

Cultural and morphological characterization of *Fusarium solani* causing Wilt of French bean in Maharashtra

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Five different isolates of *Fusarium solani* causing wilt of French bean were collected from different parts of Maharashtra. Morphological and cultural characterizations of the isolates were done along with their response to temperature and pH. Different temperature ranging from 0 to 40°C, pH from 4 to 8 and different media namely Potato dextrose agar (PDA), Richard's agar, Czapek's agar and Ashby's agar were used. Favourable temperature range for growth and sporulation for all the isolate was found to be 25 to 30°C. Thermal death point of *F. solani* was found to be 53°C. Slightly acidic medium with a pH of 5.5 to 6.5 supported profuse growth of the pathogen. In case of culture media Richard's agar was observed to be the best medium suited for growth of *F. solani*. Size of microconidia and macroconidia ranged from 5-12.4×2-3.6 µm and 26.5-48×3-4.7 µm respectively. In case of fungicidal efficacy, Carbendazim 50WP @0.1% was found to be the most effective against all the isolates. Pathogenic variation study was carried out using different wilt isolates under glass house conditions followed by field screening of French bean germplasm to locate resistant source(s) against the disease.

Key words: French bean, wilt, *Fusarium solani*, pathogenic variations

INTRODUCTION

French bean commonly known as common bean or kidney bean or snap bean (Krasu and Oz, 2011) is the third most important food legume, following soybean and peanut. It is an excellent source of protein, carbohydrate and soflavonoids. *Phaseolus vulgaris* contributes 90% of cultivated species worldwide. In India it is mainly grown in Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh and Tamilnadu. It was grown in an area of 1.34×10⁵ ha with the production of 11.64×10⁵ t (Anon., 2014-15). Wilt caused by *Fusarium solani* is one of the most complex root rots and vascular disease, affecting dry bean production worldwide (Deeksha *et al*, 2009). Yellowing and wilting of leaves are the common symptoms of this disease (Fig1a,b). The disease is difficult

control because the pathogen can persist, indefinitely in the form of mycelium in infected plant debris or in the form of chlamyospore in the soil. This disease is an economic problem in most growing locations, which shows up to 70% yield loss (Saremi *et al*, 2007; Naseri, 2008). Fungicides and tolerant varieties are the major tools to manage the disease but limited success have been obtained to manage the disease. The present study was aimed at characterizing the fungal pathogen and studying its variability along with its sensitivity to different fungicides.

MATERIALS AND METHODS

Collection, isolation, and pathogenicity of isolates

Five wilt infected samples of French bean were collected from different regions of Maharashtra i.e. Mahabaleshwar, Ganeshkhind, Koregaon, Haveli

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and Research farm of College of Agriculture, Pune. They were isolated separately under aseptic conditions and multiplied on potato dextrose agar (PDA) and named as Isolate I to Isolate V respectively. Small pieces of infected tissues of stem and root were successively washed with sodium hypochlorite solution and three times with sterile distilled water and incubated in PDA plates at $25 \pm 0.2^\circ\text{C}$ for 4 days. The isolates were purified by single hyphal tip method and preserved at 4°C on PDA slants for future use. Isolates were initially identified according to the morphological and microscopic characters and its selectivity for French bean plant was established through standard pathogenicity test. The soil inoculation method was followed for testing the pathogenicity of the isolates. Twenty earthen pots were filled with sick soil (soil previously inoculated with *Fusarium* isolates and maintained) and two pots were filled with only sterilized un-inoculated soil i.e. control. These isolates were tested on susceptible variety Kanpur-3. Thirty seeds per pot were sown and pots were kept in glass house. The observations were recorded up to 6 weeks after inoculation.

Study of cultural and morphological characters of *F. solani*

The isolated fungus was grown on different media viz. Czapek's agar, Ashby's media, PDA, Richard's agar in order to study its growth characters and ability to sporulate on different media. The above mentioned media were prepared, autoclaved and poured in Petri plates. Four replicates for each medium were maintained. Plates were then inoculated with the uniform bit (5 mm diameter) of the 7 day old culture of *Fusarium solani* with the help of sterilized cork borer and incubated at $27 \pm 1^\circ\text{C}$ for 7 days. Observation regarding growth characters and sporulation were recorded.

Effect of temperature on growth of *F. solani*

The effect of temperature on the growth and sporulation was studied on PDA medium. Triplicate plates were poured for each temperature and inoculated with a uniform bit of fungus of 7 days old culture. Plates were incubated at different temperature ranging from 5°C - 40°C for 7 days and observations on the colony diameter and sporulation were recorded 7 days after the inoculation.

Study on thermal death point of *F. solani*

For the determination of thermal death point, 10ml

of Richard's broth were poured in test tubes and inoculated with uniform mycelia bit of 7 days old culture of *F. solani*. A set of inoculated triplicate tubes were then subjected to 35°C - 55°C temperature in hot water bath for 10 minutes. Untreated inoculated tube with Richard's broth was kept as a control of each temperature. After the exposure of the tubes for 10 minutes, they were immediately immersed in water and then incubated at $27 \pm 1^\circ\text{C}$ for a period of 5 days to record fungal growth and sporulation. Initially from 35°C to 50°C observations were recorded at 5°C intervals and after 50°C observations at 1°C intervals were recorded.

Effect of pH on the growth of *F. solani*

Stock solution of Richard's medium without agar was prepared and distributed in 250 ml Erlenmeyer flasks. The pH level of medium viz 4.0-8.0 was adjusted by adding appropriate quantities of 0.1N hydrochloric acid and 0.1 sodium hydroxide solutions. After sterilization and necessary adjustment of pH, the flasks were inoculated with a uniform bit of fungus and maintained at $27 \pm 1^\circ\text{C}$. The mycelium mats were observed on 7 day after incubation.

In vitro effect of fungicides on *F. solani*

The poison food technique was adopted for the study to see the effect of fungicides on *F. solani* (Ngono *et al*, 2000). Carbendazim 50WP (0.10%), Copper oxychloride 50WP (0.25%), Carbendazim 12%WP+ Mancozeb 63% WP (0.20%), Mancozeb 75%WP (0.25%) fungicides at their recommended doses were used. 100 ml of Richard's agar was poured in 250 ml Erlenmeyer flasks and required quantities of the fungicides were weighed and added to each of the flasks. The flasks were shaken vigorously to ensure the uniform distribution of fungicides in the medium. Media were poured aseptically in Petri plates and inoculated with 5 mm disc of the fungus. Richard's agar without any fungicide served as control. Five replications were maintained for each fungicide. The inoculated plates were incubated for 7 days at $27 \pm 1^\circ\text{C}$. The linear growth of the colony was measured after 7 day of inoculation. Per cent inhibition was calculated by using the formula of Weitang *et al* (2004).

where,

$$I = \frac{(D_c - D_t)}{(D_c)} \times 100$$

I = Per cent Inhibition; Dc = Average diameter increase of fungal colony with control, Dt = Average diameter increase of a fungal colony in treatment.

Study of pathogenic variability under glass-house conditions

The sick soil of each isolate was prepared. Earthen pots of 6" diameter were disinfected by 5% copper sulphate solution and filled with wilt sick soil. Seeds of ten varieties were sown in pots (30 seeds per pot). Three replications of each variety for each isolate was maintained in the glass house. Wilting was recorded at 10th day and weekly up to 45 days after sowing and per cent wilt incidence percentage was calculated by using the following formula of (Mandhare and Patil, 1993). Based on per cent wilt the scale used were as follows:-

0 - 24% - Resistant (R); 25- 49% - Moderately resistant (MR); 50 -74% - Moderately susceptible (MS) 75% and above - Susceptible (S)

$$\text{Per cent wilt} = \frac{\text{Total number of wilted plants}}{\text{Total number of germinated plants}} \times 100$$

Varietal screening under field conditions

Thirty five germplasm of French bean viz Phule Surekha, G-13, HPR-35, EC-530909, IC-039081, GRB-9902, Jampa improved, GRB-9410, UHF-B-30, GK-(S)-1,958, PDR-14, P.Suyash, Jampa improved type, Kashmiri, Arka Suvridha, Contender, GK-06, EC-500377, Kanpur-1, GRB-9901, ACPB-11, G-I-GRB, GK-S, Kanpur-3, GK-2, EC-500354, GK-2, ACPR-94040, Sevil, Kanpur-2, GK-03-06, 3-2-701, PRJ-125, IC-28008 were screened at NARP Ganeshkhind. One sixty seeds of each variety were sown and out of the total number of germinated plants, per cent wilted plants were observed. On the basis of per cent wilting, germplasm lines were categorized as Resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) adopting the disease rating scale of Mandhare and Patil (1993), as done in case of glasshouse conditions

RESULTS AND DISCUSSION

Pathogenicity and symptomatology

Among the five isolates studied, Isolates II and IV

showed maximum percent wilting that is 45.39% and 40.00% respectively while minimum wilting percent was exhibited by Isolate I i.e. 4% (Table1). The wilted plants showed typical symptoms of wilting like yellowing and drooping of leaves after 15 days of inoculation. The time required for development of the symptoms leading to complete wilting varies from 25 to 40 days after inoculation. Isolate V and II required maximum and minimum period i.e. 40 days and 25 days respectively. Re-isolation of fungus was done from the artificially inoculated roots of plant and Koch's postulates were successfully proved for all isolates.

Table 1: Pathogenicity test of different isolates of *Fusarium solani*

No of isolates	No. of Seeds sown	No. of Seedlings Grown	No. of Seedlings Shown Symptoms	No .of days required for wilting	Mean Per cent Wilting
Isolate I	30	20	1	39	04.00
Isolate II	30	22	10	25	45.39
Isolate III	30	30	9	27	30.33
Isolate IV	30	25	10	37	40.00
Isolate V	30	28	11	40	38.28
CD (5%)					2.676

Cultural variation of different isolates of *F. solani*

Out of the different synthetic media evaluated for growth characters it was observed that Isolates I and V showed maximum growth on Richard's agar medium i.e. 75.33 and 79.33 mm respectively (Fig 2 a-e), which was followed by PDA, Czapek's agar and Ashby's agar (Table 2). It was reported that growth of *F. solani* was better on Richard's medium as compared to other media. Isolate IV and Isolate II manifested good growth on PDA i.e. 63.33 and 64.66 mm respectively, and this finding was supported by Paulkar and Raut (2004). PDA and Richard's agar also induced very good sporulation of the fungus which was in similar trends to the previous findings.

Morphological variation of different isolates

Observation on morphological characters viz. shape, size and septation of *F. solani* were recorded from 7th days old culture grown on Richard's agar media. The results indicated that the mycelium of various isolates of *F. solani* was

Table 2 : Cultural characteristic of different isolates of *Fusarium solani* on different cultural media

Name of the isolates	Media							
	Richard's agar		Ashby's agar		Potato dextrose agar		Czapek's agar	
	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation
Isolate I	75.33	+++	36.66	++	61.33	+++	59.00	++
Isolate II	72.00	++	37.53	+	64.66	++++	61.66	++
Isolate III	68.66	++	39.66	++	60.66	+++	63.00	++
Isolate IV	61.66	++	42.33	++	63.33	++++	64.00	++
Isolate V	79.33	++++	40.00	+	59.66	+++	66.33	+++
CD (5%)	1.461		1.722		1.683		1.946	

Table 3 : Effect of temperature on growth and sporulation of different isolates of *Fusarium solani*

Name of the isolates	Temperature (°C)													
	5		15		20		25		30		35		40	
	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation	Mean Colony diameter (mm)	Sporulation
Isolate I	-	-	28.66	+	64	++	72.00	++++	68	+++	39.33	++	19	-
Isolate II	-	-	30.33	+	67	+++	76.00	++++	73	++++	45.00	++	-	-
Isolate III	-	-	31.33	+	64	+++	75.33	++++	70	+++	37.66	+	-	-
Isolate IV	-	-	31.33	++	62.00	+++	70.00	++++	71.66	+++	38.00	+	-	-
Isolate V	-	-	26.00	+	68.00	++	73.00	++++	72	++++	41.33	++	21	-
CD (5%)			3.009		1.843		2.074		1.716		2.472			

- ++++ = Excellent growth /sporulation
 +++ = Good growth/sporulation
 ++ = Moderate growth/ sporulation
 + = Scanty growth/sporulation
 - = No growth/sporulation
 * = Average of three replications

hyaline, septate and profusely branched. Mycelial growth of the fungus was cottony and pinkish white in colour. Both types of conidia i.e. micro and macroconidia were observed. Microconidia were usually oval, mostly 0-1 septate and measured 5-12.4×2-3.6 µm. Macroconidia were fusiform, curved or falcate, 2-3 septate and measured 26.5-48×3-4.7 µm.

Effect of temperature on growth and sporulation of different isolates

It was observed that the *F. solani* used in the study could remain active in the temperature range of 15-35°C irrespective of the isolates. Further, it was found that the minimum temperature requirement of *F. solani* for their survival was 15°C while it could

Table 4 : Thermal death point of different isolates of *Fusarium solani* after fungal growth at 7 days

Name of the isolates	Temperature (°C)													
	35		40		45		50		52		53		54	
	Fungal growth	Sporulation	Fungal growth	Sporulation	Fungal growth	Sporulation	Fungal growth	Sporulation	Fungal growth	Sporulation	Fungal growth	Sporulation	Fungal growth	Sporulation
Isolate I	++	++	+	+	+	-	+	-	+	-	-	-	-	-
Isolate II	++	++	-	-	+	+	+	-	-	-	-	-	-	-
Isolate III	++	++	-	-	+	+	-	-	+	-	-	-	-	-
Isolate IV	++	++	+	-	+	-	-	-	-	-	-	-	-	-
Isolate V	++	++	-	-	+	-	+	-	-	-	-	-	-	-

Table 5 : Effect of pH on growth of different isolates of *Fusarium solani*

Name of the isolates	pH range (Mean mycelial growth (mm) 7 DAI)									
	4	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	
Isolate I	19.00	32.00	54.27	56.69	74.30	78.28	59.30	51.21	31.00	
Isolate II	20.00	30.24	56.34	52.65	76.18	77.31	61.40	56.15	30.87	
Isolate III	12.00	33.12	54.44	56.61	73.30	78.20	64.44	57.30	33.06	
Isolate IV	17.00	31.35	56.35	55.94	73.27	78.25	64.95	52.23	31.55	
Isolate V	16.00	31.14	58.35	56.65	74.29	75.40	66.36	55.35	31.56	
CD (5%)	1.843	1.745	1.751	2.710	1.885	1.784	1.930	2.647	1.745	

Table 6: *In vitro* effect of fungicide on growth of different Isolates of *Fusarium solani*

Name of the isolates	Treatments									
	Carbendazim 50WP (0.10%)		Mancozeb 75WP (0.25%)		Carbendazim 12+ Mancozeb 63 WP (0.20%)		Copper oxy chloride 50WP (0.25%)		Control	
	Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)
Isolate I	12.84	84.59	51.36	38.38	17.00	79.60	71.08	14.72	83.35	
Isolate II	18.31	75.94	52.30	31.23	18.36	75.86	71.37	6.16	76.06	
Isolate III	13.24	84.71	54.43	37.15	21.40	75.29	73.46	15.18	86.61	
Isolate IV	15.71	80.52	53.31	33.86	18.45	77.11	73.16	9.2	80.61	
Isolate V	13.28	85.44	52.30	38.59	18.35	78.45	71.87	15.61	85.17	
CD (5%)	3.647		1.700		2.279		1.792		3.016	

tolerate a maximum temperature up to 35°C. No colony growth was noticed at 5°C and 40°C temperatures. Maximum growth and profuse sporulation was observed at 25°C. Above this temperature the growth of *F. solani* was significantly low. The maximum colony growth of the fungus (76 mm) was observed in Isolate II at 25°C followed by Isolate III, Isolate V, Isolate I and Isolate IV respectively. Isolate V showed the least colony growth (26 mm) at 15°C while at 35°C Isolate II and Isolate III manifested maximum and minimum colony

growth of (45 mm) and (37.66 mm) respectively (Table 3). At 40°C colony growth of none of the isolates were observed. Isolates I, II, III showed abundant sporulation at temperature of 25°C but no sporulation was observed in any of the isolates at 40°C.

Study on thermal death point of different isolates

It was observed from the investigation that the fungus could grow up to a temperature of 52°C and

Table 7 : Evaluation of different isolates of *Fusarium solani* for their disease reaction in glasshouse

Cultivars	Isolate I		Isolate II		Isolate III		Isolate IV		Isolate V	
	Wilting per cent	Disease reaction	Wilting per cent	Disease reaction	Wilting per cent	Disease reaction	Wilting per cent	Disease reaction	Wilting per cent	Disease reaction
PhuleSurekha	76	S	75	S	68	MS	77	S	75	S
Contender	77	S	62	MS	80	S	79	S	79	S
GK-06	75	S	81	S	77	S	76	S	77	S
EC-500377	66	MS	79	S	39	MR	67	MS	78	S
Kanpur-1	79	S	77	S	70	MS	78	S	19	R
Kanpur-2	68	MS	15	R	18	R	39	MR	68	MS
G-13	81	S	76	S	79	S	69	MS	78	S
GK-03-06	42	MR	82	S	69	MS	70	MS	77	S
HPR-35	78	S	78	S	67	MS	66	MS	79	S
EC-530909	65	MS	41	MR	78	S	68	MS	78	S

Resistance: R, Moderate resistance:MR, Moderate susceptible: MR,Susceptible: S

sporulation up to 50°C. Further a restricted mycelial growth of *F. solani* was observed up to 52°C in Isolate I and Isolate III (Table 4). Microscopic observation revealed no sporulation beyond 45°C in any of the isolates. No fungus growth was observed at 53°C indicating thereby the thermal death point of *F. solani* was between 52 and 53°C.

Effect of pH on growth of different isolates of *F. solani*

Maximum mycelial growth of Isolates I, III, and IV was observed at pH 6.5. However, pH range from 4.5- 8 was found suitable for the growth of *F. solani* under study. Present study revealed that all isolates expressed relatively lesser growth at lowest and highest pH i.e. 4 and 8 respectively (Table 5). Fayzalla *et al*, (2008) reported 5.5-6.5 as an optimum pH range for the fungal growth, which is similar to the present study.

In vitro assay of fungicides against the pathogen

Carbendazim 50WP@ 0.10% completely inhibited the growth of all *F. solani* isolates and maximum inhibition per cent was observed in Isolate V i.e. 85.44 % (Table 6) (Fig. 3 a-f). It was followed by Carbendazim 12WP + mancozeb 63WP @0.20%. Mancozeb75WP @0.25% and Copper oxychloride

50WP@ 0.25% inhibited the fungus comparatively to a lesser extent. Isolate II and IV exhibited an inhibition of 31.23% and 9.2% respectively.

Study of Pathogenic variations and varietal screening in glass house and field conditions

Out of ten cultivars used under glass house conditions Kanpur-2 was found to be resistant to Isolate II and III, and Kanpur-1 to Isolate V. Cultivar EC-530909 was found moderately resistant to Isolate II (Table 7). Present study clearly indicated the variation among five isolates of *F. solani* regarding their virulence and pathogenicity which may be due to presence of a new race of the pathogen in the particular region. It was further proved in the study that an isolate pathogenic to one variety may not have an identical reaction with another variety, and the response of isolates varied with genotype. This suggests the presence of multi-allelic or multigenic responses towards resistance mechanism of French bean varieties against *Fusarium* wilt disease (Saxena and Cramer, 2009). However, the cause of this variation can only be ascertained by molecular studies.

Under field conditions among thirty five germplasm of French bean Kanpur-2, GK-03-06, 3-2-701, PRJ-125 were moderately resistant while only IC-28008

Table 8 : Field screening of French bean for wilt incidence under natural field conditions

Name of the germplasm	No of Germinated plant/160 plants	Wilted plant	Wilting percent	Reaction
PhuleSurekha	130	75	57	MS
Contender	117	90	76	S
GK-06	134	103	76	S
EC-500377	113	90	79	S
Kanpur-1	120	93	77	S
Kanpur-2	109	50	45	MR
G-13	136	80	58	MS
GK-03-06	116	45	38	MR
HPR-35	118	85	72	MS
EC-530909	123	78	63	MS
IC-039081	128	89	69	MS
3-2-701	132	55	41	MR
GRB-9902	110	30	27	MS
PRJ-125	131	52	39	MR
GRB-9901	144	110	76	S
ACPB-11	120	95	79	S
Jampa improved	128	90	70	MS
IC-28008	148	15	10	R
GRB-9410	134	89	66	MS
UHF-B-30	138	88	63	MS
G-II-GRB	105	82	78	S
GK-S	121	91	75	S
Kanpur-3	134	106	79	S
GK-(S)-1	131	95	72	MS
GK-1	120	90	75	S
EC-500354	106	80	75	S
958	104	75	69	MS
GK-2	125	96	76	S
PDR-14	138	95	68	MS
ACPR-94040	137	105	76	S
P.Suyash	130	75	57	MS
Jampaimproved type	112	65	58	MS
Kashmiri	118	68	57	MS

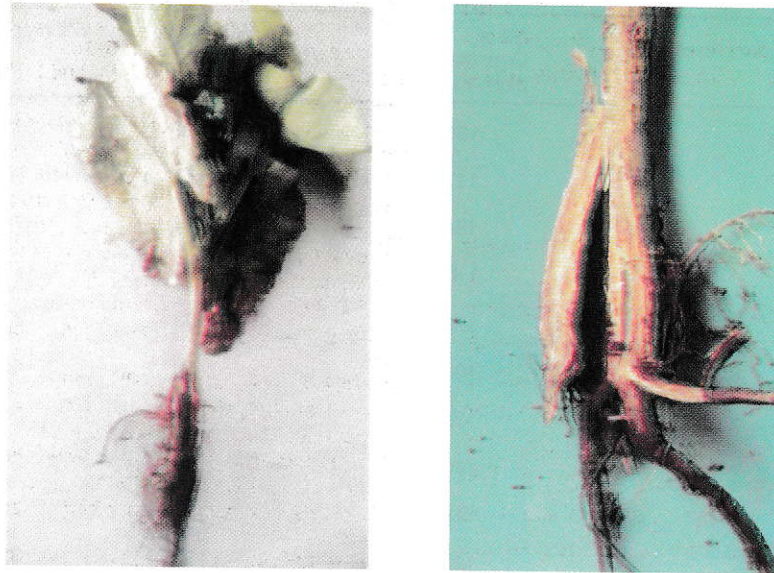


Fig. 1a-b : Symptoms of wilt infected French bean

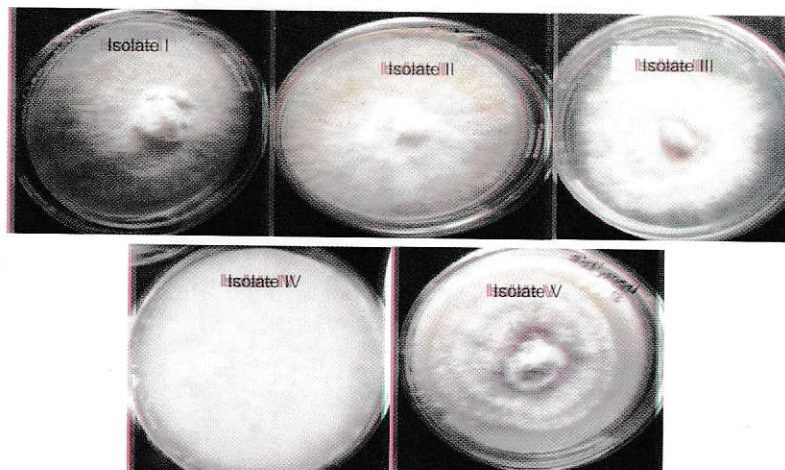


Fig. 2a-e : Growth of *Fusarium solani* isolates on Richard's agar

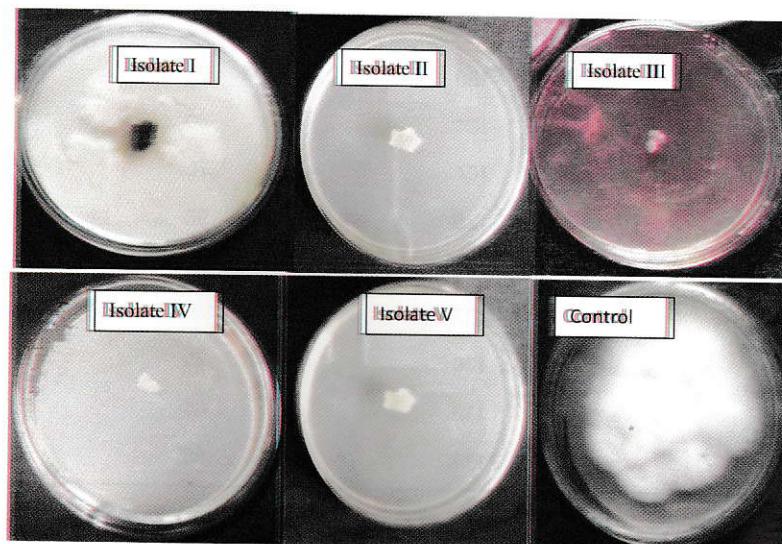


Fig. 3 a-f : *In vitro* assay of *Fusarium solani* isolates against carbendazim 50WP @0.1%

was found to be resistant (Table 8). To derive an unequivocal conclusion regarding the resistant status of these germplasm, multilocal trials need to be conducted.

CONCLUSION

The present endeavour depicts the role of different pH, temperature and media to understand ecological survival of pathogen. Molecular characterization of the pathogen, to understand the variability is essential for devising the management strategies. New generation fungicides like strobilurin should also be used to understand the vulnerability of the pathogen against chemicals. Finally, field experiments with screened chemicals should be carried out to demarcate specific management strategy against the pathogens.

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