt caused by *Fusarium udum* Butler is a serious problem. The incidence of infection ranges from 3-94% in the field (McRae, 1923; Plyman, 1933). The annual Pigeon pea crop loss due to wilt in terms of value in India alone has been estimated at US $ 36.3 millions (Kannaiyan *et al.*, 1984).

Several high yielding varieties like TTB-7, Hy 3C, Bahar *etc.*, are highly susceptible to *Fusarium* wilt. In this context, the variety *Maruthi* (ICP-8863) developed by the International Crop Research Institute for Semi Arid Tropics (ICRISAT) is a significant contribution in host resistance. But in recent days, the variety was observed succumbing to wilt in few pockets at the farmer's field. This has led to a speculation of possible occurrence of variation in the pathogenicity or virulence in the pathogen. To combat such problems, there is a need for good disease management programme. However, no single measure would be sufficient and durable in disease control. Therefore, studies were undertaken to search for effective integrated management of *Fusarium* wilt of Pigeon pea to know the possible variation in the pathogen.

MATERIALS AND METHODS

Collection, isolation and identification of pathogen

About one hundred diseased samples of Pigeon pea were collected from Pigeon pea growing areas of Karnataka and Andhra Pradesh. The usual tissue isolation technique was followed to get *Fusarium* culture from infected Pigeon pea plants showing true vascular wilt symptoms. The pure culture thus obtained was identified as *Fusarium udum* according to Booth (1971). Out of about one hundred isolates studied only six isolates, which were showing clear visible variation in cultural and morphological characters, were selected for further studies. These isolates from Bangalore, Bijapur, Chitradurga, Dharwad, Gulbarga and Hyderabad were designated as I_1, I_2, I_3, I_4, I_5 and I_6 respectively, for record and further identification.

Variation studies

Cultural and morphological studies

The cultural characteristics of different isolates of monoconidial origin were studied on different media. The observations were made on colour, septation, size and other morphological characters of conidia under high power objective lens. The size of the conidia was measured by using calibrated ocular micrometer and also cultural characters like growth, type colour of colony, *etc.*, were observed on 8th day after inoculation and 25th day for chlamydospore formation. Sporulation was studied by means of Haemocytometer under the microscope (Tuite, 1969). Five discs of fungal growth of 5 mm diameter were dissolved in 20 ml of distilled water and shaken well to get spore suspension. One drop of spore suspension was placed on Haemocytometer. After placing the glass coverslip, spores were counted microscopically from five blocks of Haemocytometer. Spores per ml of suspension were calculated using the following formulae.

$$Spores\ per\ ml = \frac{No.\ spores\ observed \times 2000 \times ml\ of\ water}{Area\ of\ the\ disc}$$

Spore germination studies

The method used by Rovira (1956) was adopted for the preparation of root exudates. Sterilised 10 cm diameter Giffy pots were filled with washed clean sand. In each pot four seeds were sown at equidistance in 2 cm depth with ICP 8863 and TTB 7 seeds. The pots were periodically watered with sterile water. The water leachate at 15th day old seedlings was collected. Then seedlings were gently removed without any injury to the roots. The removed seedlings were kept for a day in the removed leachate. Thus, collected concentrated root exudates were used for the study.

Spore germination of all six isolates of *F. udum* was studied by using conventional hanging drop method by using cavity slide. Spore suspensions were prepared in root exudates of resistant (ICP-8863) and susceptible (TTB-7) cultivars separately to each isolate. Spore suspension of each isolate was then transferred separately on to cover slips and inverted over a cavity slide, the edges of cover slips were sealed by using Vaseline. The slides were placed in Petriplates lined with moistened absorbent cotton at room temperature. Germination counts were recorded after 6, 12, 18 and 24 h. The spore germination was calculated by taking the average number of spores germinated out of the total number of spores present in ten microscopic fields under high power objective and expressed as per cent germination.

Isozyme studies

The six isolates were grown in 50 ml of Richard's broth standardized for the period of peak protein production after incubating for 5-7 days. Later the mycelium was washed 2-3 times using Whatman No. 41 filter paper. The mycelium was washed 2-3 times with sterile distilled water to remove the salt contents of the broth. Then using the blotter paper excess moisture was removed from the mycelium. Later the mycelia of the six isolates were kept in polythene bags with proper labelling and the polythene bags were sealed. These samples were kept at -70°C in an ultra freezer. At the time of Electrophoretic studies the mycelia were taken out and freeze dried (lyophilized) for 3-4 h until the moisture is removed. Later 0.2 g of the sample was taken into centrifuge tube, to which fine glass powder was added and 80 micro litre of appropriate extraction buffer was added (Pitel and Cheliak, 1984). The mycelium was ground using motorized homogenizer at 2000 rpm for 30 sec. The samples were kept cool at the time of homogenization. The extracts of the three isolates were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant solution was transferred to another centrifuge tube. This supernatant solution served as the source of sample for isozyme studies. A total of 14 enzymes were used for this study *viz.*, Catalase (CAA), Fumarase (FUM), Peroxidase (POX), Glucose-6-phosphate Dehydrogenase (G6PDH), 6-Phosphogluconate Dehydrogenase (6PGDH), Malate Dehydrogenase (MDH), Isocitrate Dehyhdrogenase (IDH), Superoxide Desmutase (SOD), Acid Phosphatase (ACP), Glyceraldehyde-3-Phosphase dehydrogenase (G3PDH), Hexokinase (HK), Malic enzyme (ME), Phosphoglucomutase (PGM) and Phosphogluco isomerase (PGI). The electrode buffer was used as described by Pitel and Cheliak (1984). After loading, the gel was run

on an average of 5-6 h with constant voltage/current of 250 volts/50 MA. After the termination of electrophorosis the gel was removed from the electrophoretic apparatus and staining of the gel for various isozymes was done following the staining recipe given by Pitel and Cheliak (1984). The gels incubated for varying periods in the staining solutions at 37°C until visible staining was observed. The isozyme bands were given loci position depending upon the number of loci expressed and polymorphic loci if any were detected by visualising alleles. The isozyme data was analyzed to calculate similarity index values between isolates according to Sokal and Sneath (1963). A dendrogram showing genetic distance was drawn for six isolates in unweighted paired group mean of averages (UPGMA) programme.

Virulence behaviour

A set of eight host differential of pigeon pea ICP-9145, ICP-8863, ICP-9144, ICP-2376, ICP-8858, C-11 and KP-8859 as recommended by ICRISAT were obtained from Indian Institute of Pulses Research (IIPR), Kanpur. The virulence behaviour was tested by regular test tube method and modified root injury method.

Modified root injury method

The seeds of the differential lines were surface sterilized using 2% sodium hypochlorite for 1 min. The earthen pots were filled with sterilized red soil and sand mixture in 3:1 ratio. In the middle of the earthen pots, 15 cm length cut pieces of PVC pipes were immersed and the soil inside was scooped out. The surface sterilized seeds of the differential lines were sown on the pot at just periphery of the PVC pipes all round. The pots were watered regularly with sterile water. The PVC pipes were gently removed after watering, when the seedlings were 8-10 days old. Due to removal of PVC pipes the entire root system of Pigeon pea is exposed to facilitate for infection. The gap created by removing the PVC pipes filled by using the giant culture of *F. udum* on Maize meal medium, sterilized red earth and sand mixture (1:1). The spore load was adjusted to 10^6 spores^{-g} soil diluting with red earth and sand.

Management of *F. udum* wilt

The field experiments on management of *Fusarium* wilt of Pigeon pea by using cultural, chemical and biological methods were conducted for two years during *kharif* 1996-97 and 1997-98 using the susceptible variety TTB-7 in a randomised complete block design (RCBD) with three replications. There were treatments of cultural practices, organic and inorganic amendments, native practices and inoculation of antagonists. The intercropping of redgram:sorghum (4:1) and mixed cropping of 10:1 (V/V); the application of organic amendments like farm yard manure (FYM), neem cake, tea waste and Vermicompost @ 7.5, 2.5, 4.25, 2.5 t ha^{-1}; the inorganic amendments dolomite (1.0 t ha^{-1}), N P K 62.5:125:62.5 kg ha^{-1}; soil antagonists @ 100 g kg^{-1} seeds along with normally recommended rhizobium treatments on the seeds and soil application in the furrow @ 25 kg ha^{-1} mixed with sand. The native practices for plant disease control like modified panchagavya (MPG-3) was prepared as per Padmodaya (1994). The seeds were treated

with 50% cow urine by soaking for 1 h and then used for sowing. The effect of treatments with respect to percent wilt was recorded 30, 60, 90, 120 and 150 days after sowing (DAS).

The soil antagonists *viz.*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from wilt affected sick soils by using the recommended selective media as per Vasudeva *et al.* (1958), Sands *et al.* (1972) and Elad and Chet (1983), respectively.

The initial and final soil pathogenic population after the experiment were analysed on colony forming units (cfu) basis by serial dilution technique at 10^{-5} dilution with 10 ml stock solution as recommended by Nash and Snyder (1962).

RESULTS AND DISCUSSION

Cultural and morphological characters studied on potato dextrose agar at room temperature showed variation among six isolates of *F. udum*. Variation observed among the isolates with respect to colony diameter, pigmentation and sporulation was distinct (Table 1). The isolate I_5 was distinct from any other with maximum spore density and chalmylospores. The isolates I_1 and I_3 were comparable, though with minor differences. Existence of such variability among the isolates are well documented by many workers. Nene *et al.* (1980) have classified *F. udum* in to 12 distinct groups based on cultural characters. Reddy and Basuchoudhary (1985) grouped six isolates of *F. udum* into three distinct groups based on colony characters.

Table 1. Conidial characters of six isolates of *Fusarium udum*.

	Conidia size (µm)		Septation		Chlamy dospore Dia (µ)	Spore density (spores/ml)		Conidia (macro)		Conidia (micro)	
	Macro	Micro	Macro	Micro		Macro	Micro	Shape	Colour	Shape	Colour
I_1	18-21×4-5 µm	6-8×2-3 µm	3-4	0-1	04	6.0	14.0	Sickle	Hyaline	Oval	Hyaline
I_2	23-25×4-5 µm	6-8×2-3 µm	2-3	0-1	14	7.0	24.0	Sickle	Hyaline	Oval	Hyaline
I_3	19-26×3-4 µm	6-7×2-3 µm	3-4	0-1	07	6.0	21.0	Sickle	Hyaline	Oval	Hyaline
I_4	20-22×4-5 µm	6-7×2-3 µm	2-5	0-1	11	10.0	21.0	Sickle	Hyaline	Oval	Hyaline
I_5	23-26×4-5 µm	6-8×2-3 µm	3-4	0-1	17	10.0	34.0	Sickle	Hyaline	Oval	Hyaline
I_6	22-24×4-5 µm	6-9×2-3 µm	3-4	0-1	11	13.0	27.0	Sickle	Hyaline	Oval	Hyaline

The results in the present study also show that the isolates varied from each other in cultural and morphological characters particularly with the spore density and accordingly they have been grouped in to four groups with I_1 and I_3 in one, I_2 and I_4 in another and remaining I_5 and I_6 as separate groups.

The isolates behaved distinctly in more or less with same trend in growth phase pH, temperature and nutritional requirements during the studies.

Root exudates of the plant influence root-infecting fungi (Schroth and Hildebrand, 1964). The exudation of compounds may be stimulatory or inhibitory (Rovira, 1969). In the present study of spore germination with susceptible cultivar TTB-7 and resistant cultivar ICP 8863, revealed that 24 h after treatment, the spore germination in susceptible cultivar has reached to the maximum (100%) (Fig. 1 and 2). There was a significant difference in the germination behaviour between the isolates in a given period of 24 h. The isolates differed significantly in the spore germination studies with root exudates. This method can be suggested as an indicator for differentiating the strains (Buxton, 1962). The analysis of the root exudates for phenols and total sugars revealed 4.34 $mg^{-100}ml$ and 6.20 mg^{-ml} respectively in resistant cultivar (ICP8863) compared to 1.73 $mg^{-100}ml$ and 2.18 mg^{-ml} respectively in susceptible cultivar (TTB-7), very clearly indicating that stimulatory factors operate in the spore germination at rhizosphere. Similar observations of high amount of flavonol an alkaloid which is a precursor for phenol synthesis in resistant cultivar C-1 1-6 and low amount in susceptible cultivar TS-136-1 of Pigeon pea were made by Murthy and Bagyaraj (1980). This study is also on line with the studies conducted in other *Fusarium* sp. by Kraft (1974).

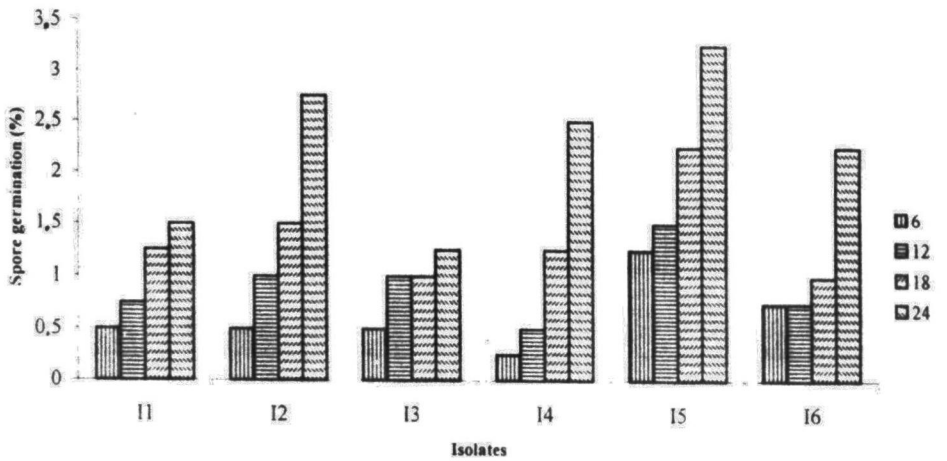

Fig. 1. Per cent spore germination of six isolates of *Fusarium udum* in root exudate of cultivar ICP8863 (Maruthi).

The gel electrophoretic technique was used as a powerful taxonomic tool to clarify and delineate specific or sub-specific groups of different fungi. The similarity index matrix (Table 2) and dendrogram (Fig. 3) was drawn based on the isozyme activity patterns of six enzymes *viz.*, IDH, PGI, G-6PDH, PGM, 6-PGDH and SOD out of 14 enzymes screened. The isolates formed into two distinct groups with isolates I_1 and I_3 in one and I_2, I_5, I_4 and I_6 in another. The latter group had three subgroups of I_2 and I_5 together in one, I_4 and I_6 separately. Thus the isolates differentiated in to only two broad groups. Similar observations were noticed in *Fusarium ciceri* affecting chickpea in different isolates as reported by Desai *et al.* (1994) and on *Fusarium* spp by Pomazi *et al.* (1993).

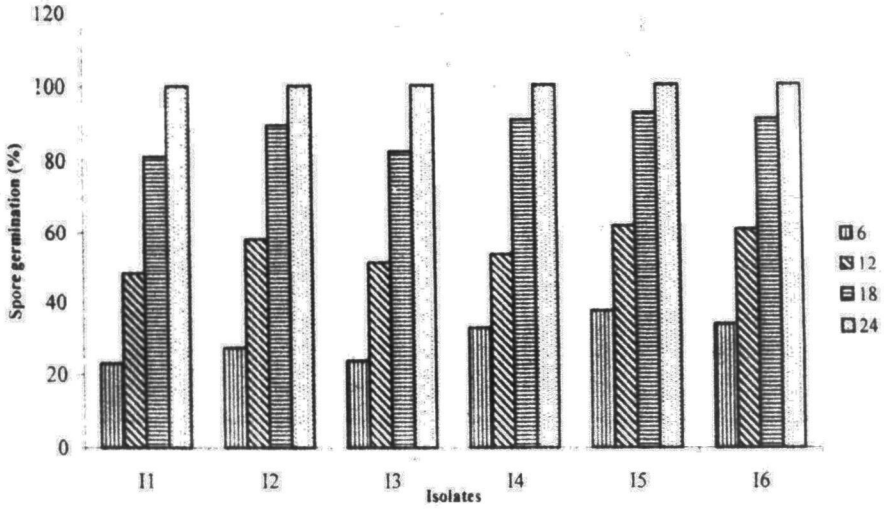

Fig. 2. Per cent spore germination of six isolates of *Fusarium udum* in root exudate of cultivar TTB7.

Table 2. Matrix of per cent similarity index values of the isolates of *Fusarium udum* studied using isozyme pattern.

Isolates	I_1	I_2	I_3	I_4	I_5	I_6
I_1	-	31.25	81.25	56.25	37.50	56.25
I_2		-	50.00	62.50	68.75	50.00
I_3			-	62.50	43.75	50.00
I_4				-	68.75	62.50
I_5					-	68.75
I_6						-

The *in-vitro* and pot culture experiments on virulence behaviour of the six isolates of *F. udum* exhibited similar reaction on the ICRISAT recommended host differentials. The isolates from various parts showed a wide range in their pathogenicity.

The isolate I_5 showed high virulence on all the host differentials except ICP 8863 in the study. All the isolates were avirulent on ICP 8863 except isolates I_5. All the isolates differed in their virulence behaviour showing their distinct nature in pathogenicity on a set of host differentials (Table 3). Earlier, Baldev and Amin (1974); Shit and Sengupta (1980) have observed variability in the isolates of *F. udum*. Based on the pathogenicity of the isolates on host differentials, they can be separated in to four distinct groups. The first group with isolates I_1 and I_3, second group with isolates I_2 and I_4, third with isolate I_5 and

fourth group I_6. Mukharjee (1956) suggested that the possibility of existence of pathogenic races of the fungus as expressed by the differential response of the same variety under different environmental conditions. The findings of the present studies indicated a clear variation among the isolates and strongly support the existence of pathotypes in *F. udum*.

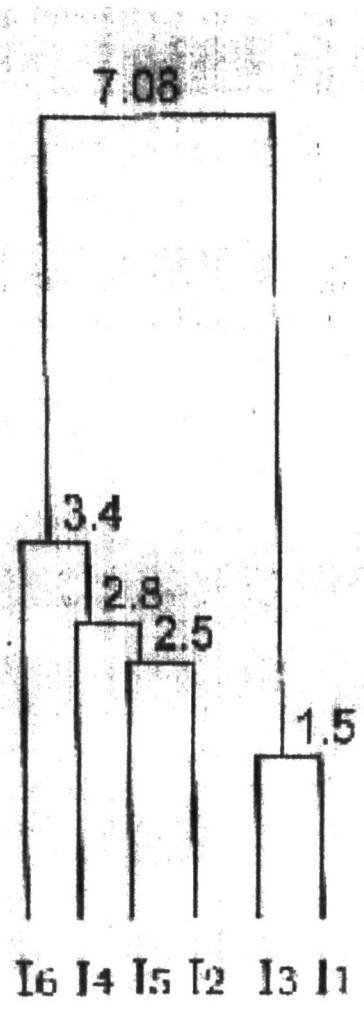

Fig. 3. Dendogram showing genetic distance of isolates of *Fusarium udum*.

In-vitro assessment of *T. viride*, *P. fluorescens* and *B. subtilis* have indicated that *T. viride* makes strong colony inhibition and wilt control in pot culture experiments followed by *P. fluorescens* and *B. subtilis* over the control (Table 4 and 5). Somashekara (1992) also reported that *T. viride* is more efficient in controlling the *F. udum* compared to *T. harzianum*, *T. haematum* and *T. koningii*. It is further supported by the fact that antagonistic organisms isolated from rhizosphere of wilted plants were highly effective in

suppressing the population of *F. udum* as evidenced by Upadhyay and Rai (1987) and Mukopadhyay (1989).

Table 3. Reaction of isolates of Fusarium udum on host differentials of Pigeon pea.

Host differentials	Per cent wilt incidence					
	Isolates					
	I_1	I_2	I_3	I_4	I_5	I_6
ICP 2376	16.6	50.0	20.0	62.5	92.0	91.6
ICP 8862	15.0	11.1	10.0	0.0	22.2	12.5
ICP 8863	0.0	0.0	0.0	0.0	12.5	0.0
ICP 9174	30.0	12.5	20.0	8.33	20.0	20.0
ICP 9145	12.5	0.0	27.27	11.11	23.0	25.0
ICP 8858	0.0	0.0	0.0	18.0	20.0	28.5
ICP 8859	0.0	12.5	0.0	0.0	22.22	20.0
C-11	5.3	25.0	0.0	37.5	80.0	33.3

Table 4. *In-vitro* effects of soil antagonists isolated from rhizosphere of wilted plants in sick plot on *Fusarium udum* expressed in per cent colony inhibition.

Treatments	Per cent colony inhibition					
	I_1	I_2	I_3	I_4	I_5	I_6
T. viride	98.16 (83.03)	95.43 (77.75)	97.20 (80.67)	96.16 (78.61)	91.33 (77.35)	96.69 (78.56)
P. fluorescens	96.46 (79.07)	94.86 (76.55)	94.13 (75.89)	95.50 (73.44)	94.66 (76.58)	94.56 (76.53)
B. subtilis	86.23 (68.14)	84.40 (66.67)	86.06 (68.01)	84.36 (66.01)	80.83 (63.67)	83.4 (65.89)
Fusarium udum (Control)	0.0	0.0	0.0	0.0	0.0	0.0
	Isolates		Treatment		I×T	
S.E. (mean)	0.35		0.44		0.89	
LSD (p=0.05)	1.04		1.28		2.56	

* Values in parentheses are arc sine transformed

Among the twenty treatments tested for two years *T. viride* + *P. fluorescens* + *B. subtilis* + Neem cake + Mixed cropping (T_{16}) treatment (Fig. 4) was the best which differed significantly from others, followed by T_{15} (Neem cake + *T. viride* + Mixed cropping) and T_{13} (*T. viride* + *P. fluorescens* + *B. subtilis* + FYM). However, the treatments T_{15} and T_{13} did not vary significantly (Table 6 and 7).

Table 5. Effects of soil antagonists isolated from rhizosphere of wilted plants in sick plot on *Fusarium* wilt of Pigeon pea.

Treatments	Isolates (per cent wilt)					
	I_1	I_2	I_3	I_4	I_5	I_6
T. viride	0.0 (6.22)	23.3 (6.22)	11.6 (5.42)	26.6 (7.33)	28.6 (6.17)	23.3 (6.62)
P. fluorescens	0.0 (4.72)	25.0 (8.61)	11.33 (8.56)	16.0 (7.33)	36.6 (6.55)	31.6 (5.75)
B. subtilis	0.0 (6.22)	31.6 (7.33)	11.6 (7.33)	23.3 (4.62)	51.0 (9.26)	36.6 (11.32)
		Treatments		Isolates		T×I
S.E. (mean)		0.53		1.26		2.53
LSD (p=0.05)		1.83		3.47		6.95
Fusarium udum (Control)	100.0	100.0	100.0	100.0	100.0	100.0

* Values in parentheses are arc sine transformed

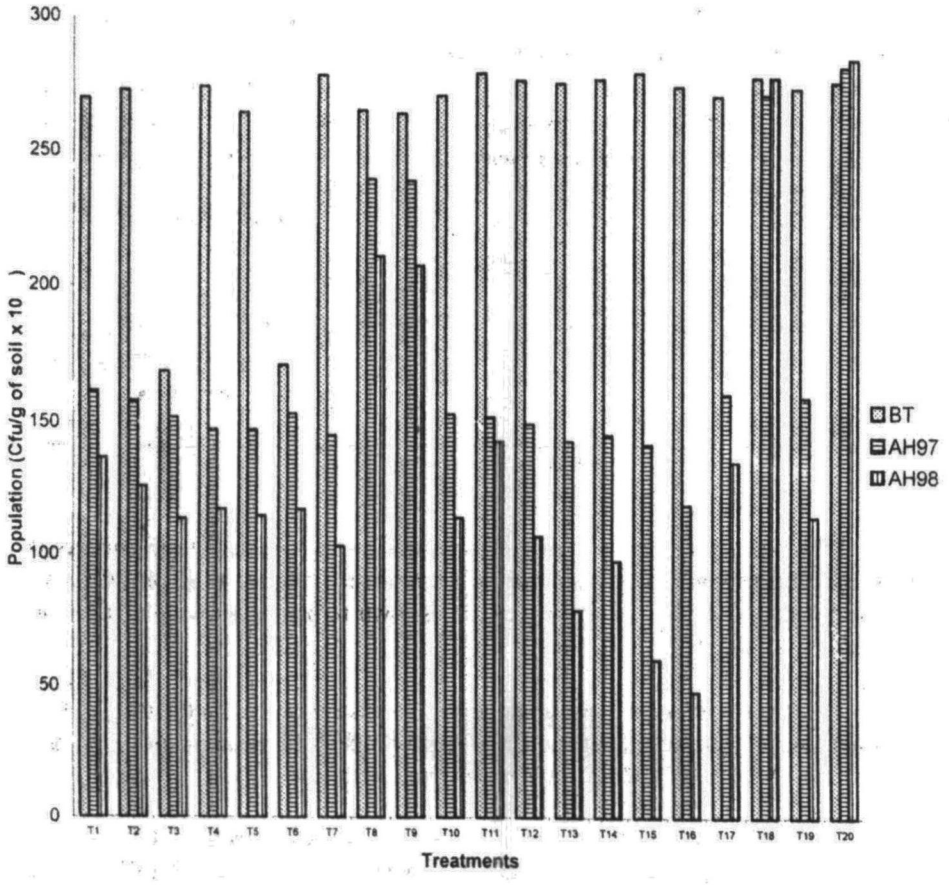

Fig. 4. Fusarium udum population before and after treatments.

Table 6. Effect of different treatments on Fusarium wilt of Pigeon pea with respect to per cent wilt and yield during 1996-97.

Treatments	Per cent wilt after*		
	60 DAS	150 DAS	Yield (kg/ha)
T_1 - *Trichoderma viride*	5.6 (13.57)	88.0 (69.71)	58.00
T_2 - *P. fluorescens* + *B. subtilis*	3.95 (11.28)	84.5 (66.77)	67.37
T_3 - *T. viride* + *P. fluorescens* + *B. subtilis*	0.0 (0.0)	80.0 (63.40)	70.37
T_4 - FYM	5.65 (13.73)	90.5 (71.96)	56.37
T_5 - Tea waste	2.9 (9.77)	89.0 (70.56)	59.50
T_6 - Neem cake	4.25 (11.80)	83.5 (65.99)	65.50
T_7 - Vermicompost	0.00 (0.00)	87.0 (68.80)	68.50
T_8 - Cow urine treat	7.5 (15 83)	93.5 (73.50)	28.75
T_9 - Panchagaya seed treat	9.4 (17.81)	92.5 (72.01)	31.37
T_{10} - Sorghum mixed cropping	4.6 (12.19)	86.0 (67.96)	46.50
T_{11} - Sorghum intercropping	9.05 (17.49)	91.0 (74.59)	31.50
T_{12} - *T. viride* + FYM	4.65 (12.43)	86.5 (68.40)	66.12
T_{13} - *T. viride* + *P. fluorescens* + *B. subtilis* + FYM	0.00 (0.00)	69.5 (63.03)	148.25
T_{14} - Neem cake + Mixed cropping	0.00 (0.00)	80.0 (63.36)	72.27
T_{15} - *T. viride* + Neem cake + Mixed cropping	0.00 (0.00)	67.5 (61.63)	156.87
T_{16} - *T. viride* + *P. fluorescens* + *B. subtilis* + Neem cake + Mixed cropping	0.00 (0.00)	58.2 (43.31)	183.25
T_{17} - Dolomite	7.8 (16.19)	83.4 (65.66)	72.12
T_{18} - Carbandazim 50 wp seed treat @ 2 g kg^{-1} seed (Chemical control)	7.7 (16.05)	91.6 (74.81)	35.62
T_{19} - Dolomite + Neem cake	4.8 (12.70)	79.8 (62.26)	74.75
T_{20} - Control	14.05 (21.07)	95.0 (78.63)	26.50
S.E. (mean)	0.83 (0.92)	1.54	3.16
LSD (p=0.05)	2.16 (2.63)	4.15	9.89

* Values in parentheses are arc sine transformed

The results are similar to that of Somashekara (1992) in the treatments as far as tea waste is concerned. It is also similar to the results of Doube *et al.* (1994 a, b) in the Vermicompost treatment. Neem cake is known to have suppressive effect on *F. udum* (Reddy *et al.*, 1990).

Application of inorganic amendments like Dolomite and NPK fertilizers did not show any significant effect; slight change of pH did not affect the pathogen unlike it was observed by Brady (1974) on *Streptomyces scabies*.

The intercropping treatment of Pigeon pea with sorghum did not yield expected results. This is probably the root zone of the sorghum may not be sufficient to reach the Pigeon pea roots. As only root zone of sorghum is known to have inhibitory effect by the production of compound similar to cyanide (Rangaswamy and Balasubramanian, 1963;

Table 7. Effect of different treatments on Fusarium wilt of Pigeon pea with respect to per cent wilt and yield during 1997-98.

Treatments	Per cent wilt after*		Yield (kg/Act)
	60 DAS	150 DAS	
T_1 - *Trichoderma viride*	3.65 (10.75)	83.5 (65.97)	90.50
T_2 - *P. fluorescens* + *B. subtilis*	2.8 (9.35)	80.5 (63.75)	103.00
T_3 - *T. viride* + *P. fluorescens* + *B. subtilis*	0.0 (0.0)	76.75 (61.12)	106.25
T_4 - FYM	3.65 (10.98)	87.0 (68.80)	88.25
T_5 - Tea waste	1.25 (6.38)	84.5 (66.75)	95.62
T_6 - Neem cake	1.55 (7.14)	77.5 (61.63)	100.12
T_7 - Vermicompost	0.0 (0.0)	80.5 (63.77)	101.24
T_8 - Cow urine treat waste	3.7 (11.05)	93.5 (71.01)	34.75
T_9 - Panchagaya seed treat	4.45 (12.16)	92.0 (68.54)	36.50
T_{10} - Sorghum mixed cropping	2.35 (8.72)	78.65 (62.42)	74.25
T_{11} - Sorghum intercropping	4.65 (12.42)	89.15 (70.68)	56.00
T_{12} - *T. viride* + FYM	2.55 (9.18)	74.4 (59.55)	108.75
T_{13} - *T. viride* + *P. fluorescens* + *B. subtilis* + FYM	0.0 (0.0)	64.64 (54.74)	211.00
T_{14} - Neem cake + Mixed cropping	0.0 (0.0)	69.50 (56.43)	114.75
T_{15} - *T. viride* + Neem cake + Mixed cropping	0.0 (0.0)	63.2 (52.61)	219.25
T_{16} - *T. viride* + *P. fluorescens* + *B. subtilis* + Neem cake + Mixed cropping	0.0 (0.0)	49.3 (44.57)	290.62
T_{17} - Dolomite	4.3 (11.92)	81.75 (64.64)	91.25
T_{18} - Carbandazim 50 wp seed treat @ 2 g kg^{-1} seed (Chemical control)	3.65 (10.98)	89.3 (69.25)	38.75
T_{19} - Dolomite + Neem cake	2.25 (8.60)	74.05(59.32)	125.50
T_{20} - Control	15.20 (22.90)	97.5 (80.82)	25.25
S.E (mean)	0.79	1.18	2.92
LSD (p=0.05)	2.36	3.50	8.65

* Values in parentheses are sine transformed

Hillocks *et al.*, 1997). The population of *F. udum*, which was present almost uniformly throughout the sick plot, reduced initial heavy inoculum load drastically after the treatments were imposed over two seasons (Fig. 4). The treatments, cow urine 50% seed treatment (T_8), Panchagavya 10^{-1} seed treatment (T_9) and carbendazim 50 wp @ 2 g kg^{-1} seed treatment (T_{18}) showed least significant effect. The reduction in pathogen population is due to the effect of treatments. This is because fluorescent Pseudomonads produce extracellular 'siderophores' (microbial iron transport agents), which complex the iron and make it less available or unavailable to pathogens and promote plant growth. The results are in line with the observation by Sakthivel *et al.* (1986). Vasudeva *et al.* (1958) reported antibiotic 'bulbiformin' production in case of *B. subtilis*, which inhibited the growth of *F. udum* in the soil. The mycoparasitism in *T. viride* by hyphal coiling, lyses and antibiosis are well documented by Cook (1990). Scarselletti and Faull (1994) attributed for Pyrone, a metabolite responsible for inhibition. The experiments were taken up under extreme sick

soil situation. Hence, the effect of any of the treatments can be conveniently extrapolated to normal wilt situation as sustainable measures. The integration of bio-inoculants will definitely have bearing on checking the variation and management of the disease.

CONCLUSIONS

The present study indicated clear cut existence of variation of *F. udum* and occurrence of four races. The soil antagonists isolated from sick soil were location specific (Table 5). The treatment with soil antagonists isolated from sick soil (*T. viride* + *P. fluorescens* + *B. subtils*) @ 100 g kg^{-1} seeds and soil application in the furrow @ 25 kg ha^{-1} along with neem cake @ 2.5 t ha^{-1} before sowing in a mixed cropping of Sorghum (either cut for fodder or for grain purpose) is effective in controlling the Pigeon pea wilt from initial cent per cent to 49.3% over two years in a intense wilt affected sick soil condition.

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