



## ***Fusarium*- a notorious pathogen of mulberry**

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### **Abstract**

Root rot disease in mulberry is a serious issue, severely affecting all varieties and threatening sustainable production. The bivoltine double hybrids of the silkworm (*Bombyx mori*, L.) are reared commercially for cocoon production in tropical southern India, hence, intensive mulberry farming is essential. Thus, the mulberry crop might be compromised due to continuous and massive biomass production. Hence, for understanding the virulence of root rot causing pathogens, their complexity, biology, studies on identification and characterization of the pathogen, etiology, symptomology and pathogenesis are important to devise effective disease management strategies and for minimizing losses. The disease incidence due to *Fusarium* was nearly 60% in the surveyed districts, which contained 40 plant samples. Most of the *Fusarium* isolates produced characteristic white to dull white mycelia with a cottony texture. Microscopical observations of *Fusarium* isolates revealed general characteristics of hyaline, thin-walled mycelia, macro and microconidia and thick walled chlamydospores. The genomic sequence of the isolates formed major clade with four distinctive sub-clades with four *F. solani* isolates and two *F. incarnatum* isolates formed sub-clade five in unrooted NJ phylogenetic tree. Further, the infection processes were also recorded *via in-vitro* pathogenicity tests and the KV1 isolate exhibited higher colonization and root decay.

**Keywords** – Chlamydospores – conidia – *Fusarium* – genomic sequence – in-vitro pathogenicity – mulberry – root-rot

### **Introduction**

Silkworm rearing and proteinaceous cocoon production was possible only with deciduous mulberry belonging to Moraceae. This plant is perennial grown and harvested throughout the year in tropical areas. It thrives in extreme climatic conditions and suited for cultivation in most of the agro-climatic conditions (Vijayan et al. 2014). There are 150 species known of mulberry comprising commercially cultivating varieties V1, MR2, G2, G4, and S36. Mulberry is cultivated around 2.62 Lakh Hectare in India with productivity of 65-67 MT/ Ha/ year mostly by small and marginal farmers. India is popular for all types of silk production including, mulberry and vanya silk-eri,

muga and tasar, nevertheless maximum raw silk production is from mulberry 31119 MT (76%) in 2025 (CSB 2025).

*Fusarium*, is a common filamentous soil-borne fungi and instigating significant threat to many plants worldwide. *Fusarium* belongs to Nectriaceae with more than 1500 recorded species. Most of the species are pathogens, producing mycotoxins and hindrance to much crop production (Arie 2019).

*Fusarium solani* and *Lasiodiplodia theobromae* were found as fungi related with mulberry root rot disease in different parts of Eastern and North Eastern India. Similarly, in Thailand, *F. solani* f.sp. *mori* had been isolated from the roots of infected mulberry (Sanoamuang et al. 1984). Dutta et al. (2017) mentioned *F. solani* as the causal agent of mulberry root rot in West Bengal. Additionally, *F. acuminatum* caused mulberry white fruit disease in China (He et al. 2025). *Fusarium* pathogens colonize and attack the vascular system of root, hindering the uptake of water and nutrients by plants (Srivastava et al. 2018). Once present, the fungus forms chlamydospores for long time, are difficult to eradicate and could easily spread through soil, water, implements, and plant debris (Arie 2019). Depending on the specific *Fusarium* pathogen and host plant, symptoms on the above-ground parts of the plant might vary significantly. The disease may cause lesions on the stems and/or leaves, wilting, stunting, and chlorosis (Coleman 2016).

*Fusarium* has been reported to cause diseases including leaf spot, leaf blight, leaf sheath rot and pseudobulb rot in Hawaii. Nevertheless, root rot is the major disease caused by numerous species and/ or sub-species of *Fusarium* in plants including mulberry, vegetables, and pulse crops. During crop rotation, some plants may not show symptoms but yield reduction was seen. Some plants like wheat, corn can serve as host for the pathogen development and multiplication (Jennifer 2025).

Root rot disease in mulberry is a serious issue, severely affecting all varieties and threatening sustainable production. Nine types of root rot diseases were reported in mulberry viz., dry rot, charcoal rot, black rot, white rot, violet rot, *Armillaria* rot, *Rhizopus* rot, *Rhizctonia* rot and bacterial rot. The root rot pathogens could cause leaf yield loss of maximum 70 per cent and complete mortality of plants (Sharma et al. 2003, Ganeshamoorthi et al. 2008, Sutthisa et al. 2010, Sowmya 2018, Gnanesh et al. 2020).

Equally, root rot disease severity in mulberry gardens can be influenced by soil, climate and environmental factors (Chowdary 2006, Saratha et al. 2021). The dry season with high levels of sunlight are the conditions for physical and biological changes in the soil that may support the growth of *Fusarium* and increase vascular wilt (Rosmana et al. 2014). *Fusarium* species are opportunistic pathogens, so they could infect weak and stressed plants.

Root diseases of mulberry, in contrast to foliar diseases, were observed year-round with varying severity. The mulberry cultivar V1 exhibited a maximum leaf loss of 39% and a disease incidence of 55% (Chowdary & Govindaiah, 2009, Rajeswari & Angappan 2018). Similarly root rot occurrence was recorded 2 to 30 per cent in Karnataka (Chamarajanagar district), Andhra Pradesh (Mandapale and Chittoor districts) and Tamil Nadu (Krishnagiri, Kanyakumari, and Salem districts) (Chandrashekar & Rajadurai 1999, Sharma 1999). Ghosh et al. (2016) reported the mortality of mulberry up to 50-60 per cent in India due to fungal infection.

Since there were no root rot resistant mulberry variety/ genotype available, disease management is difficult. Pinto et al. (2018) mentioned the difficulties in managing root rot diseases in comparison with foliar diseases and they were the major obstacles around the world. Hence, early detection of pathogen, etiology, symptomology and pathogenesis are important for minimizing losses. Management involves the combination of preventive measures, biological, cultural, chemical and physical.

## **Materials And Methods**

### **Survey and collection of samples**

A roving survey was conducted during 2019-2020 in traditional sericulture tract of Tamil Nadu (Coimbatore, Tiruppur, Erode, Dharmapuri and Krishnagiri districts). Infected mulberry root

samples were collected from two fields per block in each district, labeled and preserved for isolation of pathogens.

### **Isolation of pathogens**

Pathogens associated with mulberry root rot were isolated by tissue segment method. Diseased mulberry roots were rinsed with water, fragmented at the junction of healthy and rotten zones. Surface sterilization of root bits with sodium hypochlorite (1%), ethanol (70%) and thrice with sterile water was done. Aseptically air-dried root bits were inoculated on Potato Dextrose Agar (PDA) supplemented with streptomycin sulphate (0.03%) and incubated at  $28 \pm 2^\circ\text{C}$  for 7 days (Pappachan et al. 2020). The hyphal tip method was used to obtain pure cultures, which were then identified by spore/ sclerotia morphology and colony characteristics (Mihail 1992).

### **Morphological characterization of pathogens**

Pathogens associated with mulberry root rot disease were identified as described (Booth 1977, Dhingra & Sinclair 1973, Higgins 1992, Sowmya et al. 2018). Macromorphological characteristics of fungal pathogens such as topography, colony colour, and pigment production were recorded on PDA after seven days of incubation at  $28 \pm 2^\circ\text{C}$ . Relative growth rate was calculated (Mayek-Perez et al. 1997). Further micro-morphological characteristics including spore/ sclerotia size, and shape were recorded with the aid of phase contrast microscope (Leica DM2000, Germany) to establish the identity of the pathogens.

### **Molecular characterization of pathogens**

For molecular confirmation, the DNA of the fungal isolates was extracted using the CTAB method (Cetyl Trimethyl Ammonium Bromide method) (He 2000). Fungal mycelium (50 mg) was ground in liquid nitrogen and dissolved in CTAB buffer followed by hot water bath incubation and ice-cold centrifugation for 10 min at 13000 rpm.

The aqueous phase was mixed with double volume of phenol: chloroform (24:1) and subjected to ice-cold centrifugation for 10 min at 13000 rpm. The upper clear aqueous layer was collected, precipitated for overnight at  $20^\circ\text{C}$  using ice-cold isopropanol. Precipitated DNA pellet was washed thrice with 70 per cent ethanol, air dried and dissolved in sterile double distilled water. The presence of DNA was checked by discrete bands on 0.8 per cent agarose gel electrophoresis stained with ethidium bromide and purity using spectrophotometer (Nanodrop, USA) at 260 nm and 280 nm.

Genus and species identities of the pathogens were confirmed by amplifying the ITS 1 and ITS 4 regions of rRNA using master cycler (Eppendorf, Germany) (White et al. 1990). PCR reaction mix (40  $\mu\text{l}$ ) comprised ready to use 2x master mix (20  $\mu\text{l}$ ), primers (4  $\mu\text{l}$  each), Double distilled water (DDW) (8  $\mu\text{l}$ ), DNA sample (4  $\mu\text{l}$ ). The modified amplification conditions were (i) 10 min at  $95^\circ\text{C}$  of initial denaturation (ii) 35 cycles of denaturation at  $94^\circ\text{C}$  for 1 min, primer annealing at  $55^\circ\text{C}$  for 1 min, primer extension at  $72^\circ\text{C}$  for 1min and (iii) 10 min at  $72^\circ\text{C}$  of final extension (Sambrook et al. 1989). The amplicon was sequenced and submitted to the NCBI database.

Phylogenetic analysis was done in the MEGA program version 11.0 to confirm species identity. ITS-rRNA sequences of relative isolates of the pathogens were obtained from NCBI, GenBank. All the sequences were aligned using ClustalW algorithm and evolutionary history were analysed using the Neighbor-Joining (NJ) method. The genetic distances were computed using the Kimura-2-parameter method in a pairwise deletion manner and the nodal support was assessed by bootstrap analysis with 1000 replicates (Mahadevakumar et al. 2017).

### ***In-vitro* pathogenicity test**

Experiments to demonstrate the pathogenicity were carried out *in-vitro*. The detached root cortex method (Yoshida et al. 2001) with slight modifications was carried out as *in-vitro* study. Surface sterilized, pencil-thickness, healthy mulberry root bits of 8 cm length were used for the

study. Under aseptic conditions, 2 cm length root bark was removed using a sterile scalpel and 7 days old fungal mycelial disc of 6 mm (Ø) was inoculated. Un-inoculated samples were served as control. Three replications were maintained for each isolate and incubated at 28 ± 2°C for 20 days.

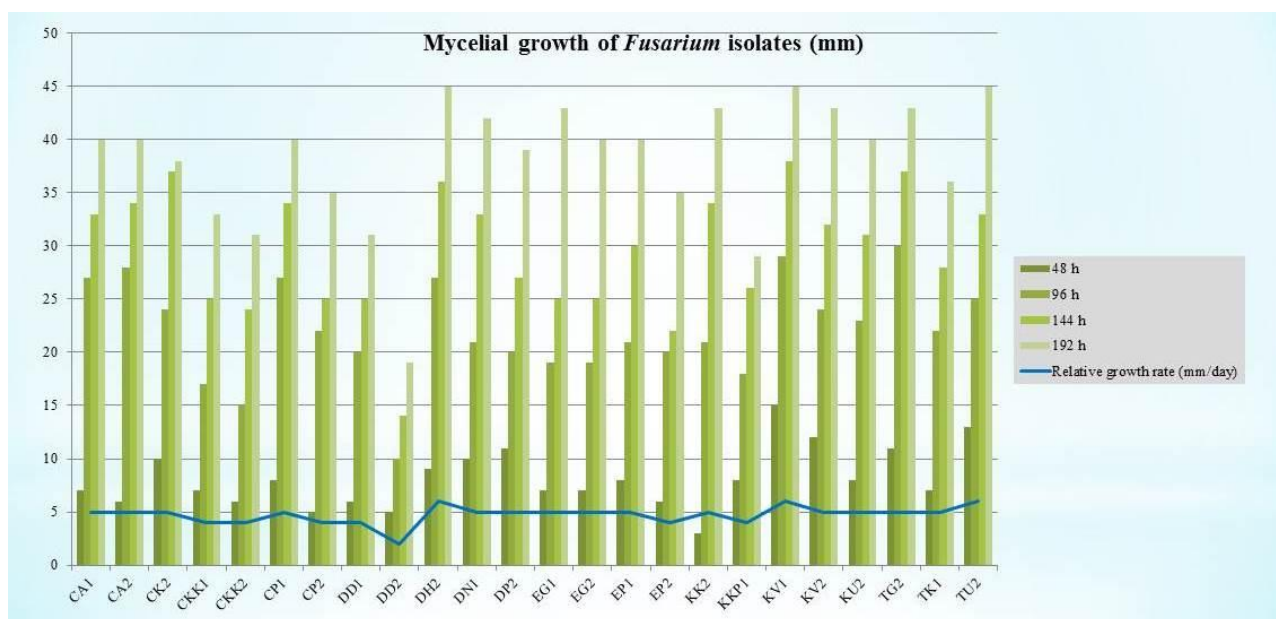
## Results

### Isolation of *Fusarium* pathogen

The disease incidence due to *Fusarium* genera was nearly 60% in the surveyed districts, which contained nearly 40 plant samples. Twenty-four isolates of *Fusarium* genera were obtained during isolation with highest number of isolates (n=7) from the Coimbatore District.

### Morphological characterization of pathogens

Relative growth rate of *Fusarium* isolates showed that 23 isolates recorded more than 4 mm/day, except the isolate DD2 recorded 2 mm/day (Fig. 1).



**Fig. 1** – Relative growth rate of *Fusarium* isolates

Only three isolates (DH2, KV1 and TU2) exceeded maximal radius in 8 DAI. Most of the *Fusarium* isolates produced characteristic white to dull white mycelia with cottony texture (Table 1; Fig. 2a-d).

**Table 1** Macro-morphology of *Fusarium* isolates

S. No	Isolate	Colony morphology in PDA
1	CA1	Dull white mycelium
2	CA2	White cottony mycelium; dark red saltation
3	CK2	Pale white mycelium; young mycelium bears tiny red dew drops; produce red pigment which darkens with age
4	CKK1	Dull white mycelium
5	CKK2	Dull white appressed mycelium
6	CP1	White cottony mycelium
7	CP2	White cottony mycelium becomes slimy on aging; rare pigment production

**Table 1** Continued

S. No	Isolate	Colony morphology in PDA
8	DD1	White mycelium; occasionally produce meroon red pigmentation and saltation
9	DD2	Creamy white appressed mycelium; produce reddish orange pigment
10	DH2	White mycelium
11	DN1	White appressed mycelim; produce purplish orange pigment
12	DP2	Creamy white mycelium
13	EG1	Creamy white mycelium; produce clear liquid/ dew drops on aging
14	EG2	Creamy white mycelium
15	EP1	Creamy white mycelium
16	EP2	Orange appressed mycelium becomes slimy on aging; occasionally observed reddish saltation
17	KK2	Grayish white appressed mycelium; produce reddish orange pigment become purple on aging
18	KKP1	Pale white dense, cottony mycelium
19	KV1	White mycelium with enormous watery sporodochia production
20	KV2	Creamy white sparse mycelium
21	KU2	White mycelium grow in wavy pattern
22	TG2	Cream colored mycelium; produce orange pigment
23	TK1	White mycelium; creamy saltations
24	TU2	White fluffy, cottony mycelium

*Fusarium* isolates exhibited occasional saltations (CA2, DD1) and some isolates produced pigments regularly in agar such as red (CK2), orange (EP2 and TG2), reddish orange (DD2), purplish orange (DN1, KK2). The colour intensity increased on aging of the culture. The observed colony morphologies were mostly normal, dense (KKP1), fluffy (TU2) and appressed for few isolates including CKK2, DN1, KV2. Colored or colorless watery dew drops were observed on the most isolates from 8 DAI (Fig. 2a-d).

### Microscopical characterization of pathogens

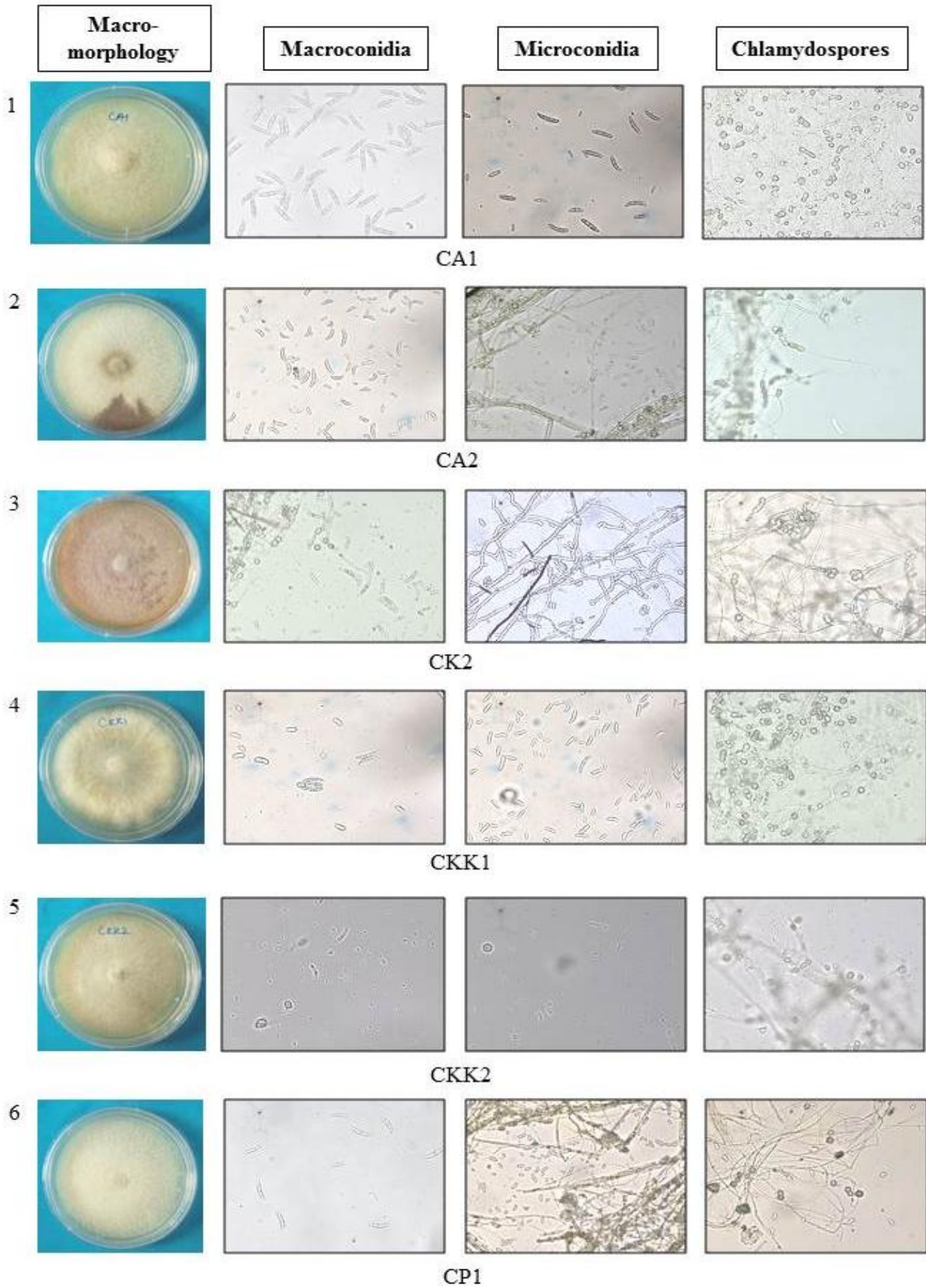
Microscopic observations revealed general characteristics of hyaline, thin-walled mycelia, conidia and thick walled chlamydospores of *Fusarium* isolates (Fig. 2a-d). Individual description of macroconidia, microconidia and chlamydospores for each isolate are presented in Table 2. Most of the *Fusarium* isolates produced abundant macro and micro-conidia. However, the isolates viz., CA2, CK2, CKK1, CKK2, CP1, DD2, KK2 and KKP1 produced sparse macro-conidia. Likewise, the isolates CA1, DN1, DP2 and TK1 had sparse production of micro-conidia. Macroconidia with maximum septations of five was observed in isolates EG1 and CKK1. The macroconidia ranged from 17-37 × 3-6 µm (MLW). The longest conidia were observed in isolate TU2 (37.47 µm), while the widest conidia in isolate CP1 (6.11 µm). However, the conidia of isolate KKP1 were the smallest and thinnest of all (Fig. 2c).

Similarly, the microconidia were mostly fusiform as single or two celled and the ranged from 10-23 × 3-6 µm (MLW). Shortest microconidia were observed in the isolate KU2 (10.19 µm) while the thinnest in CA1 (3.02 µm). The highly variable morphology of chlamydospores of the isolates was also observed (Table 2).

### Molecular characterization of pathogens

Genomic DNA of pathogens associated with mulberry root rot was isolated and internal transcribed spacer region (ITS) was amplified with appropriate primers (Table 3). PCR products

were sequenced and blasted in NCBI database. The results were augmented with morphological characters.



**Fig. 2a** – Macro and micro-morphology of *Fusarium* isolates

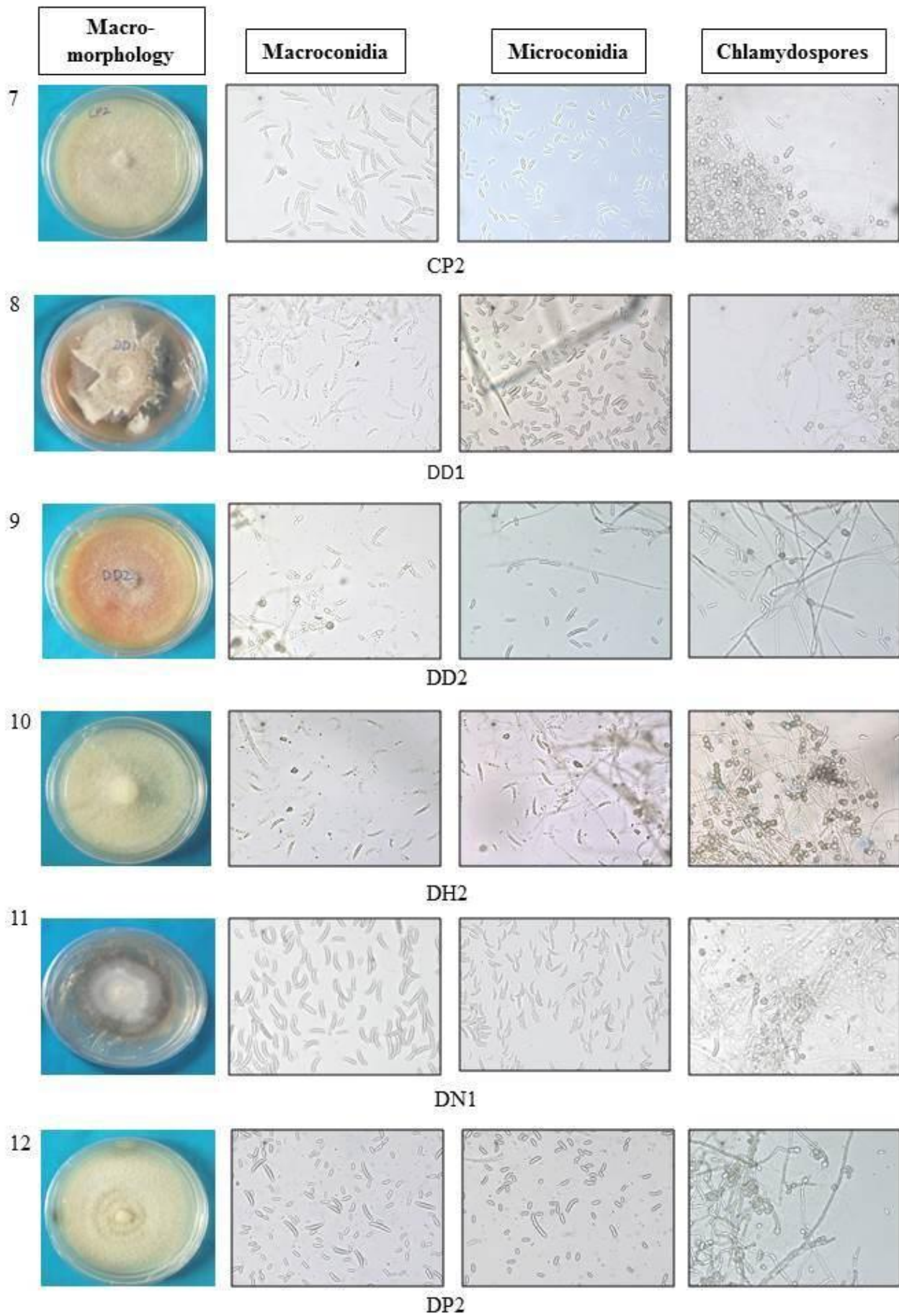


Fig. 2b – Macro and micro-morphology of *Fusarium* isolated

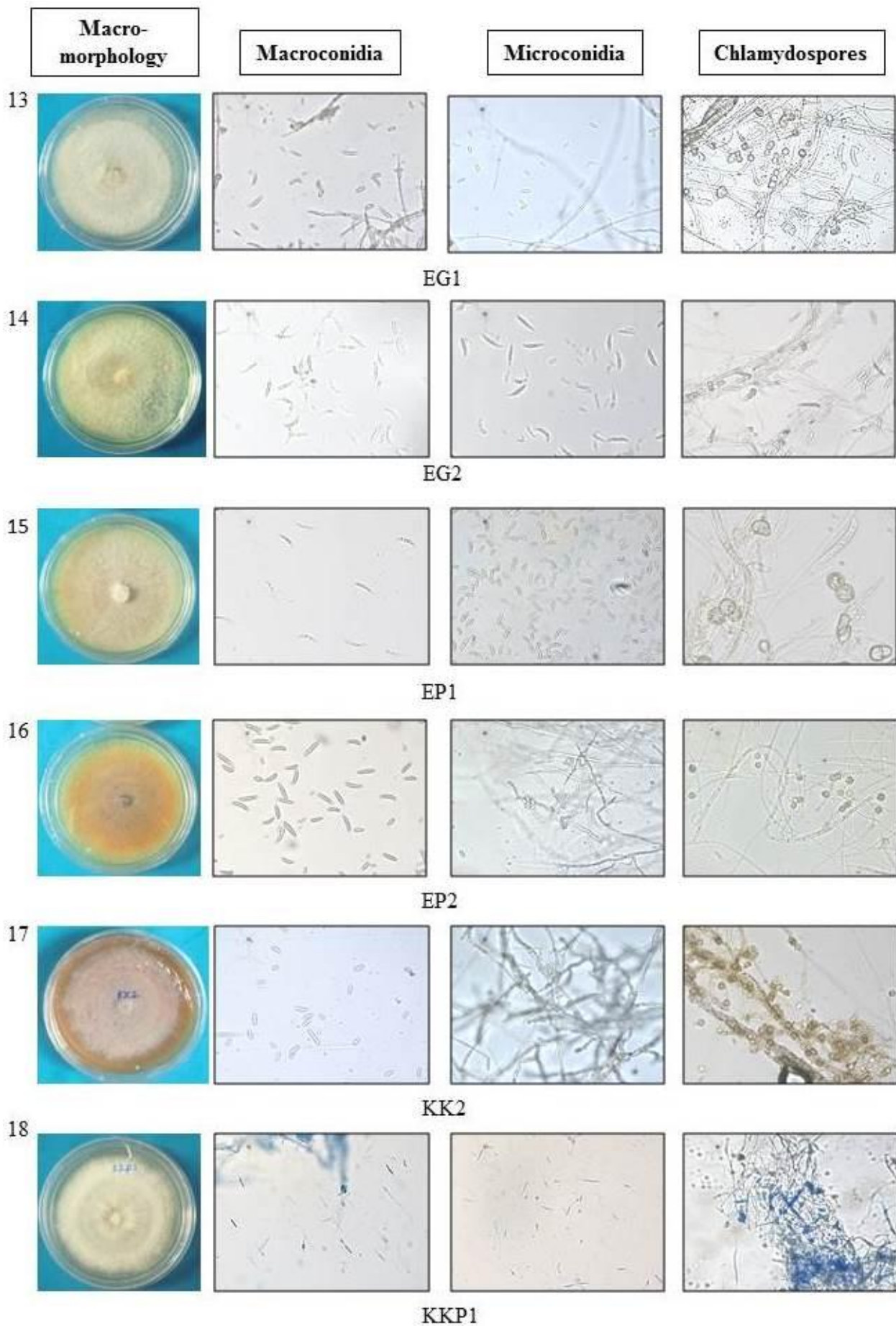


Fig. 2c – Macro and micro-morphology of *Fusarium* isolates

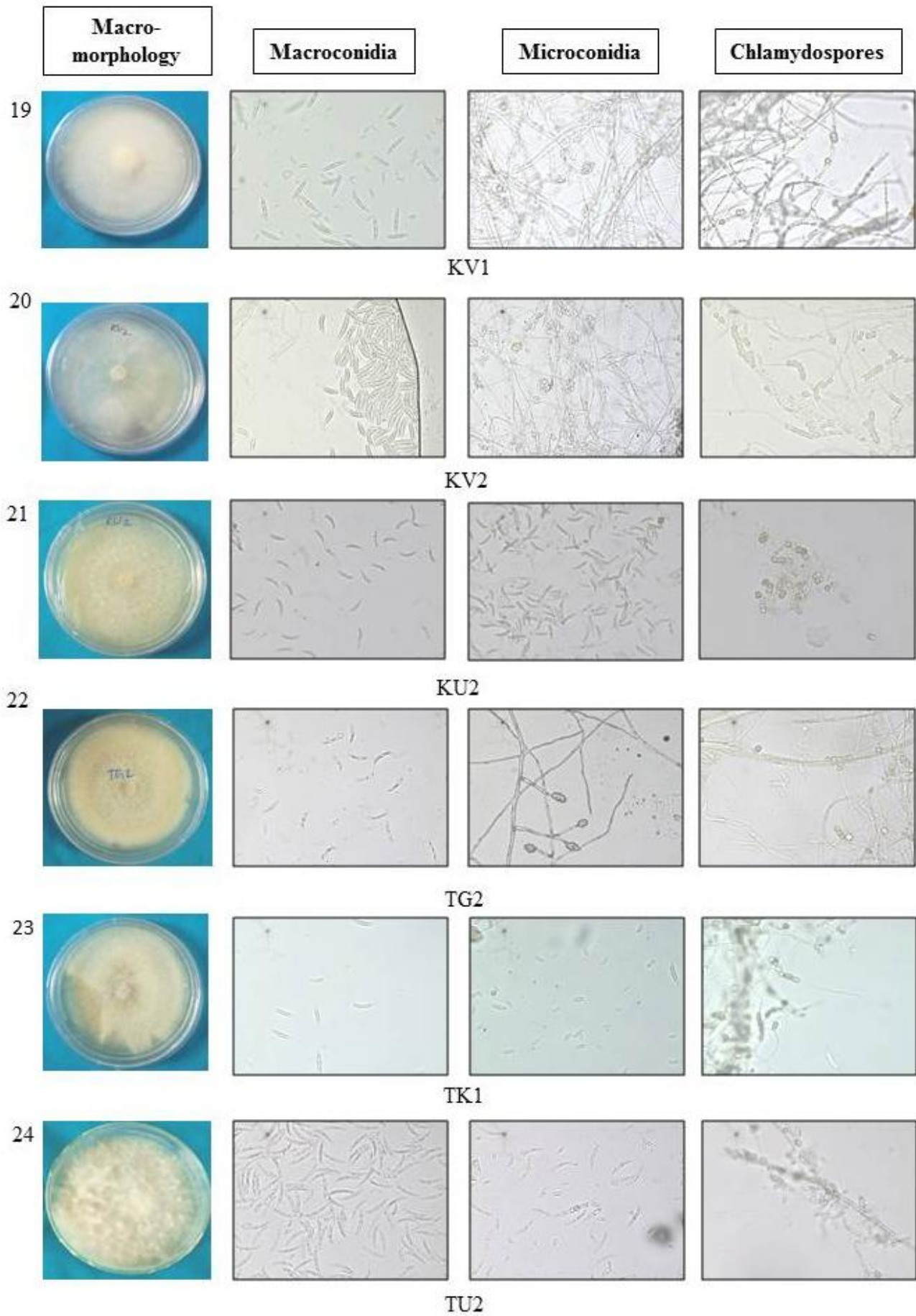


Fig. 2d – Macro and micro-morphology of *Fusarium* isolates

**Table 2** Micro-morphology of *Fusarium* isolates

S. No	Isolate	Micro-morphology (400X)		
		Macroconidia (MLW in $\mu\text{m}$ )*	Microconidia (MLW in $\mu\text{m}$ )*	Chlamydo spores (MD in $\mu\text{m}$ )*
1	CA1	32.93×4.31 Abundant; slender 0-4 septate; curved more on dorsal side; apical cell blunt; basal cell barely notched	16.07×3.02 Sparse; reniform-fusoid; 0-1 septate	9.05 Round with smooth walls; occur in single/ pairs
2	CA2	28.57×5.22 Sparse; 0-3 septate; curved more on dorsal side; apical cell hooked; basal cell barely notched	18.92×4.37 Abundant; reniform-fusoid; 0-1 septate; translucent walls	10.83 Round with smooth walls and occur in single
3	CK2	35.58× 4.91 Sparse; straight; 0-3 septate; apical cell blunt; basal cell barely notched	14.6× 5.26 Abundant; fusiform; 0-1 septate; occurred in poly-phialides	10.40 Paired; round with smooth walls; mostly found at terminal ends
4	CKK1	18.01×4.235 Sparse; 0-5 septate; long, slender almost straight; apical cell hooked; basal cell barely notched	33.55×4.20 Abundant; fusiform; 0-1 septate	8.61 Round with smooth walls; occur in chains and clusters
5	CKK2	30.06× 9.65 Sparse; 0-3 septate; curved; apical cell blunt; basal cell barely notched; translucent walls	16.84×5.16 Abundant; oval; rarely septate; translucent walls	9.77 Paired/ single; round with smooth walls
7	CP2	36.61×5.61 0-4 septate; curved; apical cell blunt; foot shaped basal cell	13.57×6.02 Reniform-fusoid; 0-1 septate; occurred in short mono-phialides	9.03 Round with smooth walls; occur in single/ pairs/ chains;
8	DD1	30.63×3.86 Long, slender almost straight; hyaline; 0-3 septate; apical cell blunt; basal cell barely notched; translucent walls	10.03×3.57 Abundant; fusiform; 0-1 septate	8.91 Round with smooth walls; occur in single/ pairs
9	DD2	30.24×5.83 Sparse; almost straight; 0-3 septate; apical cell blunt; basal cell barely notched	11.60×5.30 Abundant; oval-fusiform; 0-1 septate;	10.81 Round with verrucose walls; occur in single/ pairs
10	DH2	28.45×5.26 Abundant; 0-3 septate; slender; straight; apical cell blunt; basal cell barely notched; translucent walls	12.68×4.69 Oval-reniform; 0-1 septate; translucent walls	9.65 Round with smooth walls; occur in single/ pairs/ chains
11	DN1	34.07×5.23 Abundant; 0-3 septate; slightly curved; apical cell blunt; basal cell barely notched	15.65×5.30 Sparse; oval-reniform; 0-1 septate	8.90 Chain of verrucose chlamydo spores; rarely occur in single

**Table 2** Continued

S. No	Isolate	Micro-morphology (400X)		
		Macroconidia (MLW in $\mu\text{m}$ )*	Microconidia (MLW in $\mu\text{m}$ )*	Chlamydo spores (MD in $\mu\text{m}$ )*
12	DP2	28.39×4.38 Abundant; 0-3 septate; curved dorsoventrally; apical cell papillate; basal cell barely notched	15.68×3.82 Sparse; obvoid; 0-1 septate; occurred in false heads	6.29 Round; rarely occur in single/ pairs
13	EG1	31.42×4.43 Abundant; 0-5 septate; slightly curved; apical cell blunt; basal cell barely notched	13.73×4.25 Abundant; oval-fusiform; 0-1 septate;	8.145 Large, round, smooth walled chlamydo spores in chains
14	EG2	31.26×3.85 Abudant; 0-3 septate; curved more on dorsal side; apical cell hooked; basal cell barely notched; translucent walls	15.03×4.53 Oval; 0-1 septate	7.32 Round, smooth walled chlamydo spores either appear in chains or single
15	EP1	31.68×4.12 0-3 septate; curved more on dorsal side; apical cell tapering; basal cell barely notched; translucent walls	11.94×3.11 Abundant; 0-1 septate; oval-fusiform	10.06 Round; paired verrucose chlamydo spores
16	EP2	27.84×5.39 Abudant; 0-3 septate; slightly curved; apical cell blunt; basal cell barely notched	11.98×4.23 Abundant; 0-1 septate; oval-fusiform, reniform also observed; occurred in short monophialides	8.66 Round; verrucose chlamydo spores occur in clusters, pairs and single
17	KK2	22.49 × 4.94 Sparse; 0-3 septate; straight; apical cell blunt; basal cell barely notched	12.91×4.14 Abundant; 0-1 septate; oval-fusiform occurred in false heads	9.10 Round; single verrucose chlamydo spores
18	KKP1	17.98×3.37 Sparse; 0-3 septate, long slender; apical cell sharp; basal cell foot shaped	10.44×3.38 Oval; 0-1 septate	6.89 Round; paired smooth chlamydo spores
19	KV1	32.29×4.61 Abundant; 0-4 septate, long slender; straight/ slightly curved apical cell papillate; basal cell barely notched	13.04×4.15 Abudant; oval-reniform; 0-1 septate occurred in long monophilades	6.91 Round; paired smooth chlamydo spores
20	KV2	32.52×5.03 Abundant; 0-3 septate; apical cell blunt; basal cell barely notched	15.45×3.55 Abudant; oval; occurred in long monophialides; rarely septate	7.87 Round; verrucose chlamydo spores occurred in chains

**Table 2** Continued

S. No	Isolate	Micro-morphology (400X)		
		Macroconidia (MLW in $\mu\text{m}$ )*	Microconidia (MLW in $\mu\text{m}$ )*	Chlamydo spores (MD in $\mu\text{m}$ )*
21	KU2	29.14×4.73 Abundant; 0-3 septate; dorsoventrally curved; apical cell blunt; basal cell barely notched; translucent walls	10.19×4.30 Oval, stout; 0-1 septate	8.30 Round; smooth chlamydo spores occurred in single and chains
22	TG2	28.24×4.25 0-3 septate; dorso ventrally curved; apical cell blunt; basal cell barely notched; translucent walls	17.39×3.66 Oval; 0-1 septate; long monophialides; translucent walls	8.60 Round smooth chlamydo spores occurred in chains
23	TK1	30.74×4.72 Abudant; 0-3 septate; almost straight and few are curved more on dorsal side; apical cell hooked; basal cell barely notched	12.41×4.52 Sparse; oval and some with truncate base; single celled	9.59 Round verrucose chlamydo spores occurred in pairs and chains
24	TU2	37.47×4.15 Abudant; 0-3 septate; long, slender; curved more on dorsal side; apical cell hooked; basal cell barely notched; translucent walls	11.70×4.41 Abudant; rentiform; 0-1 septate; translucent walls	8.42 Round smooth walled paired chlamydo spores
*MLW- Mean length and width of 10 randomly observed conidia ( $\mu\text{m}$ );				
**MD- Mean diameter of 10 randomly observed chlamydo spores ( $\mu\text{m}$ )				

**Table 3** Molecular characterization of *Fusarium* isolates

S. No	Isolates	Amplicon size (bp)	Identity authenticated by NFCCI	NCBI accession number
1	CA1	587	<i>Fusarium</i> sp.	OL685020
2	CA2	535	<i>Fusarium</i> sp.#	OL685021
3	CK2	530	<i>Fusarium solani</i>	OL685024
4	CKK1	506	<i>Fusarium solani</i>	OL685025
5	CKK2	500	<i>Fusarium</i> sp.	OL685026
6	CP1	546	<i>Fusarium solani</i>	OL685022
7	CP2	430	<i>Fusarium solani</i>	OL685023
8	DD1	595	<i>Fusarium solani</i>	OL685028
9	DD2	354	<i>Fusarium solani</i>	OL684983
10	DH2	537	<i>Fusarium solani</i>	OL692809
11	DN1	529	<i>Fusarium solani</i>	OL692810
12	DP2	541	<i>Fusarium</i> sp.#	OL692811
13	EG1	488	<i>Fusarium solani</i>	OL699941
14	EG2	201	<i>Fusarium solani</i>	OL699942
15	EP1	584	<i>Fusarium</i> sp.#	OL692814
16	EP2	398	<i>Fusarium</i> sp.#	OL685027

**Table 3** Continued

S. No	Isolates	Amplicon size (bp)	Identity authenticated by NFCCI	NCBI accession number
17	KK2	525	<i>Fusarium solani</i>	OL692816
18	KKP1	495	<i>Fusarium</i> sp.	OL685029
19	KV1	535	<i>Fusarium solani</i>	OL692818
20	KV2	512	<i>Fusarium solani</i>	OL685030
21	KU2	410	<i>Fusarium solani</i>	OL692817
22	TG2	577	<i>Fusarium</i> sp.	OL692815
23	TK1	526	<i>Fusarium</i> sp.#	OL692813
24	TU2	490	<i>Fusarium solani</i>	OL692812

# Affinity with *Fusarium solani*.

The unrooted NJ phylogenetic tree exhibited the closeness between 24 *Fusarium* isolates of the present study and six other *Fusarium* isolates of mulberry. The isolates of the present study formed major clade with four distinctive sub-clades with four *F. solani* isolates and two *F. incarnatum* isolates formed sub-clade five. However, *F. solani* and *F. oxysporum* had overlapping morphological and ecological characters. Species level identification was complicated in case of *Fusarium* isolates. Hence the isolates were confirmed only up to genera level with the help of molecular tools. The phylogenetic tree depicted the relatedness among the *Fusarium* isolates obtained from mulberry of different regions. Furthermore, the identity of *Fusarium* isolates was authenticated by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune (Table 3).

#### ***In-vitro* pathogenicity test**

Pathogenicity study was carried out *in-vitro* to visualize the colonization and infestation abilities of isolated pathogens in mulberry roots. The growth of *Fusarium* isolates on mulberry roots was slow, thin-layered as compared to the above pathogens yet with enormous sporodochia production was observed from 5 DAI. The color change in root tissue was prominent around the zone of inoculation since the colonization was thin. Among 24 *Fusarium* isolates, KV1 isolate exhibited higher colonization and root decay followed by the isolate DH2.

Un-inoculated control roots remained healthy without any symptoms. The re-isolation of the pathogens from disintegrated and inoculated bits, resulted the respective pathogens, thus confirming pathogenicity.

#### **Discussion**

Root rot, the economically important disease of mulberry had been managed with various integrated strategies (Choudhari et al. 2012). When compared to foliar diseases, managing root diseases in perennial crop is quite challenging. Hence, these pathogens of mulberry have received increased attention over the past decades. Root rot pathogens are necrotrophic and have wide host range with higher surviving ability even under extreme conditions (Sharma et al. 2003). Complete information on the casual agents is necessary to develop strategies to combat root rot disease. Therefore, the present study was focused on documenting *Fusarium* associated with mulberry root rot disease. Sowmya (2018) isolated and identified 35 isolates of *M. phaseolina*, 10 isolates of *B. theobromae*, 27 isolates of *F. oxysporum* and *F. solani* associated with mulberry root rot. Including *Fusarium* sp. the above pathogens have been reported as mulberry root rot casual agents (Sharma et al. 2003, Xie et al. 2014). The complex biodiversity, interplay and the soil-borne nature of root rot

pathogens hindered disease management in almost all mulberry growing countries. Moreover, nematodes also had role in occurrence of root rot disease complex (Gnanesh et al. 2020).

Sutthisa et al. (2010) isolated 30 isolates of *Fusarium* sp. from the mulberry roots and these isolates produced sparse aerial and cottony mycelia with varied colony color from white to purple. *Fusarium* sp. in culture plates initially produced white cottony mycelium and a dark-purple undersurface on PDA medium (Trabelsi et al. 2017). Nguyen et al. 2019 isolated *Fusarium proliferatum* (73 isolates) associated with the Indian mulberry and recorded whitish mycelial growth on PDA, later becoming light orange. *Fusarium* sp. from root rot infected mulberry plants initially had white cottony mycelium turning bluish brown later (Pappachan et al. 2020). Various phenotypic traits were documented for each category of pathogen in this study and difference in morphological features across isolates were explained as diversity in cropping systems, increased genetic diversity and altered pathogenic behavior (Mayek-Perez et al. 2001). Shah et al. (2010) also mentioned that diversity assessment and characterization of fungal pathogens were based on mycelial growth patterns, colony colour, and pycnidia production/ abundance.

Conidia are the primary means of asexual reproduction, dispersal and epidemics in a wide range of pathogenic fungi (Zheng et al. 2021). The results found in accordance with the observations of Sutthisa et al. (2010) in mulberry. *Fusarium* isolates recorded differential colony morphology and four distinct macro and micro conidial shapes. Nguyen et al. (2019) isolated *Fusarium proliferatum* (73 isolates) associated with the Indian mulberry with microconidia, produced in false heads and chains, were hyaline, 0–1 septate, clavate with a truncate base whereas macroconidia were hyaline, 3–5 septate, and sub-falcate to needle-like shaped.

*Fusarium* associated with mulberry produced hyaline, thick-walled, sub-cylindrical, slightly curved, short and bent falcate, oval or cylindrical macroconidia, with predominantly 3 septa and microconidia were hyaline and smooth-walled. There was abundant production of chlamydospores with huge variations in position and morphology including terminal or intercalary, smooth, globose to sub-globose isolated from mulberry root rot (Pappachan et al. 2020). Microconidia were oval to ellipsoid or kidney shaped, while macroconidia were oval, tapering and 3-septate and  $32\text{--}56 \times 3.1\text{--}5.7 \mu\text{m}$ . Abundant chlamydospores were also produced in culture plates (Trabelsi et al. 2017).

Singha et al. (2016) identified the *Fusarium* sp. based on the conserved ribosomal internal transcribed spacer region using universal primer pairs ITS1 and ITS 4 which varied from 380 to 620 bp amplicon size and also assessed relatedness among these isolates using Random amplified polymorphic DNA (RAPD). Quazi et al. 2013 reported that the PCR products of *Fusarium* isolates amplified using Universal primers (ITS1 and ITS4) resulted in amplified products with size of 500–600 bp. Sutthisa et al. (2010) used random amplified polymorphic DNA markers (RAPD) and identified 157 isolates of *Fusarium* belonged to 11 species isolated from mulberry roots and rhizosphere including *F. solani*, *F. oxysporum*, *F. phaseoli*, *F. culmorum*, *F. moniliforme*, *F. graminearum*, *F. scirpi*, *F. anthophilum*, *F. dlamini*, *F. dimerum* and *F. beomiforme*. However, among *Fusarium* pathogens, *F. solani* was shown to be the most pathogenic and caused mulberry root rot in pathogenicity tests.

The development and infection process of root rot casual agents were visualized with the help of pathogenicity tests conducted on sterilized mulberry root parts. The infection process ensued and enhanced by wounds made in mulberry root parts (Saratha et al. 2022). Upon contact with host roots, microsclerotia germinated on the root surface and germ tubes entered the epidermis through mechanical pressure/ enzymatic digestion/ natural apertures (Bowers et al. 1996). The hyphae grew in the cortex before spreading through the taproot and lower stem of the plant, colonized the vascular tissue and produced microsclerotia for next generation (Wyllie & Scott 1988). Yoshida et al. (2001) mentioned detached root cortex method as more suitable for preliminary and quick screening. In the present study, rotting intensity including softening of root tissue followed by discoloration and disintegration occurred with age, however virulent isolates exhibited early infection symptoms. Previously, researchers (Yoshida et al. 2001, Gnanesh et al. 2020) observed rotting of cortical tissues in root segments around *Rhizopus* mycelia blocked similar to that of infection in the mulberry field.

The intensity of disease by soil-borne pathogens was determined by the host-pathogen interaction, as well as the pathogen's capacity to compete with other microorganisms in the soil/rhizosphere for survival, development, colonization and infestation of the host root (Sowmya et al. 2018). Primary infection by one pathogen followed by secondary infection by other root rot causing fungi had resulted in more significant manifestation of the disease in mulberry (Yadav et al. 2019).

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### Conflict Of Interest

The authors declare no conflicting interests

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