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Rani N
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

Yashoda R Hegde
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

Correspondence
Rani N
Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India

Culture filtrate effect and pathogenic variability of isolates of *Fusarium oxysporum* on fenugreek

Rani N and Yashoda R Hegde

Abstract
Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. Although many diseases are reported in fenugreek, wilt caused by *Fusarium oxysporum* is becoming more severe in recent years. Maximum growth of *F. oxysporum* was attained on 14th day after incubation (393.33 mg) beyond which autolysis occurred. Among 20 isolates, Fo NES (89.50%) showed maximum per cent of seed germination and also root and shoot length which differed significantly with other isolates indicating low quantity of toxin production. Complete failure of seed germination was observed in isolates *viz.*, Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM with maximum toxin production. Higher seedling vigour index was noticed in Fo NES isolate (1337.91) followed by Fo NAR (1291.78). Whereas, the isolates like Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM recorded zero vigour index, indicating high quantity of toxin production. With respect to pathogenic variability, cent per cent disease incidence was recorded in all isolates of *F. 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. However, Fo HEB and Fo NES was found to be less virulent with minimum virulence index of 1.67 and took maximum days (60) for complete wilting of plants.

Keywords: fenugreek, *Fusarium oxysporum*, toxin, seedling vigour, virulence, pathogenic variability

Introduction
Fenugreek (*Trigonella foenum graecum* L.) is an important seed spice, originated in South-Eastern Europe belonging to the family Fabaceae. It is a 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 (*Fusarium oxysporum*) (Prasad *et al.,* 2014) [3]. The present study is concerned with one of the major diseases of fenugreek called wilt complex caused by the fungi like *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii.* For the first time in India, Shivpuri and Bansal (1987) [4] reported the *Fusarium oxysporum* Schlecht as the causal agent of wilt of fenugreek from Jaipur district of Rajasthan. Although many diseases are reported in fenugreek, wilt is becoming more severe in recent years. However no study has been conducted on this disease in Karnataka. 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 to know the toxin production and its effect on fenugreek seed germination and vigour of the plant.

Material and Methods

Growth phase of *F. oxysporum* in potato dextrose broth

The growth phase study was conducted on potato dextrose broth (PDB). Thirty ml of broth was added in each of the 150 ml conical flasks and sterilized at 1.1 kg/cm^2 pressure for 15 min. These flasks were allowed to cool and 5 mm disc of *Fusarium oxysporum* was inoculated to each of the conical flask. They were incubated at 27±1 ^{0}C. Each treatment was replicated thrice. A set of three flasks were harvested at every 48 hrs starting from the 2nd day of inoculation. Culture was filtered through Whatman No. 42 filter paper disc of 12.15 cm diameter, which was dried to a constant weight at 60 ^{0}C in an electrical oven, prior to filtration. The mycelial mat on the filter paper was washed thoroughly with distilled water to remove any salts likely to be associated with it. Subsequent harvesting was done at an interval of two days up to 30th day. The filter paper along with mycelial mat was dried to a constant temperature in an electrical oven at 60 ^{0}C, cooled in a desiccator and weighed immediately in an analytical electric balance and dry mycelial weight was calculated. Results were analyzed statistically.

Effect of culture filtrate of isolates of *Fusarium oxysporum* on fenugreek seed germination and seedling vigour

Crude toxin production in different isolates

Culture filtrates of *Fusarium oxysporum* was obtained by using Potato dextrose broth as basal medium. Aliquots of 30 ml of basal medium was dispensed in 100 ml conical flask and sterilized. Each flask was inoculated with 5 mm disc of the pathogen of twenty isolates were harvested on 14th day. Each treatment was replicated two times. The culture was filtered through Whatman No. 42 filter paper. The culture filtrate obtained was used for following.

Toxin production was assessed by studying the seed germination and seedling vigour as per the procedure followed by Anahosur (1976) [5]. One hundred healthy fenugreek seeds were surface sterilized in one per cent sodium hypochlorite solution and washed with sterile distilled water to remove the traces of sodium hypochlorite. The fenugreek seeds were soaked in culture filtrates of twenty isolates for 30 minutes. They were spread on moistened germination paper. Equal numbers of healthy seeds were soaked in the sterile distilled water, which served as control.

Observations on germination of fenugreek seeds were recorded after eight days. Root and shoot lengths were recorded in each treatment and seedling vigour index was calculated by following formula.

Vigour index = (Shoot length + Root length) × Germination percentage

Pathogenic variability among the isolates of *Fusarium oxysporum*

A pot experiment was conducted in the glass house of Department of Plant Pathology, University of Agricultural Sciences, Dharwad to find out virulence index of different isolates. Each treatment was replicated thrice. The gaint culture was inoculated to each pot at the rate of eight per cent. 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) [6].

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

Results and Discussion

Growth phase of *F. oxysporum* in PDB

The growth is an irreversible change in the size and number of individuals over a period of time. Every living organism has a definite growth pattern, in which it attains a maximum growth and declines thereafter. Growth of *Fusarium oxysporum* was studied on potato dextrose broth and the results were found significant. Maximum growth of *F. oxysporum* was attained on 14th day after incubation (393.33 mg) beyond which autolysis occurred. Hence, 14 days incubation period was considered as optimum growth period for further studies (Table 1). This is in conformity with the findings of Raghu (2014) [7] in chilli wilt caused by *Fusarium solani*.

Growth of different isolates of *F. oxysporum* in potato dextrose broth

The 20 isolates of *Fusarium oxysporum* were grown in potato dextrose broth and dry mycelial weight was recorded after fourteen days of incubation. The results (Table 2 and Plate 1) indicated that, the maximum dry mycelial weight was recorded in Fo BAN (387.67 mg) which was significantly on par with the isolate Fo YAT (382.66 mg), this was followed by Fo SOM (373.68 mg). Least dry mycelial weight was recorded in Fo NAR (254.67 mg) this was followed by Fo NES (260.00 mg). However Kumar and Upadhyay (2013) [8] recorded the dry mycelial weight ranged from 98.30 to 201.30 mg in *F. udum*.

Based on dry mycelial weight, the isolates were classified into three groups. Group I consisted of five isolates like Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM with dry mycelial weight more than 300 mg. Group II consisted of nine isolates like Fo KAM, Fo KAV, Fo SHI, Fo UPP, Fo YAD, Fo HIR, Fo SUB, Fo BAI, and Fo HAN with dry mycelial weight ranging from 275-300 mg. Group III consisted of six isolates *viz.*, Fo HEB, Fo MADI, Fo NAR, Fo HIM, Fo MAD and Fo NES with dry mycelial weight ranging from 250-275mg.

Effect of culture filtrate of isolates of *F. oxysporum* on fenugreek seed germination and seedling vigour

Table 3 indicated that, the effect of culture filtrate on the vigour index was found statistically significant in all the isolates of *F. oxysporum*. A preliminary indication of toxin production by any fungus i*n vitro* has been provided by a number of bioassay methods of plant cutting (Naik *et al*., 1996) [9] seed germination bioassay, root and shoot elongation bioassay and spore germination inhibition method (Anahosur, 1976[5]; Venkataravanappa, 2002) [10]. The effect of culture filtrates were studied on seed germination, shoot and root length of fenugreek seedlings.

Seed germination

Culture filtrate of all isolates of *F. oxysporum* affected the germination of fenugreek seeds. Among the isolates, Fo NES (89.50%) was statistically significant and superior to all other isolates and which showed maximum per cent of seed germination and also root and shoot length, indicating low quantity of toxin production. This was followed by Fo HEB (77.00%) showed maximum seed germination which differed significantly with other treatments. Cent per cent inhibition of seed germination was recorded in the isolates Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM indicating maximum crude toxin production, least inhibition of seed germination was observed in Fo NES (89.50%) and Fo HEB (77.00%).

Shoot and root length

Complete inhibition (0.00 cm) of shoot and root length was noticed in Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM isolates. The least shoot (1.88 cm) and root length (2.15 cm) were recorded in Fo YAD and Fo HER isolates respectively. Maximum shoot length (9.97 cm) was recorded in Fo HIM which was followed by Fo NAR (9.68 cm). The maximum root length (8.37cm) was recorded in isolate Fo MADI followed by Fo HIM (8.14cm).

Vigour index

Seedling vigour index was calculated and presented in the table 3. All the isolates reduced the vigour index drastically and complete reduction (100%) was recorded in Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM which was statistically superior to all other isolates in inhibiting of per cent seed germination with minimum vigour index. This was followed by Fo KAM with moderate vigour index (57.95). Higher seedling vigour index was noticed in Fo NES isolate (1337.91) followed by Fo NAR (1291.78) While, the complete zero vigour index was noticed in Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM indicating high quantity of toxin production by these isolates.

Based on vigour index the isolates were classified into three groups. Group I consisted of five isolates like Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM with vigour index 0. Group II consisted of nine isolates *viz.*, Fo KAM, Fo KAV, Fo SHI, Fo UPP, Fo YAD, Fo HIR, Fo SUB, Fo BAI, and Fo HAN with vigour index ranging from 0-1000. Group III consisted of six isolates (Fo HEB, Fo MADI, Fo NAR, Fo HIM, Fo MAD and Fo NES.) with vigour index more than 1000. The present findings clearly indicated that, active metabolites released by isolates of *F. oxysporum* in culture filtrate was toxic to seed germination of fenugreek and group I isolates produced (virulent isolate) more of the toxic metabolites than group III less virulent isolates. Similarly Kaushal and Sharma (2006) [11] reported that *Cercosporin* toxin was found to have toxic effect on seed germination, root and shoot length of greengram and blackgram (Table 3 and Plate 2).

Pathogenic variability among isolates of *Fusarium oxysporum*

Degree of pathogenic variability of the *Fusarium oxysporum* was studied on susceptible variety DFC-26. All the twenty isolates were inoculated to fenugreek to study the variation in virulence. Observations on per cent disease incidence and latent period were recorded and presented in the Table 4 and Plate 3. 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 were less virulent with minimum virulence index of 1.67 and required maximum period (60 days) for wilting of plants. Isolates with maximum virulence index required less period for wilting of plants. Cent per cent disease incidence was recorded in all different isolates of *F. oxysporum* (Fig. 3).

Based on virulence index, the isolates were classified into three groups. Group 1 consisted of five virulent isolates (Fo YAT, Fo GOV, Fo BAN, Fo GAN and Fo SOM) with high virulence index (>2.75). Group II consisted of nine moderately virulent 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 less virulent 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 cleared that Group 1 isolates were highly virulent than other isolates. Present findings are in conformity with the findings of Joshi *et al.* (2013) [12] and Nirmaladevi and Srinivas (2012) [13].

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

In the present study virulence was related to number of days taken for wilting, virulence index, crude toxin production, growth rate, dry mycelial weight 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, dry mycelial weight and vigour index. The isolates with less number of days taken for wilting, high virulence index, maximum dry mycelial weight and low vigour index were highly virulent. Whereas isolates with medium number of days taken for wilting, moderate virulence index were moderately virulent. Isolates with more number of days taken for wilting and low virulence index were less virulent.

Based on above characters the isolates of *Fusarium oxysporum* 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 number of days taken for wilting varied from 20 to 35 days, virulence index of more than 2.74 and vigour index of 0.00 indicating higher quantity of crude toxin production. Group II consisted of nine moderately virulent isolates *viz.*, Fo BAI, Fo HAN, Fo HIR, FoKAM, Fo KAV, Fo SHI, Fo SUB, FoUPP and Fo YAD with number of days taken for wilting varied from 35 to 50 days, virulence index of 1.99 to 2.74, medium dry mycelial weight of 275 to 300 mg and vigour index of 0 to 1000. 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 (>50 days, less virulence index of 1.24 to 1.99, less dry mycelial weight 250 to 275 mg and vigour index of more than 1000 indicating the less quantity of crude toxin production (Fig. 1). Highly virulent isolates produced the more toxic metabolites than less virulent isolates and these variations among isolates of *F. oxysporum* may be attributed to genetic variation. Similar studies had been conducted by Venkataravanappa (2002) [10].

Table 1: Growth phase of *F. oxysporum* in potato dextrose broth

Incubation period (days)	Dry mycelial weight (mg)
2	183.33
4	203.33
6	230.00
8	260.00
10	323.33
12	360.00
14	393.33
16	373.33
18	353.33
20	343.33
22	330.00
24	320.00
26	290.00
28	250.00
30	226.67
S.Em.±	3.55
CD at 1%	13.80
CV %	2.08

Table 2: Dry mycelial weight of different isolates of *Fusarium oxysporum* in potato dextrose broth

Isolates	Dry mycelial weight (mg)
Fo BAI	297.33
Fo BAN	387.67
Fo GAN	371.67
Fo GOV	365.00
Fo HAN	289.67
Fo HEB	264.00
Fo HEM	270.65
Fo HER	285.66
Fo KAM	283.00
Fo KAV	287.32
Fo MAD	265.00
Fo MADI	263.33
Fo NAR	254.67
Fo NES	260.00
Fo SHI	299.67
Fo SOM	373.68
Fo SUB	287.66
Fo UPP	293.00
Fo YAD	286.63
Fo YAT	382.66
S.Em.±	2.05
CD at 1%	7.83
CV %	1.17

Table 3: Effect of culture filtrate of isolates of *F. oxysporum* on seed germination and seedling vigour of fenugreek

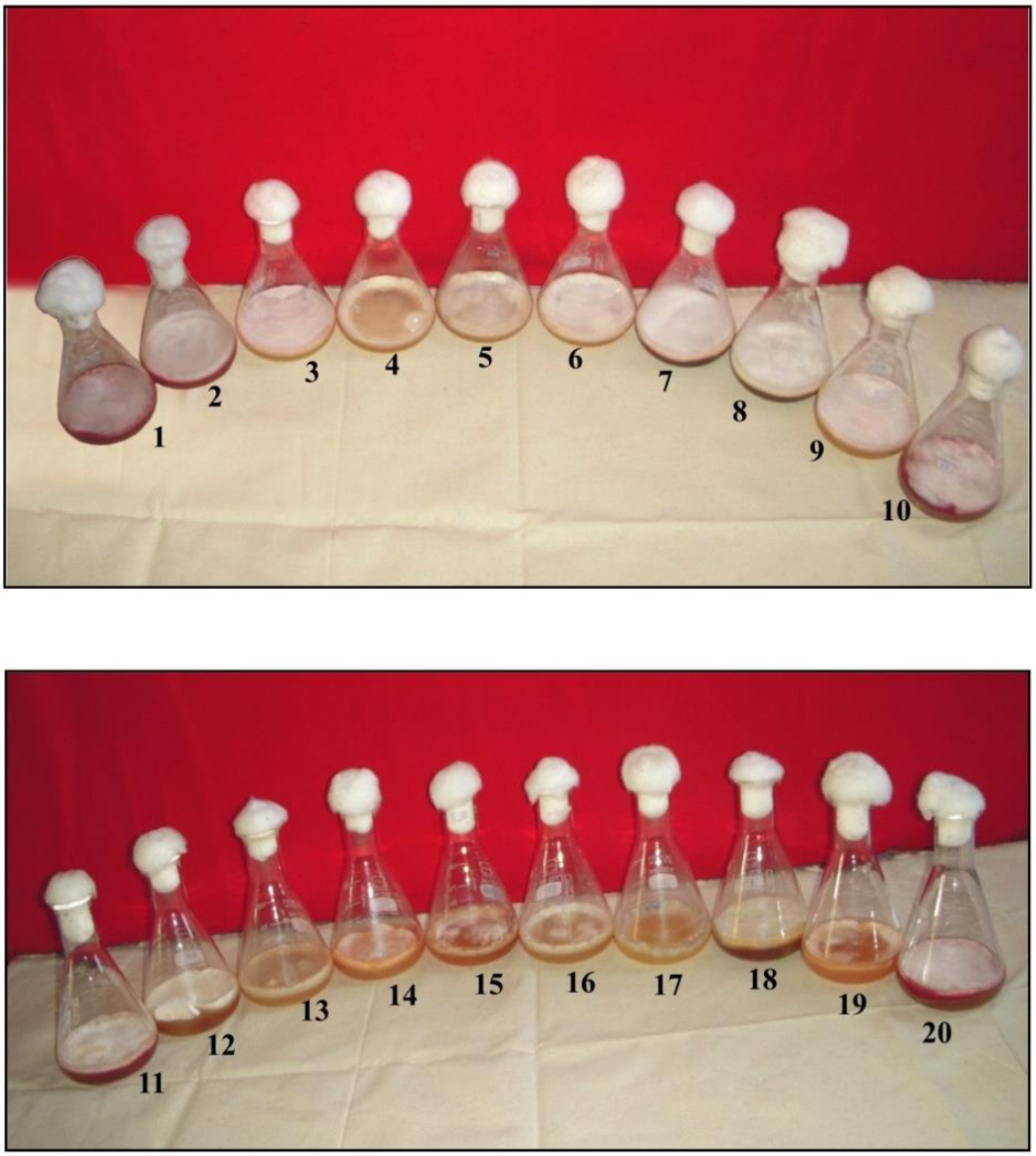
Isolates	Seed germinaton (%)	Shoot length(cm)	Root length (cm)	Vigour index
Control	100.00 (0.00)*	10.76 (0.00)	11.02 (0.00)	2178.00 (0.00)
Fo BAI	24.50 (75.50)	5.80 (46.09)	4.73 (57.07)	257.92 (88.16)
Fo BAN	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
Fo GAN	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
Fo GOV	0.00 (100.00)	0.00 (100.00)	0.00 (100.0)	(0.00 (100.00)
Fo HAN	69.50 (30.50)	8.09 (24.81)	4.22 (61.68)	855.53 (60.71)
Fo HEB	77.00 (23.00)	8.41(21.84)	7.53 (31.65)	1227.15 (43.64)
Fo HIM	65.50 (34.50)	9.75 (9.39)	8.14 (26.15)	1171.46 (46.22)
Fo HIR	31.00 (69.00)	5.64 (47.63)	2.15 (80.49)	241.34 (88.92)
Fo KAM	9.50 (90.50)	3.88 (63.94)	2.22 (79.82)	57.95 (97.34)
Fo KAV	32.00 (68.00)	7.02 (34.76)	7.06 (35.90)	450.56 (79.31)
Fo MAD	74.50 (25.50)	7.09 (38.15)	6.53 (40.68)	1014.31 (53.41)
Fo MADI	76.50 (23.50)	7.51 (30.25)	8.37 (24.04)	1213.78 (44.28)
Fo NAR	75.00 (25.00)	9.68 (10.05)	7.55 (31.43)	1291.78 (40.68)
Fo NES	89.50 (10.50)	8.71 (19.06)	6.24 (43.34)	1337.91 (38.56)
Fo SHI	45.00 (55.00)	8.06 (25.09)	6.97 (36.75)	676.30 (68.93)
Fo SOM	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
Fo SUB	30.50 (69.50)	3.75 (65.15)	3.23 (70.70)	212.68 (90.23)
Fo UPP	23.00 (77.00)	6.42 (40.34)	6.33 (42.55)	293.22 (86.53)
Fo YAD	64.00 (36.00)	1.88 (82.53)	4.68 (57.52)	419.84 (80.72)
Fo YAT	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
S.Em.±	1.10	0.59	1.43	2.71
CD at 1%	2.58	2.35	5.75	3.69
CV %	4.41	1.36	3.31	1.72

* Per cent reduction over control

Table 4: Pathogenic variability among isolates of *F. 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



1	Fo YAT	6	Fo MADI	11	Fo BAN	16	Fo BAI
2	Fo GOV	7	Fo NAR	12	Fo GAN	17	Fo HAN
3	Fo HEB	8	Fo SHI	13	Fo HIR	18	Fo MAD
4	Fo KAM	9	Fo UPP	14	Fo HIM	19	Fo NES
5	Fo KAV	10	Fo YAD	15	Fo SUB	20	Fo SOM

Plate 1: Growth of different isolates of *F. oxysporum* in PDB

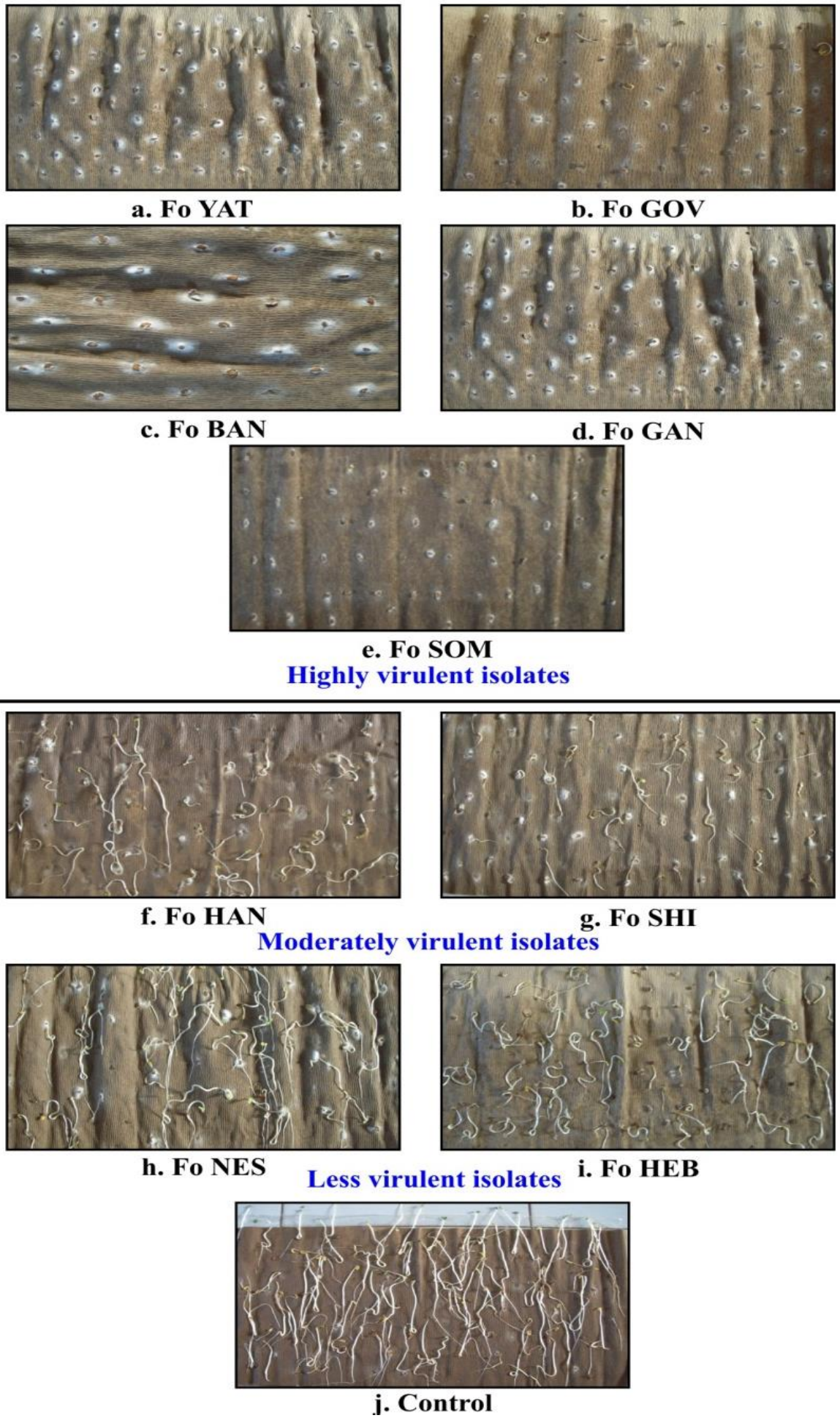
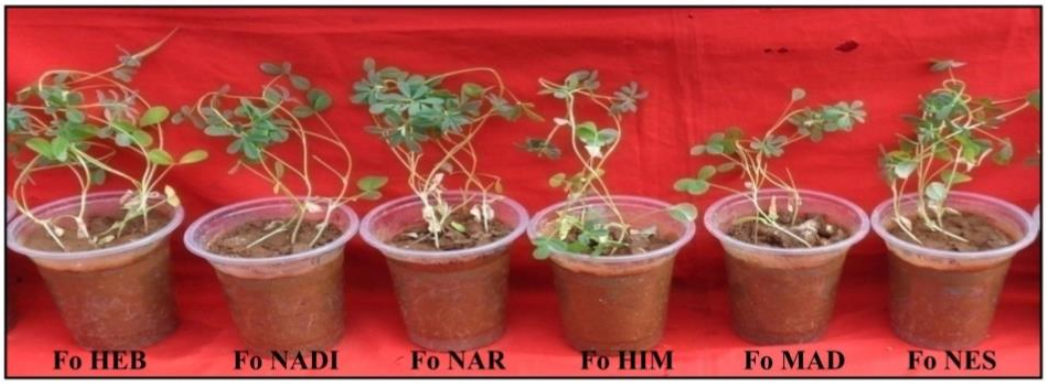


Plate 2: Effect of culture filtrate of isolates of *F. oxysporum* on seed germination and seedling vigour of fenugreek

a. General view

b. 30 DAS



c. Less virulent isolates at 30 DAS

d. Highly virulent isolates at 35 DAS

Plate 3: Pathogenic variability among isolates of *F. oxysporum*

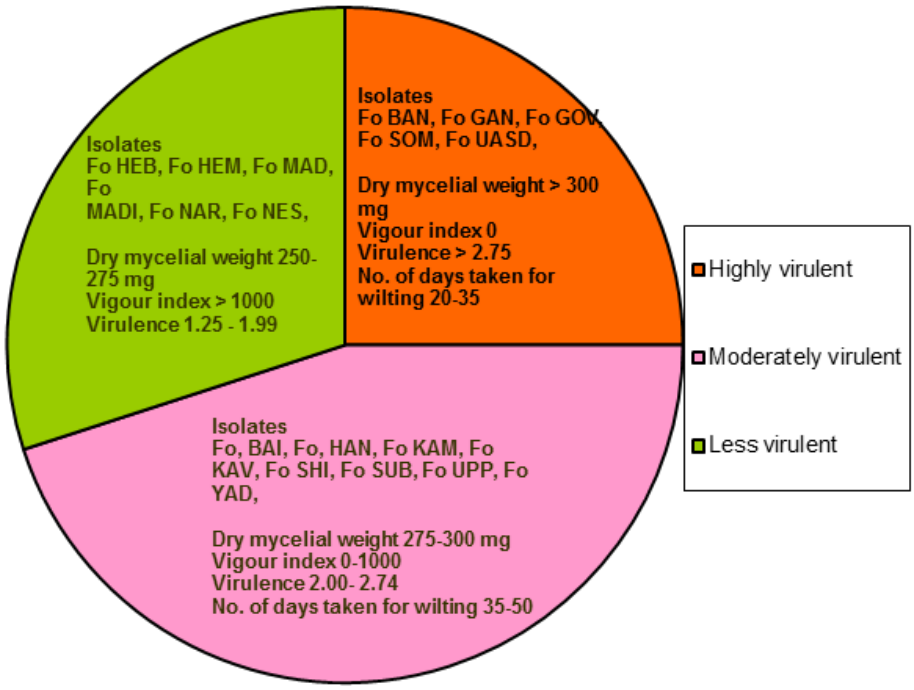


Fig 1: Grouping isolates of *F. oxysporum* based on different characters

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