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Diversity in Morphological Characteristics of *C. fimbriata* Isolates on Oat Meal Agar: Unveiling Variability and Patterns

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the important fruit crops cultivated all over the world, particularly in tropical and sub-tropics. It is affected by several diseases of which wilt one of the most important diseases caused by *Ceratocystis fimbriata.* Very little work is done on the characterization of *C. fimbriata* associated with pomegranate wilt in Karnataka. Although the morphological structures defining this species are reasonably defined. In recent years, several orchards of farmers have been severely infected by wilt and were removed in Karnataka state. This may be due to changes in the pathogenic characteristics of the fungus. Moreover, variability is the property of an organism to change its character from one generation to the other. Therefore, there is a need to study on morphological variability of *C. fimbriata*. On oatmeal agar, *C. fimbriata* produced a maximum colony diameter (90 mm) after 16 days of incubation at room temperature.

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Dark-shaded perithecia with a globose base were seen with a size of 181.1 x 131.2 µm, radiating little, hyaline and cap-formed ascospores from the summit of the perithecium which measures 5.13 x 4.27 µm. Endoconidia were hyaline, round, and hollow, and the normal size was 23.6 x 4.90 µm. Aleurioconidia were thick-walled ellipsoidal or pyriform with size of 18.5 x 10.10 µm. Morphological variability showed little variation among *C. fimbriata* with respect size of perithecia, ascospores, endoconidia, and alerioconidia.

Keywords: Ceratocystis fimbriata; endoconidia; aleurioconidia; ascospores; variability.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is an old organic product, that has a place with the family Lythraceae. Pomegranate is local to Iran, where it was first developed in around 2000 BC and spread to the Mediterranean nations. It is cultivated in India, Iran, China, Turkey, USA, Spain, Azerbaijan, Armenia, Afghanistan, Uzbekistan, the Middle East, Pakistan, Tunisia, Israel, dry regions of Southeast Asia, Peninsular Malaysia, the East Indies, and tropical Africa. The area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, and higher yield levels with excellent keeping quality, and remunerative prices in domestic as well as export markets. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status and saline soils. India is the world's leading country in pomegranate production.

It is one of the most adaptable subtropical fruit crops. In India it is regarded as a "vital cash crop", extensively grown in Maharashtra, Karnataka, Andra Pradesh, Telangana, and Gujarat, and is picking up fast in Himachal Pradesh, Rajasthan, and Madhya Pradesh. Small areas are under cultivation in Tamil Nadu, Mizoram, Odisha, Nagaland, Lakshadweep, Jharkhand, and Jammu Kashmir. total area under pomegranate in India is 1,80,640 ha out of which 1,28,650 ha is in Maharashtra only. The total production in India is 17,89,310 metric tons and 11,97,710 metric tons in Maharashtra. In Karnataka, the total area is 23,230 ha with production of 2,61,820 metric tonnes (http://nhb.gov.in).

"In Karnataka, the crop has spread across different districts *viz.,* Vijayapura, Bagalkot, Koppal, Yadgir, Raichur, Ballari, Chitradurga, Tumakuru, and Hassan. The most well-known assortments appropriate for handling and table use are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137, and Khandar. Successful cultivation of pomegranates in recent years is threatened by different pests and diseases. Bacterial blight, wilt, anthracnose, leaf spot, and root-knot nematode are important diseases. Among them, wilt caused by *Ceratocystis fimbriata* Ell. and Halst. is an emerging threat. At present the yield is seriously impacted by wilt disease and day by day the wilt disease seriousness is expanding at a quicker rate. It was first noticed in some areas of Vijayapur districts of India in 1990. By 1993, the rapid spread of this disease was observed in the entire Vijayapura district. The cause was not identified until 1995; however, in 1996 the fungus *C. fimbriata* was isolated from discoloured stem, root, and branch tissues on wilting plants. The disease is characterized by initial symptoms of yellowing and wilting of leaves on one to several branches leading to the death of affected plants in a few weeks. Cross sections of diseased plants revealed brown discoloration in the outer xylem from the roots to the main trunk" [1].

"The disease is prevalent in parts of Maharashtra, Karnataka, Telangana, Gujarat and Tamil Nadu states" [2]. "Despite many factors conducive to the high severity, seedlings selection for planting, soil borne nature, and also an association with shot hole borer and plant parasitic nematodes is noticed. This might be the reason for the current rampant spread of the disease in south Indian states. Several agents are known to cause wilt in pomegranate, but *C. fimbriata* is the major cause, hence, emphasis given is on *C. fimbriata"* [3,4]. Very little work is done on the characterization of *C. fimbriata* associated with pomegranate wilt in Karnataka. Although the morphological structures defining this species are reasonably defined, in recent years, several orchards of farmers have been severely infected by wilt and were removed in Karnataka state. This may be due to changes in the pathogenic characteristics of the fungus. Moreover, variability is the property of an organism to change its character from one generation to the other. Therefore, there is a

need to study on morphological variability of *C. fimbriata*.

2. MATERIALS AND METHODS

2.1 Isolation of the Pathogen

"Ceratocystis fimbriata, associated with wilt was isolated from the infected roots of the pomegranate plants which were collected from Ganjalli field. The sliced pieces of collected stem portions with characteristic symptoms of vascular staining were surface sterilized with 1 per cent $NaHCO_3$ (sodium hypochlorite) for about 2 minutes and washed in alcohol (70%) and twice with sterile water to remove traces of $NaHCO_3$. Pathogen isolation was made using carrot bait technique in which, stems were placed in between the carrot disks and kept in a humid chamber and incubated at 25 ± 2 °C under 12-hour photoperiod" [5]. "After perithecium development, *C. fimbriata* was moved to newly pre-arranged PDA and oat meal agar media to permit the full improvement of growths. In order to confirm the identity of the fungus, the ascospores, aleroconidia, endoconidia and perithecia were observed under the high power (40x) microscope from Raichur isolates the pure culture. The recognizable proof of investigations of microbe has been done" [4].

2.2 Hyphal Tip Isolation

This technique was followed for keeping up with unadulterated culture. Hyphal tip disengagement was finished on water plates. Weaken spore suspension of the pathogen was ready in sanitized refined water containing eight to ten spores for every ml from the 15-day-old culture. One ml of such suspension was spread consistently on two percent set water agar plates and noticed for spores under the magnifying instrument. A single spore was set apart with a marker on the posterior of the Petri plate and it was permitted to grow. Such plates were occasionally noticed for spore germination under magnifying lens. The hyphae developing from every cell of the single spore were followed and set apart with a marker. The tip of the hyphae was sliced cautiously and moved to PDA plates and brooded at 25 ± 2°C for 15 days. Later, mycelial bits of the fungus were transferred to the canter of Petri plates containing PDA and incubated at 25 ± 2°C for 15 days. Saltation or sectoring was seen in the way of life to affirm the unadulterated culture of growth.

2.3 Maintenance of the Culture

The hyphal tip cultures of the mycelia were sub-refined on potato dextrose agar inclines and kept the in lab at 25 ± 2°C for 15 days. Such mother culture slopes were safeguarded at 5°C in the fridge. Further, the pathogen was sub cultured once a month and utilized for future examinations.

2.4 Pathogenicity

Pathogenicity tests were conducted on six-month-old seedlings of pomegranate *cv*. Kesar was raised in plastic pots (30 x 45 cm). Potting mixture was sand: red soil: FYM (1:2:1) and it was tyndallized in an autoclave at 1.1 kg/cm^2 (121 ^{0}C) pressure for 30 min. successively for two days. Wounds of 1 mm depth x 0.5 mm width were made on the epidermis of the roots with a sterilized razor blade. The wounded area in each plant was inserted with *C. fimbriata* culture utilizing a disinfected needle and wrapped with cotton material (saturated with sterile refined water) and plastic film. The method was replicated thrice with inoculation on the other two plants under glasshouse conditions. Plants that were inoculated with distilled water served as control. The inoculated plants were kept in a glass house (average temperature of 27 °C) for further observation. When plants were inoculated with the pathogen and started to express symptoms, symptoms expressed plants were collected for the re-isolation on PDA culture for Koch's postulates prove.

2.5 Studies on Morphological Variability of *C. Fimbriata*

Studies on morphological variability among the isolates of *C. fimbriata* were carried out during the study. Fifty samples were collected from nine pomegranate-growing districts of Karnataka during the survey. The isolates were obtained by tissue isolation using the carrot bait technique followed by inoculation on oat meal agar. Fifty isolates were obtained from such samples and designated as Cf-1 to Cf-50 for variability studies (Table 1).

2.6 Morphological Variability of Isolates of *C. Fimbriata*

Fifty isolates of *C. fimbriata* were observed with the growth of aleurioconidia, endoconidia, ascospore, and perithecia. For this, the growth of

individual isolates was selected from 21 days old pure culture and kept on a clean sterile glass slide using a sterilized needle. With the assistance of a fluorescent magnifying lens, the length and broadness of aleurioconidia, endoconidia, ascospore, and perithecia in µm have majored. Three perceptions were recorded from the unadulterated culture of growth to keep up with replications. Ten aleurioconidia, endoconidia, ascospores, and perithecia were gotten haphazardly to decide the measurement from every replication.

Table 1. Designation of *C. fimbriata* isolates of pomegranate wilt collected from different districts of Karnataka

Sl. No.	Name of the place		Designation of the isolate
	District	Village	
1	Viajaypura	Kumtagi	Cf-1
2		Babaleshwar	Cf-2
3		Hittinhalli	Cf-3
4		Jumnal	Cf-4
5		Kannollo-1	Cf-5
6		Devara hippargi-1	Cf-6
7		Bandal	Cf-7
8	Bagalkot	Devanal	Cf-8
9		Govindkoppa	Cf-9
10		Kaladgi-1	Cf-10
11		Lokapur-1	Cf-11
12		Mahalingapur-1	Cf-12
13	Koppal	Kalkbandi	Cf-13
14		Kamanur	Cf-14
15		Kustgi	Cf-15
16		Maladgatti-1	Cf-16
17		Kodkera	Cf-17
18	Yadgir	Gogi K	Cf-18
19		Wandurga-1	Cf-19
20		Tumkur	Cf-20
21		Heggandoddi-1	Cf-21
22		Chincholi-1	Cf-22
23	Raichur	Yatgal	Cf-23
24		Chandrabanda	Cf-24
25		Karekal	Cf-25
26		Ganjhalli-1	Cf-26
27		Kurkihalli	Cf-27
28		Benkal	Cf-28
29		Arkera-1	Cf-29
30	Ballari	Kampli	Cf-30
31		Lakshmipura	Cf-31
32		Khondanhalli	Cf-32
33		Thambrahalli	Cf-33
34		Basarkodu	Cf-34
35	Chitradurga	Sirana hatti-1	Cf-35
36		Ramajjanahalli	Cf-36
37		Nagayana hatti-1	Cf-37
38		Maskal-1	Cf-38
39		Seerana katte-1	Cf-39
40		Shraranagar	Cf-40
41	Tumakur	Madana kunte-1	Cf-41
42		Karekyatana halli	Cf-42
43		Chikka halikute-1	Cf-43
44		Thogargunte-1	Cf-44
45		Hosahali	Cf-45

Sl. No.	Name of the place		Designation of the isolate
	District	Village	
46	Hassan	Mylanahalli-1	Cf-46
47		Nadakihalli	Cf-47
48		Chika bidane-1	Cf-48
49		Haranhalli-1	Cf-49
50		Goran koppal-1	Cf-50

3. RESULTS

3.1 Isolation and Identification

Standard tissue (Carrot bait technique followed by oat meal agar) isolation was followed to isolate *Ceratocystis fimbriata* culture from the diseased sample of infected root with the typical symptoms of dark grayish-brown streaks on splitting of root portion, collected from pomegranate field. Within 3-4 days after on carrot bait the white cottony growth was observed. Later 5-6 days black colour perithecia were observed when carrot the culture was transformed on oat meal agar. The pure culture was maintained on oat meal agar at 28 ± 2 °C. Sub-culturing was done at every fortnight interval. The fungus isolated was confirmed as *C. fimbriata* based on its cultural and morphological characteristics.

3.2 Pathogenicity

Pathogenicity for the local isolate (Cf-26) was carried out as discussed in 'Material and Methods'. Yellowing of leaves and some branches or twigs is the first initiation of symptoms then drooping and drooping of leaves are indicated that the pathogen developing very fast as seen during the present examination. The leaves turned pale yellow starting from the lower branches and progressed upwards. Afterward, halfway yellowing/shrinking of the plant with drying and passing of certain branches occurred. "The isolate expressed yellowing after 40 days after the inoculation again, the fungus was re-isolated from such wilted plants from pots and was found to resemble the original culture of *C. fimbriata* thus proving the pathogenicity" [6].

3.3 Morphological Variability of Isolates of *C. fimbriata*

Diversity in morphological characters such as length and breadth of aleurioconidia, endoconidia, ascospore and perithecia were close measured by using fluorescent microscope (Table 2 and Plate 1). All fifty isolates showed little variability with respect size of perithecia, ascospores, endoconidia, and alerioconidia. The length of aleuroconidia ranged from 17.2-18.8 µm and breadth 9.90-14.12 µm. Cf-44 isolate recorded maximum size of aleuroconidia (18.6 µm x 11.30 µm) followed by Cf-1 (18.6 µm x 11.10 µm) and the minimum size of aleurioconidia was found in Cf-41 (17.2 µm x 11.00 µm).

The length of endoconidia ranged from 20.5-25.6 µm and breadth 3.10-4.9 µm, Cf-36 isolate recorded maximum size of endoconidia (25.6 µm x 4.15 µm) followed by Cf-12 (25.4 µm x 4.15 µm) and minimum size of endoconidia was found in Cf-29 (20.5 µm x 3.10 µm).

The length of ascospore ranged from 3.89-5.83 µm and breadth 3.10-4.27 µm, Cf-3 isolate recorded maximum size of ascospore (5.83 µm x 3.29 µm) followed by Cf-25 (5.83 µm x 3.26 µm) and minimum size of ascospore was found in Cf-6 (3.89 µm x 3.25 µm)

The length of perithecia ranged from 164.9-193.7 µm and breadth 96.90-141 µm Cf-11 isolate recorded maximum size of perithecia (193.7 µm x 121.1 µm) followed by Cf-1 (193.1 µm x 106.4 µm) and minimum size of perithecia was found in Cf-33 (164.9 µm x 104.2 µm).

4. DISCUSSION

4.1 Pathogen

The findings of this research indicated that the growth of *C. fimbriata* started after 3-4 days on carrot bait followed by culture on oat meal agar. The mycelia growth was whitish grey in colour which changed to brown colour. The endoconidia and aleurioconidia are produced 3-4 days after incubation and perithecium was produced after 10-16 days of incubation. "The dark-hued perithecia with a globose base were observed, exuding small, hyaline and hat-shaped ascospores from the apex of the perithecium neck. The endoconidia were hyaline, cylindrical, and formed endogenously in hyphae and

aleurioconidia were thick-walled ellipsoildal or pyriform, golden-brown in colour. They had borne singly or in chain while coming out from perithecia" [7, 8, 9].

Table 2. Morphological characteristics of different isolates of *C. fimbriata* on oat meal agar

Sl. No.	Isolate	Aleurioconidia (µm) (L x B)	Endoconidia (µm) (L x B)	Ascospore (µm) (L x B)	Perithecia (µm) (L x B) *
1	Cf-1	18.6 x 11.10	21.6 x 3.50	4.83 x 3.21	193.1 x 106.4
2	Cf-2	18.1 x 10.70	20.6 x 3.10	4.33 x 3.11	190.2 x 110.6
3	Cf-3	18.0 x 14.12	20.7 x 3.10	5.83 x 3.29	183.5 x 121.5
4	Cf-4	18.5 x 11.50	21.6 x 3.70	4.43 x 3.23	175.1 x 131.5
5	Cf-5	18.6 x 10.10	22.6 x 4.10	4.13 x 3.14	183.8 x 141.2
6	Cf-6	17.6 x 10.90	23.6 x 4.90	3.89 x 3.25	186.2 x 111.8
7	Cf-7	18.5 x 11.30	20.6 x 3.40	5.82 x 3.20	179.1 x 101.9
8	Cf-8	17.9 x 10.20	22.6 x 3.80	5.13 x 4.27	174.4 x 96.90
9	Cf-9	17.4 x 9.90	20.6 x 3.12	4.83 x 3.21	181.1 x 131.2
10	Cf-10	18.1 x 10.10	22.6 x 4.13	4.89 x 3.29	183.3 x 101.9
11	Cf-11	17.4 x 10.40	21.5 x 4.14	4.89 x 3.30	193.7 x 121.1
12	Cf-12	18.5 x 10.10	25.4 x 4.15	4.89 x 3.34	181.1 x 99.60
13	Cf-13	18.1 x 11.30	21.6 x 3.16	4.50 x 3.32	164.9 x 104.2
14	Cf-14	18.3 x 10.40	22.8 x 4.17	4.43 x 3.31	175.4 x 115.3
15	Cf-15	17.7 x 11.10	23.6 x 4.18	4.83 x 3.34	172.6 x 121.9
16	Cf-16	18.6 x 9.90	22.6 x 4.19	4.83 x 3.35	187.1 x 111.1
17	Cf-17	17.3 x 11.30	21.6 x 3.50	4.81 x 3.21	181.6 x 101.4
18	Cf-18	18.1 x 11.10	24.6 x 4.30	4.81 x 3.22	179.2 x 131.5
19	Cf-19	17.9 x 10.20	22.6 x 3.80	5.13 x 4.27	173.4 x 96.90
20	Cf-20	17.4 x 9.90	20.6 x 3.12	4.83 x 3.21	181.1 x 131.2
21	Cf-21	18.5 x 11.20	21.6 x 3.50	5.80 x 3.25	172.6 x 121.9
22	Cf-22	17.9 x 10.20	20.6 x 3.10	4.43 x 3.23	187.1 x 111.1
23	Cf-23	17.4 x 9.90	20.7 x 3.10	4.12 x 3.10	181.6 x 101.4
24	Cf-24	18.1 x 10.10	21.6 x 3.70	3.89 x 3.25	179.2 x 131.5
25	Cf-25	17.4 x 10.40	22.6 x 4.10	5.83 x 3.26	177.4 x 96.90
26	Cf-26	18.5 x 10.10	23.6 x 4.90	5.13 x 4.27	181.1 x 131.2
27	Cf-27	18.1 x 11.30	20.6 x 3.40	4.89 x 3.30	179.1 x 101.9
28	Cf-28	18.3 x 10.40	22.6 x 3.80	4.89 x 3.34	176.4 x 96.90
29	Cf-29	17.7 x 11.10	20.5 x 3.10	4.50 x 3.32	181.1 x 131.2
30	Cf-30	18.6 x 9.90	21.6 x 3.50	4.43 x 3.31	183.3 x 101.9
31	Cf-31	17.7 x 11.10	24.6 x 4.30	4.83 x 3.34	192.7 x 121.1
32	Cf-32	18.6 x 9.90	22.6 x 3.80	4.83 x 3.35	181.1 x 99.60
33	Cf-33	17.3 x 11.30	21.6 x 3.50	4.81 x 3.21	164.9 x 104.2
34	Cf-34	18.1 x 10.10	22.6 x 4.13	4.81 x 3.22	175.4 x 115.3
35	Cf-35	17.4 x 10.40	21.5 x 4.14	5.13 x 4.27	172.6 x 121.9
36	Cf-36	18.5 x 10.10	25.6 x 4.15	4.50 x 3.32	179.2 x 131.5
37	Cf-37	18.1 x 11.30	21.6 x 3.16	4.43 x 3.31	178.4 x 96.90
38	Cf-38	18.3 x 10.40	22.8 x 4.17	4.83 x 3.34	181.1 x 131.2
39	Cf-39	17.7 x 11.10	23.6 x 4.18	4.83 x 3.35	179.1 x 101.9
40	Cf-40	18.6 x 9.90	22.6 x 4.19	4.81 x 3.21	173.4 x 96.90
41	Cf-41	17.2 x 11.00	21.6 x 3.50	4.81 x 3.22	181.1 x 131.2
42	Cf-42	18.1 x 11.10	24.6 x 4.30	5.13 x 4.27	183.3 x 101.9
43	Cf-43	17.9 x 10.20	22.6 x 3.80	4.83 x 3.21	190.7 x 121.1
44	Cf-44	18.6 x 11.30	20.6 x 3.12	4.50 x 3.32	179.2 x 131.5
45	Cf-45	17.9 x 10.20	22.6 x 4.13	4.50 x 3.32	191.1 x 106.4
46	Cf-46	17.4 x 9.90	21.5 x 4.14	4.43 x 3.31	190.2 x 110.6
47	Cf-47	18.6 x 9.90	22.6 x 4.19	4.89 x 3.34	179.1 x 101.9
48	Cf-48	17.7 x 11.10	21.6 x 3.50	4.50 x 3.32	174.4 x 96.90
49	Cf-49	18.6 x 9.90	24.6 x 4.30	4.43 x 3.31	181.1 x 131.2
50	Cf-50	17.3 x 11.30	22.6 x 3.80	4.83 x 3.34	183.3 x 101.9

** L x B = (Length x Breadth)*

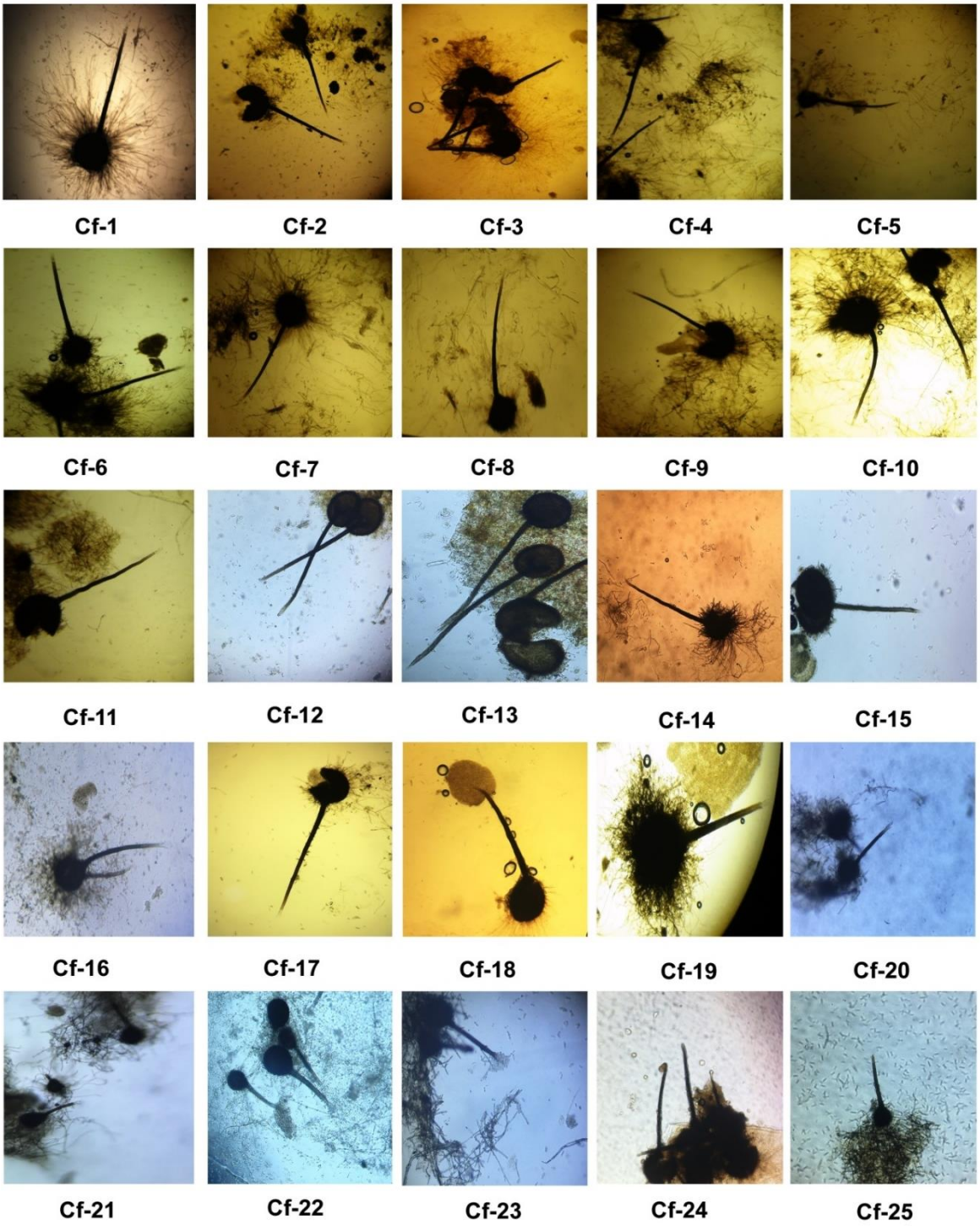
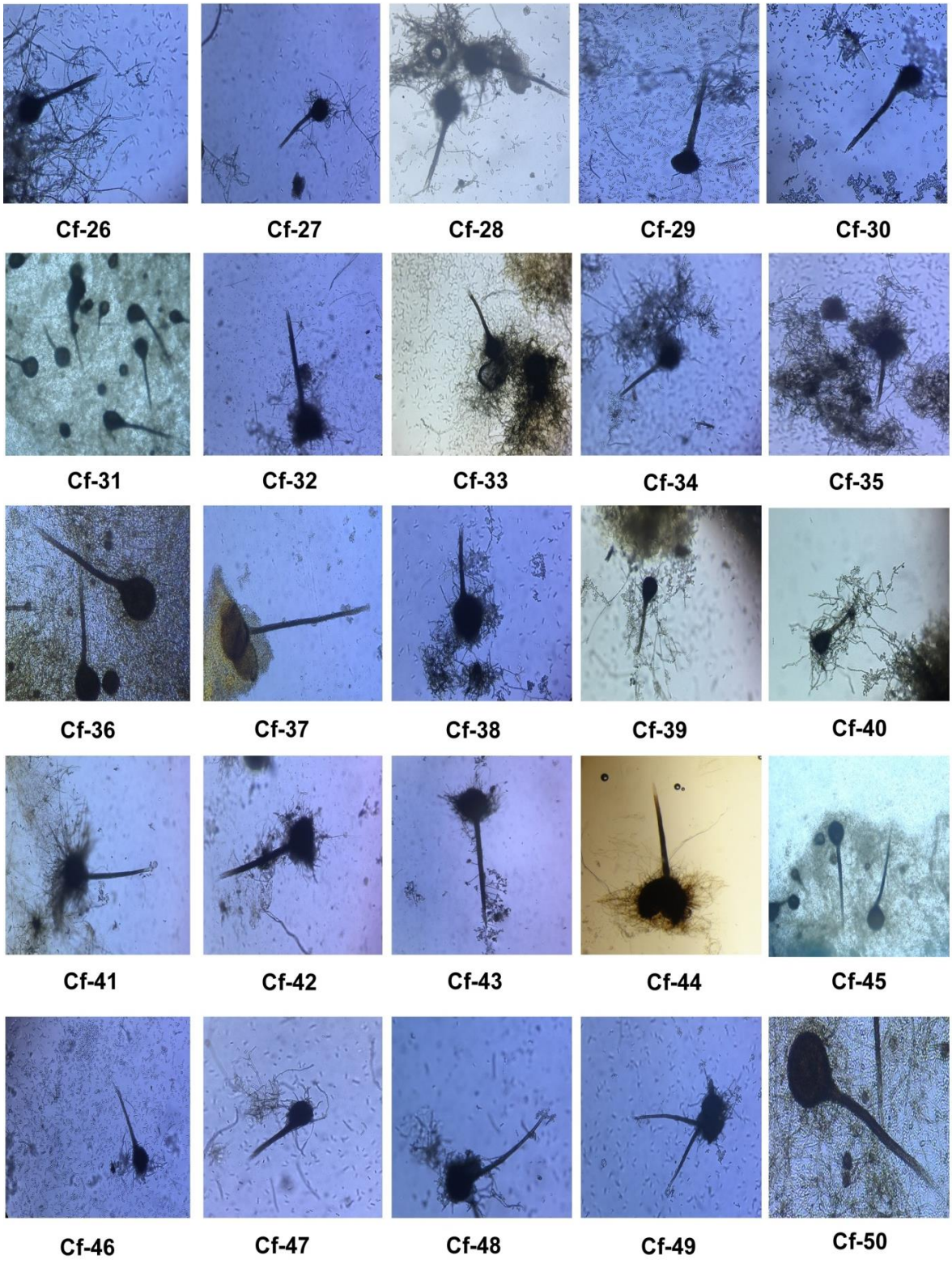


Plate 1. Morphological characteristics of different isolates of *C. fimbriata* (10x)

1=Cf-1, 2=Cf-2, 3=Cf-3, 4=Cf-4, 5=Cf-5, 6=Cf-6, 7=Cf-7, 8=Cf-8, 9=Cf-9, 10=Cf-10, 11=Cf-11, 12=Cf-12, 13=Cf-13, 14=Cf-14, 15=Cf-15, 16=Cf-16, 17=Cf-17, 18=Cf-18, 19=Cf-19, 20=Cf-20, 21=Cf-21, 22=Cf-22, 23=Cf-23, 24=Cf-24, 25=Cf-25

Plate 1 Contd…



26=Cf-26, 27=Cf-27, 28=Cf-28, 29=Cf-29, 30=Cf-30, 31=Cf-31, 32=Cf-32, 33=Cf-33, 34=Cf-34, 35=Cf-35, 36=Cf-36, 37=Cf-37, 38=Cf-38, 39=Cf-39, 40=Cf-40, 41=Cf-41, 42=Cf-42, 43=Cf-43, 44=Cf-44, 45=Cf-45, 46=Cf-46, 47=Cf-47, 48=Cf-48, 49=Cf-49, 50=Cf-50

4.2 Isolation and Identification

"The isolation of the fungus, *Ceratocystis fimbriata* was done on carrot bait technique followed by culture on oat meal agar using a diseased sample of infected root with typical symptom of dark grayish-brown streaks on root portion. The fungus isolated was confirmed as *C. fimbriata* based on its cultural and morphological characteristics such as mycelial characters, aleroconidia, endoconidia and perethecial production" [7-11].

4.3 Pathogenicity Test

"To demonstrate Koch's hypotheses, *Ceratocystis fimbriata* was detached from the impacted tissue of the root and immunized to plants under glasshouse conditions. After 40 days, the pathogen produced typical symptoms of the disease such as the yellowing of foliage of one or two branches of the plant followed by yellowing and drooping of foliage of the entire plant within 72 days. At the point when parted open the impacted root, dim grayish-earthy coloured streaks like earthy coloured dark staining in vascular and about cortex tissues were noticed. The pathogen was re-isolated from such symptoms and compared with the original culture for its conformity. *C. fimbriata* carries mycelial development which was whitish-dim in variety and endoconidia, aleurioconidia, and perithecium were built and re-separated fungi. Similarly, pathogenicity was proved by earlier workers with a description that was observed during the present study" [1, 9, 12, 13, 11]

4.4 Morphological Characters of *C. fimbriata*

The local isolate of *C. fimbriata* (Cf-26) delivered a greyish-hued province with a level kind with a standard sort of edge on oat feast agar. In the current examination, it was seen that the edge tone was greyish and the state distance across was 90 mm following 16 days of hatching at room temperature. The shade of the province changed to a greyish variety with age inferable from the creation of aleurioconidia, endoconidia, ascospores, and perithecium. Dark-shaded perithecia with globose base was seen with a size of 181.1 x 131.2 µm, radiating little, hyaline and cap-molded ascospores from the summit of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, round, and hollow, and the normal size was 23.6 x 4.90 µm. Aleurioconidia were thick-walled ellipsoidal or pyriform with size of 18.5 x 10.10 µm. Similar results with respect to morphological characters were reported by several workers [1, 9, 2], comparative morphological qualities of the parasite showed perithecia brown to dark with a globose base, necks just about 800-900 µm long with ostiolar hyphae. Ascospores curved 4-8 x 25 µm, hyaline, nonseptate, cap-formed appearance. Conidiophores hyaline, septate up to 150 µm long. Conidia barrel-shaped, some of the time in chains, shorten at the finishes [10].

4.5 Morphological Variability of Isolates of *C. fimbriata*

Morphological characters studied on oat meal agar at room temperature of all the fifty *C. fimbriata* isolates showed little variability with respect to the size of perithecia, ascospores, endoconidia and alerioconidia. The length of aleuroconidia ranged from 17.2-18.8 µm and breadth, 9.90-14.12 µm. Cf-44 isolate recorded the maximum size of aleuroconidia (18.6 µm x 11.30 µm) followed by Cf-1 (18.6 µm x 11.10 µm). Similarly, the length of endoconidia ranged from 20.5-25.6 µm and breadth 3.10-4.9 µm, Cf-36 isolate recorded the maximum size of endoconidia (25.6 µm x 4.15 µm) followed by Cf-12 (25.4 µm x 4.15 µm). The length of ascospore ranged from 3.89-5.83 µm and breadth 3.10-4.27 µm, Cf-3 isolate recorded the maximum size of ascospore (5.83 µm x 3.29 µm) followed by Cf-25 (5.83 µm x 3.26 µm). The length of perithecia ranged from 164.9-193.7 µm and breadth 96.90-141 µm Cf-11 isolate recorded maximum size of perithecia (193.7 µm x 121.1 µm) followed by Cf-1 (193.1 µm x 106.4 µm) and minimum size of perithecia was found in Cf-33 (164.9 µm x 104.2 µm) [14]. Conducted studies in *C. fimbriata* in pomegranate and reported that endoconidia measured 9.2 to 29.6 × 3.1 to 6.8 µm, aleurioconidia were brownish, thick-walled, near globose and measured 8.7 to 18.1 × 8.2 to 10.7 µm and perithecia were dark brown to black, globose, measured 90.8 to 149.8 µm in diameter and had a long thin neck, 254.4 to 533.8 µm long, through which ascospores exuded. Ascospores were small, hyaline and hat shaped, measured 3.7 to 6.5 × 3.1 to 5.7 µm and accumulated in a sticky matrix at the tip of the ascomal neck. Microscopic examination of a fifteen days old culture revealed hyaline conidia (10-15 µm long) and perithecia were black with a globose base (100-300 µm) [7].

5. CONCLUSIONS

We conclude that this study revealed morphological little variability in *C. fimbriata*

isolates from different districts of Karnataka, suggesting that the same planting material and pathogen have been distributed throughout the state this may be the one reason and By picking healthy areas for the distribution of healthy seedlings, people would be able to select the best management strategies for the future.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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