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RESEARCH ARTICLE

Prevalence, symptomatology, pathogenicity and nutritional requirements of *Fusarium oxysporum* f.sp.*phaseoli* causing Fusarium yellows of French bean in Thandikudi

■ **S. ANUSUYA, M. MUTHAMILAN, N. REVATHY AND M. ANANTHAN**

SUMMARY

Fusarium yellows /wilt caused by *Fusarium oxysporum* f.sp.*phaseoli* are one of the serious diseases in French bean in Thandikudi and Kodaikanal hills of Dindugal district was found to prevalent in all French beans growing areas under survey with maximum of 71.25 per cent incidence and 65.88 per cent disease severity at Thandikudi village of Dindugal district in Tamil Nadu. The pathogenicity test was conducted by artificial inoculation of test fungus into the young two leaf stage of French bean and the typical symptom were produced after twelve days after inoculation. Among the different solid and liquid media tested oat meal agar potato dextrose agar medium supported growth of the casual fungus. Fungus could grow well at a pH 4.0 and 5.0, respectively. This pathogenic fungus grew maximum when basal medium was supplemented with lactose and dextrose as carbon sources showed maximum growth and with potassium nitrate and sodium nitrate as nitrogen sources.

Key Words : *Fusarium oxysporum* f.sp.*phaseoli*, Pathogenecity, Thandikudi, Symptoms

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French bean (*Phaseolus vulgaris*) is an annual and herbaceous plant grown worldwide for its edible beans, used both for the tender vegetables, and are shelled green beans and dry beans which are referred to as beans. In India, vegetables occupy about an area of 9,068.30 lakhs ha and with the production of about 1,59,511.29 lakhs tonnes among which beans vegetable occupy an area of about 1,25.12 lakhs ha and with the production of about 1,292.33 lakhs tonnes. This vegetable is largely grown in Andra Pradesh, Jharkhand,

Maharashtra, Karnataka, Odisha, Uttarakhand, and Tamil Nadu states. It has the following curative effect on the diseases like diabetes, reduces oedema, sciatica, chronic rheumatism, kidney and bladder problems. Crop is susceptible to many fungal diseases, among these the Fusarium yellows/wilt, caused by *Fusarium oxysporum* (Schlecht) f.sp. *phaseoli* Kendrick and Snyder is an important disease of common beans (*P. vulgaris*). In the 1930s the disease re-appeared in the same localities, but disappeared when the affected fields were planted with other crops (Kendrick and Snyder, 1942). Despite the importance of the disease, not much is known about the ecology of the pathogen, its survival and inoculum dynamics in soil. Buy keeping in view the seriousness of the disease and importance of French bean crop in Tamil Nadu hills, it was thought to study the diseases in detail.

MATERIAL AND METHODS

Survey and disease assessment :

A survey was conducted during July 2013 on the occurrence of Fusarium yellows/wilt disease in major French bean growing areas of Tamil Nadu, *viz.*, Dindigul (Thandikudi, Perumparai, Kodaikanal) and Ooty (Jegathla, Thenadukambai) and from each field 100 plants were selected at random and the number of Fusarium wilt infected plants was recorded.

Isolation of the pathogen :

The pathogen inciting wilt in French bean was isolated from samples by tissue segment method and infected leaf tissue pieces were surface sterilized with 0.1 per cent mercuric chloride and rinsed with three times in sterile water, were plated on potato dextrose agar in sterile Petri plates and incubated at room temperature (28±2°C) for 12 days. The fungus was further purified by single spore isolation and the purified isolates were maintained on PDA slants.

Pathogenicity in glass house :

Fusarium oxysporum f. sp. *phaseoli* isolates were multiplied in sand-maize medium. The substrate containing of sand and maize powder in the ratio of 9:1 and each was inoculated with two 9 mm culture discs of the fungus. These were incubated at room temperature (28 ± 2°C) for 15 days and used as source of inoculum. The pathogenicity of the fungus was confirmed by Koch's postulates. Earthen pots of five kg of pot mixture and inoculated by mixing 10 g inoculum of fungus multiplied on sand maize medium. French bean seeds were sown in pots with proper control. The pots were maintained in glasshouse by uniform and judicious watering. The plants were observed for development of disease symptom and the pathogen was reisolated from the artificially inoculated plants and the characters were compared with the original ones.

Pathogenicity *in vitro* :

The 7 to 10 day old seedling were taken and grown under controlled condition in a Petri dish under *in vitro* condition. Above the seedling, the cotton immersed in spore suspension from 12 old cultures were placed near the root region and incubated at room temperature for 12 days. Then it was observed for the development of symptom as that of the original ones.

Effect of media :

To ascertain the suitability of different solid and liquid media on the vegetative growth of *Fusarium oxysporum* f.sp.*phaseoli* seven different liquid media *viz.*, Potato dextrose, Oat meal, Carrot dextrose, Beetroot dextrose, Czapek's Dox, Rose bengal and Richard's media were used. The sterilized and warm medium was poured into sterilized Petri dishes (9 cm) in 20 ml quantities and allowed to solidify and same medium was taken in 100 ml quantities in 250 ml conical flask. The conical flasks and Petri plate were inoculated each with 12 days old 9 mm culture disc of the isolates and incubated at room temperature (28±2°C) for 12 days with three replications. The radial growth of the mycelium and mycelial dry weight was measured.

Effect of carbon sources and nitrogen sources :

Sucrose in Czapek's Dox agar medium was substituted with different carbon sources *viz.*, dextrose, glucose, lactose, fructose, and maltose were used separately in solid and liquid media. Sodium nitrate in Czapek's Dox agar medium was substituted with different nitrogen sources *viz.*, ammonium nitrate, ammonium sulphate, urea, peptone, and potassium nitrate were used separately in solid and liquid media with three replications. The carbon and nitrogen sources were added in a way so as to provide an equal amount of carbon. Similarly, inoculation and observation followed as mentioned above.

Effect of pH :

The pH of the Potato dextrose agar medium was

adjusted with N/10 HCL or NaOH using a pH meter before addition of agar and autoclaved to get a pH of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with three replications. Similarly, inoculation and observation followed as mentioned above.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Survey and disease assessment :

French bean plants showing symptom of Fusarium yellows/wilt disease caused by *Fusarium oxysporum* (Schlecht) f.sp. *phaseoli* (Kendrick and Snyder, 1942) were observed and examined very closely in Thandikudi, Kodaikanal of Dindigul district and Jegathla and Conoor in Ooty district of Tamil Nadu. The maximum of 71.25 per cent Fusarium yellows disease incidence was observed in the plants collected from Thandikudi village. Similar trends were noticed by earlier workers while working with various plant diseases caused by *Fusarium oxysporum* f.sp. *phaseoli* (Singleton *et al.,* 1992) (Table 1).

Table 1 : Incidence of Fusarium yellows of French bean caused by *Fusarium oxysporum* f.sp. *phaseoli* in different areas of Tamil Nadu

Place of collection	District	Diseases incidence (%)
Thandikudi	Dindigul	71.25
Jegathla	Ooty	30.54
Perumparai	Dindigul	62.65
Thenadukambai	Ooty	45.88
Kodaikanal	Dindigul	40.33
C.D. (P=0.05)		1.50

Symptomatology and fungal description :

The disease symptom developed as a yellowing within leaves with brown spot surrounded by yellow halo, drying of basal leaves and slowly extended upwards and whole plant starts to wilting. The affected fruits were shrunk. In severe cases, the whole plant starts yellowing and drying, dropping of leaves were observed in final stage with the reduction in the yield or with no yield and the pathogen mycelial growth was white in colour which differed slightly from the other isolates and the spore characters were also studied (Fig., 1 and 2).

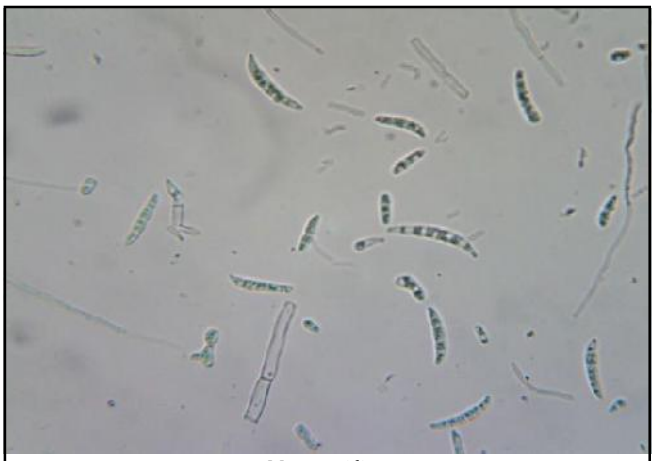
Pathogenicity of the *Fusarium oxysporum* f. sp. *phaseoli* on French bean :

Among the five isolates of *Fusarium oxysporum*

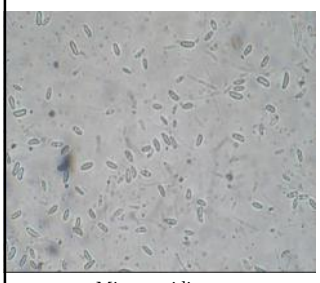
Yellowing of matured leaf

Yellowing of bottom most leaf nearest to soil surface

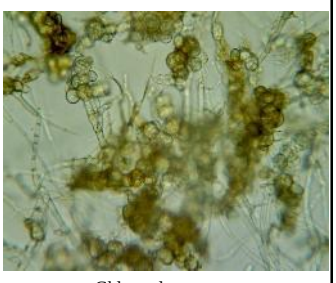
Fig. 1: Yellowing of matured leaf and yellowing of bottom most leaf nearest to soil surface



Macroconidia



Microconidia



Chlamydospores

Fig. 2 : Macroconidia, microconidia and chlamydospores

f.sp. *phaseoli*, the isolate from Thandikudi was fast in growth with 65.88 per cent, isolate was used for pathogenicity *in vitro*. The results on pathogenicity of *Fusarium oxysporum* f. sp. *phaseoli* using 20-day-old seedling furnished *in vitro* and *in vivo* with 7 day old seedling. Till sixth day, symptoms were not noticed in seedlings inoculated with or without spore suspension *in vitro* and sand maize media *in vivo*. Symptoms developed

Table 2 : Effect of different solid and liquid media on the mycelial growth of isolates of *Fusarium oxysporum* f.sp. *phaseoli in vitro*

Sr. No.	Isolates	Mycelial growth in (cm) and dry weight in (g)* at 12 DAI							
		Oats agar medium	Potato dextrose medium	Beet root dextrose medium	Carrot dextrose medium	Czapek's dox agar	Richard's agar	Rose Bengal agar	Mean
1.	I_1	8.50 (1.73)	8.15 (1.51)	8.44 (1.41)	7.66 (1.46)	7.00 (0.55)	7.20 (0.66)	6.10 (0.46)	7.57 (1.11)
2.	I_2	7.53 (1.51)	7.10 (1.38)	7.16 (1.38)	7.16 (1.09)	6.10 (0.34)	6.60 (0.47)	5.55 (0.22)	6.74 (0.91)
3.	I_3	8.42 (1.75)	7.57 (1.47)	7.39 (1.26)	7.10 (1.26)	6.67 (0.43)	6.90 (0.65)	6.12 (0.41)	7.16 (1.03)
4.	I_4	8.10 (1.56)	7.43 (1.58)	7.10 (1.35)	6.88 (1.08)	6.16 (0.36)	6.96 (0.54)	5.70 (0.31)	6.86 (0.96)
5.	I_5	7.60 (1.68)	7.30 (1.41)	7.17 (1.33)	7.17 (1.28)	6.00 (0.47)	6.57 (0.64)	5.80 (0.24)	6.80 (1.00)
Mean		8.03 (1.64)	7.51 (1.47)	7.45 (1.34)	7.19 (1.23)	6.38 (0.43)	6.84 (0.59)	5.85 (0.32)	-

DAI = Days after inoculation *Dry weight and mean of three replications

C.D. (P= 0.05)		C.D. (P= 0.05)	
Isolates	- 0.09	Isolates	- 0.05
Media	- 0.10	Media	- 0.06
Isolates × Media	- 0.24	Isolates × Media	- 0.14

Table 3 : Effect of different carbon sources in solid and liquid media on the mycelial growth of isolates of *Fusarium oxysporum* f. sp. *phaseoli in vitro*

Sr. No.	Isolates	Mycelial growth in (cm) and dry weight in (g)* at 12 DAI							
		Sucrose	Glucose	Fructose	Dextrose	Maltose	Lactose	Control	Mean
1.	I_1	7.46 (3.45)	6.96 (2.40)	7.86 (3.46)	8.40 (4.13)	8.16 (4.00)	8.96 (5.26)	5.16 (1.23)	7.56 (3.41)
2.	I_2	7.00 (2.26)	6.20 (1.00)	7.26 (2.66)	8.00 (2.86)	7.20 (2.86)	8.23 (2.86)	4.10 (1.83)	6.85 (2.33)
3.	I_3	7.30 (3.33)	6.86 (2.00)	7.50 (3.76)	8.00 (4.23)	7.73 (3.80)	8.66 (3.66)	4.33 (0.96)	7.19 (3.10)
4.	I_4	7.23 (3.06)	6.26 (1.86)	7.23 (3.33)	8.00 (3.33)	7.40 (3.46)	8.46 (3.23)	4.80 (1.33)	7.05 (2.80)
5.	I_5	7.16 (3.13)	5.80 (1.56)	7.33 (3.70)	8.00 (3.00)	7.30 (2.96)	8.10 (2.90)	4.63 (1.50)	6.90 (2.78)
Mean		7.23 (3.04)	6.41 (1.76)	7.43 (3.38)	8.08 (3.51)	7.55 (3.41)	8.48 (3.58)	4.60 (1.37)	-

*Dry weight and Mean of three replications DAI = Days after inoculation

C.D. (P= 0.05)		C.D. (P= 0.05)	
Isolates	- 0.15	Isolates	- 0.04
Different carbon sources	- 0.13	Different carbon sources	- 0.05
Isolates × Different carbon sources	- 0.35	Isolates × Different carbon sources	- 0.11

Table 4 : Effect of different nitrogen sources in solid and liquid media on the mycelial growth of isolates of *Fusarium oxysporum* f. sp. *phaseoli in vitro*

Sr. No.	Isolates	Mycelial growth in (cm) and dry weight in (g)* at 12 DAI							
		Peptone	Potassium nitrate	Sodium nitrate	Ammonium nitrate	Ammonium sulphate	Urea	Control	Mean
1.	I_1	4.33 (2.60)	8.83 (5.83)	8.50 (4.60)	7.30 (3.46)	7.83 (3.60)	7.76 (4.66)	3.90 (0.98)	6.92 (3.67)
2.	I_2	3.90 (1.76)	8.26 (4.26)	7.86 (3.40)	6.63 (2.73)	6.70 (2.43)	7.20 (3.55)	3.33 (0.81)	6.26 (2.70)
3.	I_3	4.30 (2.43)	8.73 (5.60)	8.26 (4.53)	6.96 (3.33)	7.63 (3.40)	7.53 (4.56)	3.93 (0.89)	6.76 (3.53)
4.	I_4	4.22 (2.36)	8.60 (5.40)	8.13(4.26)	6.75 (3.03)	7.40 (3.11)	7.40 (4.26)	3.63 (0.83)	6.59 (3.32)
5.	I_5	4.14 (2.30)	8.46 (4.17)	8.06 (4.03)	6.70 (2.96)	7.10 (3.00)	7.33 (3.53)	3.46 (0.82)	6.46 (3.50)
Mean		4.17 (2.29)	8.58 (5.16)	8.16 (4.16)	6.83 (3.10)	7.33 (3.11)	7.44 (4.11)	3.65 (0.86)	-

*Dry weight and Mean of three replications DAI = Days after inoculation

C.D. (P= 0.05)		C.D. (P =0.05)	
Isolates	- 0.09	Isolates	- 0.05
Different nitrogen sources	- 0.11	Different nitrogen sources	- 0.06
Isolates × Different nitrogen sources	- 0.26	Isolates × Different nitrogen sources	- 0.14

twelve days after inoculation (DAI) and the lesion length increased over time. Similar results on the pathogenicity of *F.o.* f.sp. *phaseoli* have also been reported on beans from Brazil and crucifers (Bosland and Williams, 1987). The pathogenicity in glasshouse was further confirmed in glasshouse which recorded the maximum of 65.88 per cent of Fusarium yellows disease incidence when compared to other isolates (Fig. 3). Infected plants exhibited symptoms of yellowing of leaves with brown spot, drying of leaves followed by wilting of the plants. The pathogen was reisolated and Koch's postulates were fulfilled.

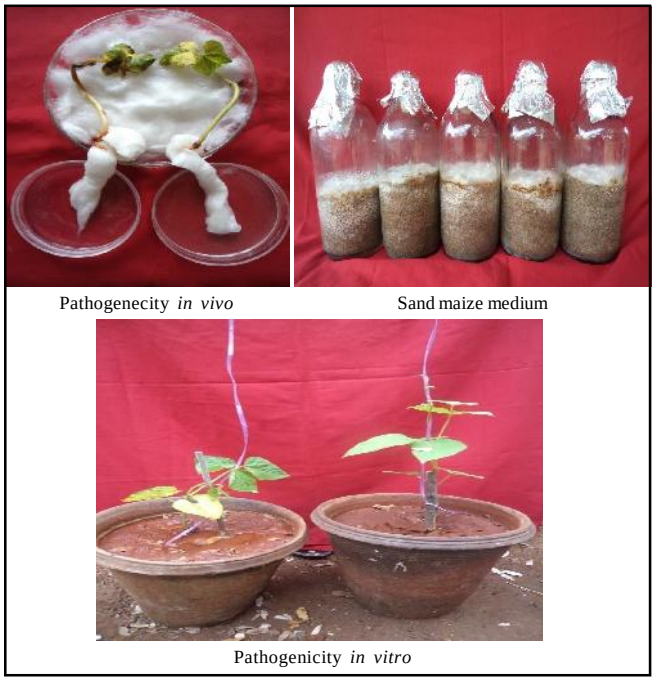


Fig. 3 : Pathogenecity *in vivo*, sand maize medium and Pathogenicity *in vitro*

Effect of media, carbon and nitrogen sources :

Among the media tested vegetative growth of the pathogen *Fusarium oxysporum* f sp.*phaseoli* and data were received on diametric mycelial growth and dry mycelial weight (Table 2). Which revealed that maximum diametric growth was observed in oats agar followed by potato dextrose agar medium 8.03 and 1.64g. The results were similar to the findings of Ingole (1995) and Jamaria (1972), the mycelial growth of *F.o.* f.sp. *phaseoli* was best in oat meal agar and PDA medium. It corroborates the findings of the mycelial dry weight of *Fusarium oxysporum* f. sp. *chrysanthemi* was maximum in oat meal agar and potato dextrose broth (Singh and Kumar, 2011). The carbon sources which recorded maximum growth and mycelial diameter was lactose with 8.48 cm and 3.58g followed by dextrose. The fungus may utilize certain complex form of carbon compound and break into simple form, which may be readily metabolized (Bais *et al.*, 1970). Nitrogen sources tested potassium nitrate followed by sodium nitrate recorded the maximum growth and mycelial diameter as 8.58 cm and 5.16g this above data were recorded after12 DAI (Ramaprasad Shresti, 2005) (Table 3 and 4).

Effect of pH :

The different pH levels tested, *Fusarium oxysporum* f. sp. *phaseoli* was found to grow well at pH 4.0 favoured excellent growth of the pathogen since hill soil is acidic in nature and recorded the maximum mean mycelial growth of 7.57cm and it was significantly different from other pH levels. The optimum pH favourable for the growth of *Fusarium oxysporum* f. sp. *ciceri* was between 4.5 to 7.0 (Table 5).

Table 5 : Effect of different pH level on the growth *Fusarium oxysporum* f.sp. *phaseoli*

Sr. No.	Isolates	Mycelial growth at 12 DAI (cm)* pH levels						
		4.0	5.0	6.0	7.0	8.0	9.0	Mean
1.	I_1	8.52	8.00	6.87	8.10	7.52	7.22	7.70
2.	I_2	6.55	6.27	5.23	6.66	5.70	5.60	6.00
3.	I_3	8.00	7.58	6.55	7.74	7.16	6.89	7.32
4.	I_4	7.80	7.14	6.00	7.48	6.59	6.42	6.90
5.	I_5	7.00	6.59	5.88	6.95	6.25	6.00	6.44
Mean		7.57	7.11	6.10	7.38	6.64	6.42	-

*Mean of three replications DAP = Days after inoculation

C.D. (P= 0.05)

Isolates - 0.12

pH level - 0.14

Isolates × pH level - 0.31

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