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Morphological and cultural variability of *Fusarium* spp. causing wilt in chilli in Karnataka

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Abstract
Morphological and cultural variability was studied among the isolates of *Fusarium* spp. collected from fusarium wilt infested chilli fields of north Karnataka. The isolates cultured on potato dextrose agar medium showed considerable cultural variabilities in colony diameter, colony morphology, pigmentation and morphological variabilities in conidial size, septation and sporulation rate. Colony diameter among *F. oxysporum* isolates ranged from 63.93- 89.87 mm and produced white fluffy cottony growth with orangish pigmentation. The isolates produced macro conidia of size 11.5-24 × 2.2-5.5 µm with 3-4 septation, micro conidia of size 5-6 × 2.7-3 µm and chlamydospore of size 10.5-11.2 µm in diameter. Colony diameter among *F. solani* isolates ranged from 57.80-89.87 mm, and produced white-creamish cottony/fluffy/submersed growth with creamish, light orangish, purplish and pinkish pigmentation. The isolates produced macro-conidia of size 10-25.5×3-6.5 with 2-3 septation, micro-conidia of size 5-12.5×2.1-3.5 µm and chlamydospores of size 10-12 µm in diameter. The isolates also showed variation in sporulation rate.

Keywords: *Fusarium solani*, *Fusarium oxysporum*, chilli and variability

1. Introduction
Chilli (*Capsicum annuum* L.) belonging to family solanaceae is a globally important spice and vegetable crop. Chillies are rich source of vitamin A, vitamin C, vitamin E, folic acid, calcium, potassium and antioxidants (*i.e*., flavonoids, capsaicinoids and carotenoids). Different varieties are cultivated for varied uses like vegetable, pickles, spice and condiments. It is being used for imparting taste, flavour, and colour to food and also used in preservative, pharmaceutical, perfumery, cosmetic products and religious rituals etc. (Raghu *et al.* 2017) [15]. The major chilli growing countries are India, China, Korea, Nigeria, U.S.S.R. Mexico, *etc*. India is one of the largest producer and exporter of this crop with an area of 2.87 lakh ha and production of 34.06 lakh metric tonnes. India ranks second among world's chilli exporting countries. The leading chilli producing states in India are Andhra Pradesh (49%), Maharastra (26%), Karnataka (15%), West Bengal (12%), Tamil Nadu (3%). The Karnataka follows Andhra Pradesh, which share 15 per cent of the production in the country with an area of 0.45 lakh ha and 6.07 lakh tonnes. In Karnataka the crop is mainly cultivated in districts like Dharwad, Haveri, Koppal, Bellary, Raichur, Kalburgi and Belgavi (Anon., 2017) [2]. A number of biotic and abiotic stresses are a constraint in chilli production. The main biotic factors are diseases due to fungi, bacteria and viruses, which have drastically restricted the yield potential and quality. Among these, fusarium wilt has emerged as a serious problem in recent years. The yield loss due to the disease is known to vary from 10-50 per cent worldwide and 10-80 percent in Karnataka. (Loganathan *et al.*, 2013) [11]. In different chilli growing states of India wilt incidence varied from 2.0 to 85.0 percent in (Anon, 2005) [1]. In Karnataka major planting seasons are *kharif*: June-July and *Rabi*: October-November. Prevalence of warm temperature, high humidity with high soil moisture coinciding with active growth period of the crop favours development of fusarium wilt (Shali, 2000). Fusarium wilt symptoms are characterised by yellowing of foliage, upward and inward rolling of leaves, progressive wilting of foliage leading to a permanent wilt and death of the plant, and a reddish or brownish discolouration of the vascular tissue (MacHardy and Beckman, 1981; Rivelli, 1989) [12, 16].

In chilli, wilt appears both in seedling and at later stages of the crop, and leads to highest mortality at flowering and fruiting stages. Although the disease first appears in patches in a field, it can extend to the entire field with repeated monocropping and growing of susceptible varieties. Understanding the present context of fusarium wilt incidences in all high yielding varieties of chilli in major chilli growing districts of Karnataka the present study was carried out to know the *Fusarium* spp. responsible and variability among different isolates.

2. Materials and Methods

2.1 Collection, isolation and identification of pathogen

Fifty one isolates were collected from major chilli growing districts of Karnataka namely Bagalkot, Bellary, Belagavi, Haveri, Raichur and Tumkur for fusarium wilt incidence. The roots and basal stem portion of completely and partially wilted plants of chilli were collected from the field. The fungal culture was isolated on PDA media from collected plant sample at temperature of 27±1ºC and by using single hyphal tip technique pure culture was obtained. Totally 51 isolates were isolated and cultured on potato dextrose agar (PDA) media and maintained at 4 ºC temperature and used for further studies. The fungal isolates were identified by comparing their morpho-cultural characters like production of spores such as micro, macro and chlamydo-spores, shape, size and septation in macro-conidia and pigmentation in culture with those described by Leslie and Summerell (2006) [9].

2.2 Cultural variability

All 51 isolates of *Fusarium* were designated as FS (*Fusarium solani*)/FO (*Fusarium oxysporum*) and characterized on the basis of their cultural and morphological characters on PDA. The type of colony, colony diameter, conidial size, septation and sporulation rate were recorded at 3rd, 5th and 7th day after incubation.

2.3 Morphological variability

Fifty one isolates of *Fusarium* spp. collected from different regions were cultured on potato dextrose agar medium and taken for morphological studies. Culture was mounted on slide in lactophenol cotton blue mounting medium and observed under microscope fitted with camera (Symbiont Technologies DM-2020®) under high power magnification (40X). Observations on conidial characters like type of conidia, size, number of septation and sporulation rate were recorded. For counting sporulation rate, spores from 3 microscopic fields were counted and averaged. Based on the average number of spores per microscopic field the isolates were categorized under high sporulation, medium sporulation and less sporulation if conidia were >50, 30-50, <30 respectively.

3. Results

3.1 Isolation, purification and identification of pathogen

A total of fifty one isolates of fungus were isolated from chilli plant samples collected from different places by standard tissue isolation procedure and they were purified and maintained on PDA as described in material and methods. The fungal isolates were identified by comparing their morpho-cultural characters like production of spores such as micro, macro and chlamydo-spores, shape, size and septation in macro-conidia and pigmentation in culture with those described by Leslie and Summerell (2006) [9]. Out of 51 isolates, three isolates (FO 22, FO 26, FO 30) were identified as *F. oxysporum* and 48 isolates were identified as *F. solani*. *Fusarium oxysporum* had thin-walled, relatively slender and 3-5 septate macro-conidia, evenly curved fusoid with the widest part in the middle and pointed at both the ends. The micro-conidia were formed on smaller false heads with floccose, sparse or abundant mycelia, which ranged from white to pale violet in pigmentation. Whereas, *Fusarium solani* had thick-walled, curved, dorsoventrally straight, relatively wider, stout and robust macro-conidia with the widest diameter in the upper half of the spore. The micro-conidia were monophilidic and formed on relatively longer false heads with white to cream colour pigmentation with sparse mycelium. The chlamydospores were formed singly, in pairs, in chains or in clumps in both the species. Chlamydospores of *F. solani* were smooth textured whereas; in *F. oxysporum* they were rough textured.

3.2 Cultural characters of isolates on PDA

Three *F. oxysporum* isolates FO 22, FO 26 and FO 30 produced white fluffy cottony growth. Among 48 isolates of *F. solani* 19 isolates (FS 3 to FS 7, FS 10, FS 14, FS 20, FS 31, FS 32, FS 39, FS 40, FS 41 to FS 46) produced white cottony growth, 15 isolates (FS 1, FS 2, FS 8, FS 17, FS 21, FS 23, FS 27, FS 28, FS 29, FS 34 to FS 47 produced white fluffy cottony growth, seven isolates (FS 11 to FS 13, FS 18, FS 19, FS 24, FS 38 produced white submersed cottony growth, seven isolates (FS 9, FS 15, FS 16, FS 25, FS 33, FS 50 and FS 51 creamy white cottony growth (Table 1).

Isolates produced creamish, light orangish, orangish, pinkish and light pinkish pigmentation. *F. oxysporum* isolates FO 22 produced light pinkish, isolate FO 26 produced purplish and FO 30 produced pinkish pigmentation. Among 48 *F. solani* isolates 13 isolates (FS 1 to FS 4, FS 7, FS 8, FS 9, FS 15, FS 16, FS 21, FS 32, FS 47 FS 48 and FS 49) produced creamish pigmentation and rest of the isolates (FS 5, FS 6 FS 33 to FS 37, FS 39 to FS 46) produced orangish pigmentation (Table 1).

After seven days incubation, *F. oxysporum* isolates showed highest radial growth of 89.87 mm in isolate FO 30, 75.33 mm in isolate FO 26 and least mycelial growth of 63.93 mm in isolate FO 22 respectively. However, among the 48 isolates, 18 isolates (FS 4, FS 8, FS 17, FS 18,FS 27, FS 28, FS 33, FS 34, FS 36, FS 38 to FS 40, FS 42 to FS 44, FS 49 to FS 51) showed highest radial growth in range of 85.53- 89.93 mm, 25 isolates (FS 1 to FS 3, FS 5 to FS 7, FS 9, FS 11 to FS 13, FS 16, FS 19 to FS 21, FS 23 to FS 25, FS 31, FS 35, FS 37, FS 41, FS 45 to FS 48) showed radial growth of 73.47-79.93 mm, 5 isolates (FS 10, FS 14, FS 15, FS 29, FS 32) showed least radial growth of 57.80- 65.67 mm (Table 1).

3.3 Morphological characters of isolates on PDA

Studies on morphological variability of 51 isolates of *Fusarium* on potato dextrose agar indicated that, all the isolates produced both micro and macro-conidia and chlamydopores. However, isolates varied with rate of production of spore, size of macro and microspores. In isolates of *F. oxysporum*, the mean size of macro-conidia ranged from 13.5-24.5 × 2.25-5.5 µm and the mean size of micro-conidia ranged from 5-6×2.7-3 µm with 3 septation. In case of F. solani isolates, the mean size of the macro-conidia ranged from 8-29 × 2.5-6.5 µm and that of micro-conidia ranged from 5-18.5× 2.2-4.5 µm with 2-3 septa. The chlamydospores produced in different isolates were either

smooth and/or rough with size range of 9.5-12.5 µm in diameter. The overall sporulation rate was high in one isolate of *F. oxysporum* (FO 26) and 17 isolates of *F. solani*, whereas, ten isolates were found to have medium sporulation and 21 isolates had less sporulation.

4. Discussion

A total of fifty one isolates of fungus were isolated from wilted chilli plant samples collected from different locations of Karnataka, purified and identified based on their morpho-cultural characters. By comparing the morpho-cultural characters of 51 isolates with that described by Leslie and Summerell (2006) [9], three isolates (FO 22, FO 26 and FO 30) were identified as *F. oxysporum* and 48 isolates were identified as *F. solani*. The micro-conidia produced by the pathogens were monophilidic and these were formed on relatively long false heads in *F. solani* and on smaller false heads in *F. oxysporum* isolates. The *F. oxysporum* isolates produced thin-walled, relatively slender, indistinctly septate macro-conidia, evenly curved, fusoid with the widest part in the middle. They were thin-walled with 3-5 septa and pointed at both the ends with purple or pinkish pigmentation. Similarly, *F. solani* isolates produced thick-walled, curved, dorsoventrally straight, relatively wider, stout and robust macro-conidia, which had the widest diameter in the upper half of the conidia. Micro-conidia of *F. solani* isolates were wider, more oval in shape with thicker walls. It produced cream to orangish pigmentation. Similar descriptions were also reported by (Rozlianah and Sariah, 2006; Patil *et al*., 2014; Isaac *et al*., 2018) who differentiated and identified *F. solani* and *F. oxysporum*. The progressive and relative radial growth of the fungus recorded at 3rd, 5th and 7th day of incubation on PDA showed significant variations among isolates. Present findings are in agreement with many other reports. The colony of *F. solani* isolates were white cottony with aerial mycelia and produced purple, pink or orangish pigmentation and *F. oxysporum* isolates produced white cottony aerial mycelia and purple, pink or pale violet pigmentation (Devika Rani *et al*., 2007; Gogoi *et al*., 2017; Ferniah *et al*., 2014; Mohammed *et al*., 2016) [4, 6, 5, 13]. Kadam *et al*. (2012) [8] reported that potato dextrose agar supported maximum mycelial growth in different isolates of *Fusarium oxysporum* f.sp.*ciceri*. The differential colour of the *F. oxysporum* and *F. solani* isolates may be due to the presence of specific pigments (*viz*., javanicin, bostrycoidin, solanione and lycopersin) produced by these isolates (Booth, 1971) [3]. Lilly and Barnet (1951) [10], reported that nutritional requirement of different fungi differs and best growth may be obtained in particular media. Variations in growth might be due to differential ability of the isolates to utilize different nutritional source. Variations in size of macro and micro-conidia in different *Fusarium* spp. and isolates has also been reported by many other workers (Nirmaladevi and Srinivas, 2012; Ferniah *et al*., 2014; Devika Rani *et al*., 2007; Hafizi *et al*., 2013; Gogoi *et al*., 2017) [14, 5, 4, 6, 7]. Nirmaladevi and Srinivas (2012) [14] reported that size of macro-conidia was 15.0-37.5 x 2.5-4.0 µm and that of micro-conidia was 2.5-15.0 x 2.0-3.0 µm in *F. oxysporum* f.sp. *lycopersici*. In *F. solani* size of macro-conidia varied from 27.0-46.0 x 3.0-4.5 µm with 3-5 septations and 16.8-66.0 x 4.0-6.28 µm with 1-5 septations; and that of microconidia varied from 5.0-15.0 x 2.2-3.5 µm and 5.75-15.2 x 3.8-7.4 µm (Ferniah *et al*., 2014; Devika Rani *et al*., 2007) [5, 4].

Table 1: Growth characters of *Fusarium* isolates on Potato dextrose agar medium after seven days of incubation

Isolate Name	Place of collection	Colony characters	Mean Colony diameter(mm)			Pigmentation
			3	5	7	
FS 1	Kaginele	White fluffy cottony	79.33	43.33	59.20	Creamish
FS 2	Kaginele	White fluffy cottony	77.33	35.33	55.13	Creamish
FS 3	Chikningdalu	White cottony	79.87	38.67	53.67	Creamish
FS 4	Chikningdalu	White cottony	89.53	48.67	78.67	Creamish
FS 5	Dasanakoppa	White cottony	75.87	39.33	53.67	Orangish
FS 6	Dasanakoppa	White cottony	79.87	44.67	57.87	Orangish
FS 7	Haladakatti	White cottony	79.73	45.33	63.33	Creamish
FS 8	Haladakatti	White fluffy cottony	89.47	47.33	66.67	Creamish
FS 9	Madihalli	Creamy white submersed cottony	79.60	45.00	62.67	Creamish
FS 10	Madihalli	White cottony	69.53	39.20	55.67	Orangish
FS 11	Beniwad	White submersed cottony	79.93	39.33	57.33	Light Orangish
FS 12	Beniwad	White submersed cottony	77.80	34.67	49.33	Light Orangish
FS 13	Hexambada	White submersed cottony	73.47	39.33	53.67	Creamish
FS 14	Hexambada	White cottony	65.67	28.67	42.67	Light Orangish
FS 15	Karoshi	Creamy white submersed cottony	63.87	37.33	49.53	Creamish
FS 16	Karoshi	Creamy white submersed cottony	75.60	45.33	57.07	Creamish
FS 17	Shaktinagar	White fluffy cottony	85.53	39.33	69.53	Light Orangish
FS 18	Shaktinagar	White submersed cottony	89.73	38.67	69.27	Light Pinkish
FS 19	Devsoogoor	White submersed cottony	79.20	38.00	59.87	Light Orangish
FS 20	Devsoogoor	White cottony	73.80	38.67	59.07	Orangish
FS 21	Neeramanvi	White fluffy cottony	79.60	39.33	53.73	Creamish
FO 22	Neeramanvi	White cottony fluffy	63.93	39.00	47.60	Light Pinkish
FS 23	Kapgal	White cottony fluffy	79.73	38.53	49.67	Orangish
FS 24	Kapgal	White submersed cottony	79.67	39.67	63.47	Orangish
FS 25	Hospet	Creamy white cottony	79.27	38.47	59.20	Orangish
FO 26	Hospet	White fluffy cottony	75.33	36.87	57.80	Purplish
FS 27	Kamalapura	White fluffy cottony	87.60	38.80	63.93	Pinkish
FS 28	Kamalapura	White fluffy cottony	89.73	49.67	71.53	Pinkish
FS 29	Lakshmipura	White fluffy cottony	57.80	24.80	33.53	Purplish
FO 30	Lakshmipura	White fluffy cottony	89.87	46.67	69.47	Pinkish

FS 31	Kampli	White cottony	77.87	41.33	59.13	Light Orangish
FS 32	Kampli	White cottony	67.67	35.80	47.47	Creamish
FS 33	Hasundi	Creamy white submersed cottony	87.87	38.60	61.33	Orangish
FS 34	Hasundi	White fluffy cottony	89.87	41.27	67.33	Orangish
FS 35	Halageri	White fluffy cottony	79.53	39.07	59.27	Orangish
FS 36	Halageri	White fluffy cottony	89.73	46.67	65.27	Orangish
FS 37	Kakola	White cottony growth	77.80	45.67	63.93	Orangish
FS 38	Kakola	White cottony submersed	89.93	42.67	63.80	Pinkish
FS 39	Alagur	White cottony	89.87	49.67	79.33	Orangish
FS 40	Alagur	White cottony	89.33	49.00	78.67	Orangish
FS 41	Guledagudda	White cottony	77.80	45.67	63.53	Orangish
FS 42	Guledagudda	White cottony	89.53	47.53	67.53	Orangish
FS 43	Kaladgi	White cottony	89.87	49.67	79.20	Orangish
FS 44	Kaladgi	White cottony	89.73	47.87	66.93	Orangish
FS 45	Siddapura	White cottony	77.93	45.93	63.93	Orangish
FS 46	Siddapura	White cottony	77.73	45.67	61.87	Orangish
FS 47	Rampura	White fluffy cottony	79.73	38.67	57.80	Creamish
FS 48	Jangere	White cottony	79.80	37.20	57.93	Creamish
FS 49	Kolaramanahalli	White cottony	89.87	39.27	49.53	Creamish
FS 50	Kotaldinnae	Creamy white fluffy cottony	85.87	49.53	60.93	Light Orangish
FS 51	Ramestripalya	Creamy white fluffy cottony	87.87	39.13	59.93	Orangish
Mean			41.45	60.36	80.64	
CD (0.01)			0.013	0.105	0.051	
S.Em ±			0.048	0.037	0.018	

Table 2: Morphological variability of *Fusarium* isolates of chilli collected from different locations of Karnataka

Isolate name	Overall sporulation rate	Macrospores				Microspores			Chlamydospore	
		Length (µm)	Width (µm)	Septation	Macrospore rate	Length (µm)	Width (µm)	Microspore rate	Diameter (µm)	Chlamydospore rate
FS 1	+	17	4.1	2-3	+	12.5	3.5	+	10.5	+
FS 2	++	16	4.3	2-3	+	9.5	3.1	+	11.35	+
FS 3	+++	15	3.9	2-3	++	8	3.2	+++	10.5	++
FS 4	+++	13	4.1	2	++	7.5	3.1	+++	12	++
FS 5	+++	23.5	6.1	2-3	+	10.5	2.9	++	10	++
FS 6	+++	15.5	4.1	2-3	+	7	2.2	+	12	++
FS 7	++	15.5	5	2	+	8	2.9	++	10.5	++
FS 8	+++	14	4	2	+	9.5	3.5	+++	9.5	+
FS 9	+	12	3.5	2-3	+	8.2	3.2	+	12	+++
FS 10	+	11	3	2-3	+	7.3	3	+	10	+++
FS 11	++	13.5	4	2-3	+	7	3.2	+	11	+++
FS 12	++	12	3.5	2-3	+	8	3.1	+	10.25	+++
FS 13	+++	18	4.5	2-3	+	7	3.1	+++	11.5	++
FS 14	+++	17	3.2	2-3	++	9.5	3.2	+++	12.5	++
FS 15	+++	11.5	3.2	2	+	8	3	+++	12.25	++
FS 16	++	11	3.2	2	+	9	3.2	+	11.5	+++
FS 17	+	12	3	2	+	7.5	3.1	+	10.85	+++
FS 18	+	12.5	3	2-3	+	6.5	2.1	+	11.2	+++
FS 19	++	11.5	2.5	2-3	+	9.3	3.3	++	11	++
FS 20	+	25.5	6	2-3	+	8	2.2	+	9.5	+++
FS 21	+++	12.5	3.5	2-3	+	6	2.9	+++	11.25	+
FO 22	+	13.5	2.2	3	+	5	2.7	+	10.5	+
FS 23	+++	12	3.2	2	+	11	3	+++	11.5	+++
FS24	+	13	3.2	2-3	+	10.5	3.2	+	11	+++
FS25	+++	14	4.5	3	++	9.5	2.8	+++	12	+++
FO26	+++	24	5.5	3-4	++	6	3	+++	11.2	+
FS27	+	13	3.4	2-3	+	7	3.2	+	10.8	+++
FS28	+	12	3	2	+	6	2.9	+	9.5	+++
FS29	+	13	3	2-3	+	5	2.5	+	12	+++
FO30	+	11.5	3	3	+	6	2.9	+	11.2	+++
FS31	+	14	3.5	2-3	++	7	2.8	++	10.5	+++
FS32	+	13	4	2	++	12	2.9	++	11.2	+++
FS33	+++	13	3.2	2	++	8	3.1	+	11.8	+++
FS34	+	11	3	2	+	7.5	2.5	+	12	+++
FS35	+++	16	4.5	2-3	++	8.5	3.1	++	10.5	+++
FS36	+++	17.5	4	2-3	++	7	3	++	11.5	+++
FS37	+++	11.5	3.5	2	+	9.5	3.3	+++	12.2	+++
FS38	++	18.5	4.5	2	+	8.5	3.2	+	11.5	+
FS39	+	10	3	2	+	8.5	2.5	+	10.5	+++

FS40	+	11.5	3	2	+	6	2.2	+	10	+++
FS41	+	14	4	2-3	+	4	2.9	+	11.2	+++
FS42	+	13	3.5	2	+	8	3.5	+	10.5	+++
FS43	+	10	3.5	2-3	+	7	2.8	+	11.5	+++
FS44	+	12	2.5	2	+	6	3.2	+	12	+++
FS45	++	10.5	3	2	+	7.2	3	++	10.5	++
FS46	+	11	3	2	+	5.8	2.5	+	10.25	++
FS47	+++	13.5	3.5	2-3	+	9.2	3	+++	12	+
FS48	++	17.5	4.5	2-3	+	7.5	3	+	9.5	+
FS49	++	15	4.5	2	++	7.5	3	+	11.5	++
FS50	+++	16	4.9	2	++	8	2.9	+++	10.2	+
FS51	+++	15	4.8	2	+	11.5	3.3	+++	11.25	+

+++ = High sporulation (> 50 spores per microscopic field) ++ = Medium sporulation (30-50 spores per microscopic field) + = Less sporulation (30 spores per microscopic field)

5. Conclusion

In the respective study, we isolated and identified 51 isolates of *Fusarium*. Among them three isolates (FO 22, FO 26, FO 30) were identified as *F.oxysporum* and rest of the isolates was identified as *F. solani*. The isolates showed lot of cultural and morphological variabilities on PDA media and thus variation among the isolates was existed and confirmed.

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