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 chlamydompospores 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%), Maharashtra (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 discoulouration 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±1°C 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 °C 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 maintained 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 septe 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 monophyllic 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) produced 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 chlamydospores. 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 5-6x2, 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 monomorphic 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; Fernia 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, bostrycodin, 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; Fernia 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 (Fernia 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 |
|--------------|---------------------|-------------------|--------------------------|--------------|
| FS 1         | Kaginelle           | White fluffy cottony | 79.33 43.33 59.20       | Creamish     |
| FS 2         | Kaginelle           | 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       | Orangeish    |
| FS 6         | Dasanakoppa         | White cottony      | 79.87 44.67 57.87       | Orangeish    |
| 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         | Madhiali            | Creamy white submersed cottony | 79.60 45.00 62.67   | Creamish     |
| FS 10        | Madhiali            | White cottony      | 69.53 39.20 55.67       | Orangeish    |
| FS 11        | Beniwad             | White submersed cottony | 79.93 39.33 57.33       | Light Orange |
| FS 12        | Beniwad             | White submersed cottony | 77.80 34.67 49.33       | Light Orange |
| 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 Orange |
| 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 Orange |
| FS 18        | Shaktinagar         | White submersed cottony | 89.73 38.67 69.27       | Light Pinkish|
| FS 19        | Deveoogor           | White submersed cottony | 79.20 38.00 59.87       | Light Orange |
| FS 20        | Deveoogor           | White cottony      | 73.80 38.67 59.07       | Orangeish    |
| FS 21        | Neeramanvi          | White fluffy cottony | 79.60 39.33 53.73       | Creamish     |
| FS 22        | Neeramanvi          | White cottony fluffy | 63.93 39.00 47.60       | Light Pinkish|
| FS 23        | Kappal              | White cottony      | 79.73 38.53 49.67       | Orangeish    |
| FS 24        | Kappal              | White submersed cottony | 79.67 39.67 63.47       | Orangeish    |
| FS 25        | Hospet              | Creamy white cottony | 79.27 38.47 59.20       | Orangeish    |
| FS 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        | Lakshimipura        | White fluffy cottony | 57.80 24.80 33.53       | Purplish     |
| FS 30        | Lakshimipura        | White fluffy cottony | 89.87 46.67 69.47       | Pinkish      |
### Table 2: Morphological variability of *Fusarium* isolates of chilli collected from different locations of Karnataka

| Isolate name | Overall sporulation rate | Length (µm) | Width (µm) | Sporeulation rate | Length (µm) | Width (µm) | Microspore | Chlamydospore (µm) | 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         | ++                | 10.3            | ++               |
| 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           | +                |
| FS 22        | +                        | 13.5        | 2.2        | 3                 | +           | 5          | 2.7         | +                 | 10.5            | +                |
| FS 23        | +++                      | 12          | 3.2        | 2                 | +           | 11         | 3           | +++               | 11.5            | +++              |
| FS 24        | +                        | 13          | 3.2        | 2-3               | +           | 10.5       | 3.2         | +                 | 11             | +++              |
| FS 25        | +++                      | 14          | 4.5        | 3                 | ++          | 9.5        | 2.8         | +++               | 12             | +++              |
| FS 26        | +++                      | 24          | 5.5        | 3-4               | ++          | 6          | 3           | +++               | 11.2            | +                |
| FS 27        | +                        | 13          | 3.4        | 2-3               | +           | 7          | 3.2         | +                 | 10.8            | +++              |
| FS 28        | +                        | 12          | 3          | 2                 | +           | 6          | 2.9         | +                 | 9.5             | +++              |
| FS 29        | +                        | 13          | 3          | 2-3               | +           | 5          | 2.5         | +                 | 12             | +++              |
| FS 30        | +                        | 11.5        | 3          | 3                 | +           | 6          | 2.9         | +                 | 11.2            | +++              |
| FS 31        | +                        | 14          | 3.5        | 2-3               | ++          | 7          | 2.8         | ++                | 10.5            | +++              |
| FS 32        | +                        | 13          | 4          | 2                 | +           | 12         | 2.9         | ++                | 11.2            | +++              |
| FS 33        | ++                       | 13          | 3.2        | 2                 | +           | 8          | 3.1         | +                 | 11.8            | +                |
| FS 34        | +                        | 11          | 3          | 2                 | +           | 7.5        | 2.5         | +                 | 12             | +++              |
| FS 35        | +++                      | 16          | 4.5        | 2-3               | ++          | 8.5        | 3.1         | ++                | 10.5            | +++              |
| FS 36        | +++                      | 17.5        | 4          | 2-3               | ++          | 7          | 3           | ++                | 11.5            | +++              |
| FS 37        | +++                      | 11.5        | 3.5        | 2                 | +           | 9.5        | 3.3         | +++               | 12.2            | +++              |
| FS 38        | ++                       | 18.5        | 4.5        | 2                 | +           | 8.5        | 3.2         | +                 | 11.5            | +                |
| FS 39        | +                        | 10          | 3          | 2                 | +           | 8.5        | 2.5         | +                 | 10.5            | +++              |
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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