

A comparative assessment of the efficacy of conidia and chlamydospores of *Fusarium udum* in inducing wilt of pigeonpea

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In potted fallow soil, inoculated with *Fusarium udum* (the causal organism of wilt of pigeonpea) abundant chlamydospores were produced in 1.5 months time. Both conidia and chlamydospores of *F. udum*, when inoculated to pigeonpea seedlings in water and soil cultures, initiated wilt. Disease severity increased with increase in concentration of the conidia/ chlamydospores. Chlamydospores had more inoculum potential in water culture and also in soil after longer inoculum incubation. Germination of chlamydospores was higher than conidia in the rhizosphere of a susceptible pigeonpea cultivar.

Key words: *Fusarium udum*, pigeonpea wilt, infective potential of conidia and chlamydospores

INTRODUCTION

Wilt of pigeonpea caused by *Fusarium udum* Butler, a serious and destructive disease in India, is estimated to cause annual yield losses of about 36.3 million rupees (Kannaiyan *et al.*, 1981). *F. udum* produces three types of propagules, namely, microconidia, macroconidia and chlamydospores. While conidia are produced during the active growth stage of the fungus in presence of suitable nutrients, in absence of the host and under unfavourable conditions hyphae and macroconidia are converted into chlamydospores which act as resting structures (Krikum and Wilkinson, 1963; French and Nielson, 1968; Van Eck, 1978). In presence of a suitable host, both conidia and chlamydospores may initiate infection. But they may not have same inoculum potential. The object of the present study is to study the relative efficacy of the conidia and chlamydospores of *F. udum* in initiating wilt in pigeonpea seedlings.

MATERIALS AND METHODS

An isolate of *F. udum* obtained from the stock culture of the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, was used throughout the investigation.

For preparation of conidial inoculum the test fungus was grown in 30 ml of sterilized potato dextrose broth in 150 ml Erlenmayer flasks for 10 days at 28°C. The mycelial mat was then taken out in 10 ml of sterile distilled water in 50 ml Erlenmayer flask and shaken in an electric shaker for 5 minutes. The mycelial mat was then discarded and the concentration of conidia in the suspension was measured by a haemocytometer and the required dilutions were made by adding distilled water.

For getting chlamydospores, the test fungus was grown under identical condition for 30 days by which time most of the mycelial cells were converted into chlamydospores (Chakraborty, 1987). The mycelial mat was taken out and washed twice in sterile distilled water by thoroughly shaking, so that the small amount of conidia that were present were washed out. The mycelial mat was then taken in 110 ml of sterilized distilled water and blended in a waring blender for six minutes to separate the chlamydospores. The chlamydospore suspension was first filtered through a thin layer of absorbent cotton and then centrifuge at 3000 r.p.m. for 5 minutes to precipitate the chlamydospores. Required concentrations of chlamydospore suspension were prepared in sterile distilled water.

Fifteen days old seedlings of susceptible pigeonpea cv. ICP-2376, raised in sand culture, were used for inoculation. The fungal inocula (conidia/chlamydospores) were prepared in nutrient solution (McLean and Cook, 1941) in sterilized black coloured bottles. One seedling was placed in each bottle in such a way that the root portion remained dipped within the fungal inoculum and the upper portion containing the leaves remained outside. A piece of nonabsorbent cotton wool kept the plant in position. After 24 h the seedlings were removed and placed in sterile nutrient solution. Appearance of symptoms was recorded periodically and finally after 13 days. Six replications were kept for each treatment and control.

For soil inoculation, the soil was collected from the University experimental farm at Kalyani (silty-loam, pH-7.8). The soil upto plough depth (15 cm) was collected, dried in air, sieved to remove all coarse particles and sterilized at 15 psi for 1 h for 2 consecutive days. Surface sterilized seeds of pigeonpea cv. ICP-2376 were sown forth in each pot. When the seedlings were 15 days old, they were inoculated by adding 10 ml of conidial or chlamydospore suspension at the root region of the seedlings by means of sterilized pipettes. The seedlings were regularly observed for appearance of symptoms and final reading was taken after 40 days. Three replications were made for each treatment and uninoculated control. For chlamydospores production in soil, 1 kg of natural soil was thoroughly mixed with 100 g of fungal inoculum grown in sand-maize-meal medium and incubated for 15 days at 28°C (Nash *et al.*, 1961). Fifteen days old seedlings of the susceptible cv. were transplanted to the chlamydospores infested soil in small earthen pots @ 120 g/pot. Six replications were maintained. The pots were lightly watered regularly and observed periodically for appearance of symptoms.

Disease symptoms in inoculated seedlings were assessed by using 0 (no symptoms) to 4 (complete wilting and death of seedlings) scale following Kaiser and Sen Gupta (1977) and disease severity was expressed as disease index following the formula : $(D.I.)_p = (D.I.)_T / N$ where, $(D.I.)_p$ = Average disease index per seedlings, $(D.I.)_T$ = Total disease index in a particular set, N = The number of seedlings in a particular set.

For studying the germination of conidia and chlamydospores in the rhizosphere, seeds of

susceptible (ICP-2376) and resistant (ICP-8863) cultivars of pigeonpea were surface sterilized in sodium hypochlorite solution for 5 min and allowed to germinate and grow for 3-4 days on moistened filter papers in sterile petridishes. Soil (with 30% M.H.C) was infested with conidia and chlamydo spores (10 ml of 1×10^3 /ml per 100 g soil) separately. Then the infested soil was spread as a 1.5 mm thick layer at the bottom of plastic plates and the soil surface was smoothen with a spatula. The seedlings of each susceptible and resistant cultivars were gently laid on the soil layer parallel to each other and overlaid with another layer of infested soil and incubated at 28°C for the periods of 1, 3 and 5 days. Five roots were carefully removed from each cultivar and the soil particles was taken on a glass slide and observed under microscope for germination of conidia and chlamydo spores. Three replicates were kept for each treatment and control with inoculated soil but without the seedlings.

For studying the population of conidia and chlamydo spores in the rhizosphere, seven days old pigeonpea seedlings (both susceptible and resistant cvs.) grown in the previous were experiment were carefully removed and the root portions were suspended in 9 ml of sterile distilled water and shaken vigorously to make the total volume upto 10 ml. This gave soil dilution of 1 : 10. The population of *F. udum* was detected by dilution plate count technique in Papavizas medium (Papavizas, 1967). Six replications were taken for each treatment and control of uninoculated natural soil.

RESULT AND DISCUSSION

Production of chlamydo spores in soil

Two phases in the life cycle of soil borne plant pathogenic *Fusaria* are known, (i) actively growing phase during which conidia are produced on actively growing mycelium and (ii) dormant phase during which the fungus perennates as thick walled chlamydo spores in soil in absence of a suitable host. The chlamydo spores germinate only in presence of suitable nutrient which is provided by root exudates of a suitable host plant. Existence of chlamydo spores of *F. solani* f.sp. *phaseoli* in soil has been demonstrated by Nash *et al.* (1961). Schippers and Voetberg (1969) demonstrated that both conidia and hyphal stages were ultimately transformed into chlamydo spores in soil in absence of a suitable host. In the present study it has been demonstrated that in inoculated potted soil, *F. udum* was ultimately converted into chlamydo spores after about 1.5 months. In soil smears on glass slides stained with lactophenol cottonblue, 4 to 5 chlamydo spores were observed. No other fungal structures were distinguished. The chlamydo spores were thick walled, round to subglobular, hyaline at first, later becoming deep brown and measured 27.6 to 39.3 μ (diameter).

Disease production in inoculated seedlings

In liquid culture, there was an increasing disease index with corresponding increase in inoculum concentration, as well as with increase in duration of incubation period both with conidial and chlamydo spore inocula (Table 1). However, disease indices were much higher in seedlings inoculated with chlamydo spores as compared to those with conidia in identical concentrations. Similar observation was reported by Couteaudier and Alabouvette (1990) in case of *F. oxysporum* f.sp. *lini* in inoculated soil. The higher infective potential of chlamydo spores was probably due to presence of stored nutrients.

Table 1. Disease rating of pigeonpea seedlings inoculated with different concentrations of conidia and chlamydospores of *F. udum* at different time intervals (in liquid culture)

Period after inoculation (days)	Average disease index in different concentration of conidia and chlamydospores (per ml)							
	Conidia			C.D (P=0.05)	Chlamydospores			C.D (P=0.05)
	1x10 ³	1x10 ⁴	1x10 ⁵		1x10 ³	1x10 ⁴	1x10 ⁵	
7	0.17	0.25	0.83	0.21	0.33	0.50	0.75	0.14
9	0.67	0.67	1.50	0.36	1.33	1.67	2.75	0.33
11	1.00	1.50	2.50	0.33	1.75	2.08	3.08	0.51
13	1.67	1.92	2.83	0.24	2.33	2.75	3.58	0.51

Table 2. Disease rating of pigeonpea seedlings inoculated with different concentration of conidia and chlamydospores of *F. udum* at different time intervals (in soil)

Period after inoculation (days)	Average disease index in different concentrations of conidia and chlamydospores (per ml)							
	Conidia			C.D (P=0.05)	Chlamydospores			C.D (P=0.05)
	1x10 ³	1x10 ⁴	1x10 ⁵		1x10 ³	1x10 ⁴	1x10 ⁵	
25	1.00	1.08	1.75	0.04	0.84	0.96	1.41	0.22
30	1.50	1.66	2.20	0.09	1.08	1.25	1.58	0.16
35	1.63	1.70	2.20	0.18	1.95	2.16	2.70	0.35
40	2.12	2.54	2.70	0.14	2.37	2.92	3.29	0.38

Table 3. Germination of conidia and chlamydospores of *F. udum* at different time intervals in the rhizosphere of pigeonpea seedlings

Treatments	Germination(%) at different periods of inoculation (days)					
	Conidia			Chlamydospores		
	1	3	5	1	3	5
Susceptible cultivar	5.66 (13.73)*	9.33 (17.59)	11.00 (20.10)	11.00 (19.30)	14.33 (22.21)	22.66 (26.72)
Resistant cultivar	3.66 (10.97)	5.66 (13.73)	6.33 (14.52)	4.60 (12.40)	6.00 (14.17)	8.33 (16.75)
Control (without any seedling)	3.16 (10.23)	5.00 (12.92)	5.83 (13.97)	0.0	0.0	0.0
C.D. (P=0.05)	0.95	1.68	1.09	0.79	0.72	2.04

*Figures in parentheses are transformed angular values

Table 4. Population of *F. udum* in the rhizosphere of pigeonpea grown in *F. udum* conidia and chlamydo spores inoculated soil (MHC of soil 30%)

Treatments	Number of <i>Fusarium</i> propagules ($\times 10$)	
	Conidia	Chlamydo spores
Susceptible cultivar	18.33	22.00
Resistant cultivar	5.33	6.33
Control (Uninoculated soil)	0.00	0.00

In inoculated soil also similar trend of result was obtained (Table 2). However, in earlier stages, disease severity was higher in seedlings inoculated with conidia as compared to those inoculated with chlamydo spores, particularly in higher concentrations. In later stages (longer incubation period) disease severity was higher with chlamydo spore inoculum.

Chlamydo spores require a ready source of energy for germination and host penetration and stimulus for germination of the chlamydo spores of soil borne *Fusarium* spp. is provided by the nutrients in the root exudates of crops growing in the vicinity (Schroth *et al.*, 1963; Chakraborty *et al.*, 1992). Fairly good germination of the chlamydo spores of *F. udum* was observed in the rhizosphere of a susceptible pigeonpea cultivar and it was higher than those of conidia (Table 3). Similar results was observed in case of population of *F. udum* in the rhizosphere of inoculated soil (Table 4).

Thus, although the chlamydo spores are considered primarily as dormant structures for survival in absence of the host in soil, in presence of a suitable host, these rapidly germinate and have more inoculum potential than the conidia due to the presence of stored nutrients. Later on, however, in presence of continuous supply of nutrients from root exudates the fungus actively grows in soil, produces abundant conidia which become the main infective structure.

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