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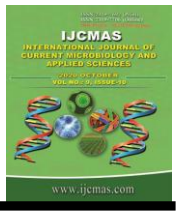

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Pathogenic Variability and Fungicidal Sensitivity of *Fusarium* spp. Causing Wilt in Chilli in Karnataka

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A B S T R A C T

Pathogenic variability and fungicidal sensitivity of the *Fusarium* isolates collected from the major chilli growing districts of Karnataka was studied. The isolates were studied for their pathogenic variability by inoculating to susceptible chilli variety by root dip inoculation method and categorized based on their virulence. Three fungicides *viz.*, Carbendezim, Mancozeb and Propiconazole at 0.2, 0.25 and 0.05 per cent concentrations were used to test the sensitivity of isolates against them. Majority of the *Fusarium* isolates showed high sensitivity for both carbendezim and propiconazole.

Introduction

Chilli (*Capsicum annuum* L.) belonging to family solanaceae is a important spice and vegetable crop. Chillies are good source of vitamin A, vitamin C, vitamin E, folic acid, calcium, potassium and antioxidants like flavonoids, capsaicinoids and carotenoids. Several varieties of chilli 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. Universally, major chilli producing countries are China, Mexico, Turkey, Indonesia, India, Spain and United States. India is one of the largest producers and exporters of this crop and ranks second among world's chilli exporting countries.

In India, area under green chilli accounts to 2.87 lakh ha with a production of 34.06 lakh metric tonnes and that under red chilli is 8.11

lakh ha with a production of 15.20 lakh metric tonnes. Within the country, the leading chilli producing states are Andhra Pradesh, Telangana, Maharastra, Karnataka, Madhya Pradesh, West Bengal and Tamil Nadu. Karnataka accounts to 15 per cent share of the total green chilli production in the country with an area of 0.45 lakh ha and production of 6.07 lakh tonnes. In Karnataka, the crop is mainly cultivated in districts like Dharwad, Haveri, Koppal, Bellary, Raichur, Kalburgi and Belagavi (Anon., 2017). Chilli production in the country is increasingly constrained by several abiotic and biotic factors.

Among the biotic factors, diseases caused by fungi, bacteria and viruses are responsible for drastic reduction of yield and quality of chilli. In recent years, wilt caused by the fungus *Fusarium* spp. has emerged as a serious disease in chilli. Incidence of this disease has been reported to vary from 2 to 85 per cent in different chilli growing regions of India (Anon., 2005).

The yield loss due to the disease is known to vary from 10 to 80 per cent worldwide (Loganathan *et al.*, 2013), 5 to 57 per cent in South India (Raghu *et al.,* 2014) and 20 per cent in black cotton soils of northern Karnataka (Devika Rani *et al.,* 2007). *Fusarium* spp. are soil borne in nature and produce chlamydospores which enables the fungus to persist in soil for long time even in the absence of host plant. Hence, once soil is infested with the pathogenic *Fusarium*, it becomes difficult to cultivate susceptible varieties and lot of fungicides need to be drenched to manage the disease. *Fusarium* spp. exhibit lot of pathogenic, variability and also varied sensitivity to different fungicides. Hence, the present study was aimed to study both these aspects to understand the pathogenic variability of *Fusarium* spp. collected from different infested chilli fields and their responses towards tested fungicides.

Materials and Methods

Collection, pathogen isolation and identification

Fifty one isolates of *Fusarium* spp. were collected from major chilli growing districts of Karnataka namely Bagalkot, Bellary, Belagavi, Haveri, Raichur and Tumkur. For isolation of the pathogen, the roots and basal stem portion of completely and partially wilted chilli plants were cut into small pieces of 1-2 cm size and surface-sterilized with sodium hypochlorite solution for one to two minute and washed repeatedly in sterilized water and placed in Petriplates containing PDA medium and incubated at 27±1°C. The purified culture of the pathogen was obtained by hyphal tip method and maintained at 4 ºC temperature and used for further studies. The pathogen isolates were mainly identified on the basis of cultural and morphological characters of *Fusarium* as described by Leslie and Summerell (2006).

Pathogenic variability

To study pathogenic variability of different isolates, cultivar of chilli Byadagi Dabbi was inoculated by root dip technique. Twenty five days old seedlings of *Fusarium* susceptible chilli variety Byadagi Dabbi were taken for the study. Briefly, the roots of the seedlings were thoroughly washed in tap water followed by sterile water and trimmed at root hair region. Then the seedlings were inoculated by dipping the roots for 30 min in spore suspension of fungus (at the rate of 1×106 spores/ml) which was cultured on PDB for ten days.

Inoculated seedlings were transferred to pots containing sterilized soil and maintained in poly house. Set of seedlings without inoculation was maintained as control. Symptom expression was recorded regularly

from third day of inoculation upto 3 weeks. Disease was scored according to scale, 1- No symptoms, 2- Slight chlorosis, wilting or stunting of the plant, 3- Moderate chlorosis, wilting or stunting of the plant, 4- Severe chlorosis, wilting or stunting of the plant, 5- Death of the plant as described by Nirmaladevi and Srinivas (2012).

Sensitivity to fungicides

Different isolates of fusarium were tested for their sensitivity to fungicides viz., Mancozeb (contact), Carbendezim and Propiconazole (systemic) at concentration 0.25, 0.2 and 0.05 per cent respectively. Poisoned food technique was adopted to test the sensitivity. Required quantity of individual fungicide was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of fungicides. Later 20 ml of the poisoned medium was poured into sterile Petriplates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the center of each agar plate.

Control was maintained without adding any fungicides to the medium. Each treatment was replicated twice. Then such plates were incubated at room temperature for ten days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent (1947).

$$I = \frac{C \times T}{C} \times 100$$

Where,

I = Per cent inhibition
C = Radial growth in control (mm)
T = Radial growth in treatment (mm)

Results and Discussion

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). 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.

Pathogenic variability

Pathogenic variability of different isolates was tested on susceptible cultivar Byadagi dabbi by root dip technique as explained in material and methods.

Table.1 Pathogenic variability of *Fusarium* isolates of chilli on *cv.* Byadagi Dabbi

Isolate name	Symptoms (DAI)				Per cent wilt incidence (DAI)			
	3rd	7th	15th	22nd	3rd	7th	15th	22nd
FS 1	CL	CL and MW	SW	SW	-	-	-	-
FS 2	CL	CL and MW	SW	SW	-	-	100	-
FS 3	CL	CL and MW	SW	SW	-	-	100	-
FS 4	CL	CL and MW	SW	SW	-	-	100	-
FS 5	CL	CL and MW	SW	SW	-	-	100	-
FS 6	CL	CL and MW	SW	SW	-	-	100	-
FS 7	CL	CL and MW	SW	SW	-	-	100	-
FS 8	CL	CL and MW	SW	SW	-	-	100	-
FS 9	CL	SW	SW	SW	-	100	-	-
FS 10	CL	SW	SW	SW	-	100	-	-
FS 11	CL	SW	SW	SW	-	100	-	-
FS 12	CL	SW	SW	SW	-	100	-	-
FS 13	CL	SW	SW	SW	-	100	-	-
FS 14	CL	SW	SW	SW	-	100	-	-
FS 15	CL	SW	SW	SW	-	100	-	-
FS 16	CL	SW	SW	SW	-	100	-	-
FS 17	CL	CL	SW	SW	-	-	100	-
FS 18	CL	CL	SW	SW	-	-	100	-
FS 19	CL	SW	SW	SW	-	100	-	-
FS 20	CL	CL	CL	CL	-	-	-	-
FS 21	CL	SW	SW	SW	-	100	-	-
FO 22	CL	SW	SW	SW	-	100	-	-
FS 23	CL	CL	SW	SW	-	-	100	-
FS 24	CL	SW	SW	SW	-	100	-	-
FS 25	CL	CL	CL	CL	-	-	100	-
FO 26	CL	SW	SW	SW	-	100	-	-
FS 27	CL	CL	CL	CL	-	100	-	-
FS 28	CL	SW	SW	SW	-	100	-	-
FS 29	CL	CL	SW	SW	-	-	100	-
FO 30	CL	SW	SW	SW	-	100	-	-
FS 31	CL	CL	SW	SW	-	-	100	-
FS 32	CL	CL	SW	SW	-	-	100	-
FS 33	CL	CL	SW	SW	-	-	100	-
FS 34	CL	CL	SW	SW	-	-	100	-
FS 35	CL	CL	SW	SW	-	-	100	-
FS 36	CL	CL	SW	SW	-	-	100	-
FS 37	CL	CL	SW	SW	-	-	100	-
FS 38	CL	CL	SW	SW	-	-	100	-
FS 39	-	-	-	CL and W	-	-	-	-
FS 40		-	SW	SW	-	-	100	-
FS 41	-	-	-	CL and W	-	-	-	-
FS 42	-	-	-	CL and W	-	-	-	-
FS 43	CL	CL	SW	SW	-	-	100	-
FS 44	CL	CL	SW	SW	-	-	100	-
FS 45	CL	CL	CL	CL	-	-	-	-
FS 46	CL	CL	SW	SW	-	-	100	-
FS 47	CL	CL	CL	CL	-	100	-	-
FS 48	CL	CL	CL	CL	-	100	-	-
FS 49	CL	CL	CL	CL	-	100	-	-
FS 50	CL	CL	CL	CL	-	100	-	-
FS 51	CL	CL	CL	CL	-	100	-	-

CL = initial symptoms of chlorosis,, CL and W = leaf chlorosis and wilting, CL and MW = moderate wilting and chlorosis SW= severe wilting, - = no incidence / no symptoms, DAI = Days after inoculation

Table.2 Grouping of *Fusarium* isolates of chilli based on pathogenicity

Virulence grade	Isolate name	Number of isolates
Highly pathogenic	FS 9, FS 10, FS 11, FS 12, FS 13, FS 14, FS 15, FS 16, FS 19, FS 21, FO 22, FS 24, FO 26, FS 27, FS 28, FO 30, FS 47, FS 48, FS 49, FS 50, FS 51	21
Moderately pathogenic	FS 2, FS 3, FS 4, FS 5, FS 6, FS 7, FS 8, FS 17, FS 18, FS 20, FS 23, FS 25, FS 29, FS 31, FS 32, FS 33, FS 34, FS 35, FS 36, FS 37, FS 38, FS 40, FS 43, FS 44, FS 46	25
Weakly pathogenic	FS 39, FS 41, FS 42, FS 45	4

Table.3 Sensitivity of *Fusarium* isolates of chilli to three different fungicides

Isolate name	Per cent inhibition of colony growth		
	Mancozeb (0.25%)	**Carbendezim (0.2%)**	**Propiconazole (0.05%)**
FS 1	60.00 (50.75)	91.11 (72.62)	80.56 (63.81)
FS 2	76.11 (60.72)	99.73 (86.98)	82.44 (65.20)
FS 3	73.33 (58.90)	97.89 (81.61)	89.44 (71.09)
FS 4	73.33 (58.90)	99.71 (86.87)	89.11 (70.70)
FS 5	73.33 (58.90)	95.56 (77.80)	82.44 (65.20)
FS 6	60.00 (50.75)	93.33 (75.00)	80.89 (64.05)
FS 7	73.33 (58.90)	91.11 (72.62)	82.44 (65.20)
FS 8	56.11 (48.49)	92.22 (73.77)	86.78 (68.65)
FS 9	100.00 (90.00)	91.11 (72.62)	80.56 (63.81)
FS 10	100.00 (90.00)	93.33 (75.00)	84.67 (66.92)
FS 11	100.00 (90.00)	97.78 (81.39)	89.11 (70.70)
FS 12	56.11 (48.99)	95.56 (77.80)	82.78 (65.45)
FS 13	61.11 (51.40)	99.73 (86.98)	84.67 (66.92)
FS 14	61.11 (51.40)	99.69 (86.77)	100.00 (90.00)
FS 15	71.22 (57.53)	99.64 (86.52)	82.44 (65.23)
FS 16	47.22 (43.39)	91.11 (72.62)	87.22 (69.03)
FS 17	67.89 (55.46)	99.67 (86.67)	100.00 (90.00)

FS 18	75.78 (60.49)	99.71 (86.87)	100.00 (90.00)
FS 19	61.11 (51.40)	99.69 (86.77)	89.11 (70.70)
FS 20	73.56 (59.02)	99.73 (86.98)	82.78 (65.45)
FS 21	77.22 (61.48)	93.33 (75.00)	80.56 (63.81)
FO 22	59.11 (50.23)	99.76 (87.15)	100.00 (90.00)
FS 23	55.22 (47.97)	95.56 (77.80)	84.67 (66.92)
FS 24	60.00 (50.75)	97.78 (81.39)	87.22 (69.03)
FS 25	68.89 (56.09)	99.73 (86.98)	91.67 (73.19)
FO 26	58.33 (49.77)	99.78 (87.27)	82.44 (65.20)
FS 27	47.78 (43.71)	99.73 (86.98)	85.00 (67.19)
FS 28	47.78 (43.71)	99.67 (86.67)	87.22 (69.03)
FS 29	75.00 (59.98)	99.73 (86.98)	89.44 (71.01)
FO 30	39.11 (38.69)	97.78 (81.39)	87.33 (69.13)
FS 31	58.33 (49.77)	93.33 (75.00)	85.00 (67.19)
FS 32	58.89 (50.10)	93.33 (75.00)	84.67 (66.92)
FS 33	78.33 (63.23)	82.22 (65.03)	89.78 (71.34)
FS 34	85.00 (67.19)	80.00 (63.40)	85.00 (67.19)
FS 35	74.44 (59.61)	82.22 (65.03)	86.89 (68.74)
FS 36	100.00 (90.00)	99.71 (86.87)	85.00 (67.19)
FS 37	100.00 (90.00)	99.69 (86.77)	89.11 (70.70)
FS 38	100.00 (90.00)	80.00 (63.40)	89.11 (70.70)
FS 39	91.22 (72.73)	99.73 (86.98)	91.22 (72.73)
FS 40	90.56 (72.08)	93.33 (75.00)	84.67 (66.92)
FS 41	52.22 (46.25)	80.00 (63.40)	89.44 (71.02)
FS 42	59.00 (50.16)	99.71 (86.87)	96.11 (78.62)
FS 43	45.78 (42.55)	99.73 (86.98)	95.78 (78.11)

FS 44	56.11 (48.49)	99.71 (86.87)	97.89 (81.61)
FS 45	53.89 (47.20)	99.69 (86.77)	95.78 (78.11)
FS 46	54.44 (47.53)	81.11 (64.22)	93.56 (75.26)
FS 47	65.56 (58.06)	99.71 (86.87)	78.00 (62.00
FS 48	58.89 (50.10)	73.33 (58.88)	84.67 (66.92)
FS 49	100.00 (90.00)	82.22 (65.03)	73.89 (59.24)
FS 50	57.89 (49.52)	86.67 (68.55)	80.56 (63.81)
FS 51	67.22 (55.05)	80.00 (63.40)	80.56 (63.81)
Control	-	-	-
Mean	67.63	92.20	85.57
CD (0.01)	3.612 (2.221)	0.438 (0.321)	1.436 (1.199)
SE.m±	1.269 (0.780)	0.154 (0.113)	0.505 (0.421)

Inoculated plants showed gradual increase in expression of wilt symptoms starting from 3rd day to fourth week. Pathogenic variability among the isolates was ascertained on the basis of the ability of each isolate to cause disease and the temporal variation in appearance of the specific symptoms *viz.*, leaf chlorosis, yellowing and drooping, moderate wilting, severe wilting and death of plants. The appearance of the specific symptom varied depending on the virulence level of each isolate (Table 1). Based on pathogenicity reaction the isolates were categorized into 3 groups viz., highly pathogenic (isolates that caused complete wilting by seventh day), moderately pathogenic (isolates that caused complete wilting by 15th day) and weakly pathogenic (isolates that caused chlorosis and moderate wilting even after 22nd day) (Table 2). All the three isolates of *F. oxysporum* (FO 22, FO 26 and FO 30) were highly pathogenic as the symptoms appeared in the most severe form and complete wilting and death of the plant was seen within first week after inoculation. Among 48 isolates of *F. solani* nineteen isolates (FS 1, FS 9 to FS 16, FS 19, FS 21, FS 24, FS 27, FS 28, FS 47 to FS 51) were highly pathogenic. Twenty five isolates (FS 2 to FS 8, FS 17, FS 18, FS 20, FS 23, FS 25, FS 29, FS 31 to FS 38, FS 40, FS 43, FS 44, FS 46) were moderately pathogenic and four isolates (FS 39, FS 41, FS 42 and FS 45) were weakly pathogenic. The present results are also in conformity with the findings of Ferniah *et al.*, (2014) who reported that the *F. oxysporum* isolate P1a was highly pathogenic that caused wilting in two chili cv. TM999 and Gantari with 40 and 63 per cent disease severity respectively. Similarly, Nirmaladevi and Srinivas (2012) identified 18 isolates of *Fusarium oxysporum* as highly pathogenic, 40 as moderately pathogenic and 20 as weakly pathogenic in their study in tomato cultivars. Raghu *et al.*, (2016) also reported variations in pathogenicity of 36 isolates of *Fusarium* spp. on chilli. The pathogenic variability in *Fusarium* isolates might be brought about by either natural mutation, natural selection, genetic drift, gene flow or mating system (McDonald and Linde, 2002). The variation in the *F. oxysporum* isolates, in turn can occur by means of natural mutations, through

parasexuality or heterokaryosis (Buxton, 1962; Kuhn *et al.*, 1995).

Sensitivity to fungicides

Among the fungicides tested, irrespective of the isolates, maximum inhibition of growth was observed in carbendezim (0.2%) followed by propiconazole (0.05%), and least inhibition was observed in mancozeb (0.25%). However, the isolates varied for their sensitivity to different fungicides. For carbendezim, majority of the isolates showed highest sensitivity with 80-90 per cent inhibition of growth and isolate FS 48 was least sensitive with 73.83 per cent inhibition of growth. For mancozeb, nine isolates (FS 9, FS 10, FS 11, FS 30, FS 37, FS 38, FS 39, FS 40, FS 49) showed high sensitivity with 90-100 per cent inhibition of growth, followed by isolates (FS 1 to FS 7, FS 13, FS 14, FS 15, FS 17 to FS 21, FS 24, FS 25, FS 29, FS 33 to FS 35, FS 47, FS 51) which showed 60-80 per cent inhibition of growth and 19 isolates (FS 8,, FS 12, FS 16, FO 22, FO 23, FO 26, FS 27, FS 28, FS 30, FS 31, FS 41 to FS 46, FS 48, FS 50) showed least sensitivity with 45-60 per cent inhibition of growth. For propiconazole, four isolates (FS 14, FS 17, FS 18, FO 22, FS 42, FS to FS 46) showed highest sensitivity with 90-100 per cent inhibition of growth and rest of the isolates showed 78-90 per cent inhibition of growth (Table 3). The isolates which showed least sensitivity might have developed resistance against those fungicides which needs to be considered while managing the disease. Sensitivity to fungicides may indicate usefulness of particular fungicide in management of the pathogen. Resistant individuals within in a fungal population may develop due to repeated application of fungicides (Eckert, 1988). In the present study the isolates collected from different locations varied for their sensitivity to different fungicides. Variations in sensitivity against fungicides in different *Fusarium* spp. and isolates have also been reported by Rajput *et al.*, 2006; Amini *et al.*, 2010; Andrabi *et al.*, 2011; Maitlo *et al.*, 2014; Dahal and Shrestha 2018.

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