

Original Research Article

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Cultural and Morphological Variations of *Fusarium solani* (Mart.) Sacc. Causing Root Rot of Patchouli in Assam, India

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ABSTRACT

Root rot disease of patchouli is caused by *Fusarium solani* (Mart.) Sacc. Eight isolates were collected from patchouli growing areas of Assam to study the cultural and morphological characteristics of *F. solani*. The colony diameter of eight isolates ranged from 81.55 mm to 90.00 mm at ten days after inoculation on PDA medium. The fastest radial growth (90.00 mm) was observed in isolates JFS₁, JFS₂ and NFS₄ followed by isolates GFS₈ (87.56 mm), BFS₆ (87.46 mm), NFS₃ (85.34 mm) and NFS₅ (84.78 mm) whereas minimum radial growth was observed in isolate BFS₇ (81.88 mm). All isolates produced micro, macro-conidia and chlamydo spores. The size of macro conidia was ranged from 13-15 x 3-4 µm to 27-29 x 4-5 µm, size of micro conidia was ranged from 3-4 x 1-2 µm to 9- 10 x 1-3 µm, the number of septa in macro and micro conidia are 2-4 and 0-1 respectively and the colour is hyaline. All conidia were hyaline and macro conidia were sickle shaped with the blunt end, micro conidia was cylindrical and round to oval and chlamydo spore were intercalary, terminal, globose to oval shaped in all the isolates. The septation of macro conidia is 2-4 and micro conidia were 0-1. Conidiophores were elongated and sparsely branched, each branch usually terminated with a spore bearing phialide. The most distinguished characters of long monophialidic conidiogenous cells were observed in all the isolates.

Keywords

Cultural and morphological variations, Root rot.

Article Info

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Introduction

Patchouli (*Pogostemon cablin* (Blanco) Benth.) plant is an important perennial aromatic plant belongs to lamiaceae family. Patchouli is commercially cultivated in India, Bangladesh, Nepal, Sri Lanka, Myanmar, Thailand and China. It also grows wild in Singapore, Indonesia and Malaysia but owing to high demand of its essential oil, used in cosmetics, breath fresheners, flavoring baked foods, sausages, etc. (Singh and Rao, 2009; Puttana *et al.*, 2005). In India, Commercial cultivation of patchouli plant was first initiated and practiced by Tata Oil Mills in the

year 1942. However, after its initial stray attempts of cultivation, an organized cultivation practice was established by CIMAP (Central Institute of Medicinal and Aromatic Plants), Bangalore, India in the year, 1962 (Kumar *et al.*, 1986). Patchouli is cultivated in Assam, Madhya Pradesh, Tamil Nadu, Kerala and Karnataka. Currently, India is producing a small quantity of patchouli oil against its requirements of 40 tonnes of pure oil and about 70 tonnes of formulated oil. Hence, majority of these oils are being imported from Indonesia (Jadhav *et al.*,

2003). In northeast, Patchouli cultivation is started in the first instance on about 1000 ha land by farmers and entrepreneurs and planned to go up to five times in future (Das, 2001). Root rot of patchouli caused by *F. solani* is severe, destructive, wide spread and found in all patchouli growing areas of India. The typical symptoms of the disease are yellowing of lower leaves, drooping, and wilting of plant. Sreedevi *et al.*, (2009) reported that the presence of cultural and morphological variability among the *Fusarium solani* isolated from different areas of Karnataka state, India. In the present studies isolates of *F. solani* (Mart.) Sacc. collected from four districts of Assam were studied for the presence of cultural and morphological variations among these isolates.

Materials and Methods

Collection, isolation and identification of *F. solani* isolates

Eight isolates of *F. solani* were collected from different areas of Assam namely Herbal garden (AAU), Pokamura, Kaliabor Nursery, Seuj Nagar, Uluoni Gaon, Bijoypur, Madhupur, Sugarcane Research Station (SRS) (Buralikson) and were designated as JFS₁, JFS₂, NFS₃, NFS₄, NFS₅, BFS₆, BFS₆ and GFS₈. For isolation of the pathogen, infected tissues of the root were cut into small pieces of 1-2 cm size and surface-sterilized with 0.1% sodium hypochlorite solution for one to two minute and washed repeatedly in sterilized water and placed in petri-plates containing PDA medium and incubated at 27±1°C. The purified culture of the pathogen was obtained by hyphal tip method and sub-cultures from peripheral growth were made on PDA slants. The pure *F. solani* cultures were stored at 4°C in refrigerator and used for further studies. Isolation of *F. solani* from different plants by following the standard tissue isolation techniques has been reported by several

workers (Satyaprasad and Rama Rao, 1981; Kore and Patil, 1985; Kore and Mashalkar, 1987).

The pathogen isolates were mainly identified on the basis of cultural and morphological characters as *Fusarium solani* (Mart) Sacc. as described by Booth (1971). Further, the identity of *F. solani* was confirmed from National Center of Fungal Taxonomy, New Delhi (I.D. No.-9080.17). The fungus also produced wilt and typical root rot symptoms under pathogenicity test. Thus, *Fusarium* sp. understudy was identified and confirmed as *F. solani*.

Symptomatology

Following symptoms of patchouli root rot caused by *F. solani* were observed during the study.

Root rot of patchouli

Patchouli plants showed wilting at all stages of their growth. Yellowing of the older leaves was the first external symptom which later advanced to middle and terminal leaves. Drooping of green parts followed exactly as if there was not enough moisture in the soil. Brown to black discolouration of stem and roots was observed followed by dark brown discolouration of vascular tissue. Severe disintegration of primary and secondary roots surface was also observed. Ultimately, the whole plant dried and there was premature death of the plant.

Cultural variability

Eight isolates of *F. solani* (Mart.) Sacc. were collected from different places were grown on PDA for variability studies. The petri-plates (90 mm) containing 15-20 ml of PDA medium were inoculated aseptically with 5 mm mycelial disc of the fungus from the periphery of actively growing culture and

incubated at $27\pm 1^{\circ}\text{C}$ for 10 days. The observations were recorded on colony diameter, colony color, colony margin, pigmentation and surface elevation. The colony diameter of the isolates (mm) was measured at 48, 72, 96, 144, 168, 216 and 240 hours after inoculation. All the isolates showed wide variations in respect of colony colour and pigmentation. The experiment was laid out in completely randomized design (CRD) with five replications. Colony diameter (mm) was measured from three sides of the colony growth.

Morphological variability

Spores of *F. solani* of different isolates were measured from the pure culture of the pathogens. Spores were mixed with lacto phenol cotton blue thoroughly so that, a uniform spread is obtained and then cover slips was placed over it. Spores were measured under high power objectives (40x) using ocular and stage micrometer. The average size of the spores were then determined and shape of the spores were recorded. For micro and macroconidia, length and breadth of fifty (50) spores for each of three replications of all the isolates were measured using ten day old culture.

Results and Discussion

Cultural characteristics based on colony diameter (mm) among the *F. solani* isolates

The colony diameter of eight isolates of *F. solani* was observed up to ten days and incubated at $27\pm 1^{\circ}\text{C}$. The highest colony diameter (90 mm) was observed in isolates JFS₁, JFS₂ and NFS₄ followed by isolates GFS₈, BFS₆, NFS₅ and NFS₃ whereas minimum colony diameter was observed in isolate BFS₇. Similar type of observations were also made by earlier worker Sreedevi *et al.*, (2009) who studied cultural and morphological variability among eight

isolates of *F. solani* causing fungal wilt of patchouli. Chandran and Kumar (2012) studied that radial growth of thirteen isolates of *F. solani* incitant of dry root-rot of citrus in Karnataka (Table 1).

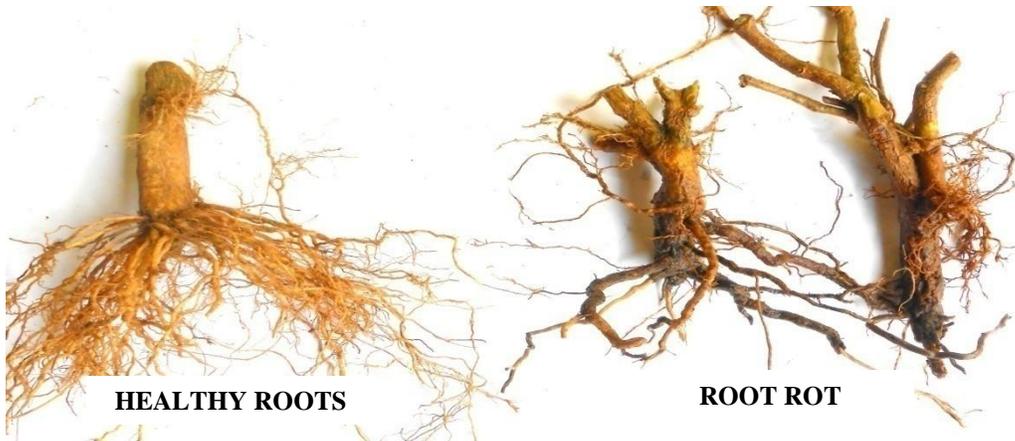
Colony characteristics on PDA

The results presented in the Table 2 revealed that significant differences between isolates with respect to colony character, colony margin and pigmentation in culture plates. The colony coloured of the isolates were white in colour, colony margin of the isolates were vary from smooth to irregular. White colour cottony growth and raised mycelium with smooth margins was observed in JFS₁ isolates. JFS₂ isolates showed white cottony with fluffy growth mycelium. NFS₃ isolates observed white dense growth with smooth margin. White sparse growth was observed in NFS₄ isolates. NFS₅ isolates showed white colour fluffy growth and irregular margin. White fluffy mycelium with irregular margins was observed in BFS₆ isolates. Isolates BFS₇ showed white fluffy mycelium with concentric ring and irregular margin. White raised cottony mycelium and irregular mycelium was observed in GFS₈. Chandran and Kumar (2012) while studying variability of *F. solani* isolates found six isolates as white sparse growth, three as white cottony growth, two as white fluffy and three as white dense growths. Similar types of results were also made by earlier workers (Champawat and Pathak, 1989; Madhukeshwara, 2000; Vettrano *et al.*, 2009 and Sreedevi *et al.*, 2009).

Based on colony pigmentation isolates were assigned to three groups as par "Royal Horticultural Society Colour Chart, London". They were classified as Yellow, Purple and Orange. Two isolates were found purple (JFS₁ and JFS₂), four isolates were found yellow (NFS₃, NFS₄, NFS₅, NFS₆, NFS₇) and one isolate (GFS₈) was orange.



A. Initial wilting and dropping of lower parts



HEALTHY ROOTS

ROOT ROT

B. Healthy roots and infected root rot of patchouli plant



C. Black vascular discoloration of infected stem



D. Isolation of *F. solani* from infected roots

Plate.1 (A-D) Typical symptoms of wilting, root rot and vascular discoloration of patchouli plants



JFS₁



JFS₂



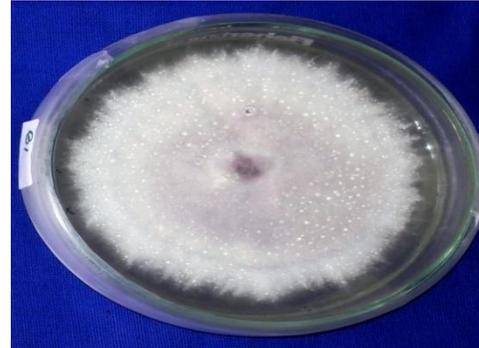
NFS₃



NFS₄



NFS₅



BFS₆



BFS₇



GFS₈

Plate.2 Pure culture of different isolates of *F. solani* on PDA

FRONT VIEW REVERSE VIEW



A. JFS₁

FRONT VIEW REVERSE VIEW



B. JFS₂



C. NFS₃



D. NFS₄



E. NFS₅



F. BFS₆

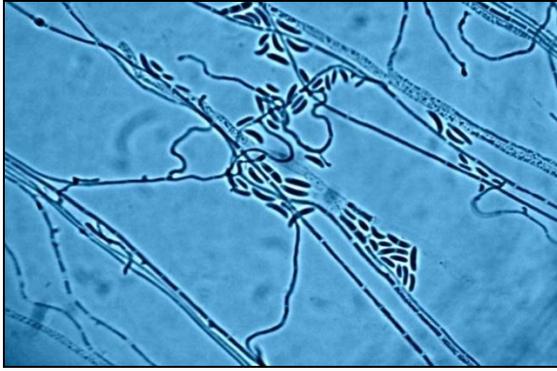


G. BFS₇

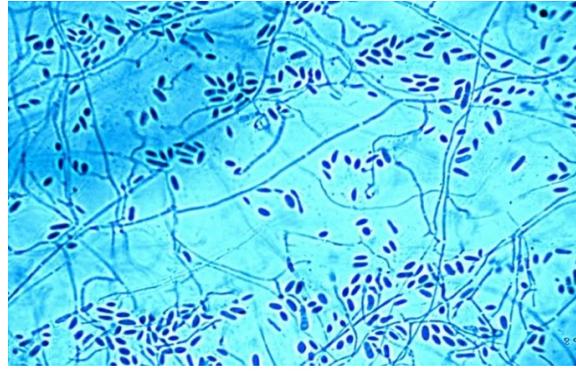


H. GFS₈

Plate.3 (A-H) Cultural variability of different isolates of *Fusarium solani*



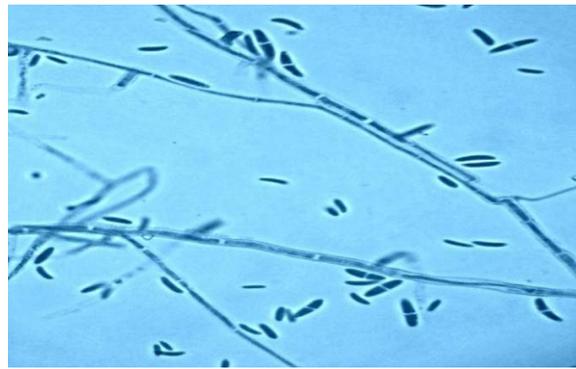
A. JFS₁



B. JFS₂



C. NFS₃



D. NFS₄



E. NFS₅



F. BFS₆

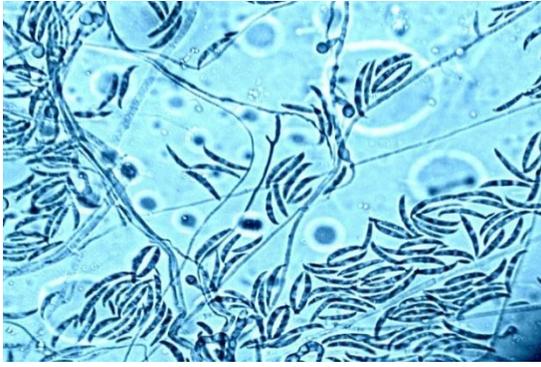


G. BFS₇

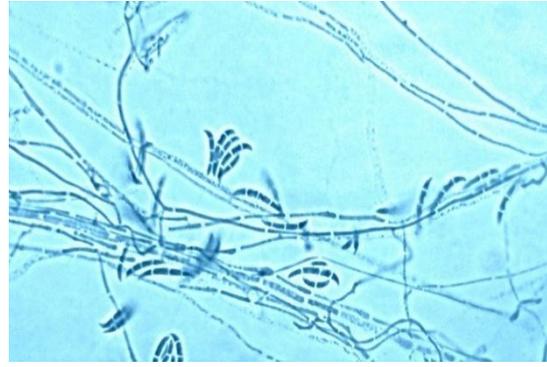


H. GFS₈

Plate.4 (A-H) Photomicrograph of micro-conidia of different isolates of *F. solani* (40X)



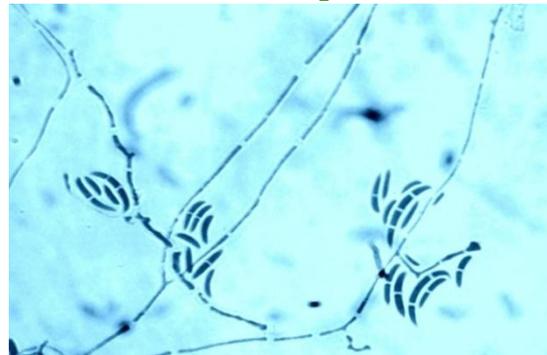
A. JFS₁



B. JFS₂



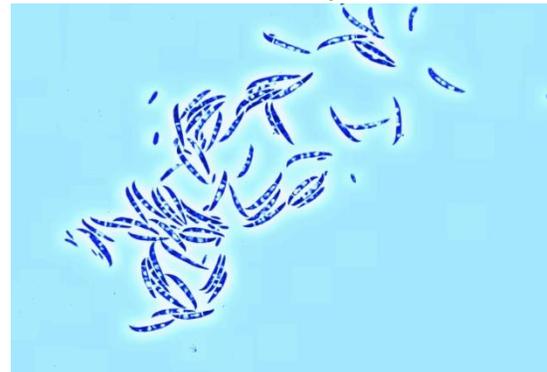
C. NFS₃



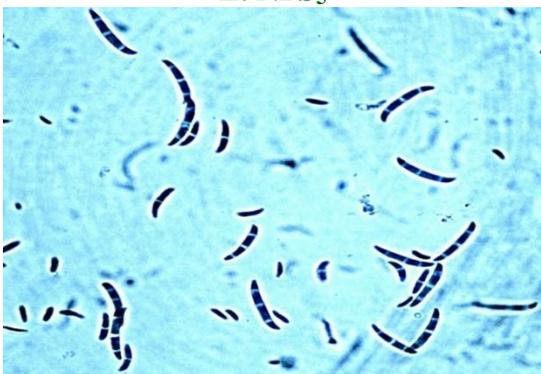
D. NFS₄



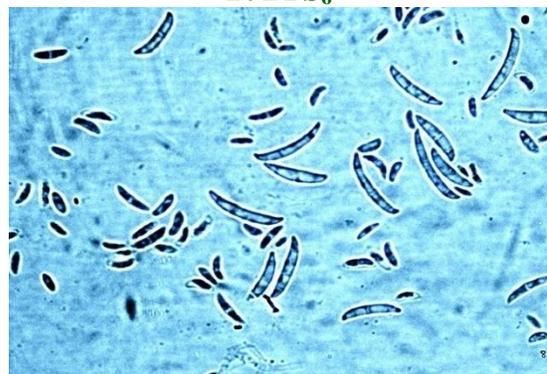
E. NFS₅



F. BFS₆



G. BFS₇



H. GFS₈

Plate.5 (A-H) Photomicrograph showing different isolates of macro-conidia of *Fusarium solani* (40X)



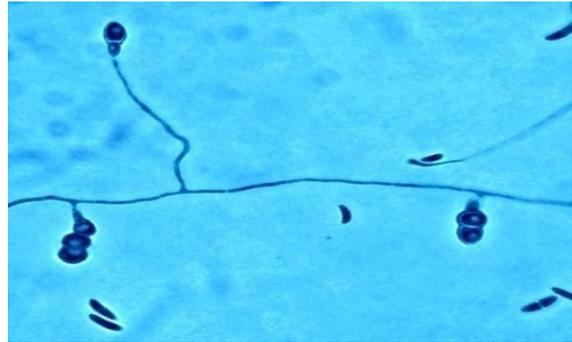
A. JFS₁



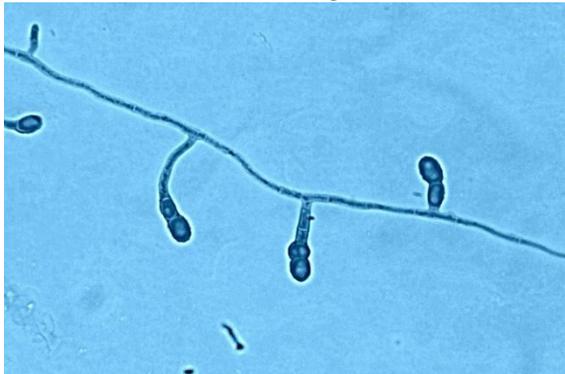
B. JFS₂



C. NFS₃



D. NFS₄



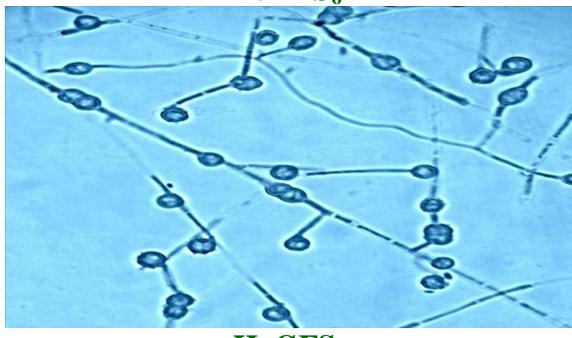
E. NFS₅



F. BFS₆



G. BFS₇



H. GFS₈

Plate.6 (A-H) Photomicrograph showing chlamydospores of different isolates of *Fusarium solani* (40X)

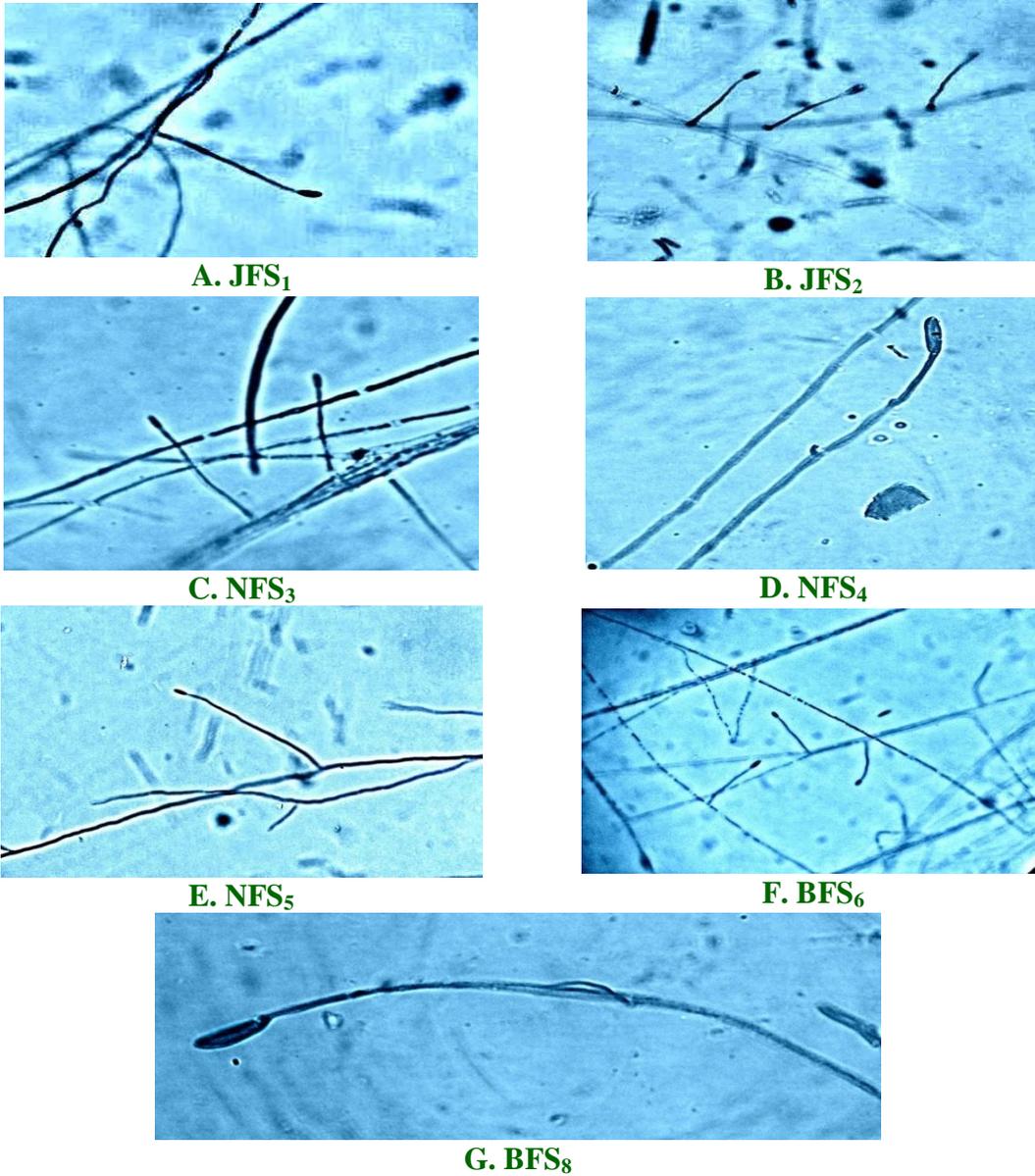


Plate.7 (A-G) Long monophialidic conidiogenous cells in different isolates of *F. solani* (40X)

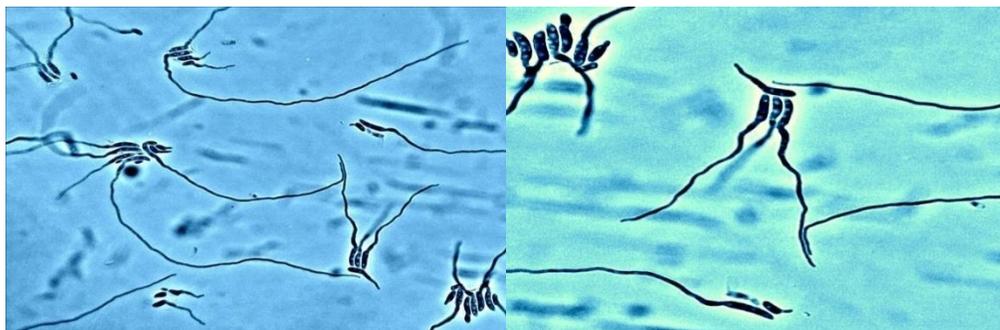


Plate.8 Germination of macro-conidia from single polar cell (40X)

Table.1 Colony diameter (mm) of *F. solani* isolates up to 10 days after inoculation on PDA

Isolates No	Place of isolation	*Colony diameter (mm)						
		48 hrs	72 hrs	96 hrs	144 hrs	168 hrs	216 hrs	240 hrs
JFS ₁	Jorhat	18.06	32.48	40.64	61.76	68.32	89.92	90.00
JFS ₂	Jorhat	16.38	28.10	35.60	52.69	62.28	84.86	90.00
NFS ₃	Nagaon	16.02	26.16	36.36	54.22	63.36	75.64	85.34
NFS ₄	Nagaon	17.84	27.84	36.62	59.54	67.40	85.46	90.00
NFS ₅	Nagaon	14.71	20.27	27.56	45.66	50.60	76.58	84.78
BFS ₆	Biswanath	14.56	19.37	24.46	52.30	60.30	84.88	87.46
BFS ₇	Biswanath	15.61	23.52	28.50	48.32	57.70	70.52	81.88
GFS ₈	Golaghat	16.60	27.54	33.50	56.46	64.70	76.76	87.56
SEm(±)		0.57	0.57	0.15	0.17	0.36	0.26	0.27
CD (0.05)		1.16	1.17	0.29	0.35	0.72	0.55	0.55

Means within columns separated by Duncan's multiple range test P=0.05

* Values are mean of five replications

Table.2 Colony characteristics of eight isolates of *F. solani* on PDA

Isolates No	Colony characters	*Pigmentation	Colony margin
JFS ₁	White colour cottony growth and raised mycelium	Purple group 75 (B)	Smooth margin
JFS ₂	White cottony with fluffy growth mycelium	Purple group 75 (A)	Smooth margin
NFS ₃	White dense growth	Yellow group 12 (D)	Smooth margin
NFS ₄	White sparse growth	Yellow orange 14 group (B)	Smooth margin
NFS ₅	White colour fluffy growth	Yellow group 12 (C)	Irregular margin
BFS ₆	White fluffy mycelium	Yellow group 12 (C)	Irregular margin
BFS ₇	White fluffy mycelium with concentric ring	Yellow orange 14 group (A)	Irregular margin
GFS ₈	White raised cottony mycelium	Orange group 28 (C)	Irregular margin

* Royal Horticultural Society Colour Chart

Table.3 Morphological characteristics of different isolates of *F. solani* on PDA

Isolates No	Spore size (µm)		Septation		Shape		Colour
	Micro conidia (L x B)	Macro conidia (L x B)	Micro conidia	Macro conidia	Macro conidia	Micro conidia	
JFS ₁	(5.53-8.59) X (2.33-2.63)	(22.36-28.92) X (3.78-4.32)	0-1	3-4	Elongated with blunt ends	Round to oval	Hyaline
JFS ₂	(3.94-7.04) X (1.98-2.40)	(21.16-25.44) X (2.65-3.87)	0-1	3-4	Sickle shaped with blunt ends	Round to oval	Hyaline
NFS ₃	(5.3-8.52) X (2.27-2.59)	(17.39-23.17) X (2.91-4.51)	0-1	2-3	Sickle shaped	Round to oval	Hyaline
NFS ₄	(9.85-12.69) X (2.60-4.14)	(23.88-29.24) X (3.21-4.97)	0-1	3-4	Elongated with blunt ends	Round to oval	Hyaline
NFS ₅	(9.54-11.56) X (2.74-4.04)	(26.42-32.46) X (3.24-4.74)	0-1	3-4	Elongated with blunt ends	Round to oval	Hyaline
BFS ₆	(7.58-11.47) X (2.68-3.56)	(23.99-32.63) X (5.10-6.26)	0-1	3-4	Sickle shaped	Round to oval	Hyaline
BFS ₇	(9.72-13.04) X (1.40 - 2.68)	(25.32-29.78) X (4.35 - 5.81)	0-1	2-3	Sickle shaped	Round to oval	Hyaline
GFS ₈	(7.82-9.60) X (2.26 - 3.14)	(22.55-26.11) X (3.63-4.95)	0-1	2-3	Sickle shaped with blunt ends	Round to oval	Hyaline

Morphological characteristics on PDA

The fungus *F. solani* produced two types of asexual spores viz., micro and macro conidia. The resting spores namely chlamydospores also were observed in 10-15 days age old cultures. The size of micro and macro conidia was ranged from (3-4 x 1-2) to (9-10 x 1-3) µm and (26.42-32.46) X (3.24-4.74) µm to (17.39-23.17) X (2.91-4.51) µm respectively, the number of septa in micro and macro conidia are 0-1 and 2-4 respectively and the colour is hyaline. The shape of macroconidia is sickle shaped and elongated with blunt ends and microconidia is round to oval shaped. The chlamydospores were oval, intercalary and terminal among the isolates (Table 3). The most

distinguished characters of long monophialidic conidiogenous cells were observed in all the isolates. Conidiophores were elongated and sparsely branched, each branch usually terminated with a spore bearing phialid. The maximum size of macroconidia was observed in isolates NFS₅ (26.42-32.46) X (3.24-4.74) µm and BFS₆ (23.99-32.63) X (5.10-6.26) µm followed by isolates BFS₇ (25.32 -29.78) X (4.35-5.81) µm, NFS₄ (23.88-29.24) X (3.21-4.97) µm, JFS₁ (22.36 - 28.92) X (3.78-4.32) µm, GFS₈ (22.55-26.11) X (3.63-4.95) µm and JFS₂ (21.16-25.44) X (2.65-3.87) µm whereas minimum macro conidia was observed in isolate NFS₃ (17.39-23.17) X (2.91-4.51) µm. The maximum size of micro conidia was observed in isolates NFS₄ (9.85-12.69) X (2.60-4.14) µm

and BFS₇ (9.72-13.04) X (1.40-2.68) µm followed by isolates NFS₅ (9.54-11.56) X (2.74-4.04) µm, GFS₈ (7.82-9.60) X (2.26-3.14) µm, BFS₆ (7.58-11.47) X (2.68-3.56) µm, JFS₁ (5.53-8.59) X (2.33-2.63) µm, and NFS₃ (5.3-8.52) X (2.27-2.59) µm whereas minimum microconidia was observed in isolate JFS₂ (3.94-7.04) X (1.98-2.40) µm. Sreedevi *et al.*, (2009) observed that the size of micro and macro conidia of *F. solani* causing root rot of patchouli ranged from (6.60-19.80 x 3.30-6.60) µm and (29.70-47.85 x 4.95-6.60) µm, respectively. The description of the pathogen is in agreement with that of *F. solani* (Mart.) Sacc. Booth (1971) as observed by Chandran *et al.*, (2012).

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