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Cultural, Morphological and Pathogenic Variability among the Isolates of *Fusarium solani* causing Wilt Disease of Chilli (*Capsicum annuum* L.)

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Variability among 44 isolates of *Fusarium* spp. the Chilli wilt pathogen, collected from different locations of south India was studied in respect of Cultural and morphological and pathogenic variability. The colony diameter ranged from 60 mm to 90 mm after 8 days of inoculation incubated at 27±1°C. The colony colour varied from white, cream and violet with yellow coloured pigmentation on Potato dextrose agar. The growth on different solid media revealed that the best media was Potato dextrose agar (90 mm) with abundant sporulation followed by oat meal agar and V-8 juice agar. The colour of the colony also varied from white, cottony, cream and greyish in different media. As for the pathogenic variability is concerned the isolates showed variations in causing disease in different varieties of chilli and these isolates were clustered into three groups based the disease reaction.

Keywords: Cultural, morphological, pathogenic variability, chilli wilt, *Fusarium* spp.

Chilli (*Capsicum annuum* L.) is one of the most important commercial spice and vegetable crop of India. Chilli is used for different purposes such as vegetable, spice, condiments, sauce and pickles. Chilli is a tropical and subtropical crop, is one of the major vegetable and spice crops grown in the country and is popularly known as *wonder spice***.**

The chilli area, production, productivity is in decreasing trend even though it is a highly profitable commercial spice and vegetable crop.

Many factors operate in successful cultivation, production, marketing and exporting of the quality chilli, of which diseases play an important role.

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Vascular wilt of chilli caused by many species of *Fusarium* especially *Fusarium solani* and *Fusarium oxysporum* is the most important disease. The pathogen can survive on infected plant debris and Soil for many years causing severe damage to the yield. Fusarial wilt has become a serious problem in recent years in almost all chilli growing tracts of India. Especially in black cotton soils leading upto 20% yield loss (Devika Rani, 2007).

Use of resistant varieties cultivars is the most practical and economical method of disease management practice. But the pathogen is highly variable and poses a major obstacle to resistant breeding, hence present investigation was undertaken to know the cultural, morphological and pathogenic variability among the isolates of *Fusarium* collected from major growing areas of south India.

MATERIALS AND METHODS

Survey and collection of isolates

An intensive roving survey for disease incidence was conducted in major chilli growing areas of Karnataka, Andhra Pradesh and Maharashtra during *kharif* 2011-12 to 2013-14. Chilli plant samples showing typical wilting symptoms along with rhizosphere soil were collected during survey was subjected for the isolation. A total of 45 isolates were collected from all the surveyed areas and isolation of the pathogen was done by standard tissue isolation method on PDA and single spore isolation was made thus obtained pure culture was used for further variability studies.

Cultural and Morphological variability among the isolates of *Fusarium* spp

A total of forty four single spore isolates established and maintained on PDA slants were used for the study of various morphological characters by growing these isolates on Potato dextrose agar media. Kurgod isolate (*Fs* -4) was further studied on various solid media. Seven day old culture (5mm disc) of each isolate was inoculated separately and incubated at 27±1°C. In the study of different solid media, after the growth reached 90 cm in any of the solid media, the observations like fungal radial growth, colony characters like coloure, pigmentation, texture, and appearance of colony, sporulation and number of macroconidia, microconidia, and chlamydospores along with number of septa were recorded. The same characters were also recorded for various isolates isolated on Potato dextrose agar media. The isolates were grown in Potato dextrose broth and incubated for 14 days at 27±1°C and dry mycelial weight was recorded.

Pathogenic variability among the isolates of *Fusarium* spp.

The Pathogenicity tests of selected 36 isolates of *Fusarium* spp. was conducted on three popular chilli varieties *viz.* Sitara, Byadgi kaddi and Byadgi dabbi under artificially inoculated condition under glasshouse condition. The isolates were multiplied in using sand corn media. The twenty day old giant culture was used to inoculate into sterilized soil at @10w/w. the surface sterilized seeds of three varieties of chilli were sown @20 seeds/pot with two replications. The pots were maintained with optimum moisture at field capacity by watering regularly. The final observations like germination percentage, Pre-emergent and post emergent seedling death and percent disease incidence was calculated using the formula

$$\text{Pre emergence seedling death} = \frac{\text{Number of seedlings emerged in healthy soil} - \text{Number of seedlings emerged in sick soil}}{\text{Total number of seedlings emerged in healthy soil}} X100$$

$$\text{Post emergence seedling death} = \frac{\text{Numberof seedlings wilted}}{\text{Total number of seedlings emerged}} X100$$

Based on these observations the isolates were categorized on the basis of death of seedlings into three groups as, slightly pathogenic (1), moderately pathogenic (2) and, highly pathogenic (3).

RESULTS AND DISCUSSION

Morphological and Cultural variability among the isolates of *Fusarium* spp

On PDA medium the maximum colony diameter (90.00 mm) was observed in isolates such as *Fs*1,*Fs*3,*Fs*11,*Fs*13,*Fs28*,*Fs29*,*Fs34*,*Fs35*,*Fs38* and *Fs43*. followed by *Fs20*,*Fs21*,*Fs44* and *Fs45* with 88 mm of colony diameter and the least growth (60 mm , 62.5 mm and 65 mm) was recorded in *Fs12*,*Fs2* and *Fs*14 and they were statistically on par with each other. Based on colony diameter the isolates were grouped, the isolates *Fs*1,*Fs*3,*Fs4*, *Fs10*,*Fs*11,*Fs*13,*Fs25*,*Fs28*,*Fs29*,*Fs34*,*Fs35*, *Fs38*,*Fs20*,*Fs21*,*Fs44* and *Fs43* form one cluster and isolates *Fs7*,*Fs9*,*Fs22* and *Fs40* formed another cluster whereas the remaining isolates formed a separate cluster. Majority of the isolates produced white mycelia, regular margin, smooth texture with flat growth with light brown pigmentation, while some of the isolates formed creamy, violet mycelia, irregular to regular margin with fluffy growth with light or dark yellow pigmentation.

The observation on the production of sporulation was recorded. The results revealed that abundant sporulation (>200 spores per microscopic field) was observed in isolates like *Fs2*, *Fs4*, *Fs6*, *Fs7*, *Fs15*, *Fs29*, *Fs30*, *Fs38*, *Fs39*. The other isolates were grouped were also grouped as categories like good sporulation (100-200 spores per microscopic field) and moderate sporulation (0-100 spores per microscopic field).

Observations on the growth of pathogen on different solid media revealed that the maximum growth was observed in case of potato dextrose agar with 90 mm growth followed by oat meal agar (69.66 mm) and V-8 juice agar which are statistically on par with each other. The least mycelial growth was observed in case of corn meal agar. Jhamaria (1972) reported that, potato dextrose agar,

Table 1. List of isolates of *Fusarium* spp collected from different locations of South India

S.No	Location	State	Name of the Isolate
1	Emmiganore	Andhra Pradesh	*Fs1*
2	Sangolala	Maharashtra	*Fs2*
3	Garag	Karnataka	*Fs3*
4	Kurgod	Karnataka	*Fs4*
5	Basavarajappa nagar	Karnataka	*Fs5*
6	Birur	Karnataka	*Fs6*
7	Saidapur	Karnataka	*Fs7*
8	Bylahongal	Karnataka	*Fs8*
9	Ananthapur	Andhra Pradesh	*Fs9*
10	Basavapura	Karnataka	*Fs10*
11	Madire	Andhra Pradesh	*Fs11*
12	Sindnur	Karnataka	*Fs12*
13	Kumbapur farm	Karnataka	*Fs13*
14	Annigeri	Karnataka	*Fs14*
15	Haveri	Karnataka	*Fs15*
16	Chukkanakal	Karnataka	*Fs16*
17	Emmiganore-2	Andhra Pradesh	*Fs17*
18	Nayanagar	Karnataka	*Fs18*
19	Joida	Karnataka	*Fs19*
20	Pandrapura	Maharashtra	*Fs20*
21	Dharwad	Karnataka	*Fs21*
22	Davanagere	Karnataka	*Fs22*
23	Guntur-1	Andhra Pradesh	*Fs23*
24	Guntur-2	Andhra Pradesh	*Fs24*
25	Jagalore	Karnataka	*Fs25*
26	Shakthi nagar	Karnataka	*Fs26*
27	Emmigana hatti	Karnataka	*Fs27*
28	Madire	Karnataka	*Fs28*
29	Byadgi	Karnataka	*Fs29*
30	Adoni	Andhra Pradesh	*Fs30*
31	Kampli	Karnataka	*Fs31*
32	Kalghatgi	Karnataka	*Fs32*
33	Kaladgi	Karnataka	*Fs33*
34	Hebballi	Karnataka	*Fs34*
35	UAD(D) Hort plot	Karnataka	*Fs35*
36	Byalalu	Karnataka	*Fs36*
37	Navalagund	Karnataka	*Fs37*
38	Budugumpa	Karnataka	*Fs38*
39	Nelahal	Karnataka	*Fs39*
40	Shanavasapura	Karnataka	*Fs40*
41	Manvi	Karnataka	*Fs41*
42	Mandapura	Karnataka	*Fs42*
43	Somasamudra	Karnataka	*Fs43*
44	Manthralaya	Andhra Pradesh	*Fs44*

Richards's agar and Czapek's agar provided maximum growth and sporulation of *F. oxysporum.* f .sp. *niveum*. Patel (1991) reported considerable variation among 13 isolates of *F. solani*. Sharma and Agnihotri (1972) recorded morphological and pathogenic variation among three isolates of *Fusarium orthocerus* App. & Wr.

Krishnarao and Krishnappa (1997) reported that *Fusarium* spp. isolated from wilted chickpea plants collected from different locations of Karnataka differed in growth pattern, pigmentation, sporulation and pathogenicity.

As for the sporulation is concerned, the abundant sporulation was recorded in Potato dextrose agar (>250 spores) and the least sporulation in case of Asthana hawker's medium. In all the cases we observed 1-3 septa of macroconidia and the chlamydospores were produced both solitary and in Chains of 1-4 cells produced at intercalary or at terminal end of hyphe. Nirmala Devi and Srinivas (2012) studied the Cultural variability among 112 isolates of *Fusarium oxysporum* f sp *lycopersici* causing wilt of tomato. The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet. The isolates were categorized into two groups i.e., fluffy growth and adherent smooth growth. Most of the isolates showed fluffy growth while other isolates revealed adherent growth on the medium. The septation of the macroconidia was 3 to 5 and the microconidia were usually aseptate or single septate. The chlamydospores were present at the terminal or intercalary positions, usually single or in pairs. Sporulation of the macroconidia, microconidia and the chlamydospores varied highly among the iaolates. The isolates thus exhibited a high level of diversity in terms of culture and morphology.

The observations on dry mycelial weight of different isolates revealed that the maximum dry mycelial weight was recorded in Kurgod isolate (*Fs*-4) with 410 mg of dry mycelial weight followed by *Fs*-14(Kumbapur farm) isolate with 400 mg of dry mycelia weight and the least dry mycelia weight (160 mg) recorded in Hebballi farm isolate (*Fs*-34).

Pathogenic variability

Observations on Pathogenicity of *Fusarium* spp on three different varieties of chilli revealed considerable variations. The pathogen caused the pre-emergent and post-emergent seedling death due to artificial inoculation of different isolates of the pathogen. The percent disease incidence was ranged between 50-100 percent on different varieties of chilli. The isolates were further groped into three categories *viz*, highly pathogenic, moderately pathogenic and slightly pathogenic based on their reaction in causing the disease.

On sitara variety, the highly pathogenic isolates such as *Fs1, Fs3, Fs4, Fs6, F8,Fs10,Fs11, Fs13,Fs17, Fs20, Fs22, Fs23,Fs24, Fs26,Fs27, Fs30, Fs32, Fs38,Fs39,Fs40,Fs43* formed one cluster with 91 to 100 percent disease incidence. moderately pathogenic isolates *Fs5,Fs7,Fs9, Fs12, Fs16, Fs18, Fs19, Fs25,Fs28,Fs29,Fs41* formed second cluster with 81-90 percent disease incidence, slightly pathogenic isolates *Fs2,Fs14,Fs15,Fs35* Formed separate cluster with less than 80 percent disease incidence. similar results were obtained in case of variety Byadgi Dabbi. Where as in case of Variety Byadgi Kaddi, The highly pathogenic isolates such as *Fs1, Fs3, Fs6, F8, Fs10, Fs11, Fs13, Fs15, Fs17, Fs20, Fs22, Fs23, Fs24, Fs26,Fs30,Fs32,Fs39,Fs41* formed one cluster, moderately pathogenic isolates *Fs4, Fs5,Fs7,Fs12, Fs14,Fs18, Fs19,Fs25, Fs28, Fs35,Fs38,Fs43* formed second cluster, slightly pathogenic isolates Fs2, Fs9,Fs16,Fs29 separate cluster.

With these results it is clear that the pathogen shows a high degree of variability in Pathogenicity which may be slight to complete death of the host plants at any stages of the crop growth. This will be helpful further in identifying the most virulent isolate of the pathogen.

The pathogen is very tricky which exhibits pathogenic variability frequently, besides infecting other hosts. While such variations are to be explored further by studying these characters at cultural, morphological, pathogenic and molecular levels to give a logical conclusion. Hence there is a need to take up variability study at the pathogen at molecular level to identify at genomic level.

Kumar *et al* (2007) observed 13.3 to 100 percent wilt when seedlings of pigeonpea (*var*.bahar) were inoculated with 104 isolates of *F.udum.* Nirmala Devi and Srinivas (2012) Observed Pathogenic variation in symptoms on aerial parts

and within the stem tissues of tomato plants infected with *F. oxysporum* f. sp. *lycopersici*. At early stage, symptoms appeared as yellowing of the lower leaves and in later stages, drooping of the leaves was observed. In severe infection, the pith of the stem was turned brown in colour. In severely infected plants lower leaves dried, ultimately the aerial parts of the tomato plant showed loss of turgidity and drooped down Pathogenicity of 114 isolates was studied on five susceptible varieties by root cut and dip inoculation method. The isolates were categorized into 4 groups viz., highly pathogenic moderately pathogenic, weakly pathogenic and non pathogenic based on the symptomatological variations in the test tomato varieties, whereas un- inoculated tomato seedlings showed no symptoms.

Variability among 15 isolates of *F.udum* revealed that the colony diameter ranged from 42.3 to 70.30 mm 8 days after inoculation, colony colour varied from white to pink and pink of the plate showed light to dark yellow pigmentation. The dry mycelial weight ranged from 98.30 to 201.30 mg. The wilt incidence ranged from 14.3 to 61.9 percent on a susceptible cultivar bahar (Kumar and Upadhyay, 2013).Fifteen isolates of *Fusarium oxysporum* f.sp.*ciceri w*ere collected and observed for morphological characters like pigmentation, size, septation of mico, macro-conidia and chlamydospores. Potato dextrose agar and Czapek's Dox agar supported maximum growth. Based on pathogenic reaction and region the isolates were grouped as different races like Race 1, Race 2, Race 4, and Race 6 (Kadam *et al*.2012).

Current study also revealed the variation in cultural morphological and pathogenic characters of *Fusarium* spp. which are in confirm with earlier workers based on cultural, morphological and pathogenic characters.

REFERENCES

1. Devika Rani, G. S., Naik, M. K., Raju, K. and Prasad, P. S., Prevalence of Wilt of Chilli and assessment of population dynamics of *Fusarium* in predominant Chilli (*Capsicum annuum* L) growing regions of Karnataka. *Soil. Biol.*, 2007; **27**(1-2): 50-61.
2. Jhamaria, S. L., Nutritional requirement of *Fusarium oxysporum* f.sp. *niveum*, *Indian Phytopath.*, 1972; **25**: 29-32.
3. Kadam, N., Mane, S. S. and Katkar, M., Pathogenicity, nutrition and host differential reaction of isolates of *Fusarium oxysporum* f.sp. *ciceri* causing chickpea wilt from Maharashtra. *J. Pl. Dis. Sci*. 2012; **7**(2): 185-189.
4. Krishnarao, V. and Krishnappa, K., Variation in the isolates of *Fusarium* spp. From chickpea in Karnataka. *J. Mycol. Pl. Pathol*, 1997; **27**: 25-28.
5. Kumar, V., Chauhan, V. B. and Srivastava, J. P., Pathogenic and biochemical variability *in F.udum*, causing pigeon pea wilt. *Indian Phytopathol*, 2007; **60**: 281-288.
6. Kumar, S. and Upadhyay, J. P., Cultural, Morphological and Pathogenic variability in isolates of *Fusarium udum* causing wilt of Pigeonpea, *J. Mycol. Pl. Pathol* 2013; **43**(1): 76-79.
7. Nirmaladevi, D. and Srinivas, C., Cultural, morphological, and pathogenicity variation in *Fusarium oxysporum* f sp. *lycopersici* causing wilt of tomato, *Batman Univ. J. Life. Sci*., 2012; **2**.
8. Patel, S. T., Studies on some aspects of wilt of chickpea. Ph.D. Thesis, *Uni Agril. Sci. Dharwad* (India), 1991.
9. Sharma, R. C. and Agnihotri, J. P., Pathogenicity and variability of lentil wilt *Fusarium*. *Indian J. Mycol. Pl. Path.*, 1972; **2**: 170.