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STUDIES ON THE TAXONOMIC POSITION AND NOMENCLATURE OF PIGEON PEA *(CAJANUS CAJAN* (L.) MILLSP.) WILT *FUSARIUM*

BY

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The taxonomic position and nomenclature of pigeon pea wilt *Fusarium* have been studied on the basis of its cultural and morphological relationships with other two *Fusaria* of the section Elegans, namely *F.oxysporum* f sp. *vasinfectum* (incitant of cotton wilt) and *F. oxysporum* f. sp. *ciceri* (incitant of gram wilt). In their cultural characters the three wilt *Fusaria* did not show any marked difference except that pinnotes and sporodochia were produced by pigeon pea and cotton wilt *Fusaria* only. The conidia of pigeon pea and cotton wilt *Fusaria* did not show much difference in their size. In shape also the conidia of both the *Fusaria* showed some similarities, and in both of them the conidia were kidney shaped (microconidia) to falcate (macroconidia). The conidia of pigeon pea wilt *Fusarium* were, however, markedly curved at one or both ends to a somewhat apical cell. The conidia of gram wilt *Fusarium* were little larger in size. Chalmydospores of pigeon pea wilt *Fusarium* were mostly terminal, rarely intercalary, whereas in cotton and gram wilt *Fusaria* they were both terminal and intercalary. Cross inoculation studies, however, showed that the three wilt *Fusaria* were highly host specific. Earlier works by the present authors, as already mentioned in this paper, on the serological and electrophoretical patterns of buffer-soluble proteins have shown that the pigeon pea and cotton wilt pathogens are very closely related serologically. Thus from morphological as well as serological studies placing of pigeon pea and cotton wilt *Fusaria* in seperate species does not seen to be very justified and they should be included in one species, F. *oxysporum*. Therefore, from the evidences as produced in the present study the naming of pigeon pea wilt fungus as *Fusarium oxysporum* f. sp. *udum* seems to be highly justified.

INTRODUCTION

The foundation for all subsequent studies on *Fusarium* was laid by Appel and Wollenweber (1910) which was later modified by Wollenweber and Reinking (1935). The main demerit of this system was the creation of species within the range of normal cultural variants. Pointing out the wide range of cultural

variations in the genus *Fusarium*, Synder and Hansen (1940, 1941, 1945) proposed a new species concept for this genus. Since then various modifications of these systems and newer systems of classifications of the genus *Fusarium* have been proposed by various workers. But Snyder and Hansen's (1940) treatment of Wollenweber and Reinking's section Elegans, all members of which were included in one species *F. oxysporum*, have been accepted by most of the workers except for a few pathogens such as the pigeon pea wilt *Fusarium*.

Wilt of pigeon pea was first reported by Butler (1910) who described the causal fungus as *F. udum*. Later Butler (1926) came to the conclusion that *F. udum* could not be distinguished from *F. vasinfectum*, the cotton wilt pathogen. Wollenweber and Reinking (1935) listed *F. udum* and *F. vasinfectum* separately within the section Elegans. Snyder and Hansen (1940) renamed these two fungi respectiveiy as *F. oxysporum* f. sp. *udum* and *F. oxysporum* f. sp. *vasinfectum*. Due to certain specific cultural and morphological characters inclusion of the pigeon pea wilt pathogen in *F. oxysporum* have been objected by several workers (Gordon, 1954 ; Booth, 1971 : Subramanian, 1971).

The taxonomic treatment of a species of the genus *Fusarium* should be based on its relation to allied members of the genus. Studies were, therefore, undertaken on the cultural and morphological reiationships of pigeon pea wilt *Fusarium* with other two *Fusaria* of the section Elegans, namely *F. oxysporum* f. sp. *vasinfectum* (the incitant of cotton wilt) and *F. oxysporum* f. sp. *ciceri* (the incitant of gram wilt), the results of which are presented and discussed in this paper.

MATERIALS AND METHODS

The fungal cultures were grown in sucrose casamino acid medium (sucrose, 15 g : KH_2PO_4 1 g : $MgSO_4$ 0.5 g ; hydrolysed casein, 4.6 g ; agar, 20 g ; distilled water 1000 ml) in 10 cm petriplates at 27±1°C for 10 days after which their cultural and morphological characters were studied, For measuring the conidia and chlamydospores the fungal cultures were mounted in lactophenol - cotton blue in glass slides and measurements of chlamydospores were taken under a microscope by means of micrometer.

For inoculation studies susceptible varieties of pigeon pea *(Cajanus cajan* var. E. B. - 3), cotton (*Gossypium herbaceum* var ALF. 1027) and gram (*Cicer arietinum* var. S - 26) were used. Cross inoculation of the three hosts with the different fungal cultures wese made in 10 cm long reagent bottles containing 10 ml of sterile nutrient solutions. For this purpose 8 discs of 5 mm diameter fungal growths were cut at random from different places of 10 days old fungal cultures at 27+1°C on petriplates and added to the reagent bottles. The bottles were

shaken for 15 minutes in a shaker for thorough mixing of the spores. To this 50 ml of the nutrient solution was further added to make the total volume 50 ml. 10 doys old seedlings of the hosts, grown in sterilized sand, were carefully removed and a single seedling was transferred to each af the reagent bottles containing the inoculum in sterile nutrient solution and held in position by loosely plugging the reagent bottles with nonabsorbant cotton. After 5 days the seedlings were taken out and transferred to another set of reagent bottles containing 60 ml of sterile nutrient solution. The nutrient solution was replaced every three days with fresh solution. The bottles were kept in room temperature. For each treatment 12 replications were kept and a set of uninoculated seedlings were kept as control. Disease symptoms started appearing 6 days onwards. The seedlings were observed regularly and the final data were recorded after 20 days. Each seedling was assessed by using different disease indices ranging from 0 (no-symptoms) to 4 (complete wilting) and average disease index for each treatment was calculated.

RESULTS AND DISCUSSION

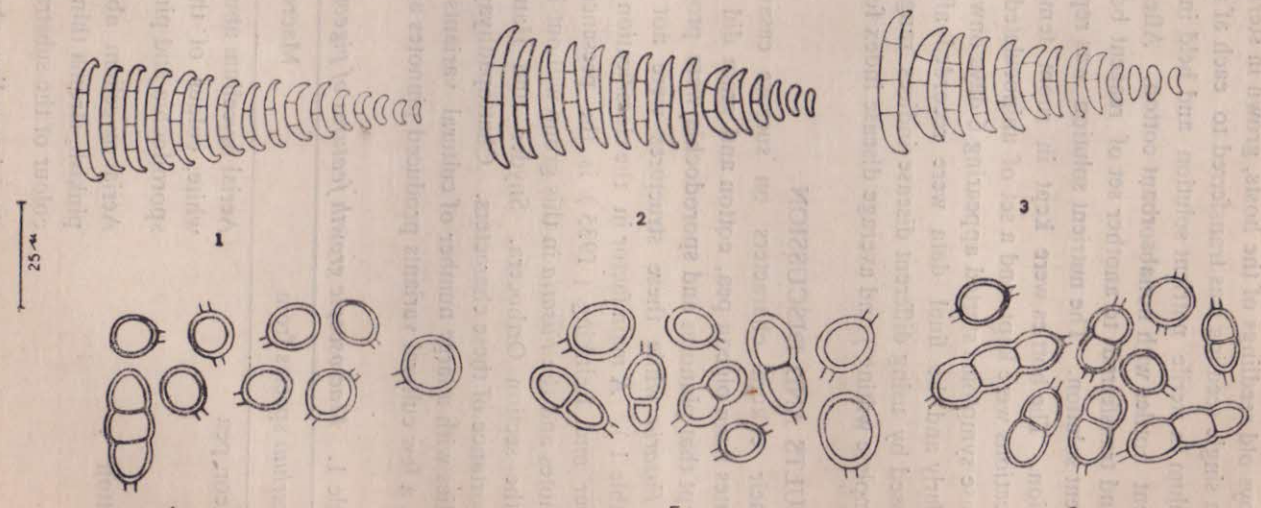
In their cultural characters on sucrose casamino acid medium the *Fusarium* isolates from pigeon pea, eotton and gram did not show any marked difference except that pinnotes and sporodochia were produced by pigeon pea and cotton wilt *Fusaria* while these structures were not produced by gram wilt *Fusarium* (Table 1). A main factor in the classification of the section Elegans by Wollenweber and Reinking (1935) is the presence or absence of sporodochia and pinnotes and the *Fusaria* in this group without having these structures were placed in the section Orthocera. Snyder and Hansen I940) did not accord any importance of these characters. Chattopadhyay and Sen Gupta (1967) in their sludies with a large number of cultural variants of gram wilt *Fusarium* observed that a few cultural variants produced pinnotes and sporodochia.

Table 1. *Macroscopic growth features of pigeon pea, cotton and gram wilt* Fusarium

Fusarium species from	Macroscopic growth feature
Pigeon pea	Aerial mycelium abundant, wooly, stroma thin, mycelium white, colour of the substratum lemon-yellow (4/3), sporodochia and pinnotes present.
Cotton	Aerial mycelium abundant, wooly, mycelium white to pinkish, stroma thin, pinnotes and sporodochia present, colour of the substratum pansy-violet (033).
Gram	Aerial mycelium abundant, white, cottony, stroma thin, pinnotes and sporodochia absent, substratum colour Indian-yellow (6/3).

Table 2. *Comparative differences in size and septation of conidia and chlamydospores of pigeon pea, cotton and gram wilt* Fusarium

Fusarium species from	Conidia							Chlamydospores						
	Range of septation	Range of length (in μ)	Average length (in μ)	Length of average conidia (in μ)	Range of breadth (in μ)	Average breadth (in μ)	Breadth of average conidia (in μ)	Range of septation	Range of length (in μ)	Average length (in μ)	Length of average chlymydospores (in μ)	Range of breadth (in μ)	Average breadth (in μ)	Breadth average chlamydospores (in μ)
Pigeon pea	0-3	5.2-32.5	19 2	30.0	1.5-3.0	2.4	3.0	0	4.5-12 5	9.0	8.5	4.5-12.5	9.0	8.5
Cotton	0-3	6.2-30.0	18.3	28.5	2.0-3.5	2.6	3.25	0-1	7.5-17 5	10.0	9.0	5.0-12.5	9.5	8.5
Gram	0-3	8.7-40.0	23.4	35.0	2.5-5 0	3.9	4.5	0-1	10.0-20.0	15.0	12 5	7.5-16.0	12.0	11.0



Figs. 1 to 6. Conidia (Figs. 1 to 3) and chlamydospores (Figs. 4 to 6) of F. *oxysporum* fs. sp. *udum*, *vasinfectum* and *ciceri* respectively.

The size (Table 2) of conidia and chlamydospores of pigeon pea and cotton wilt *Fusaria* did not show much difference (Figs. 1, 2, 4, and 5). In shape (Table 3) also the conidia and chlamydospores of both the fungal isolates showed some similarities, and in both of them the conidia were kidney shaped (microconidia) to falcate (macroconidia). The conidia of pigeon pea wilt *Fusarium* were, however, markedly curved at one or both ends to a somewhat apical cell. The conidia of gram wilt *Fusarium* were, however, little larger in size (Fig. 3). On the other hand, the chlamydospores of pigeon pea wilt *Fusarium* were mostly terminal, rarely intercalary, whereas in cotton and gram wilt *Fusaria* they were terminal and intercalary (Figs. 4, 5 and 6).

Table 3. *Shape and colour of conidia and chlamydospores of pigean pea, cotton and gram wilt* Fusaria

F. oxysporum f. sp.	Conidia	Chlamydospores
udum	Kidney shaped (macroconidia) to falcate (microconidia) with curvature long and narrow, typically thin walled, markedly curved at one or both ends to a somewhat hooked apical cells colour hyaline.	Round, rarely intercalary, mostly terminal, thick walled, light yellowish in colour, and occurred singly or in chains.
vasinfectum	Microconidia kidney shaped, macroconidia fusiform to falcate, slightly curved at one or both ends with a narrowly or smoothly rounded tip ; thin walled and hyaline.	Round to spherical, both terminal and intercalary, thick walled, light yellowish in colour, occurred singly or in chains, single celled or 1-septate.
ciceri	Microconidia oval to oblong or kidney shaped ; macroconidia ovoid to spindle shaped, slightly curved to curved or almost cylindrical at the middle, sometimes curved at one or both ends with a narrowly or smoothly rounded tip ; thin walled and hyaline.	Round to spherical, both terminal and intercalary, slightly warty, thick walled (thicker than the other two f.sp.), light brown, occurred singly or in chains and single celled or 1-septate.

The characteristic features of Butler's (1910) *F. udum* were production of salmon-yellow coloured pinnotes, hooked macroconidia, and terminal and intercalary chlamydospores. Padwick (1940) pointed out that the pigeon pea wilt

Fusarium combines the characters of both the section Lateritium and Elegans. The hooked conidia and pigmentation resemble more closely to the section Lateritium where Gordon (1952) has placed it and named it *F. lateritium* f. sp. *cajani*. Booth (1971) also included the fungns in the sectian Lateritium, but due to some characteristic features like production of intercalary chlamydospores in the mycelium and abundant microconidia preferred to treat it as a separate species, *F. udum*. But the characters like abundant production of microconidia, formation of both terminal and intercalary chlamydospores and the ability to cause vascular wilt by the pigeon pea wilt fungus are typical of the section Elegans where Padwick (1940) and Subramanian (1971) have placed it. Due to formation of hooked macroconidia Subramanian (1971), however, treated the fungus as a separate species *F. udum* and did not support its merger with *F. oxysporum* as proposed by Snyder and Hansen (1940).

Predominance of hooked conidia in the pigeon pea wilt fungus was also observed in the present studies. Except this character the conidial morphology of this fungus was, however, very similar to *F oxysporum* f. sp. *uasinfectum*. It may be mentioned here that at least a few isolates of pigeon pea wilt fungus studied by Padwick (1940) did not produce hooked macroconidia. On the other hand, Chattopadhyay and Sen Gupta (1967) observed that in some variants of gram wilt pathogen, *F. oxysporum* f. sp. *ciceri*, there was a tendency to produce hooked conidia. Thus overemphasis should not be given on this character in creation of a species in the genus *Fusarium*.

Earlier works by the present authors (Kaiser and Sen Gupta, 1976) on the serological and electrophoretical studies of the buffer soluble proteins of the three fungal isolates have shown that serologically all of them have some relations and the pigeon pea and cotton wilt *Fusaria* are very closely related. Thus from morphological as well as serological studies placing of pigeon pea and cotton wilt *Fusaria* in separate species does not seem to be very justified. Cross inoculation studies (Table 4), however, showed the three wilt *Fusaria* under study to be

Table 4. *Cross inoculation in pigeon pea, cotton and gram*

Inoculated with *Fusarium* species from	Pigeon pea		Cotton		Gram	
	Number of seedlings killed	Disease index	Number of seedlings killed	Disease index	Number seedlings killed	Disease index
Pigeon pea	12	4.00	0	0.46	0	0.38
Cotton	0	0.80	10	3.67	0	0.34
Gram	0	0.60	0	0.46	8	3.84
Control (uninoculated)	0	0.00	0	0.00	0	0.00

highly host specific. Therefore, from the evidences as produced here and also from the preceeding discussions it may be concluded that naming of the pigeon pea wilt fungus as *Fusarium oxysporum* f. sp. *udum* according to Snyder and Hansen (1940) species concept seems to be highly justified.

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