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Investigations on Cultural, Morphological and Pathogenic Variability among Isolates of *Fusarium oxysporum* Causing Wilt of Fenugreek

N. Rani and Yashoda R. Hegde

Department of Plant Pathology, University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India.

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Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, belonging to the family Fabaceae, commonly known as Methi. Wilt caused by *Fusarium oxysporum* is an important disease in fenugreek. Cultural, morphological and Pathogenic variability studies were conducted among 20 isolates of the pathogen and isolates were classified in to three groups. Group I consisted of five highly virulent isolates *viz.,* Fo BAN, Fo GAN, Fo GOV, Fo SOM and Fo YAT with less number of days taken for wilting (20 to 35 days), higher virulence index of more than 2.74, growth rate of more than 18.00 mm/day, dry mycelial weight of more than 300 mg. Group II consisted of nine moderately virulent isolates *viz.*, Fo BAI, Fo HAN, Fo HIR, Fo KAM, Fo KAV, Fo SHI, Fo SUB, Fo UPP and Fo YAD with number of days taken for wilting ranged from 35 to 50 days, virulence index of 1.99 to 2.74 and medium growth rate of growth(11.00 to 14 mm/day), medium dry mycelial weight of 275 to 300 mg. Group III consisted of six less virulent isolates *viz.,* Fo HEB, Fo HIM, Fo MAD, Fo MADI, Fo NAR and Fo NES with more number of days taken for wilting (more than 50 days), less virulence index of 1.24 to 1.99, slow growth rate 11 to 14 mm/ day, less dry mycelial weight of 250 to 275 mg.

Keywords: Fenugreek, wilt, virulence, *Fusarium oxysporum,* variability.

Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. It is native of India and leading fenugreek producing country in the world. It is the third largest seed spice in India after coriander and cumin. In India, it is grown in about 66,000 ha with an annual production of about 90,000 tonnes (Anon., 2014)[1]. Rajasthan is the fenugreek bowl of country, contributing 90 per cent to the country's production. It has some pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity. (Kor and Moradi, 2013)[2].

Fenugreek is mainly grown as leafy vegetable throughout Karnataka and there is ample scope for its cultivation as seed spice. But fenugreek suffers from many of fungal diseases *viz.,* Cercospora leaf spot caused by *Cercospora traversiana,* root rot (*Rhizoctonia solani),* leaf spot (*Ascochyta sp.),* powdery mildew (*Erysiphe polygoni),* downy mildew (*Peronospora trigonellae)* and Fusarium wilt (Prasad *et al.,* 2014)[3]. Although many diseases are reported in fenugreek, wilt caused by *Fusarium oxysporum* is becoming more severe in recent years(Shivapuri and Bansal,1987)[4]. Variability studies help for understanding the pathogen complexity and virulence helps in identifying highly virulent isolate

* To whom all correspondence should be addressed.
E-mail: uasyashoda@rediffmail.com

of the pathogen. Hence present study was under taken to know the pathogenic and its morphological variability of different isolates of *Fusarium oxysporum.*

MATERIALS AND METHODS

Cultural and morphological variability among isolates of *F. oxysporum*

Twenty three different isolates of *Fusarium oxysporum* collected from different locations of fenugreek growing areas were studied for variability with respect to morphological, cultural characters and pathogenic variability. All isolates were cultured on potato dextrose agar. Observations on growth parameters of each isolates like growth rate, colour of the colony, pigmentation and texture were recorded. Microscopic observations were recorded for microconidia, macroconidia and chlamydospore formation after 15 days of inoculation.

Pathogenic variability among the isolates of *Fusarium oxysporum*

A pot experiment was conducted in the glasshouse of Department of Plant Pathology, University of Agricultural Sciences, Dharwad to find out virulence index of different isolates. The giant culture was inoculated to each pot at the rate of eight per cent and five seeds were sown in each pot. Each treatment was replicated thrice. Observations were recorded on symptoms of wilting and number of days taken for wilting (latent period).

The numerical values of per cent disease incidence and latent period were used to calculate the virulence index using the following formula (Thakur and Rao, 1997)[5].

Virulence index (VI) = Per cent disease incidence (PDI) × Latent period^{-1}

RESULTS AND DISCUSSION

Growth of different isolates on PDA

All twenty three isolates were grown on PDA and radial growth, colony characters were measured at four days of incubation. The variation was observed among 23 isolates of *F. oxysporum* on potato dextrose agar. The maximum colony diameter 90 mm was observed in the isolates of Fo YAT, Fo GOV, Fo BAN, Fo GAN, Fo SOM, Fo ATH, and Fo IND which was significantly superior to all other isolates, this was followed by Fo KAM, Fo KAV, Fo MADI, Fo SHI, Fo UPP, Fo YAD, Fo HIR, Fo HIM, Fo SUB, Fo BAI and Fo HAN (70-85 mm). The minimum colony diameter was observed in remaining isolates *i.e.,* Fo HEB, Fo NAR, Fo MAD, Fo NES and Fo NAG (55-70 mm). Flat type of colony character was recorded in eight isolates *viz.*, Fo ATH, Fo BAI, Fo HAN, Fo HIR, Fo KAM, Fo KAV, Fo NAR and Fo YAD. Remaining isolates showed raised type of colony. Colour of the colonies were varied with different shades *viz.,* dark pink to light pink, light orange, light yellow, greyish black, cottony white, whitish and off white. Colonies margin was circular in all the isolates. Pigmentation varied from red to orange, light orange, light yellow, deep yellow, brownish, cream white to off white. Majority of the isolates showed the cottony type of texture than velvety texture (Table1 and plate 1). The present findings receive support from Nirmaladevi and Srinivas (2012)[6] who showed the colour and pigmentation of the isolates of *Fusarium oxysporum* on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet.

The observation on sporulation of pathogen revealed that higher sporulation (> 40 conidia/microscopic field) was observed in isolates like Fo YAT, Fo NAR, Fo NES, Fo SOM and Fo ATH. Less sporulation (< 20 conidia/microscopic field) was observed in isolates of Fo GOV, Fo HEB, Fo KAM, Fo UPP, Fo HIR, Fo HIM, Fo HAN, Fo MAD and Fo IND. Based on sporulation, isolates were grouped in to three groups as high sporulators (>40 conidia/microscopic field, viz.,isolates like Fo YAT, Fo NAR, Fo NES, Fo SOM and Fo ATH.) moderate sporulators(20-40 conidia/microscopic field, isolates like Fo BAI, Fo BAN, Fo YAD , Fo SHI, Fo KAV, Fo MADI, Fo GAN, Fo SUB and Fo NAG.) and low sporulatiors d”20 conidia/ microscopic field (*viz.,* Fo GOV, Fo HEB, Fo KAM, Fo UPP, Fo HIR, Fo HIM, Fo HAN, Fo MAD and Fo IND)

Morphological variability

Spore morphology of different isolates of *F. oxysporum*

Tremendous variation was observed with respect to spore size and arrangement of chlamydospore. Both bigger spore size of macroconidia (37.24-45.16 × 4.74-6.28 mm) and

microconidia (9.10-11.43 × 2.55-3.54 mm) were observed in isolate Fo YAT and also with maximum number of septa (3), which was superior to all other isolates. This was followed by Fo SOM with 1-3 septa in macroconidia with spore size of 25.63-43.74×3.58-5.82 µm. Both small sized macro conidia (3.78-8.01 × 1.00-1.52 mm) with 1-2 septa and micro conidia (1.70-2.52 × 1.05-1.55 mm) were observed in Fo NES. The chlamydospores were produced both solitary and in chains of 1-8 cells produced at intercalary or at terminal end of hyphae. Most of the cases the chlamydospores arrangement was observed in chain of 2 (Table 2 and Plate 2 and 3.).

Growth rate of different isolates of *F. oxysporum* on PDA

The growth rate of different isolates of *F. oxysporum* was recorded from 2nd day to 8th day. The growth rate ranged from 11.25 to 22.50 mm per day. The maximum growth rate per day (22.5 mm) was recorded in isolate Fo BAN which is statistically superior to all other isolates. This was followed by Fo YAT, Fo GOV, Fo GAN and Fo SOM (18.00 mm/day) and least growth rate (11.25 mm/day) was recorded in four isolates *viz.,* Fo HEB, Fo NAR, Fo MAD and Fo NES. The observations were summarized in table 3.

The isolates were classified based on growth rate, into three groups. Group I consisted of five fast growing isolates *viz.,* Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM with growth rate more than 18 mm per day. Group II consisted of nine isolates with moderate growth rate ranging from 14-18 mm per day *viz.,* Fo KAM, Fo KAV, Fo SHI, Fo UPP, Fo YAD, Fo HIR, Fo SUB, Fo BAI, and Fo HAN with growth. Group III consisted of six slow growing isolates *viz.,* Fo HEB, Fo MADI, Fo NAR, Fo HIM, Fo MAD and Fo NES with growth rate ranging from 11-14 mm per day. Present observations are in agreement with the findings of Nirmaladevi and Srinivas (6) who reported that out of the 114 isolates of *F. udum*, 85 isolates were rated as fast growing, 19 as moderate growing and

Table 1. Cultural characters of different isolates of *Fusarium oxysporum* in PDA

Isolates	Colony characters					
	Diameter (mm)	Margin	Colour	Pigmentation	Texture	Sporulation
Fo ATH	90.00	Flat	Greyish white	Off white	Velvety	++++
Fo BAI	78.85	Flat	Light grey	Light yellow	Velvety	+++
Fo BAN	90.00	Raised	Dark pink	Red	Cottony	+++
Fo GAN	90.00	Raised	Cottony white	Orange	Cottony	++
Fo GOV	90.00	Raised	Dark pink	Light orange	Velvety	+
Fo HAN	74.00	Flat	Hyaline white	Off white	Cottony	+
Fo HEB	67.55	Raised	Whitish	Brownish	Velvety	+
Fo HEM	71.06	Raised	Cottony white	Light yellow	Cottony	+
Fo HER	79.88	Flat	Off white	Light yellow	Velvety	+
Fo IND	90.00	Raised	Cottony white	Deep yellow	Cottony	+
Fo KAM	81.95	Flat	Light pink	Creamish white	Cottony	+
Fo KAV	79.88	Flat	Greyish black	Creamish white	Velvety	++
Fo MAD	64.49	Raised	Whitish	Deep yellow	Velvety	+
Fo MADI	71.34	Flat	Greyish black	Creamish white	Velvety	++
Fo NAG	67.55	Raised	Off white	Creamish white	Cottony	++
Fo NAR	60.60	Flat	Greyish black	Orange	Velvety	++++
Fo NES	68.40	Raised	Whitish	Orange	Cottony	++++
Fo SHI	81.69	Raised	Dark pink	Red	Cottony	+++
Fo SOM	90.00	Raised	Dark pink	Red	Cottony	++++
Fo SUB	81.25	Raised	Light pink	Orange	Cottony	++
Fo UPP	80.00	Raised	Whitish	Creamish white	Cottony	+
Fo YAD	76.10	Flat	Whitish	Light yellow	Velvety	+++
FoYAT	90.00	Raised	Dark pink	Red	Cottony	++++

Sporulation; Conidia /microscopic field (400X)
++++ >40; +++30-40; ++ 20-30; + <20

Table 2. Morphological variability among isolates of *Fusarium oxysporum*

Name of the isolate	Size of Macroconidiamm (400X)	No. of septa	Size of Microconidia mm (400X)	Arrangement of Chlamydospores
Fo ATH	5.77-12.8 x 1.97-2.83	1	4.96-5.80 x 1.97-3.14	Solitary and terminal
Fo BAI	12.76-24.67 x 3.91-4.70	1-2	5.87-13.23 x 2.27-4.49	Both solitary and in chain of 2
Fo BAN	11.16-34.46 x 3.67-5.37	1-2	6.67-7.57 x 2.53-3.91	In chain of 2-3
Fo GAN	16.21-20.2 x 3.44-4.57	1	6.77-10.15 x 2.1-4.44	Solitary and terminal
Fo GOV	12.32-17.34 x 3.71-5.27	1	6.41-9.55 x 2.50-3.68	Solitary and terminal
Fo HAN	11.50-28.23 x 2.60-4.46	1-2	5.71-7.62 x 1.19-2.50	In chain of 2
Fo HEB	9.26-13.78 x 2.50-3.97	1-2	6.14-8.58 x 2.62-3.85	Solitary and intercalary
Fo HEM	15.04-18.86 x 3.40-4.88	1	4.91-7.23 x 2.20-4.10	Solitary
Fo HER	12.15-28.26 x 3.61-5.43	1	8.56-9.66 x 3.05-3.48	Both solitary and in chain of 2
Fo IND	12.96-24.00 x 4.22-4.59	1	5.23-7.68x1.84-3.62	Solitary
Fo KAM	18.46-36.01 x 3.86-5.83	1	7.57-10.76 x 2.28-5.28	Solitary, both terminal and intercalary
Fo KAV	7.62-26.71 x 3.75-5.39	1-2	6.25-12.06 x 2.12-3.82	In chain of 2
Fo MAD	29.41-32.92 x 4.52-5.35	1-2	6.31-10.47 x 1.99-4.88	Both solitary and in chain of 2
Fo MADI	13.58-24.12 x 3.77-5.7	1	7.75-13.51 x 3.32-4.92	In chain of 6-8
Fo NAG	9.60-17.84 x 3.01-4.96	1	5.22-10.27X1.12-6.03	Solitary and terminal
Fo NAR	18.99-27.60 x 3.90-5.36	1	8.65-11.15 x 3.18-3.80	Solitary
Fo NES	3.78-8.01 x 1.00-1.52	1-2	1.70-2.52 x 1.05-1.55	In chain of 2
Fo SHI	9.02-25.04 x 2.95-5.61	1	8.66-10.64 x 2.92-4.09	In chain of 2-3
Fo SOM	25.63-43.74 x 3.58-5.82	1-3	5.44-13.76 x 2.39-5.36	In chain of 2-4
Fo SUB	9.54-24.35 x 2.64-4.49	1	4.13-5.88 x 2.13-3.12	Solitary and terminal
Fo UPP	11.00-35.87 x 1.80-5.01	2	4.94-7.87 x 1.87-4.39	Solitary and intercalary
Fo YAD	10.16-16.53 x 1.87-4.40	1	5.71-7.12 x 1.28-3.28	Solitary and intercalary
Fo YAT	37.24-45.16 x 4.74-6.28	3	9.10-11.43 x 2.55-3.54	Both solitary and in chain of 2-4

Table 3. Growth rate of different isolates of *Fusarium oxysporum*

Isolate	Colony diameter in mm							Growth rate
	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	/day (mm)
Fo BAI	18.74	26.12	56.10	78.85	90.00	90.00	90.00	15.00
Fo BAN	39.46	68.86	90.00	90.00	90.00	90.00	90.00	22.50
Fo GAN	36.50	63.85	79.74	90.00	90.00	90.00	90.00	18.00
Fo GOV	30.88	58.90	80.73	90.00	90.00	90.00	90.00	18.00
Fo HAN	22.65	52.65	68.65	74.00	86.00	90.00	90.00	15.00
Fo HEB	19.63	30.39	52.00	67.55	76.08	81.14	90.00	11.25
Fo HEM	19.63	31.30	58.35	71.06	81.87	90.00	90.00	12.86
Fo HER	26.50	39.35	55.30	79.88	90.00	90.00	90.00	15.00
Fo KAM	19.13	36.70	64.00	81.95	90.00	90.00	90.00	15.00
Fo KAV	26.50	39.35	55.30	79.88	90.00	90.00	90.00	15.00
Fo MAD	18.00	25.05	41.52	64.49	74.37	82.17	90.00	11.25
Fo MADI	21.50	44.11	59.94	71.34	83.55	90.00	90.00	12.86
Fo NAR	18.00	29.04	47.20	60.60	76.40	85.60	90.00	11.25
Fo NES	19.75	38.60	52.40	68.40	78.00	85.70	90.00	11.25
Fo SHI	17.63	36.40	69.65	81.69	90.00	90.00	90.00	15.00
Fo SOM	30.27	67.26	75.50	90.00	90.00	90.00	90.00	18.00
Fo SUB	26.00	44.15	67.40	81.25	90.00	90.00	90.00	15.00
Fo UPP	25.05	46.05	69.21	80.00	90.00	90.00	90.00	15.00
Fo YAD	17.85	30.61	57.08	76.10	90.00	90.00	90.00	15.00
Fo YAT	32.13	67.50	82.38	90.00	90.00	90.00	90.00	18.00

Table 4. Pathogenic variability among isolates of *Fusarium oxysporum*

Isolates	No. of days taken for wilting	Virulence index
Fo BAI	48	2.08
Fo BAN	32	3.13
Fo GAN	35	2.86
Fo GOV	34	2.94
Fo HAN	50	2.00
Fo HEB	60	1.67
Fo HIM	52	1.92
Fo HIR	41	2.44
Fo KAM	38	2.63
Fo KAV	49	2.04
Fo MAD	53	1.89
Fo MADI	52	1.92
Fo NAR	53	1.89
Fo NES	60	1.67
Fo SHI	48	2.08
Fo SOM	32	3.13
Fo SUB	45	2.22
Fo UPP	39	2.56
Fo YAD	44	2.27
FoYAT	28	3.57
Control	No wilting	0.00

10 isolates were slow growing.

Pathogenic variability among isolates of *Fusarium oxysporum*

The genus *Fusarium* will cause a high degree of variation with respect to pathogenicity on its host. The degree of pathogenic variability varies from location to location and virulence nature of the pathogen. On host, the pathogen produces various types of symptoms at different growth stages such as pre-emergent and post-emergent seedling death and foliar yellowing and drooping of the leaves at later stages. In the present study an attempt was made to study the degree of pathogenic variability of the *Fusarium oxysporum* on susceptible variety DFC-26. Cent per cent disease incidence was recorded in all different isolates of *F. oxysporum*. There was cent per cent disease incidence in all the isolates of *Fusarium oxysporum*. Maximum degree of virulence index was recorded in Fo YAT (3.57) which took less number of days (28) for complete wilting of plant followed by Fo BAN and Fo SOM (3.13) required 32 days for wilting. Fo HEB and Fo NES was less virulent with minimum virulence index of 1.67 and required maximum period (60 days) for wilting of

Table 5. Grouping of isolates of *Fusarium oxysporum* based on different characters

Group	Isolate	Location	No. of days taken for wilting	Virulence index	Growth rate	Pathogenic reaction
Group I	Fo BAN	Bankapura	32	3.13	22.50	Highly virulent
	Fo GAN	Ganjigatti	35	2.86	18.00	
	Fo GOV	Govinkoppa	34	2.94	18.00	
	Fo SOM	Somanahattti	32	3.13	18.00	
	Fo YAT	Yattinagudda	28	3.57	18.00	
Group II	Fo BAI	Bhilahongal	48	2.08	15.00	Moderatelyvirulent
	Fo HAN	Hanabarahatti	50	2.00	15.00	
	Fo HIR	Hirebendigeri	41	2.44	15.00	
	Fo KAM	Kamalapura	38	2.63	15.00	
	Fo KAV	Kavalageri	49	2.04	15.00	
	Fo SHI	Shikaragatti	48	2.08	15.00	
	Fo SUB	Subaragatti	45	2.22	15.00	
	Fo UPP	Uppinabetageri	39	2.56	15.00	
	Fo YAD	Yadawada	44	2.27	15.00	
Group III	Fo HEB	Hebbali	60	1.67	11.25	Less virulent
	Fo HIM	Hiremallur	52	1.92	12.86	
	Fo MAD	Madanabavi	53	1.89	11.25	
	Fo MADI	Madhihal	52	1.92	12.86	
	Fo NAR	Narendra	53	1.89	11.25	
	Fo NES	Nesergi	60	1.67	11.25	

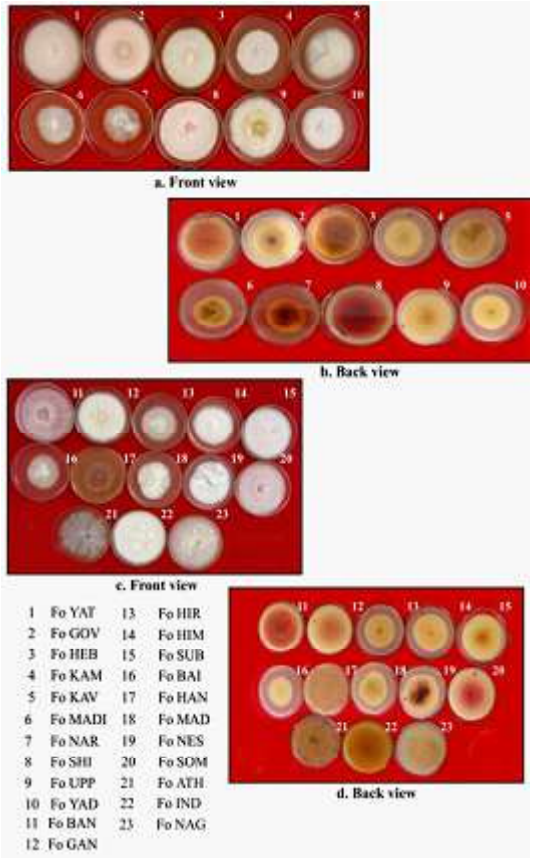

Plate 4. Growth of different isolates of *F.oxysporum* on PDA

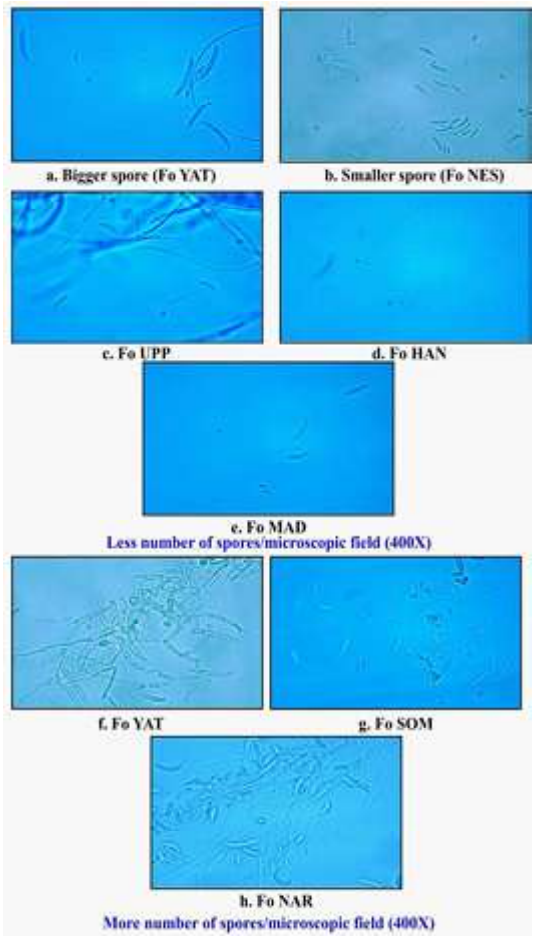

Plate 2. Spore morphology of different isolates of *F.oxysporum*

plants. Isolates with maximum virulence index required less period for wilting of plants. Observations on per cent disease incidence and latent period were recorded and presented in the Table 4 Plate 4

Based on virulence index, the isolates were classified into three groups. Group 1 consisted of five isolates (Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM) with high virulence index (>2.75). Group II consisted of nine isolates (Fo KAM, Fo KAV, Fo SHI, Fo UPP, Fo YAD, Fo HIR, Fo SUB, Fo BAI, and Fo HAN) with virulence index ranging from 2,00 to 2.74. Group III consisted of six isolates (Fo HEB, Fo MADI, Fo NAR, Fo HIM, Fo MAD and Fo NES) with low virulence index ranging from 1.25 to 1.99. It is clear that group 1 isolates were highly virulent than other isolates. Present findings are in conformity with the findings of Nirmaladevi and Srinivas, (2012)[6] and Joshi *et al.* (2013)[7]

Grouping of *F. oxysporum* isolates based on different cultural characters

In the present study virulence was correlated to number of days taken for wilting, virulence index, growth rate, and vigour index. Because of variation in cultural studies, isolates of *F. oxysporum* were grouped based on number of days taken for wilting, virulence index and growth rate. The isolates with less number of days taken for wilting, high virulence index and fast growth rate were highly virulent isolates. Whereas isolates with medium number of days taken for wilting, moderate virulence index and medium growth rate

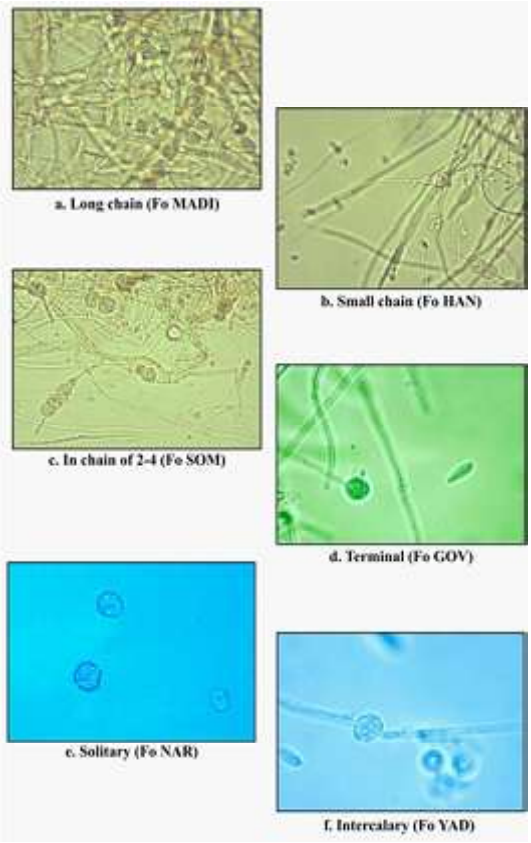

Plate 3. Arrangement of chlamydospores of different isolates of *F.oxysporum*

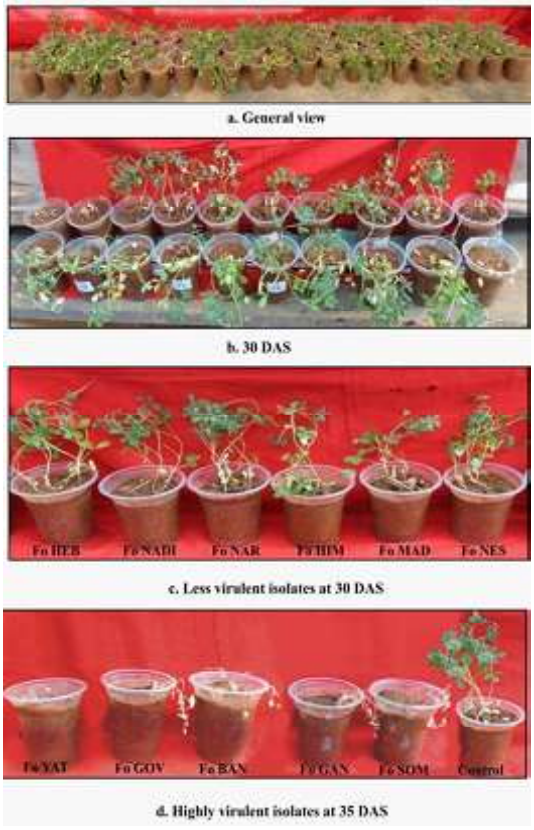

Plate 4. Pathogenic variability among isolates of *F. oxysporum*

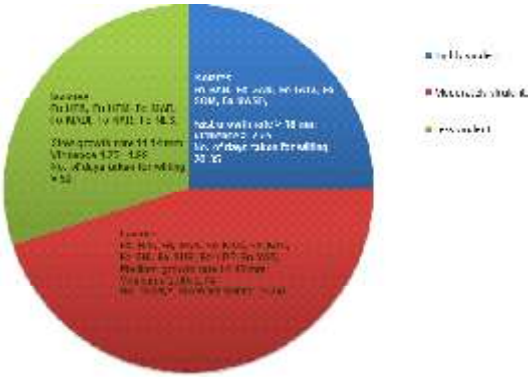

Fig. 1. Grouping isolates of *F. oxysporum* based on different characters

were moderately virulent. While isolates with low virulence index, slow growth rate and more number of days taken for wilting were less virulent.

Based on above characters the isolates were grouped into three groups. Group I consisted of five highly virulent isolates *viz.,* Fo BAN, Fo GAN, Fo GOV, Fo SOM and Fo YAT with less number of days taken for wilting ranged from 20 to 35 days, virulence index of more than 2.74, growth rate of more than 18.00 mm/day, dry mycelial weight of more than 300 mg. Group II consisted of nine moderately virulent isolates *viz.*, Fo BAI, Fo HAN, Fo HIR, Fo KAM, Fo KAV, Fo SHI, Fo SUB, Fo UPP and Fo YAD with number of days taken for wilting ranged from 35 to 50 days, virulence index of 1.99 to 2.74 and medium growth rate of 11.00 to 14 mm/day, medium dry mycelial weight of 275 to 300 mg. Group III consisted of six less virulent isolates *viz.,* Fo HEB, Fo HIM, Fo MAD, Fo MADI, Fo NAR and Fo NES with number of days taken for wilting was more than 50 days, less virulence index of 1.24 to 1.99, slow growth rate 11 to 14 mm/day, less dry mycelial weight of 250 to 275 mg (Table 5 and Fig. 1). Similar studies were conducted by Shwetha and Yashoda Hegde,2015(8) in case of *Sclerotium rolfsii* causing wilt of stevia.

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