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

Abstract:

Pomegranate (*Punica granatum* L.) is a one of the important fruit crop cultivated all over the world particularly in the tropical and sub-tropics. It is affected by several diseases of which wilt one of the most important disease caused by *Ceratocystis fimbriata.* Very little work is done on 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 change in pathogenic characters of the fungus. Moreover, variability is the property of an organism to change its characters from one generation to the other. Therefore, there is a need to study on morphological variability of *C. fimbriata*. On oat meal agar *C. fimbriata* produced maximum colony diameter (90 mm) after 16 days of incubation at room temperature. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average 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.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, belongs to the family lythraceae. Pomegranate is native to Iran, where it was first cultivated in about 2000 BC and spread to the Mediterranean countries. 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. Area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, higher yield levels with excellent keeping quality and remunerative prices in domestic as well as export market. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status as well as on 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 total area is 23,230 ha with production 2,61,820 metric tonnes.

In Karnataka, the crop has spread across different districts *viz*., Vijayapura, Bagalkot, Koppal, Yadgir, Raichur, Ballari, Chitradurga, Tumakuru and Hassan. The most popular varieties suitable for processing and table use are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137 and Khandar. Successful cultivation of pomegranate in recent years is threatened with different pest 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 crop is severely affected by wilt pathogen and day by day the wilting severity is increasing at faster rate. It was first noticed in some areas of Vijayapur districts of India during 1990. By 1993, rapid spread of this disease was observed in entire Vijayapura district. The cause was not identified until 1995; however in 1996 the fungus *C. fimbriata* was isolated from discolored stem, root and branch tissues on wilting plants. Disease is characterized by initial symptoms of yellowing and wilting of leaves on one to several branches leading to death of affected plants in a few weeks. Cross sections of diseased plants revealed brown discoloration in the outer xylem from roots to the main trunk (Somasekhara and Walli, 2000).

The disease is prevalent in parts of Maharashtra, Karnataka, Telangana, Gujarat and Tamil Nadu states (Jadhav and Sharma, 2009). Despite many factors conducive for the high severity, seedlings selection for planting, soil borne nature and also 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 (Sharma, 2009 and Sharma *et al.*, 2010), hence, emphasis given be on *C. fimbriata*. Very little work is done on 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 change in pathogenic characters of the fungus. Moreover, variability is the property of an organism to change its characters from one generation to the other. Therefore, there is a need to study on morphological variability of *C. fimbriata*.

MATERIAL AND METHODS

Isolation of the pathogen

Ceratocystis fimbriata, associated with wilt was isolated from the infected roots of pomegranate plant 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 (Moller and DeVay, 1968) 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 (Moller and DeVay, 1968). After perithecium formation, a portion of the fungi was transferred to freshly prepared PDA and oat meal agar media to allow the full development of fungi. 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 identification of studies of pathogen has done as explained by Sharma *et al*. (2010).

Hyphal tip isolation

This method was followed for maintaining of pure culture. Hyphal tip isolation was done on water plates. Dilute spore suspension of the pathogen was prepared in sterilized distilled water containing eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and observed for spores under the microscope. Single spore was marked with a marker on backside of the Petri plate and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae growing from each cell of the single spore was traced and marked with marker. The tip of the hyphae was cut carefully and transferred to PDA plates and incubated at 25 ± 2 ^{o}C for 15 days. Later, mycelial bits of the fungus were transferred in the centre of petri plates containing PDA and incubated at 25 ± 2 ^{o}C for 15 days. Saltation or sectoring was observed in the culture to confirm the pure culture of the fungus.

Maintenance of the culture

The hyphal tip cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in laboratory at 25 ± 2 ^{o}C for 15 days. Such mother culture slants were preserved at 5 ^{o}C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Pathogenicity

Pathogenicity tests were conducted on six-month-old seedlings of pomegranate *cv*. Kesar, raised in plastic pots (30 x 45 cm). Potting mixture was sand: red soil: FYM (1:2:1) and it was tyndallized in autoclave at 1.1 kg/cm^2 (121 ^{0}C) pressure for 30 min. successively for two days. Wound of 1 mm depth x 0.5 mm width were made on the epidermis of the roots with sterilized razor blade. The wounded area in each plant was inserted with *C. fimbriata* culture using a sterilized needle and wrapped with cotton cloth (moistened with sterile distilled water) and plastic film. The method was replicated thrice with inoculation on other two plants under glasshouse condition. Plants which were inoculated with distilled water served as control. The inoculated plants were kept in glass house (average temperature of 27 °C) for further observation. Once the artificially inoculated plants develop typical symptoms, the disease samples were collected and the organism was re-isolated on potato dextrose agar medium thus confirming the Koch's postulates to establish the pathogen and proving pathogencity.

Studies on morphological variability of *C. fimbriata*

Studies on morphological variability among the isolates of *C. fimbriata* was 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 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).

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

Contd….

Sl. No.	Name of the Place		Designation of the isolate
	District	Village	
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
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

Morphological variability of isolates of *C. fimbriata*

Fifty isolates of *C. fimbriata* were characterized for production of aleurioconidia, endoconidia, ascospore and perithecia. For this, the growth of individual isolate was selected from 21 days old pure culture and kept on a clean sterile glass slide using sterilized needle. With the help of fluorescent microscope, the length and breadth of aleurioconidia, endoconidia, ascospore and perithecia in µm was measured. Three observations were recorded from the pure culture of fungus to maintain replications. Ten aleurioconidia,

endoconidia, ascospores and perithecia were picked up randomly to determine the diameter from each replication.

RESULT

Isolation and identification

Standard tissue (Carrot bait technique followed by oat meal agar) isolation was followed to isolate *Ceratocystis fimbriata* culture from diseased sample of infected root with typical symptom 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 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 their cultural and morphological characters.

Pathogenecity

Pathogenecity for the local isolate (Cf-26) was carried out as discussed in 'Material and Methods'. The time of appearance of first symptoms like yellowing of leaves in some twigs or branches, followed by drooping and drying of leaves as the external signs as observed during present investigation. The leaves turned pale yellow starting from lower branches and progressed upwards. Later, partial wilting of the plant with drying and death of some branches took place. The isolate produced wilting symptoms 40 days after 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 pathogenecity.

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 the 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 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).

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

Contd…..

Sl. No.	Isolate	Aleurioconidia (µm) (L x B)	Endoconidia (µm) (L x B)	Ascospore (µm) (L x B)	Perithecia (µm) (L x B) *
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)

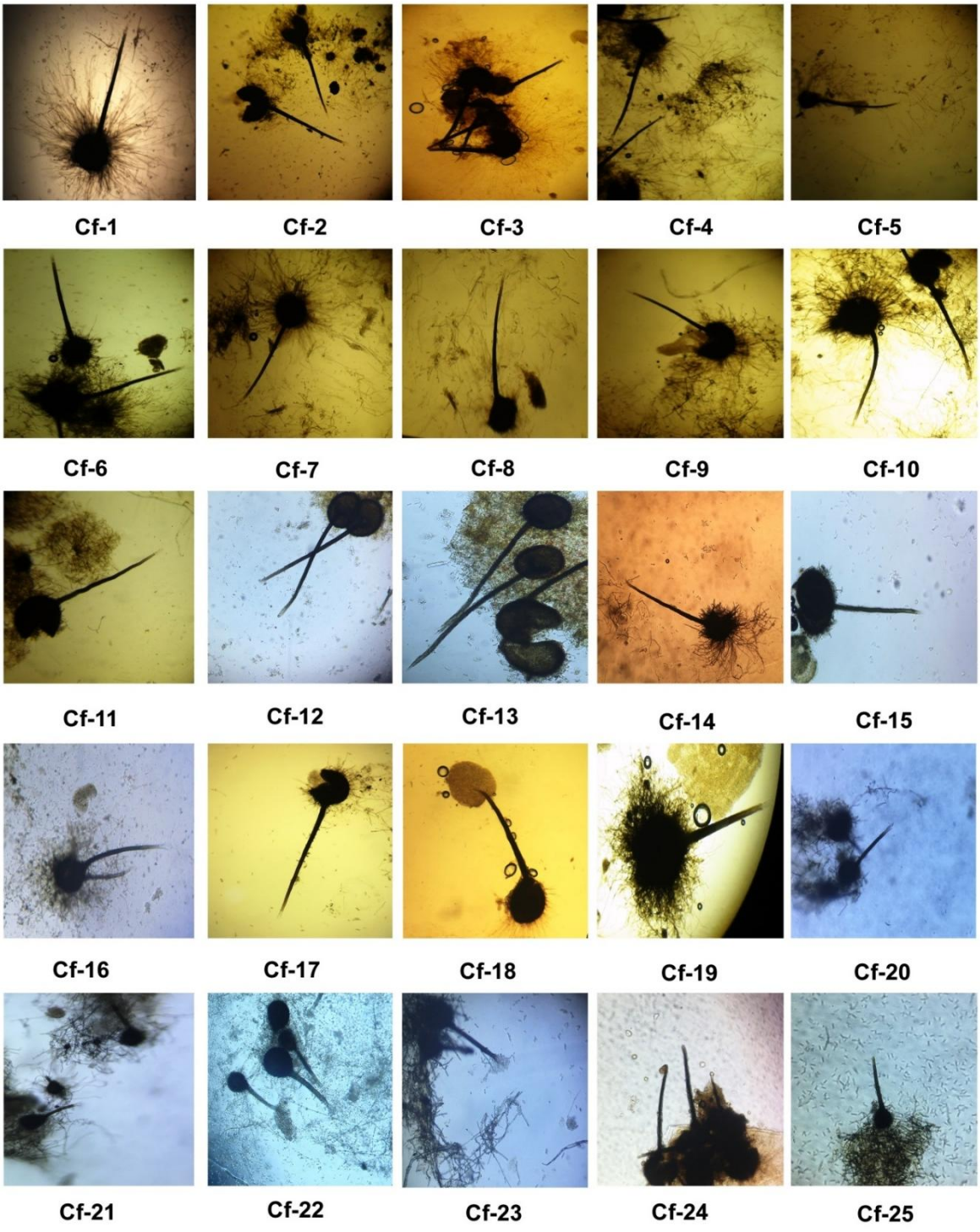
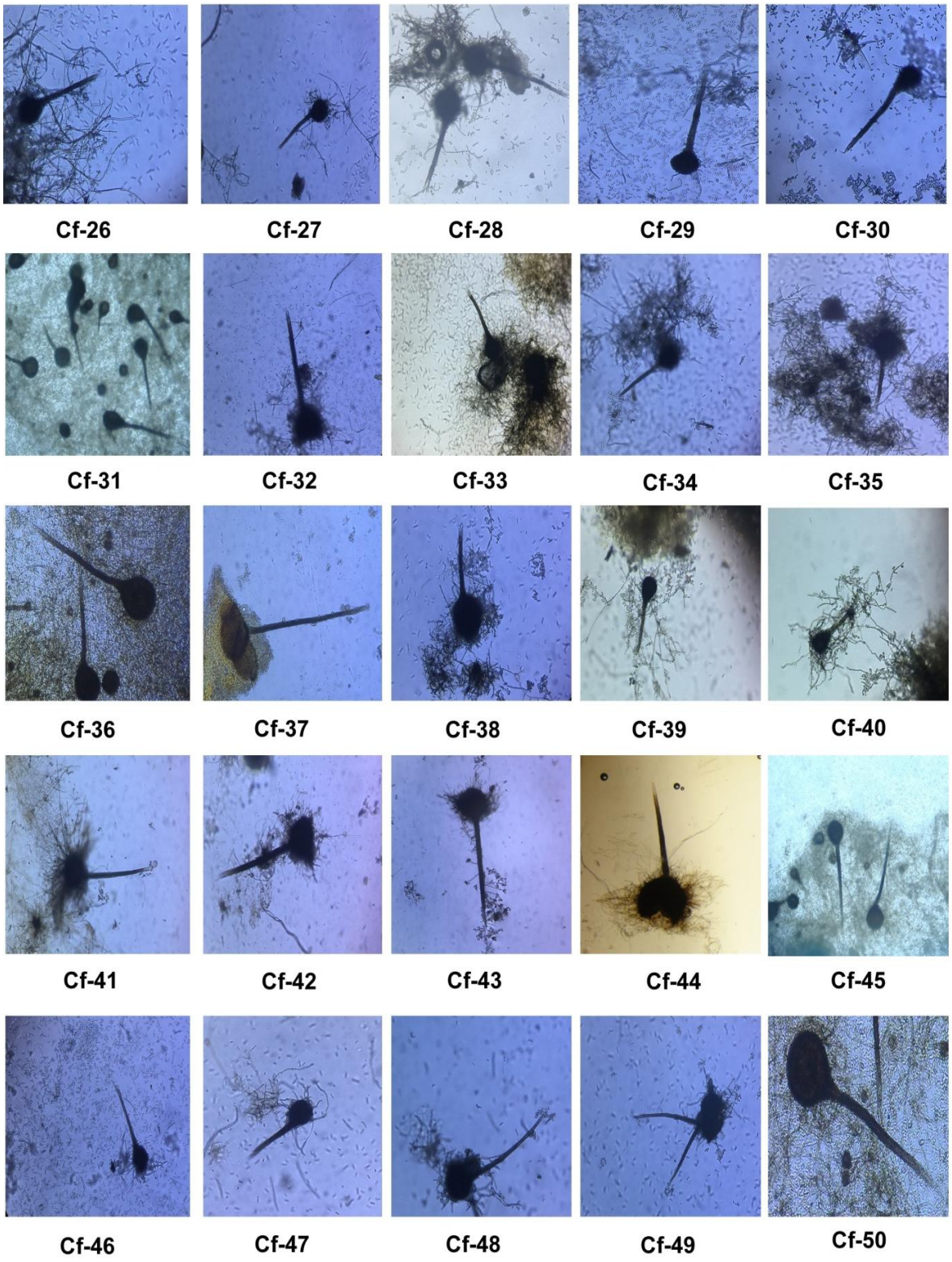


Plate 1. Morphological characteristics of different isolates of *C. fimbriata* (10x)

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

DISCUSSION

Pathogen

The studies 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 were produced after 10-16 days of incubation. The black colored 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 perethecia. These results are in conformity with Somasekhara and Wali (2000), Sonyal *et al.* (2015) and Chaudhari *et al*. (2016).

Isolation and identification

The isolation of fungus, *Ceratocystis fimbriata* was done on carrot bait technique followed by culture on oat meal agar using 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 their cultural and morphological characters such as mycelial characters, aleroconidia, endoconidia and perethecial production as reported by as reported earlier workers (Huang *et al.*, 2003; Faisal *et al*., 2006; Sonyal *et al.*, 2015; Chaudhari *et al*., 2016 and Soni and Kanwar, 2016).

Pathogenicity test

To prove the Koch's postulates, *Ceratocystis fimbriata* was isolated from affected tissue of root and inoculated to plants under glass house condition. After 40 days, pathogen produced typical symptoms of the diseases such as yellowing of foliage of one or two branches of plant followed by yellowing and drooping of foliage of the entire plant within 72 days. When split open the affected root, dark grayish-brown streaks like brown black discoloration in vascular and adjoin cortex tissues were observed. The pathogen was re-isolated from such symptoms and compared with original culture for its conformity. Pathogen produced mycelial growth which was whitish grey in colour and endoconidia, aleurioconidia and perithecium were also produced by re-isolated pathogen. Similarly pathogenecity was proved by earlier workers with description that was observed during the present study

(Somasekhar and Wali, 2000; Huang *et al.*, 2003; Khosla *et al.*, 2013; Valdetaro *et al*., 2015 and Chaudhari *et al*., 2016).

Morphological characters of *C. fimbriata*

The local isolate of *C. fimbriata* (Cf-26) produced grayish coloured colony with flat type with regular type of margin on oat meal agar. In the present investigation, it was observed that the margin colour was grayish and colony diameter was 90 mm after 16 days of incubation at room temperature. The colour of colony changed to grayish colour with age owing to production of aleurioconidia, endoconidia, ascospores and perithecium. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average 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 (Went, 1893; Somasekhara and Wali, 1999; Huang *et al.*, 2003 and Jadhav and Sharma., 2009). Faisal *et al*. (2006) explained similar morphological characteristics of the fungus which showed perithecia brown to black with globose base, necks almost 800-900 µm long with ostiolar hyphae. Ascospores elliptical 4-8 x 25 µm, hyaline, non septate, hat shaped appearance. Conidiophores hyaline, septate up to 150 µm long. Conidia cylindrical, sometimes in chains, truncate at the ends.

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 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). Similarly, 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). 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). 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).

Xu *et al*. (2011) 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) (Soni and Kanwar, 2016).

SUMMARY AND CONCLUSIONS

In morphological variability, all the fifty *C. fimbriata* isolates showed little variability with respect size of perithecia, ascospores, endoconidia and alerioconidia, The length of aleuroconidia ranged from 17.2-18.8 µm in length and breadth, 9.90-14.12 µm, Cf-44 isolate recorded maximum size of aleuroconidia (18.6 µm x 11.30 µm); 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); ascospore ranged from 3.89-5.83 µm in length and breadth 3.10-4.27 µm, Cf-3 isolate recorded maximum size of ascospore (5.83 µm x 3.29 µm) and 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). On oat meal agar *C. fimbriata* produced maximum colony diameter (90 mm) after 16 days of incubation at room temperature. Black colored perithecia with globose base was observed with size of 181.1 x 131.2 µm, exuding small, hyaline and hat shaped ascospores from the apex of the perithecium which measure 5.13 x 4.27 µm. Endoconidia were hyaline, cylindrical and average 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.

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