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Survey, isolation and pathogenicity on cucumber anthracnose caused by *Colletotrichum lagenarium* (Pass.) Ellis & Halst in Tamil Nadu

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Abstract

Colletotrichum is one of the major plant pathogen causing anthracnose disease in variety of plant species. Anthracnose is a devastating plant disease that can cause cucurbitaceous crop losses up to 30 per cent. Survey was conducted in major cucumber growing districts of Tamil Nadu viz., Thoothukudi, Tirunelveli, Tenkasi, Kanyakumari and Namakkal. The maximum disease incidence was recorded in Marukalkurichi village of Tirunelveli district (70.37 PDI) and the minimum disease incidence was recorded in Serukalai village of Namakkal district (40.74 PDI). Samples were collected from the surveyed areas, from which nine isolates of *Colletotrichum lagenarium* were isolated and CI I₂ from Marukalkurichi village sample was found to be virulent.

Keywords: Cucumber, *Cucumis sativus* L., anthracnose, *Colletotrichum lagenarium*

1. Introduction

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family. *C. sativus* is an indeterminate, tender creeping vine and having hairy leaves with three to five pointed lobes. It bears roughly cylindrical fruits and may be as large as 60 cm long and 10 cm in diameter with dark-green skin, crispy moisture, rich flesh and small edible seeds concentrated at its core. At present, cucumber is the fourth most widely cultivated vegetable crop next to tomatoes, onions and cabbage in the world (Maheshwari *et al.*, 2014)^[8]. Cucumber is a crop with a short growing season (90-100 days) and yield about 8-10 t/ha.

In India's total vegetable production, cucurbitaceous crops occupy around 5.6 per cent. Cucumber production in India ranges from 16,52,000 MT to 16,08,000 MT in the year of 2020-2021 and 2021-2022 respectively. According to 2020-21 data, cucumber cultivated area in Tamil Nadu was 2750 hectares and the production was 24,920 MT (India Stat, 2021)^[17].

Cucumber crop is affected by several pest and diseases. Anthracnose (*Colletotrichum lagenarium*) is a deadly disease that infects all parts of the cucumber plant. The first report has been documented in Shalimar, Kashmir. Anthracnose (water-soaked) like symptoms were discovered on cucumber vines in Shalimar, Srinagar, Jammu & Kashmir (Yousuf *et al.*, 2018)^[15]. When the environment is humid, the lesions become dotted with pinkish conidial masses.

The current study has been carried out on survey of cucumber anthracnose disease incidence in cucumber growing areas of Tamil Nadu, pathogenicity studies of *C. lagenarium* and morphological and cultural characterization of the various isolates of *C. lagenarium*.

2. Materials and Methods

2.1 Survey and collection of diseased sample

Roving survey was conducted during 2021-2022 to assess the severity of anthracnose disease incidence in Thoothukudi, Tirunelveli, Tenkasi, Kanyakumari and Namakkal districts of Tamil Nadu. Nine villages viz., Srivenkateshwarapuram (Thoothukudi), Marukalkurichi (Tirunelveli), Salaipudhur (Thoothukudi), Serukalai (Namakkal), Maniyarampudhur (Namakkal), Naduvapatti (Tenkasi), Krishnapuram (Tenkasi), Nanguneri (Tirunelveli) and Eraviputhur (Kanyakumari) were surveyed. The anthracnose infected leaf samples were collected and labelled properly in a clean polythene bag. The severity of the disease was determined in each of the selected villages by rating on a 0-9 scale (Montri *et al.*, 2009)^[10].

Grade	Description
0	No visible symptom
1	< 1% infection
3	1-10% infection
5	11-25% infection
7	26-50% infection
9	>50% infection

Per cent Disease Index (PDI) of anthracnose was calculated by using formula given by McKinney (1923) [9].

$$\text{Percent Disease Index} = \frac{\text{Sum of all individual disease ratings} \times 100}{\text{Total No. of plants observed} \times \text{Maximum disease grade}}$$

2.2 Isolation of *Colletotrichum lagenarium* and cultural characterization

The infected leaf samples collected from nine villages viz., Srivenkateswarapuram (Thoothukudi), Marukalkurichi (Tirunelveli), Salaipudhur (Thoothukudi), Serukalai (Namakkal), Maniyarampudhur (Namakkal), Naduvapatti (Tenkasi), Krishnapuram (Tenkasi), Nanguneri (Tirunelveli) and Eraviputhur (Kanyakumari) were used for the isolation of pathogen. The diseased tissue was cut into small pieces and surface sterilized for 30 seconds with 0.1 per cent mercuric chloride solution followed by two to three washings with sterile distilled water. Sterilized Potato Dextrose Agar media was poured into sterilized Petri plates under aseptic condition. Infected cucumber anthracnose samples were placed over the solidified media in three places at equal distances, then

incubated at 28 ± 2 °C for ten days. By following the hyphal tip procedure, the developing young fungal hyphal tip was aseptically transferred into the sterile plates containing PDA medium for purification (Sinclair and Dhingra, 2017) [4]. By following the same method, remaining eight pathogenic isolates were obtained. The pure cultures of all the nine isolates were maintained in PDA slants. The identification of the pathogen was confirmed by examining the conidia under the microscope. The plates were observed for the morphological and cultural characters viz., colour of mycelium, radial growth of mycelium, colour and shape of the conidia, length and width of the conidia.

2.3 Pathogenicity

Pathogenicity of the fungus was proved by Koch postulates. Potato dextrose broth medium was autoclaved and inoculated with a nine mm fungal disc of *Colletotrichum lagenarium*. The broth medium was incubated for 5-7 days at 28 ± 2 °C. The spore suspension was adjusted to 8×10^5 using a haemocytometer and the inoculum was sprayed over the healthy cucumber plant using hand sprayer. The inoculated plants were covered with perforated polythene bags and symptom development was monitored and recorded on a regular basis. The plants were examined up to 14 days and the severity of the disease was assessed using 0–9 point scale based on the disease assessment method given by Dasgupta (1981) [3].

Grade	Description
0	No infection (Highly Resistant)
1	Necrotic lesion or large water-soaked lesion (Resistant)
3	Acervuli present (Moderately Resistant)
5	Necrotic lesion, surface covered 25% of leaf surface, possible acervuli (Susceptible)
7	>25% of the leaves showing necrosis (Moderately Susceptible)
9	>25% of the leaves showing necrosis, abundant acervuli (Highly Susceptible)

2.4 Statistical Analysis

WASP was deployed to record and analyse the experimental findings (Web Agri Stat Package). The percentage values were transformed into an arc sine at critical difference levels with 5% significance levels.

3. Results and Discussion

3.1 Survey on the incidence of anthracnose of cucumber

The conducted roving survey revealed that the disease was present in five districts during 2021-2022. The overall disease severity ranged from 40.74 PDI to 70.37 PDI. The highest PDI of 70.37 was noticed in Marukalkurichi village of Tirunelveli district followed by Nanguneri village of Tirunelveli district (67.40 PDI). The least PDI of 40.74 was recorded in Serukalai village of Namakkal district. Cucumber anthracnose pathogen *Colletotrichum lagenarium* isolated from all the nine samples were named as CI I₁, CI I₂, CI I₃, CI I₄, CI I₅, CI I₆, CI I₇, CI I₈ and CI I₉ (Table 1; Fig 1).

Mundkar (1937) [11] originally documented the disease in India on 'Kakri' (*Cucumis melo* var. *utilissima* Roxb) and 'kaddu' (*Lagenaria vulgaris* Ser.) from Punjab. Roy (1965) [12] discovered it on cucumber and Wax gourd (*Bemincasa cerifera*) leaves in Assam.

3.2 Symptomatology

All the aboveground parts of cucumber plants were exhibited

anthracnose symptoms. First instance, lesion appeared as small and water-soaked on cotyledons. They were limited and pale in colour with chlorotic (yellow) or necrotic (brown) lesion. The lesion enlarged and the cotyledons were dry up eventually and die as the condition worsen. Similar symptoms start showing up on the leaves. The early indications might be water-soaked, have vein delimitation and be angular like angular leaf spot or downy mildew. However, lesions enlarge, becoming more necrotic, rounded and frequently lose their centres as they progress (Fig. 2). Infected petioles and stems results in vine defoliation, fruit decline and plant death. Fruit lesions were sunken, round, wet and black in colour (Crouch and Beirn, 2009) [2].

Water-soaked lesions appeared on the stems and leaves at six days after inoculation followed by necrosis and tissue shrivelling (Keinath, 2015) [7].

3.3 Pathogenicity

By inoculating artificially using pinprick technique, all the nine isolates of *C. lagenarium* were capable of manifesting the characteristic anthracnose symptoms on the leaves. The Koch's postulate was proved when the pathogenic isolates were reisolated from the artificially infected cucumber leaves and reproduced the same type of symptom on leaves. Among the nine isolates, CI I₂ showed highest disease severity followed by CI I₈. The least disease severity was observed in

CI I₄ and CI I₅. Based on disease severity level, CI I₂ was considered as highly virulent isolate. (Table 2; Fig 4).

Keinath (2015) [7] conducted inoculation studies on cucumber seedlings and kept inoculated plants in a humid environment.

3.4 Morphological and Cultural variations of *C. lagenarium*

On PDA medium, the morphological and cultural characteristics of different isolates of *C. lagenarium* were examined. The results revealed that all the isolates of *C. lagenarium* exhibited fluffy mycelial growth. The mycelial mat of CI I₁ exhibited dark brown colour, CI I₂ and CI I₄ showed light brown colour, CI I₅ showed blackish grey colour, whereas CI I₃, CI I₆ and CI I₇ showed greyish brown colour CI I₈ and CI I₉ showed greyish white colour. The maximum radial growth was observed in CI I₂ (80.72 mm) and found to be fast growing which has taken 10 days to completely cover the plate. The minimum radial growth of 56.32 mm was observed in CI I₄ and it has taken 14 days to cover the full plate. Among the isolates, excellent sporulation was found to be in CI I₂, good sporulation in CI I₁ and CI I₇, moderate sporulation was observed in CI I₃, CI I₅, CI I₆, CI I₈ and CI I₉. The isolate CI I₄ showed poor sporulation (Table 3; Fig 3). All the isolates produced hyaline conidia. The shape of the conidia is falcate (CI I₁, CI I₃ and CI I₅), ovate (CI I₄, CI I₆

and CI I₉) and oblong (CI I₂, CI I₇ and CI I₈). Significant variations in conidial dimensions were noticed among the isolates. The length of the conidia ranged from 12.15 µm to 18.50 µm. The highest conidial length was observed in CI I₂ (18.50 µm) followed by 17.32 µm in CI I₈. The minimum conidial length of 12.15 µm in CI I₄. Width of the conidia was ranged from 3.73 µm to 5.79 µm. The maximum width of conidia was observed in CI I₂ (5.79 µm) followed by 5.56 µm in CI I₈. The minimum conidial width (3.73 µm) was noticed in CI I₄ (Table 4; Fig 5).

Conidial size of *Colletotrichum lagenarium* ranged from 13-19 x 4.6µm (Gardner, 1918). Goode (1958) compared the pathogenic and morphological differences between several isolates of *C. lagenarium* on Potato dextrose agar medium. He reported that, submerged mycelia of *C. lagenarium* formed greenish black to black colonies which sporulate abundantly. Size of the conidia varied from 11-16 x 4-6 µm and 13.8 x 4.8 µm (Bose *et al.*, 1973). Broad oblong, round end conidia with the size of 14.0 x 3.7 µm was reported by Simmonds (1966) [14]. Transparent, oval or pill-shaped conidia without cross wall were reported by Zitter (1987) [16].

According to Shen *et al.*, (2001) [13] the conidia of *C. orbiculare* were to be straight to slightly curved with obtuse ends, measuring 13.1±1.6 µm in length and 5.4±0.7 µm in width.

Table 1: Survey and incidence of cucumber anthracnose in major cucumber growing areas of Tamil Nadu

S. No	Isolate No	Latitude and Longitude	Village Name	District	Variety Name	Per cent Disease Index (PDI)*
1	CI I ₁	8.534844 °, 77.849244 °	Srivenkateshwara puram	Thoothukudi	Local	57.77 ^d
2	CI I ₂	8.51031°, 77.676819°	Marukalkurichi	Tirunelveli	CO 1	70.37 ^a
3	CI I ₃	8.534458 °, 77.851017 °	Salaipudhur	Thoothukudi	Asoka	47.40 ^g
4	CI I ₄	11.281351°, 77.996823°	Serukalai	Namakkal	Local	40.74 ^h
5	CI I ₅	11.079017 °, 78.156173 °	Maniyarampudhur	Namakkal	Local	50.37 ^f
6	CI I ₆	9.4158 °, 77.9203 °	Naduvapatti	Tenkasi	Local	48.14 ^{fg}
7	CI I ₇	9.1506 °, 76.5311 °	Krishnapuram	Tenkasi	Local	53.33 ^e
8	CI I ₈	8.4961 °, 77.6465 °	Nanguneri	Tirunelveli	Local	67.40 ^b
9	CI I ₉	8.23738 °, 77.33989 °	Eravipudhur	Kanyakumari	Local	64.44 ^c
S.E(d)						1.829
CV						2.435
CD (P = 0.05)						2.341

*Mean of three replications

The treatment mean was compared by using Duncan's Multiple Range Test

In a column, mean followed by same superscript is not significantly different at P = 0.05

Table 2: Severity levels of various isolates of *C. lagenarium* (Pathogenicity test)

S. No	Isolates	Grade	Description
1	CI I ₁	5	Necrotic lesion, surface covered 25% of leaf surface, possible acervuli
2	CI I ₂	9	>25% of the leaves showing necrosis, abundant acervuli
3	CI I ₃	3	Acervuli present
4	CI I ₄	1	Necrotic lesion or large water-soaked lesion
5	CI I ₅	1	Necrotic lesion or large water-soaked lesion
6	CI I ₆	5	Necrotic lesion, surface covered 25% of leaf surface, possible acervuli
7	CI I ₇	3	Acervuli present
8	CI I ₈	7	>25% of the leaves showing necrosis
9	CI I ₉	5	Necrotic lesion, surface covered 25% of leaf surface, possible acervuli

Table 3: Cultural characters of different isolates of *Colletotrichum lagenarium*

S. No	Isolates	Color of the mycelial mat	Growth pattern	Topography	Colony diameter (mm)*	Days to cover full plate	Sporulation
1	CI I ₁	Dark brown	Wavy	Mycelial fluffy growth	64.00 ^e	12	+++
2	CI I ₂	Light brown	Circular	Mycelial fluffy growth	80.72 ^a	10	++++
3	CI I ₃	Greyish brown	Wavy	Mycelial fluffy growth	70.19 ^c	13	++
4	CI I ₄	Light brown	Wavy	Mycelial fluffy growth	56.32 ^g	14	+
5	CI I ₅	Blackish grey	Circular	Mycelial fluffy growth	67.29 ^{cd}	13	++
6	CI I ₆	Greyish brown	Circular	Mycelial fluffy growth	64.42 ^{de}	12	++

7	Cl I ₇	Greyish brown	Circular	Mycelial fluffy growth	59.91 ^f	13	+++
8	Cl I ₈	Whitish Grey	Circular	Mycelial fluffy growth	77.66 ^a	11	++
9	Cl I ₉	Greyish white	Circular	Mycelial fluffy growth	74.12 ^b	11	++
SE(d)					3.326		
CV					2.670		
CD (P = 0.05)					3.128		

*Mean of three replications

The treatment mean was compared by using Duncan’s Multiple Range Test

In a column, mean followed by same superscript is not significantly different at P = 0.05

- + Poor sporulation
- ++ Moderate sporulation
- +++ Good sporulation
- ++++ Excellent sporulation

Table 4: Conidial variations among the isolates of *Colletotrichum lagenarium*

S. No	Isolate	Colour of conidia	Shape of conidia	Length of conidia (µm)*	Width of conidia (µm)*
1	Cl I ₁	Hyaline	Falcate	13.58 ^d	4.57 ^e
2	Cl I ₂	Hyaline	Oblong	18.50 ^a	5.79 ^a
3	Cl I ₃	Hyaline	Falcate	14.27 ^d	4.82 ^d
4	Cl I ₄	Hyaline	Ovate	12.15 ^e	3.73 ^g
5	Cl I ₅	Hyaline	Falcate	15.74 ^c	4.36 ^e
6	Cl I ₆	Hyaline	Ovate	16.05 ^c	4.03 ^f
7	Cl I ₇	Hyaline	Oblong	15.29 ^c	5.07 ^c
8	Cl I ₈	Hyaline	Oblong	17.32 ^b	5.56 ^b
9	Cl I ₉	Hyaline	Ovate	15.97 ^c	4.94 ^h
S.E(d)				0.274	0.016
CV				3.392	3.047
CD (P = 0.05)				0.898	0.220

*Mean of three replications

The treatment mean was compared by using Duncan’s Multiple Range Test

In a column, mean followed by same superscript is not significantly different at P = 0.05



Highest disease incidence (Marukalkurichi)

Least disease incidence (Serukalai)

Fig 1: Survey for the incidence of anthracnose disease of cucumber

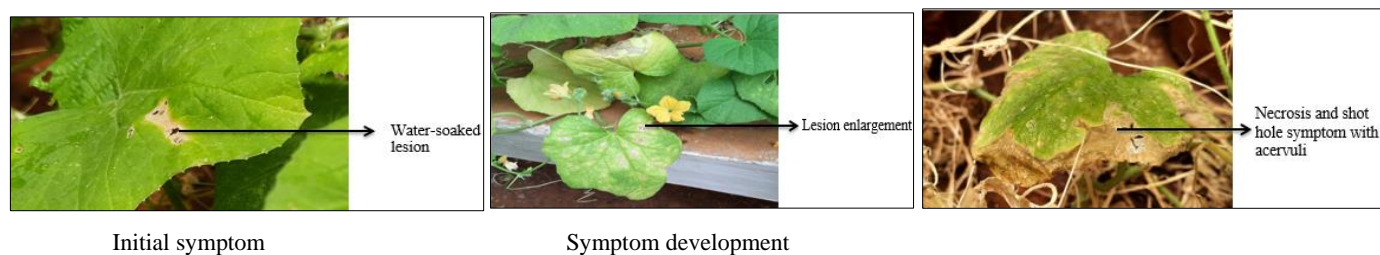


Fig 2: Anthracnose symptoms in cucumber leaves

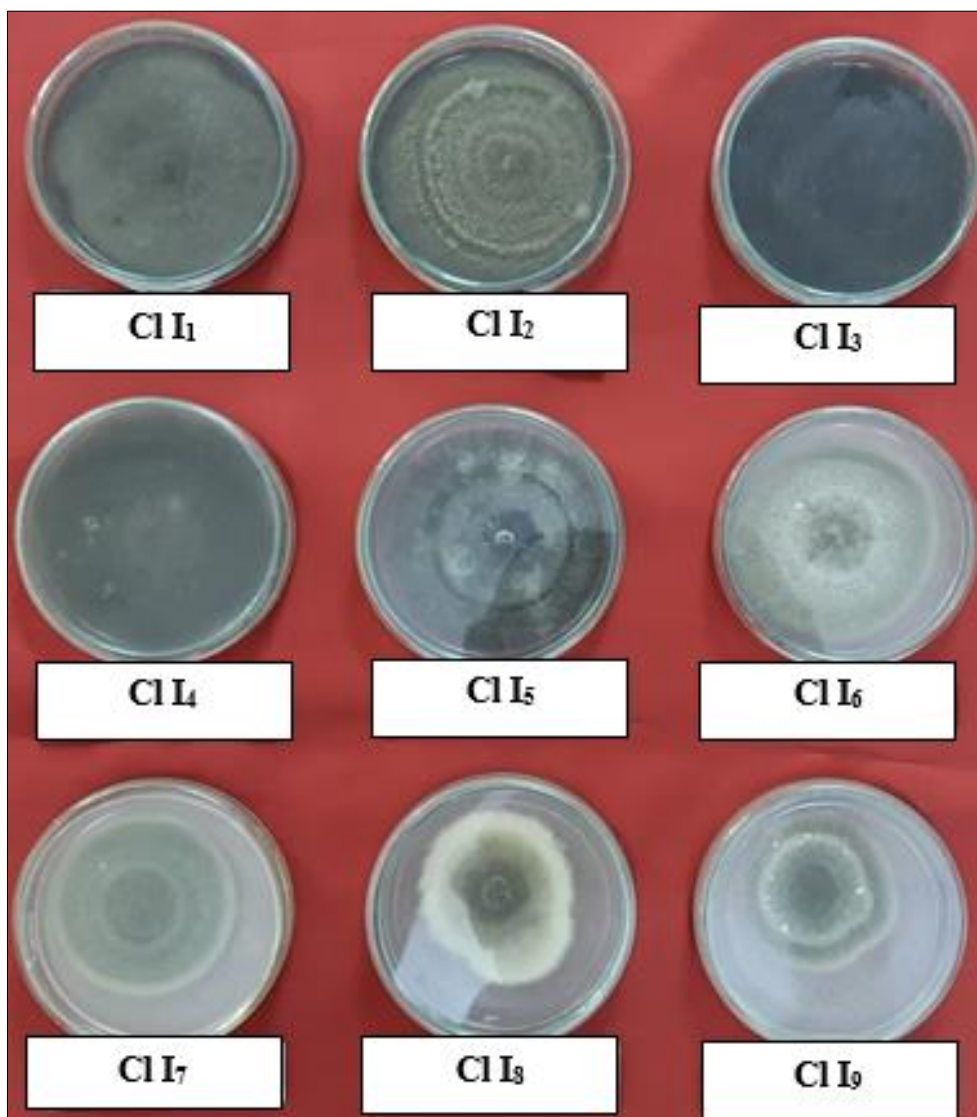


Fig 3: Cultural characteristics of various isolates of *Colletotrichum lagenarium*



Fig 4: Pathogenicity test

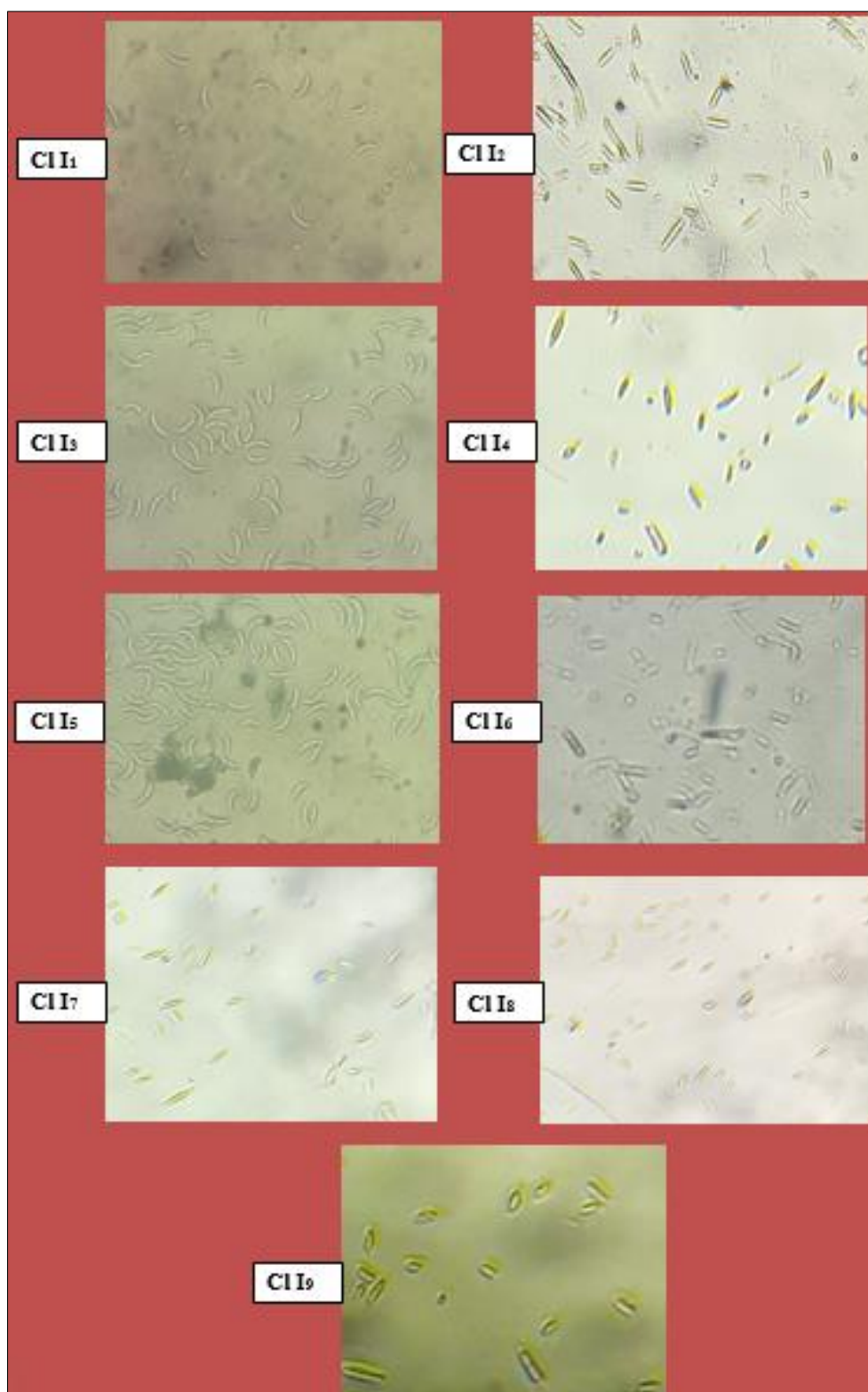


Fig 5: Conidial variations of various isolates of *Colletotrichum lagenarium*

4. Conclusion

The highest level of anthracnose disease incidence (70.37 PDI) was noticed in Marukalkurichi village of Tirunelveli district and the least level of disease incidence (40.74 PDI) was observed in Serukalai village of Namakkal district. *C. lagenarium* (CI I₂) isolated from Marukalkurichi village of Tirunelveli district was identified as a virulent one and excellent sporulation was also found in the same (CI I₂) isolate.

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