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STUDIES ON MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIABILITY AMONG THE ISOLATES OF *FUSARIUM* SPP. INCITING COWPEA WILT IN KERALA

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ABSTRACT:

Twelve isolates of *Fusarium* spp., incitant of cowpea wilt were studied for their morphological, cultural and pathogenic variability. Among the isolates, F7, F8, F10 and F11 were non – pathogenic whereas all others were wilt producing type. The growth rate of the isolates varied from 0.86-1.42 cm/day. Macro conidia produced were falcate, fusoid to sub cylindrical, mostly 3-5 septate and the size ranged from 18.8-7.9 x 3.3-1.8 µm in size. Microconidia were oval to ellipsoidal, cylindrical, straight or curved. Intercalary and terminal chlamydospores were observed among the isolates.

KEY WORDS: *Fusarium* spp., Variability, Pathogenic, Morphological, Cultural, Cowpea.

INTRODUCTION:

Cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) is an important legume crop of the tropics used as vegetable and grain as well as for fodder purposes. It is an inexpensive source of vegetable protein, and a hardy crop well adapted to relatively dry environments. Due to favorable agro climatic conditions, the crop has gained much importance in Kerala and has come to occupy a prime position among the vegetable crops raised in the State. Several diseases have been noticed in this vegetable crop, leading to economic losses and among which *Fusarium* wilt causes major concern.

Fusarium wilt of cowpea caused by *Fusarium* spp. is noticed as yellowing, withering and drooping of leaves, blackening and drying of veins and

abnormal flattening of the stem along the growing tip in the affected plants. The base of the stem swells up to the size of a small cassava tuber. The taproot and lateral roots are affected. The basal swollen portions becomes disintegrated and shredded. Occasionally, the flowers produced become reduced in size and sterile resulting in severe yield reduction (Gokulapalan *et al.*, 2006). In nature, plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence. To understand the present plant disease situations and to predict the possible future development it is essential to learn as much as possible about the variability in fungi that are pathogenic to plants (Chandran and Kumar, 2012). Keeping in view, the importance of this crop and the severity of the disease, present investigation was taken up to study the variability in cultural and morphological characters among the different isolates of *Fusarium* spp. inciting wilt in cowpea.

MATERIAL AND METHODS:

A survey was conducted in major cowpea growing areas of Thiruvananthapuram district of Kerala and diseased samples were collected randomly from farmer's fields. Isolation of the pathogen was attempted from the roots of infected cowpea plants by tissue segment method (Rangaswami and Mahadevan, 1999) on potato dextrose agar medium (PDA, Hi-Media, India) and incubated at $28 \pm 1^\circ\text{C}$ for 7 days. Axenic culture of the different isolates of the pathogen was obtained by single hyphal tip method and maintained on PDA throughout the present investigation. For testing the pathogenicity of the isolates, healthy cowpea seedlings were planted in pots filled with sterile soil, artificially infected by pouring 50 ml conidial suspension of each isolate into each pot. Three replications were maintained. The isolates capable of producing wilt and the number of days taken for the initiation of symptoms were recorded. The morphological and cultural characters of different isolates were studied by growing them on PDA and compared with those mentioned by Booth (1971). Micro morphological characters of conidia and conidiophores were studied by following slide culture technique as described by Riddel (1950). Cultural characters such as the growth of mycelium, colour / pigmentation of mycelium and sporulation were recorded. The mode of chlamydospore production *viz.*, solitary, pairs, chains and location were also observed.

RESULTS AND DISCUSSION:

A total of 12 isolates of *Fusarium* spp. were obtained from the infected plants collected from the major cowpea growing areas of Thiruvananthapuram district of Kerala and designated as F1 to F12. They were tentatively identified into four species based on the morphological and cultural characters as, *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium solani* and *Fusarium proliferatum* as shown in Table. 1. Among the 12 species, *F. oxysporum* was the prevalent species and widely distributed as it was found to be present in six locations examined. On the contrary, three species namely, *F. equiseti*, *F. solani* and

F. proliferatum were noted as less frequent species with three, two and one isolate each. The pathogenicity studies using the *Fusarium* isolates showed that all isolates except F7, F8, F10 and F11 produced symptoms of wilt on artificial inoculation. The isolate F2 from Palapoor took only seven days for the development of disease symptoms and was found to be highly pathogenic followed by the isolates F3 and F5 (Table 1).

The isolates F2, F3, F4, F6, F8 and F9 had medium growth rate of 0.98 – 1.42 cm/day. Colonies were appressed to floccose in texture, white on the upper surface, reddish brown or faint pink on the lower side of the petridish. Conidiophores consisted of single phialides, arising laterally on the hyphae or short branched conidiophores. Single, subcylindrical to slightly obclavate phialides arose from primary or secondary conidiophores. Macroconidia were 3-5 septate, 18.8-11.6 x 3.0-1.9 µm in size, thin walled, fusoid, pointed ends, occasionally falcate with terminal cell, hooked and pedicellate basal cell. Microconidia were abundant, oval to ellipsoidal, cylindrical, straight or curved. Chlamydospores when present were terminal in position.

The isolates F7, F11 and F12 had medium growth rate of 0.86 -1.05 cm/day. Colonies appeared floccose in texture, dull white on the upper surface and yellowish to dark brown on the lower side of the petridish. Conidiophores consisted of single lateral phialides in aerial mycelium. Phialides were short, compact, obclavate to doliiform. Macroconidia were mostly 3-5 septate, 13.6-11.2 x 2.9-2.1 µm in size, thick walled, typically falcate, tapering towards both ends, bent at central part with elongated apical cell and pedicellate basal cell. Microconidia were oval to ellipsoidal in shape. Chlamydospores when present were intercalary, solitary or in chains.

The isolates F5 and F10 had a fast growth rate of 1.3-1.04 cm/day. Colonies appeared sparse with creamy white upper surface and dirty white on the lower side of the petridish. The primary conidiophores arose laterally from hyphae on aerial mycelium which was unbranched or sparsely branched. Short, subcylindrical, obclavate monophialides produced slender macroconidia and long microconidia. Macroconidia were 3-5 septate, thick walled, subcylindrical, slightly curved, short and bent apical, indistinctly pedicellate basal cell and measured 13.8-7.9 x 3.0-1.8 µm in size. Microconidia were ovoid and straight or rarely ellipsoidal to curved. Chlamydospores were abundant, terminal or intercalary in position formed in single, pairs or chains.

The isolate F1 had a slow growth rate of 0.9 cm/day. Colonies appeared sparse dull white on the upper surface and dark brown on the lower side of the petridish. The primary conidiophores consisted of monophialides as well as polyphialides. Macroconidia were 1-5 septate, thin walled, with curved apical cell and little developed basal cell measuring 12.4 x 2.5 µm in size. Microconidia were rod shaped, single celled resembling a pear.

Variation in *Fusarium* isolates associated with legumes in different parts of the country has been reported by other workers as well; (Honnareddy and Dubey, 2007; Mandhare *et al.*, 2007; Lezcano *et al.*, 2012).

Hence, the present investigation clearly indicates that variations did exist in isolates of *Fusarium* spp. inciting cowpea wilt in Kerala.

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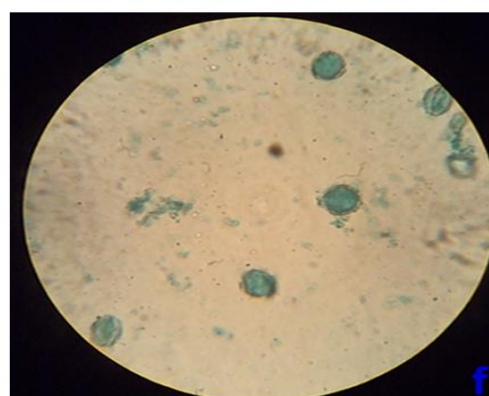
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Table. 1. Pathogenic and colony characters of isolates of *Fusarium* spp. from cowpea

Sl. No.	Isolate	Location	Fungi identified	Colony characters			Pigmentation	Pathogenicity	
1	F1	Vellayani	<i>F. proliferatum</i>	White sparse mycelial growth			Dark brown	Wilt type	producing
2	F2	Palapoor	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Wilt type	producing
3	F3	Pappanchani	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Wilt type	producing
4	F4	Pappanchani	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Wilt type	producing
5	F5	Venniyoor	<i>F. solani</i>	Sparse creamy white			Dirty white	Wilt type	producing
6	F6	Balaramapuram	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Wilt type	producing
7	F7	Muttacaud	<i>F. equiseti</i>	Dull white floccose			Yellowish-dark brown	Non -pathogenic	
8	F8	Thazhava	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Non -pathogenic	
9	F9	Pothencode	<i>F. oxysporum</i>	White	dense	floccose	Reddish brown/ Faint pink	Wilt type	producing
10	F10	Kattakada	<i>F. solani</i>	Sparse creamy white			Dirty white	Non -pathogenic	
11	F11	Kazhakkuttom	<i>F. equiseti</i>	Dull white floccose			Yellowish-dark brown	Non -pathogenic	
12	F12	Pakaloor	<i>F. equiseti</i>	Dull white floccose			Yellowish-dark brown	Wilt type	producing

Table.2. Morphological characters of macroconidia and microconidia of different isolates of *Fusarium* spp. from cowpea

Sl.No.	Isolate	Macroconidia			Shape of microconidia
		Size (μm) L X B	Septation	Shape	
1	F1	12.4 x 2.5	1-5	Sub cylindrical	Ellipsoid
2	F2	15.6 x 2.7	3-5	Fusoid	Oval to ellipsoid
3	F3	15.6 x 2.7	3-5	Fusoid	Oval to ellipsoid
4	F4	11.6 x 3.3	3-5	Fusoid	Oval
5	F5	13.8 x 3.0	3-5	Sub cylindrical	Oval
6	F6	18.8 x 2.7	3-5	Fusoid	Oval
7	F7	13.6 x 2.9	3-5	Falcate	Ellipsoid
8	F8	12.6 x 3.0	3-5	Fusoid	Oval to ellipsoid
9	F9	16.6 x 1.9	3-5	Fusoid	Oval to ellipsoid
10	F10	7.9 x 1.8	3-5	Sub cylindrical	Oval
11	F11	11.2 x 2.1	3-5	Falcate	Ellipsoid
12	F12	12.3 x 2.1	3-5	Falcate	Ellipsoid



**a) Flowering Twig, b) Fruit, c) Seed, d) Anther, e) Control
f) Stained Pollen, g) Control, h) Stained Stigma**