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# Morphological, cultural and physiological characters of *Fusarium solani* causing wilt of cluster bean

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**Abstract**
Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], commonly called guar is an important legume crop belongs to family *Fabaceae*. It is cultivated mainly in the rainy season and major producing states in India are Rajasthan, Haryana, Gujarat, Maharashtra and to a limited extent in Uttar Pradesh and Madhya Pradesh. Cluster bean is a highly commercial, industrial and medicinally important leguminous crop due to presence of glactomannan gum in endosperm of seeds. The crop is known to suffer from many diseases which are responsible for its inferior quality and poor yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange. In recent years *Fusarium* wilt of cluster bean has assumed a serious problem and causing considerable yield losses. Although wilt diseases has assumed importance in Maharashtra, no systematic work has been carried out so for the disease may appear in an epidemic form in future and may threaten the cultivation of cluster bean. Present studies were undertaken with objectives *viz*., isolation, identification, symptomatology, pathogenicity test, morphological, cultural and physiological characteristics of wilt disease. In cultural studies, it was observed that the test pathogen *Fusarium solani* shows maximum radial growth on Richard's media and good sporulation was recorded on Richard's, PDA and V8 juice agar media. In physiological studies, it was observed that the maximum growth and sporulation of *Fusarium solani* ranged from 25 °C to 30 °C and grew well from pH levels 6.5 to 7.

**Keywords:** *Fusarium* solani, *Cyamopsis tetragonoloba*, culture media, *in vitro*, sporulation, fungal colony, microconidia, macroconidia

## Introduction
Cluster bean commonly called guar is an important legume crop belongs to family *Fabaceae*. Cluster bean meal is the main by-product of cluster bean gum production. It is a highly commercial, industrial and medicinally important leguminous crop due to presence of glactomannan gum in endosperm of seeds.The crop is known to suffer from many diseases which are responsible for its inferior quality and poor yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange (Chand and Gandhi 1978). The major disease causing low planting value of the crop includes fungal, bacterial and viral diseases i.e. *Fusarium* wilt (*Fusarium solani* Mart. Sacc.), Dry Root Rot (*Macrophomina phaseolina* Tassi Goid.), Powdery Mildew (*Leveillula taurica* Lev. Arn.), *Alternaria* Leaf Spot (*Alternaria cucumerina* var. *cyamopsidis* Ellis. &Everh.), Bacterial Blight (*Xanthomonas axonopodis* pv. *Cyamopsidis* H.). Among the different pathogens attacking to the crop *F. solani* is the most common fungus causing considerable yield losses. The pathogen caused wilt at seedling to maturity. At later stages of plant growth, the infected plants exhibit girdling at collar region which results in wilting of host plant (Pareek & Varma, 2014). In recent years *Fusarium* wilt of cluster bean has assumed a serious problem.

## Material and Methods
### Collection of diseased sample and isolation of pathogen
Infected plants of cluster bean showing different symptoms of wilt were collected from Post Graduate Institute field, Mahatma Phule Krishi Vidyapeeth, Rahuri and samples were brought

to the laboratory, Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri and stored in refrigerator for further work.

**Morphological characters**

The morphology of the fungus was studied from seven days old culture grown on potato dextrose agar medium by adoption of slide culture technique. Morphological characters *viz*., size, shape and septation of different morpho structures like mycelium micro and macro conidia, chlamydospores etc. were recorded with the help of stage and filarmicrometer.

**Cultural characters**

The cultural characters of fungus were studied on the different solid media for their growth characters. These media were prepared as per the standard laboratory procedure described by Twite (1969). Twenty ml of each of the sterilized liquid medium was poured in each Petri plate. Four replications were kept for each treatment. These plates were inoculated with 5 mm disc of fungal growth at the center and incubated at 27 ± 1˚C. Colony diameter was recorded by averaging the growth of the colony in three directions for each plate after seven days of inoculation. The fungal colony color, surface elevation and sporulation were also recorded at the end of the incubation period. The data on radial growth was analysed statistically. Following culture media were used for the study *viz*. Potato Dextrose Agar, Corn Meal Agar, Richard's Agar, V-8 juice Agar, Carrot extract Agar etc.

**Physiological characters**

**Effect of different temperature on growth and sporulation of *Fusarium solani***

The effect of temperature on growth and sporulation was studied on potato dextrose agar medium. For this purpose, the media was prepared, sterilized and poured in Petri plates. The Petri plates were inoculated with 5 mm mycelial discs of inoculum cut with the help of sterilized cork borer from eight days old culture of pathogen grown on PDA media. Four replications were kept for each treatment. The inoculated Petri plates were incubated for seven days at different temperatures *viz*., 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC maintained in separate BOD incubators. The observations on colony diameter were recorded after seven days by averaging the growth of colony in three directions for each plate of each culture.

**Effect of different pH levels on growth and sporulation of *Fusarium solani***

The effect of different pH levels on growth and sporulation was studied on potato dextrose agar medium. For this purpose, The media was prepared, 100 ml of the PDA media was dispensed in seven Erlenmeyer flasks of 250 ml capacity and different pH levels of 5.5, 6, 6.5, 7, 7.5, 8 and 8.5 were adjusted with the help of digital pH meter (ELTOP 3030) by adding HCL or NaOH solutions. The flasks were plugged with non-absorbent cotton wrapped in muslin cloth and sterilized in an autoclave at 15 psi pressure for 15 minutes. After autoclaving, when the media was lukewarm, 20 ml of medium was poured in the Petri plates under aseptic conditions in the laminar air flow cabinet. The Petri plates were inoculated with 5 mm mycelial discs of inoculum cut with the help of sterilized cork borer from eight days old culture grown on PDA media. Three replications were kept for each treatment. The inoculated Petri plates were incubated at 25 ± 1 $^0$C maintained in BOD incubator. The observations on colony diameter were recorded after seven days by averaging the growth of colony in three directions for each plate of each culture and sporulation was measured with the help of haemocytometer.

**Result and Discussion**

**Morphological characters**

The mycelium was initially white and abundant. The growth of the mycelium was fluffy with raised margins in uniform circular fashion. Later the mycelium became creamy white with light brown pigmentation. The pure culture of the fungus obtained in Petri plates after incubation at 25 ± 1 ºC was examined under the microscope for morphological characteristics *viz*., size, shape and septation. The microscopic observation made by the slides prepared directly from active cultures. Observed under low (10X) and higher (40X) power magnification the fungus revealed that mycelium was hyaline, slender, branched and septate. Two types of the conidia were observed *viz*., microconidia and macroconidia. The microconidia were hyaline, oval to cylindrical and slightly sickle shaped with blunt ends without any septation. The size of microconidia ranged from 4.03 to 10.45 µm in length and 1.84 to 2.24 µm in width. The macroconidia were also hyaline, sickle shaped with septation and the size varied from 13.87 to 16.44 µm in length and 1.88 to 1.92 µm in width and chlamydospores were globose to subglobose, smooth or rough walled, 6-9 x 5-8 µm.The fungus was identified as *Fusaium solani* after comparing with the characteristics illustrated by Ellis (1971) [5] and morphological characters were also compared with the available descriptions from mycological books such as Hypomycetes (Subramanium, 1971), *Fusarium* laboratory manual (Leslie *et al*., 2000). According to Satyaprasad and Ramarao (1981) [10] the fungus isolated from diseased roots of cluster bean also formed both microconidia and macroconidia. The microconidia were cylindrical and 5.0-10.0 x 3.0-4.0 µm in size, whereas macroconidia were up to 5 septate with size of 13.0-27.5 x 3.0-4.5 µm. He classified the fungus as *Fusarium solani* (Mart.) Sacc. (IMI 236629).

**Cultural characters**

The variation in cultural characters of *Fusarium solani* was studied on the five different semisolid media *viz*., potato dextrose agar, corn meal agar, carrot extract agar, V8 juice agar and Richard's medium. And the results thus obtained are presented in Table 1 and Fig. 1 and 2, the mean colony diameter varied from 69.80 to 87 mm at 7 DAI on all the tested media. The maximum colony diameter (87 mm) was recorded on Richard's medium after seven days of inoculation. This was followed by V8 juice agar (80 mm), PDA medium (72 mm), corn meal agar (70 mm) and carrot extract agar (69.80 mm). All the tested media differed statistically and significantly among themselves. As regards sporulation, good sporulation was observed on Richard's, PDA and V-8 juice agar media, while moderate sporulation was observed on carrot extract agar and corn meal agar media. The results are in conformity with Sreedevi S. Chavan (2007) [11] found that radial growth of *F. solani* among thirteen solid media tested, maximum radial growth (90 mm) was observed on Richards's agar and potato dextrose agar media (90 mm).

**Table 1:** Effect of different media on growth and sporulation of *Fusarium solani*

| Sr. No. | Medium | Mean colony diameter in mm after 7 DAI (*) | Growth type and colour of mycelium | Sporulation |
|---|---|---|---|---|
| 1. | PDA Media | 72 | Cottony white | +++ |
| 2. | V-8 juice agar | 80 | White cottony and fluffy growth | +++ |
| 3. | Carrot extract agar | 69.80 | White sparse growth | ++ |
| 4. | Richard's medium | 87 | White cottony and fluffy growth, smooth margin | +++ |
| 5. | Corn meal agar | 70 | Pink sparse growth | ++ |
| | SE | 0.85 | | |
| | CD at 5% | 2.56 | | |

(*) = Average of four replications, DAI = Days after inoculation, +++: Good sporulation, >50 conidia/microscopic field, ++: Moderate sporulation, 30-50 conidia/microscopic field, +: Scanty sporulation, <30 conidia/microscopic field

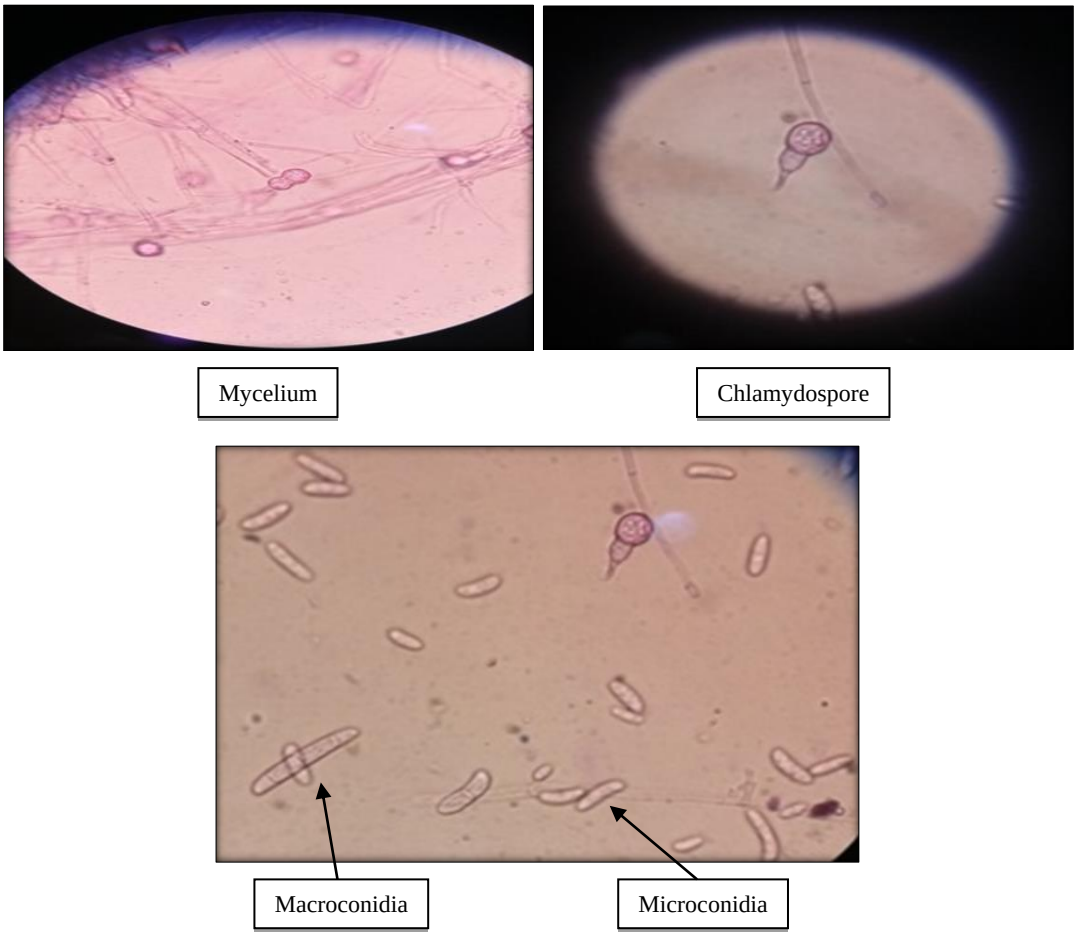



**Fig 1:** Photomicrograph showing mycelium, microconidia, macroconidia and chlamydospores of *Fusarium solani*


1. Richard's medium, 2. V-8 juice agar, 3. PDA media,
4. Corn meal agar, 5. Carrot extract agar

**Fig 2:** Growth of *Fusarium solani* on different solid media

**Physiological characters**

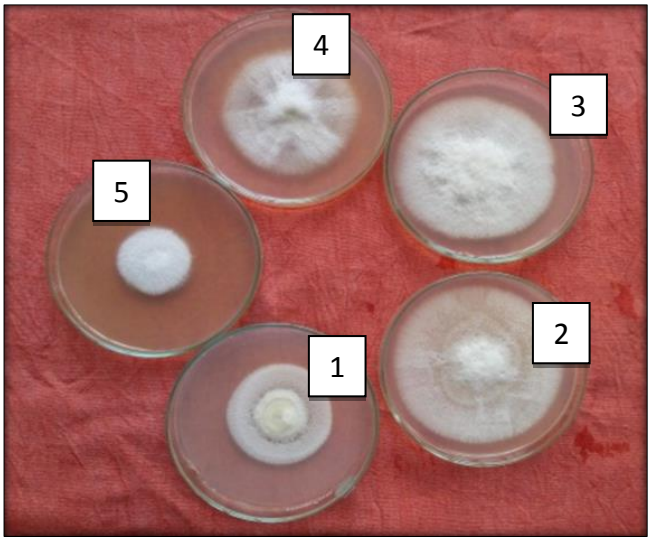
**Effect of different temperature on growth and sporulation of *Fusarium solani***

The effect of different temperatures i.e. 20 °C, 25 °C, 30 °C, 35 °C and 40 °C on the growth and sporulation of *Fusarium solani* was studied on potato dextrose agar medium. The data is presented in Table 2 and Fig. 3, the mean colony diameter of the fungus was found to be significantly different at all the tested temperatures as temperature has marked effect on growth and sporulation of the fungus. The maximum colony diameter (76.20 mm) and (73.70 mm) was observed at 25 °C and 30 °C on seven days of incubation respectively. Least radial growth of (58.50 mm), (50 mm) and (35.10 mm) was recorded at 20 °C, 35 °C and 40 °C respectively. As regard sporulation, good sporulation was observed at 25 °C and 30 °C and moderate sporulation was observed at 20 °C and 35 °C while scanty sporulation was observed at 40 °C temperature.The results are in conformity with Chaturvedi *et al*., (2003) [1] found that optimum temperature for growth of *F. oxysporum* and *F. solani* was 25 °C.El-Sayed *et al*., (2008) [6] observed maximum mycelial growth of *Fusariumsolani* was obtained at 25 °C and 30 °C. Merlin *et al*., (2013) and Kausar *et al*., (2009) [8, 12] also found that temperature of 25±2 °C supported the maximum growth of *F. solani*.

**Table 2:** Effect of different temperature on growth and sporulation of *Fusarium solani*

| Sr. No. | Temperature (°C) | Mean colony diameter in mm after 7 DAI (*) | Sporulation |
|---|---|---|---|
| 1 | 20 | 58.50 | ++ |
| 2 | 25 | 76.20 | +++ |
| 3 | 30 | 73.70 | +++ |
| 4 | 35 | 50.00 | ++ |
| 5 | 40 | 35.10 | + |
| | SE± | 0.63 | |
| | CD at 5% | 1.90 | |

(*) = Average of four replications, DAI = Days after inoculation, +++: Good sporulation, >50 conidia/microscopic field, ++: Moderate sporulation, 30-50 conidia/microscopic field, +: Scanty sporulation, <30 conidia/microscopic field




Temperature: 1. 20 °C, 2. 25 °C, 3. 30 °C, 4. 35 °C, 5. 40 °C

**Fig 3:** Effect of different temperature on growth of the *F. solani*

**Effect of different pH levels on growth and sporulation of *Fusarium solani***

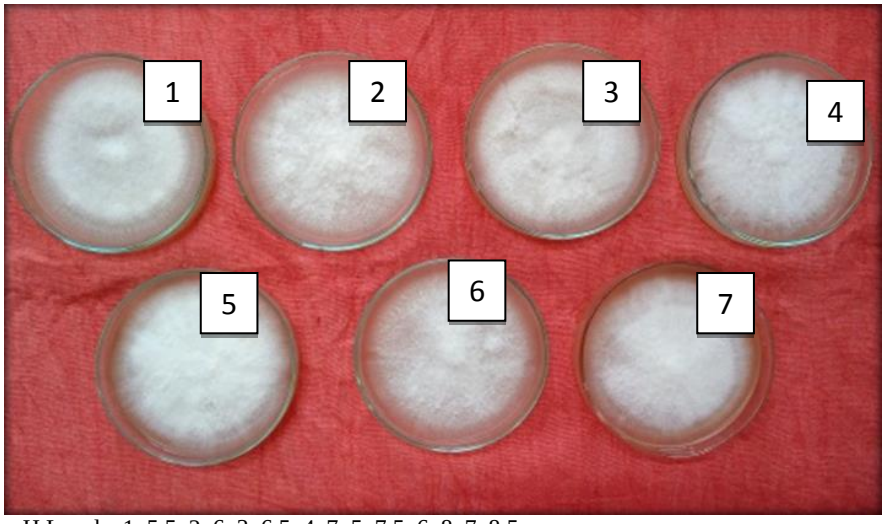
*Fusarium solani* was grown on Potato dextrose agar at seven different pH levels *viz*., 5.5, 6, 6.5, 7, 7.5, 8 and 8.5 to study their effect on the growth and sporulation of the pathogen is presented in the Table 3 and Fig. 4, The result showed that there were significant differences among pH levels and the mean colony diameter of the pathogen. Increasing trend in colony diameter was noticed from pH 5.5 to 7 and thereafter growth showed a decreasing trend from pH 7.5 to 8.5. Maximum colony diameter of 90 mm was recorded at pH 7 after seven days of incubation while minimum colony diameter of 64.30 mm was found at pH 8.5.As regard sporulation, good sporulation recorded at pH 6 to 7.5 while moderate sporulation was recorded at pH 5.5, 8 and 8.5.The results are in conformity with Chauhan (1997) [2] proved optimum pH 6.5 and 7 for growth and sporulation of *F. solani*. Tarun Birla (2014) found that pH 7 was suitable for the growth of all the isolates of *Fusarium solani.*

**Table 3:** Effect of different pH levels on growth and sporulation of *Fusarium solani*

| Sr. No. | pH | Mean colony diameter in mm after 7 DAI (*) | Sporulation |
|---|---|---|---|
| 1 | 5.5 | 70.00 | ++ |
| 2 | 6.00 | 75.00 | +++ |
| 3 | 6.5 | 81.00 | +++ |
| 4 | 7.00 | 90.00 | +++ |
| 5 | 7.5 | 74.00 | +++ |
| 6 | 8.00 | 70.00 | ++ |

| | | | |
|---|---|---|---|
| 7 | 8.5 | 64.30 | ++ |
| | SE± | 0.57 | |
| | CD at 5% | 1.75 | |

(*) = Average of four replications, DAI = Days after inoculation, +++: Good sporulation, >50 conidia/microscopic field, ++: Moderate sporulation, 30-50 conidia/microscopic field, +: Scanty sporulation, <30 conidia/microscopic field




pH Levels: 1. 5.5, 2. 6, 3. 6.5, 4. 7, 5. 7.5, 6. 8, 7. 8.5

**Fig 4:** Effect of different pH levels on growth of the *F. solani*

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