CULTURAL VARIABILITY AMONG *CERATOCYSTIS FIMBRIATA* ISOLATES CAUSING WILT DISEASE OF POMEGRANATE

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 cultural 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 cultural variability of *C. fimbriata*. Cultural variability showed variation among the *C. fimbriata* isolates. On the bases of colony color, type of colony growth, type of margin, margin color and colony growth, fifty isolates were categorized.

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 cultural variability of *C. fimbriata*.

MATERIAL AND METHODS

Cleaning of glasswares

Borosil and Corning glasswares were used for all the laboratory experimental studies. They were kept for a day in cleaning solution, prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in one liter of water. Each of these chemicals dissolved separately in 500 ml of water and finally mixed. Then glassware's were cleaned by washing with detergent solution followed by tap water and finally rinsing in distilled water.

Sterilization

All the glassware used in the study wrapped were sterilized in an autoclave at 15 p.s.i pressure for 20 minutes and kept for drying in hot air oven at 60^{o} C for two hours. Both solid and liquid media were sterilized at 15 p.s.i pressure for 15 minutes.

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.

Morphological Characters *C. fimbriata*

C. fimbriata was characterized for production of aleurioconidia, endoconidia, ascospore and perithecia. For this, the growth of Cf-26 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 were measured. Three observations were recorded from the pure culture of fungus. Ten aleurioconidia, endoconidia, ascospores and perithecia were picked up randomly to determine the diameter and *C. fimbriata* (Cf-26) was characterized for colony color and growth pattern on oat meal agar. The mycelial disc of 5 mm diameter was cut from periphery of actively growing culture of Cf-26 and transferred aseptically to a 90 mm Petri dish containing 20 ml of oat meal agar and incubated for a period till the fungal growth covered the complete petri plate in the media at 26 ± 2 ^{o}C. The colony was characterized for phenotype and growth pattern. Different morphotypes colony colour, type of colony, type of margin, margin colour and colony growth were observed *in vitro*.

Cultural characters of *C. fimbriata* on oat meal agar

The cultural characters of *C. fimbriata* were studied on oat meal agar. The composition and preparation of the above mentioned media were obtained from Ainsworth

and Bisby's "Dictionary of the Fungi" by Hawksworth *et al*. (1983). The composition and preparation of the media are as fallows

Oat meal agar

Oat meal powder : 40.00 g

Agar - agar : 20.00 g

Distilled water : 1000 ml (volume to make up)

The oat meal powder was dissolved in 500 ml of distil water and Agar agar was melted in 500 ml of distilled water separately. Both the solutions were mixed thoroughly. Then volume was made up to one liter and sterilized.

Twenty ml of each medium was poured in to the Petri dishes for solidification. Five mm discs of *C. fimbriata* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 26 ± 2 ^{o}C, Observations were taken on parameters such as growth type, mycelial colour, type of margin and radial growth (mm) when the fungus covered complete petriplate in the media. The results were analyzed statistically.

Studies on cultural 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

RESULT

Pathogen

On carrot bait followed by oat meal agar, the growth of *C. fimbriata* started after 3-4 days and the mycelium was whitish grey colour which changed to brown colour. As the growth progresses, production of endoconidia, aleurioconidia and perithecium was observed. 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 borne singly or in chain.

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.

Cultural variability of different isolates of *C. fimbriata*

To study the variability, cultural characters such as colony color, type of colony growth, type of margin, margin color and colony growth of fifty isolates was assessed on Oat meal agar as described in 'Material and Methods' and results are presented in Table 2, Table 3 and Plate 1.

Diversity in cultural characters on oat meal agar at room temperature showed variation among the *C. fimbriata* isolates, such as colony color (grayish, brown and light gray), type of colony growth (flat and fluffy), type of margin (regular and irregular) margin color (light gray and brown) and colony growth in mm were closely observed in 50 isolates of *C. fimbriata.* Most of the isolates showed gryish colour with flat type of colony growth and regular margin. The margin colour was light gray in many isolates and colony growth ranged from 70 to 90 mm.

Grouping of *C. fimbriata* isolates based on cultural characteristics

Diversity in cultural characters such as colony color (grayish/brown/light gray), type of colony growth (flat/fluffy), type of margin (regular/irregular) margin color (light gray/brown) and colony growth in mm were closely observed in 50 isolates of *C. fimbriata* and categorized as described in Table 3a and Table 3b.

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

Sl. No.	Isolate	Colony color	Type of colony growth	Type of margin	Margin color	Colony growth (mm)
1	Cf-1	Grayish	Flat	Regular	Light gray	90
2	Cf-2	Grayish	Flat	Regular	Light gray	90
3	Cf-3	Brown	Flat	Regular	Brown	88

4	Cf-4	Grayish	Flat	Regular	Light gray	90
5	Cf-5	Grayish	Flat	Regular	Light gray	83
6	Cf-6	Grayish	Flat	Regular	Light gray	90
7	Cf-7	Light gray	Flat	Regular	Light gray	90
8	Cf-8	Brown	Flat	Regular	Brown	85
9	Cf-9	Grayish	Fluffy	Irregular	Light gray	90
10	Cf-10	Brown	Flat	Regular	Brown	80
11	Cf-11	Grayish	Fluffy	Regular	Light gray	89
12	Cf-12	Light gray	Flat	Regular	Light gray	81
13	Cf-13	Grayish	Flat	Irregular	Light gray	90
14	Cf-14	Grayish	Flat	Regular	Light White	90
15	Cf-15	Light gray	Flat	Regular	Light white	70
16	Cf-16	Light gray	Fluffy	Irregular	Light gray	70
17	Cf-17	Grayish	Flat	Regular	Light gray	90
18	Cf-18	Grayish	Flat	Regular	Light gray	85
19	Cf-19	Grayish	Flat	Regular	Light White	90
20	Cf-20	Grayish	Flat	Regular	Light gray	88
21	Cf-21	Light gray	Flat	Regular	Light gray	90
22	Cf-22	Brown	Flat	Regular	Brown	81
23	Cf-23	Grayish	Flat	Regular	Light gray	90
24	Cf-24	Grayish	Fluffy	Irregular	Light gray	90
25	Cf-25	Grayish	Flat	Regular	Light white	90
26	Cf-26	Brown	Flat	Regular	Brown	90

Contd……

Sl. No.	Isolate	Colony color	Type of colony growth	Type of margin	Margin color	Colony growth (mm)
27	Cf-27	Grayish	Flat	Regular	Light gray	82
28	Cf-28	Light gray	Flat	Regular	Light gray	90
29	Cf-29	Light gray	Flat	Regular	Light gray	83

30	Cf-30	Brown	Fluffy	Irregular	Light gray	90
31	Cf-31	Grayish	Fluffy	Irregular	Light gray	70
32	Cf-32	Grayish	Flat	Regular	Light gray	90
33	Cf-33	Brown	Flat	Regular	Brown	86
34	Cf-34	Grayish	Flat	Regular	Light gray	90
35	Cf-35	Grayish	Flat	Regular	Light white	90
36	Cf-36	Light gray	Flat	Regular	Light gray	81
37	Cf-37	Grayish	Flat	Regular	Light gray	85
38	Cf-38	Grayish	Flat	Regular	Light gray	70
39	Cf-39	Grayish	Fluffy	Irregular	Light gray	90
40	Cf-40	Grayish	Flat	Regular	Light gray	84
41	Cf-41	Grayish	Flat	Regular	Light white	90
42	Cf-42	Brown	Flat	Regular	Brown	90
43	Cf-43	Grayish	Flat	Regular	Light gray	79
44	Cf-44	Grayish	Fluffy	Irregular	Light gray	70
45	Cf-45	Brown	Flat	Regular	Light gray	90
46	Cf-46	Grayish	Flat	Regular	Light gray	78
47	Cf-47	Grayish	Flat	Regular	Light gray	90
48	Cf-48	Grayish	Flat	Regular	Light gray	90
49	Cf-49	Light gray	Flat	Regular	Light gray	90
50	Cf-50	Light gray	Flat	Regular	Light gray	90

Table 3a. Categorization of isolates based on colony color, type of colony growth and type of margin

i. Based on colony color

Colony color	Isolate number	Total
Grayish	Cf-1, Cf-2, Cf-4, Cf-5, Cf-6, Cf-9, Cf-11, Cf-13, Cf-14, Cf-17, Cf-18, Cf-19, Cf-20, Cf-22, Cf-24, Cf-25, Cf-26, Cf-27, Cf-31, Cf-32, Cf-34, Cf-35, Cf-37, Cf-38, Cf-39, Cf-40, Cf-41, Cf-43, Cf-44, Cf-46, Cf-47, Cf-48	32
Light gray	Cf-7, Cf-12, Cf-15, Cf-16, Cf-21, Cf-28, Cf-29, Cf-36, Cf-49, Cf-50	10
Brown	Cf-3, Cf-8, Cf-10, Cf-23, Cf-30, Cf-33, Cf-42, Cf-45	08
Total		**50**

ii. Based on type of colony growth

Topography	Isolate number	Total
Fluffy growth	Cf-9, Cf-11, Cf-16, Cf-25, Cf-30, Cf-31, Cf-39, Cf-44	8
Flat	Cf-1, Cf-2, Cf-3, Cf-4, Cf-5, Cf-6, Cf-7, Cf-8, Cf-10, Cf-12, Cf-13, Cf-14, Cf-15, Cf-17, Cf-18, Cf-20, Cf-21, Cf-22, Cf-23, Cf-24, Cf-26, Cf-27, Cf-28, Cf-29, Cf-32, Cf-33, Cf-34, Cf-35, Cf-36, Cf-37, Cf-38, Cf-40, Cf-41, Cf-42, Cf-43, Cf-45, Cf-46, Cf-47, Cf-48, Cf-49, Cf-50	42
Total		**50**

iii. Based on type of margin

Type of margin	Isolate number	Total
Irregular	Cf-9, Cf-13, Cf-16, Cf-25, Cf-30, Cf-31, Cf-39, Cf-44	8
Regular	Cf-1, Cf-2, Cf-3, Cf-4, Cf-5, Cf-6, Cf-7, Cf-8, Cf-10, Cf-11, Cf-12, Cf-14, Cf-15, Cf-17, Cf-18, Cf-19, Cf-20, Cf-21, Cf-22, Cf-23, Cf-24, Cf-26, Cf-27, Cf-28, Cf-29, Cf-32, Cf-33, Cf-34, Cf-35, Cf-36, Cf-37, Cf-38, Cf-40, Cf-41, Cf-42, Cf-43, Cf-45, Cf-46, Cf-47, Cf-48, Cf-49, Cf-50	42
Total		**50**

Table 3b. Categorization of isolates based on margin color and on rate of growth

i. Based on margin color

Color	Isolate number	Total
Light gray	Cf-1, Cf-2, Cf-4, Cf-5, Cf-6, Cf-7, Cf-9, Cf-11, Cf-12, Cf-13, Cf-16, Cf-17, Cf-18, Cf-20, Cf-21, Cf-22, Cf-24, Cf-25, Cf-27, Cf-28, Cf-29, Cf-30, Cf-31, Cf-32, Cf-34, Cf-36, Cf-37, Cf-38, Cf-39, Cf-40, Cf-43, Cf-44, Cf-45, Cf-46, Cf-47, Cf-48, Cf-49, Cf-50	38
Brown	Cf-3, Cf-8, Cf-10, Cf-23, Cf-33, Cf-42	6
Light white	Cf-14, Cf-15, Cf-19, Cf-26, Cf-35, Cf-41	6
Total		**50**

ii. Based on rate of growth

Rate of growth	Isolate number	Total
I-Slow growing (<50 mm growth)	-	0
II-Moderately growing (51-70 mm growth)	Cf-15, Cf-16, Cf-31, Cf-38	4
III-Fast growing (71-90 mm growth)	Cf-1, Cf-2, Cf-3, Cf-4, Cf-5, Cf-6, Cf-7, Cf-8, Cf-9, Cf-10, Cf-11, Cf-12, Cf-13, Cf-14, Cf-17, Cf-18, Cf-19, Cf-20, Cf-21, Cf-22, Cf-23, Cf-24, Cf-25, Cf-26, Cf-27, Cf-28, Cf-29, Cf-30, Cf-32, Cf-33, Cf-34, Cf-35, Cf-36, Cf-37, Cf-39, Cf-40, Cf-41, Cf-42, Cf-43, Cf-44, Cf-45, Cf-46, Cf-47, Cf-48, Cf-49, Cf-50	46
Total		**50**

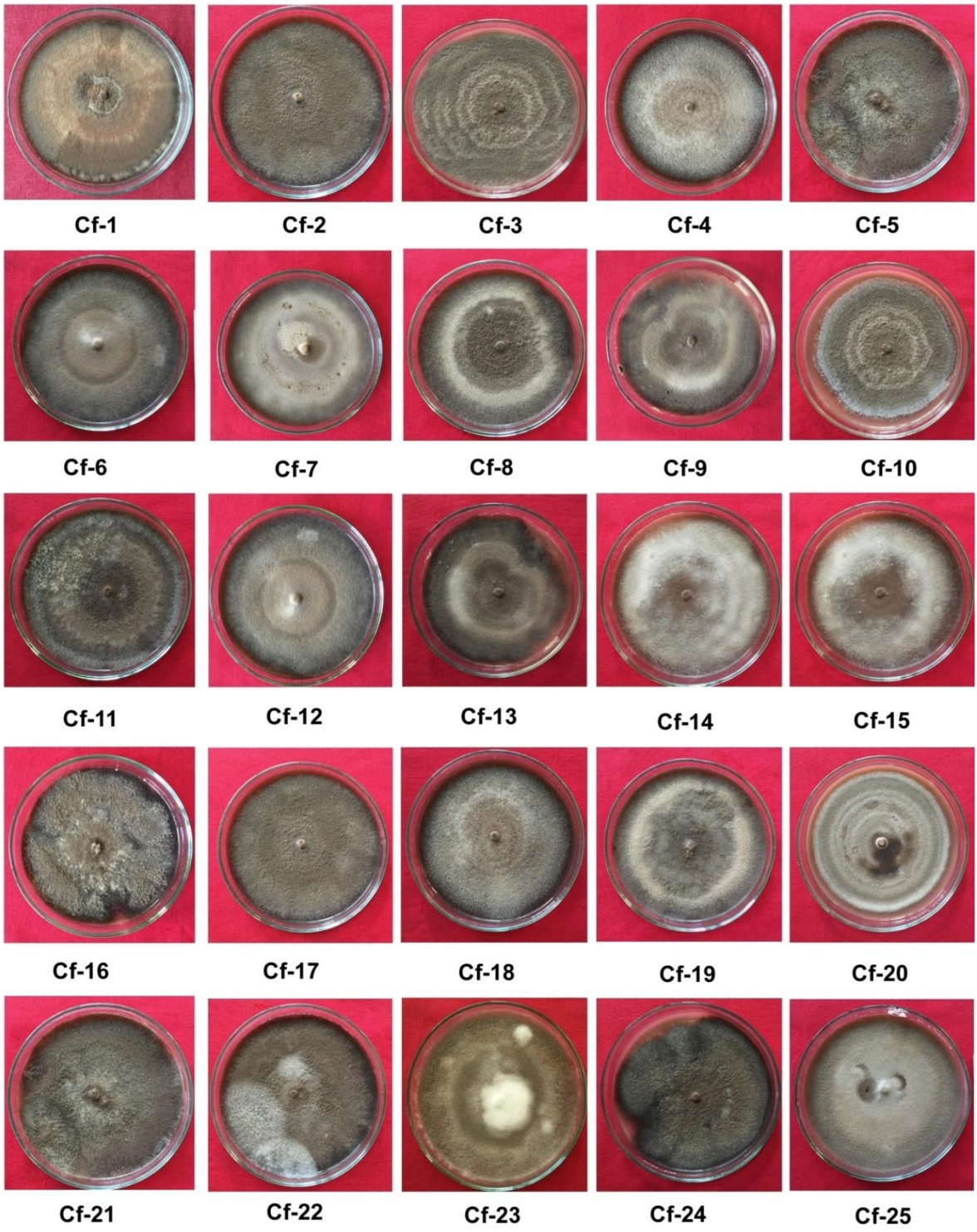

Plate 1. Cultural characteristics of different isolates of *C. fimbriata* on oat meal agar

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

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

In nature, new strains may arise by mutation, hybridization, differential cytoplasmic inheritance (Worthington *et al.,* 2011), heterokaryosis (Milgroom *et al.,* 2009) and by parasexual life cycle (Rosada *et al.,* 2010). Study of pathogenic variability is essential for breeding disease resistance in crop improvement programme. A potential pathogen is often blessed with biodiversity within its population. Basically, variation in pathogen is desirable trait for its existence in nature. This variability among the pathogens underlies their diverse nature and ability to withstand the host environment. Variability of pathogens was studied with cultural, morphological and molecular to focus on the existence of variation in *C. fimbriata* collected from different location.

In the present investigation, diversity in cultural characters showed variation among the *C. fimbriata* isolates. The variation was observed with respect to colony color (ranged from grayish, brown to light gray), type of colony growth (flat and fluffy), type of margin (regular to irregular) margin color (light gray to brown) and colony growth were closely observed in fifty isolates of *C. fimbriata.*

In the present investigation, based on colony color, isolates were categorized into three groups: grayish, light gray and brown. Thirty two isolates showed grayish, ten isolates light gray and eight isolates showed brown colour. Similar results with respect to variation in margin colour were reported by several workers (Gupta Meenu *et al*., 2014 and Sonyal *et al.*, 2015). Lal and Kandhari (2009) reported that six isolates are light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown in case of *R. solani*. Further, nineteen isolates of *Fusarium oxysporum* f.sp. *zingiberi* causal organism of Fusarium yellows in ginger were collected from different ginger growing areas of Himachal Pradesh and designated as I1 to I19. Morphological variations with respect to mycelial colour, conidial size and formation of chlamydospores and pathogenic variation in terms of disease incidence among different isolates was studied in these isolates. The mycelial colour varied from white to dull white with slightly pinkish tinge (Gupta Meenu *et al*., 2014).

Based on colony growth, isolates were categorized into two groups: fluffy and flat in the present study. Forty two isolates which showed flat growth and colonies of eight isolates fluffy growth. Similar results with respect to variation in colony growth were reported by several workers (Rasu *et al.,* 2013; Kumar *et al.,* 2014; Gupta Meenu *et al*., 2014; Manashi

Debbarma and Pranab Dutta, 2015; Poornima, 2015 and Sonyal *et al*, 2015). Prasad *et al.* (2010) studied the variation among the isolates of *S. rolfsii* collected from groundnut and reported that out of 20 isolates, colonies of 11 isolates showed fluffy growth, whereas 9 isolates were flat. Ravi Chandran and Reddi Kumar (2012) studied the variation among the isolates of *Fusarium solani* (Mart.) Sacc., collected from citrus and reported that *F. solani* isolates, AFS1, AFS2, AFS4, CFS9 and CFS13 grew more than 85 mm after 7 days of inoculation and considered as fast growing category. Among all the isolates of *F. solani* CFS9 isolate showed significant variation in radial growth (90 mm) on PDA medium

Based on type of margin, isolates were categorized into two groups: irregular and regular, in the present study. Forty two isolates which showed regular type of margin, eight isolates irregular. Similar results with respect to variation in type of margin were reported by Manashi Debbarma and Pranab Dutta (2015) in case of variability *R. solani* in which they reported that Colony size, colony growth, colour and texture (smooth or rough) varied in six isolates they studied.

With respect to diversity in margin color, isolates were categorized into three groups: light gray, brown and light white. Thirty eight isolates showed light gray colour, eight isolates brown colour and six isolates light white colour. Similar results with respect to variation in margin colour were reported by several workers (Gupta Meenu *et al*., 2014 and Sonyal *et al*, 2015). Further, Lal and Kandhari (2009) reported that six isolates were light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown in case *R. solani.*

The rate of growth was closely observed in fifty isolates of *C. fimbriata.* Isolates were found fast growing, moderately and slow growing. Forty six isolates which were fast growing with an average growth rate (71-90 mm), four intermediate growing isolates with an average growth rate (51-70mm) and none of the isolates were slow growing with an average growth rate of <50 mm. The findings are in accordance with Prabhu and Patil (2005).who reported that among twelve isolates of *S. rolfsii* 11 are fast growing and one isolate is moderate in growth. Further, Hussain *et al*. (2012) classified the isolates of *S. rolfsii* based on morphological variation into fast growing, moderately and slow growing and reported that the isolates AT-1, AT-2 and RW-2 represented significantly fast growing, isolates SR-1, CH-1 and DL-2 intermediate and SR-2, CH-2, CH-3, DL-1, AT-3 and RW-1 under slow radial colony growth.

SUMMARY AND CONCLUSIONS

Cultural variability studied on oat meal agar showed variation among *C. fimbriata* isolates. On the basis of colony color, type of colony growth, type of margin, margin color and colony growth, fifty isolates were categorized *viz.*, based on colony color of mycelia among fifty isolates *C. fimbriata*, thirty two isolates were found grayish color, ten isolates, light gray colour and brown colony color in reaming eight isolates. Based on type of colony growth, eight isolates showed fluffy growth and fifty two isolates showed flat type of colony growth. With respect to type of margin, eight isolates irregular and forty two isolates showed regular type of margin. Based on margin color, three categories of isolates, light gray in thirty eight isolates, brown in six isolates and light white in remaining six isolates and based on rate of growth, four isolates were found moderately growing (51-70 mm), forty six were fast growing (71-90 mm) and none of isolates were slow growing (< 50 mm) covering the oat meal agar within 13-16 days. Cultural variability showed variation among the *C. fimbriata* isolates. On the bases of colony color, type of colony growth, type of margin, margin color and colony growth, fifty isolates were categorized.

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