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Morphological Variation among Different Isolates of *Colletotrichum gloeosporioides* Isolated from Various Crops in Western Maharashtra, India

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A B S T R A C T

Present investigation reveals the study of morphological variation among different isolates of *Colletotrichum gloeosporioides* isolated from various crops. Forty one diseased specimens were collected from different localities of Western Maharashtra and subjected to tissue isolation on PDA. Out of 41 specimens obtained from different hosts, 14 isolates from 14 various hosts were found to be pathogenic when inoculated on respective plant part. These 14 isolates were used for further study and further abbreviated as Cg-1 to Cg-14. *Colletotrichum gloeosporioides* isolated differed significantly in all morphological traits except the type of mycelium. Mycelium of all isolates was septate. Maximum average mycelial growth rate of 12.14 mm day^{-1} was observed in isolates Cg-1 and Cg-14, isolated from pomegranate and sweet orange respectively. Almost all these 14 isolates were referred as fast growing. Isolates *viz.* Cg-7, Cg-9, Cg-10, Cg-11, Cg-12 and Cg-13 do not produce acervuli in culture. In rest of the 8 isolates, acervuli formation was noticed within 10-25 days after inoculation. The early development of acervuli after 10 days of inoculation was observed in Cg-3, Cg-5 and Cg-8. Cg-1 from pomegranate was poor in acervuli formation ability and required more time (18-20 days). The maximum length of acervuli were recorded in Cg-2, Cg-3 and Cg-5 (175µm) respectively. While it was minimum (137.67 µm) in Cg-4 and was statistically undifferentiable from Cg-1, Cg-6, Cg-8 and Cg-14.The average size of acervuli ranged between 137.67 – 175.00 X 87.67 – 170 µm. Extremely large acervuli was produced in culture of Cg-5 isolated from mango. There was a considerable variation in average length of the setae. The average maximum length of setae was (87.67µm) recorded in isolate Cg-8, which was isolated from chilli. The shortest setae were observed in Cg-2 and Cg-6 having average length of 60.00µm, isolated from custard apple and strawberry respectively. The average length of setae among all these isolates was recorded in between 60.00 – 87.67 µm. All these 14 isolates produced conidia in pure culture within 7 days after inoculation. The average length and width of conidia of *C. gloeosporioides* ranged between10.00-12.33 X 3.00-4.33 µm and this difference was statistically significant. Large sized conidia (12.33 X 4.33 µm) was recorded in Cg-8, isolated from chilli. While that of small sized conidia (10.00 X 3.00 µm) were recorded in Cg-2 and Cg-9, isolated from custard apple and ginger respectively. The maximum L X B ratio (3.33) was observed in Cg-2 and Cg-9, both these isolates were from custard apple and ginger. While it was minimum (2.75) in Cg-14 *i.e.* isolated from sweet orange. There was great deal of variation in the colony characters within *C. gloeosporioides* isolates. The colony colour was the most variable factor and none of the isolate was found to be identical with each other. The most predominant colour was creamy white to gray with various intermediate shades. While in case of colony colour at reverse, most of the isolates had dark gray to black colour with slightly different shades. Out of 14 isolates, eight isolates (*viz.*, Cg-3, Cg-4, Cg-6, Cg-8, Cg-9, Cg-10, Cg-13 and Cg-14) had fluffy mycelium growth, while that of six isolates (*viz.*, Cg-1, Cg-2, Cg-5, Cg-7, Cg-11, and Cg-12) had tight mycelium growth. Isolates (*viz.*, Cg-1, Cg-2, Cg-3, Cg-4, Cg-5, Cg-10, Cg-11 and Cg-12) had entire margin with regular shape. Entire margin with irregular shape of colonies were noticed in the isolates Cg-7 and Cg-8 respectively. While that of Cg-6, Cg-9, Cg-13 and Cg-14 had undulate or wavy margin with irregular shaped colonies.

Introduction

The Western Maharashtra region majorly contributes for the production of fruit, vegetable, cereal, flower, medicinal and ornamental crops. The production of these agricultural crops has many problems particularly, fungal diseases. Among these, anthracnose or fruit rot caused by *Colletotrichum gloeosporioides* is most destructive disease and known to cause great losses to the fruit growers in terms of both quality and quantity (Phoulivong *et al*., 2010). It causes anthracnose, die back, whither tip, shot hole, leaf blight and post harvest rots in many economically important crops such as cereals, pulses, vegetables, fruits, spices and cash crops. *Colletotrichum gloeosporioides* cause typical disease symptoms known as anthracnose, characterized by sunken necrotic tissue, produced in lesions on petioles, leaves, mummified inflorescences, flower bracts and on fruits (Dodd *et al*., 1992) and can acts as continuous sources of inoculums. The most significant damage of this fungus occurs upon its attack on fruiting stage (Baily *et al*., 1992). Under such circumstances, the nature of the *C. gloeosporioides* will be the decisive factor in the epidemic development. Therefore, investigation on the basic and applied aspects of population biology of *C. gloeosporioides* is the need of time. It is also necessary to understand how the existing population of the pathogen interacts with the emerging population of the host species and varieties. Similarly, behaviour of *C. gloeosporioides* under intensive management system in crops needs to be investigated in relation with fungicides commonly used as well as newly available. The different isolates of *Colletotrichum gloeosporioides* isolated from different host plants, shows variation in their morphological characters.

The ambiguous taxonomic status of *Colletotrichum* species has resulted in inaccurate identification which may cause practical problems in plant breeding and disease management. Hence there is great potential for augmentation of morphological characteristics for a better classification and identification system, such data will allow the development of proper, objective and automated identification techniques for *Colletotrichum gloeosporioides*.

Considering all these points the investigation on morphological variability was carried out.

Materials and Methods

The present investigation was carried out during August 2014 to December 2017 at Department of Plant Pathology and Agricultural Microbiology, PGI, M.P.K.V. Rahuri, 413 722. The material used and methods and procedures followed to investigate the morphological variability within the isolates of *Colletotrichum gloeosporioides* were as follows.

Collection, isolation and pathogenicity

Collection of disease samples

Diseased specimens from different crops in the form of infected fruits as well as leaves and shoots were periodically collected on the basis of symptoms from Western Maharashtra region in the state by personal visit. The details of the same are as described in Table 1.

Isolation

With exception of pomegranate fruits all other specimens were subjected to tissue isolation. Infected specimens showing typical anthracnose, leaf blight, fruit rot or die back symptoms were brought to laboratory, washed thoroughly with distilled water and dried in the folds of blotting paper. The diseased

patches on the surface of leaf, fruit or shoot were cut in to pieces of about 1 sq. cm in such a way that the cut piece will carry enough diseased portions along with some healthy part. The cut pieces were surface sterilized with 0.5% sodium hypochlorite for 30 sec followed by rinsing with three changes of distilled sterile water. The excess water on the surface of pieces was removed by placing them in the folds of pre sterilized blotting paper. Such pieces were then transferred aseptically on PDA previously poured in the petriplates. In each plate three pieces at equal distance were placed and such plates were incubated at 28^0 C temperature with 95% RH. After 48 hrs. of incubation, the plates were examined for development of visible mycelium growth. The typical growth around individual bit was transferred to PDA slant and incubated for 90-96 hrs. Temporary mounts were prepared to confirm the involvement of the fungus *C. gloeosporioides*. The growth obtained other than the desired fungus was discarded. In case of some fruit specimens showing pink-salmon coloured conidial growth on necrotic patches was directly transferred aseptically on the surface of PDA with the help of sterilized needle. Inoculated plates were inocubated at 28^o C temperature and 95 per cent RH for 48 hrs. The distinct mycelial growth was aseptically transferred to PDA slant. The cultures of C. *gloeosporioides* obtained were subjected to serial dilution to get monoconidial pure cultures. Such cultures were maintained further for confirmation of the pathogenicity (Table 2).

Pathogenicity

For each isolates a separate pathogenicity test was carried out by detached leaf or fruit technique. The plant species and plant parts (*i.e.* fruits or leaves) from which actually the organism was isolated were used. *C. gloeosporioides* isolates from ginger, turmeric, garlic, jasmine (Mogra) and onion were inoculated on leaves of healthy plants of respective hosts in glasshouse.

While that of isolates from pomegranate, custard apple, papaya, guava, mango, strawberry, lime, chilli and sweet orange were inoculated on healthy fruits of respective hosts. Leaves or fruits were washed with tap water, air dried, surface disinfected with 0.1 % mercuric chloride solution one minute followed by thoroughly but gentle rinsing with sterilized water for three times to remove the traces of disinfectant and subjected to fine injury on abaxyl surface with the help of carborundum powder so as to facilitate the entry of the germ tube. Thereafter, the fruits were kept on flask or beaker (100ml) by inserting the fruit stalk in sterilized water, which is previousely filled in them. The fruits were covered with sterilized polythene bags.

It was done to provide 24 hours pre-inoculation incubation of fruits as suggested by Manandhar*et al.*, (1995). Next day the bags were removed and inoculation was made at the site of fruits. Seven to ten days old culture of respective isolate showing abundant salmon coloured conidial masses was added with little quantity of sterile water and conidial masses were separated from the culture with the help of scalpel. The conidial load in the suspension was adjusted to 10^6 conidial ml^{-1}. Such freshly prepared suspension was automised on the injured surface of leaves or fruits by micro-droplet inoculation technique (MDIT). A fine wet cotton swab was then placed on the inoculated portion of the leaves or fruits. A suitable control was maintained separately at every time wherein instead of conidial suspension, sterilized water was sprayed on the injured leaf or fruit. A set of inoculated plants was maintained in the glasshouse as well as in moisture chamber according to the inoculated plant parts i. e. leaves and fruits respectively,

at 27^0C with more than 90 per cent RH for two weeks.

Reisolation was made from inoculated surface upon development of symptoms. The cultures obtained upon reisolation were compared with the respective original culture. Isolates fulfilling the pathogenicity test were accessed according to host. Cg was the common prefix representing the test fungus. All cultures were maintained in duplicate sets and stored in the refrigerator at temperature of 6-8^0C. All cultures were periodically sub cultured every after two months and maintained during the course of investigation.

Identification

Isolates fulfilling the pathogenicity test were tentatively identified on the basis of morphological and cultural characters with the help of available literature.

Variation in morphological characters

To study the morphological variation within the isolates, all isolates were grown on PDA for seven to ten days at 28^0C with 90 per cent humidity. Temporary mounts of different fungal structures viz., mycelium, acervuli, setae and conidia were prepared and observed under light microscope at magnification of 45 X.

The filarmicrometer was calibrated with the help of stage micrometer prior to measurements. Measurements of each structure were recorded from five to eight slides at three microscopic fields randomly. For conidial measurements, 50 observations were taken while for all other structures, 25 observations were recorded and mean value was calculated.

Observations on acervuli measurements of different isolates were recorded as and when they were formed within 25-45 days. The observations on morphological measurements of different structures were further analysed collectively by agglomerative hierarchial cluster analysis (Aldenderfer and Blashfield, 1984). Observations on colony morphology viz., colour, colour at reverse, distinct feature, margin and shape were recorded by growing all isolates of C. *gloeosporioides* on PDA for seven days at 28^0C with 90 per cent humidity. Variation in these colony characters was determined qualitatively. The colour ratings were made with the help of Munsell's colour chart and accordingly isolates were grouped.

Growth measurements were recorded at right angle from the reverse side of the plate with the help of scale and expressed as average growth rate in mm after 3^{rd}, 5^{th} and 7^{th} days of incubation for each isolate in three replications. The data obtained was analysed by using Completely Randomized Design (CRD).

Results and Discussion

Isolation

In the present study, *C. gloeosporioides* was isolated in the laboratory on PDA. Isolations were made from fruit samples of pomegranate, custard apple, papaya, guava, mango, strawberry, acid lime, chilli and sweet orange which were collected from different parts of the Western Maharashtra. Similarly, *C. gloeosporioides* was also recovered from leaf samples of ginger, turmeric, garlic, jasmine and onion. *C. gloeosporioides* initially produced white profuse cottony growth around the host tissue placed for isolation within 72 hrs of incubation which later turned gray with formation of acervuli in some of the isolates within next 72 hrs. These findings are in similar lines as those reported by Hasabnis (1984), Korade *et al.,* (2001).

Pathogenicity

Fruits of pomegranate, custard apple, papaya, guava, mango, strawberry, acid lime, chilli and sweet orange expressed initiation of typical fruit rot symptoms at the point of inoculation within 3-6 days after inoculation. Large necrotic round to irregular lesions developed in further seven to ten days. Symptoms development was rapid in pomegranate, custard apple, guava, mango and chilli fruits (72-90 hrs) followed by papaya, strawberry, acid lime and sweet orange (105 hrs). However, symptoms initiation was comparatively late in ginger, turmeric, garlic, jasmine and onion. It was observed that development of symptoms upon artificial inoculation was comparatively rapid when fruits were inoculated in comparison with the inoculation on leaves.

These results are in confirmation with the findings of Talhinhas *et al.,* (2005). They have proved the pathogenicity of *C. accutatum* and *C. gloeosporioides* with a representative set of isolates using fruits of 11 different olive cultivars and 'Camarosa' strawberry and obtained symptoms after 7 and 11 days on strawberry and olive fruits respectively. However, Hasabnis (1984) reported a period of 48 – 72 hrs for development of artificial infection of *C. gloeosporioides.* In present study, it took a minimum period of 72 hrs for getting initial infection on either fruit or leaf which was inoculated artificially (Table 3).

Identification

The tentative identification of all 14 isolates was made on the basis of conidial morphology and cultural characters.

Variation in the morphological characters

All these 14 isolates of *C. gloeosporioides* were produced the septate mycelium. The maximum average growth rate of 12.14 mm day^{-1} was observed in isolates Cg-1 and Cg-14 and it was statistically on par with isolates *viz.*, Cg-7 and Cg-11 followed by Cg-2, Cg-3, Cg-4, Cg-5, Cg-6, Cg-8, Cg-9, Cg-10, Cg-12 and Cg-13. These isolates were referred as fast growing. Similar trends in the colony morphology was reported earlier byAbnang *et al.,* (2006) who classified isolates of *C. gloeosporioides* based on colony colour and growth rate and categorized them into four groups. Similar results were also reported by Mathur *et al.,* (2001) and Lopez and Lucas (2010) (Table 4).

Acervuli

Out of 14 isolates under study, 8 isolates produced acervuli in culture. Isolates *viz.* Cg-7, Cg-9, Cg-10, Cg-11, Cg-12 and Cg-13 do not produce acervuli in culture. In rest of the 8 isolates, acervuli formation was noticed within 10-25 days after inoculation. The early development of acervuli after 10 days of inoculation was observed in Cg-3, Cg-5 and Cg-8. Acervuli formation was noticed within 10-25 days after inoculation.

The early development of acervuli after ten days of inoculation was observed in Cg-3, Cg-5 and Cg-8.

The acervuli were formed profusely and regularly during the course of study with these isolates and therefore these isolates were referred as profuse acervuli producers. Cg-1 from pomegranate was poor in acervuli formation ability and required more time (18-20 days) to form acervuli. Remaining isolates *viz.*, Cg-2, Cg-4, Cg-6 and Cg-14 formed acervuli less frequently when grown on PDA. In general the shape of acervuli was elliptical globose to irregular. The colour of acervuli was light brown to dark in general. The differences in acervuli measurements were

statistically significant.

The maximum length of acervuli were recorded in Cg-2, Cg-3 and Cg-5 (175 µm) respectively. While it was minimum (137.67 µm) in Cg-4 and was statistically undifferentiable from Cg-1, Cg-6, Cg-8 and Cg-14. The average size of acervuli ranged between 137.67 – 175.00 X 87.67 – 170 µm. Extremely large acervuli was produced in culture of Cg-5 isolated from mango. The range of acervuli dimensions in the present study is comparatively narrow when compared to that of acervuli measurements reported earlier by Joshi (2008). While studying the morphological variation within *C. gloeosporioides* isolates infecting custard apple. He has mentioned that the average size of acervuli ranged between 104.04 – 412.92 µm X 54.36 – 147.80 µm. These findings are in conformity with Gaikwad and Sawant (2005) (Table 5).

Length of setae

Out of 14 isolates, eight isolates could produce setae as an integral part of acervulus. Setae differed with respect to colour, shape and measurements. Light brown to dark brown coloured setae were predominantly observed in all isolates. There was a considerable variation within the *C. gloeosporioides* isolates in average length of the setae. The average maximum length of setae was (87.67µm) recorded in isolate Cg-8, which was isolated from chilli. The shortest setae were observed in Cg-2 and Cg-6 having average length of 60.00 µm, isolated from custard apple and strawberry respectively. The average length of setae among all these isolates was recorded in between 60.00 – 87.67 µm.

These findings agree with Rohana *et al.,* (2006) who studied the comparative characterization of seven isolates of *C. gloeosporioides* and stated the formation of sclerotia in one isolate but setae formation was not recorded. However, the findings in relation with setae morphology are in conformity with those recorded by Gaikwad and Sawant (2005) and Joshi (2008) (Table 6).

Conidia

All these 14 isolates produced conidia in pure culture within 7 days after inoculation. In general, conidia were hyaline, unicellular, cylindrical to allantoidal with an obtuse apex. Conidia of all isolates were more or less identical in shape. The average length and width of conidia of *C. gloeosporioides* ranged between10.00-12.33 X 3.00-4.33 µm and this difference was statistically significant.

Large sized conidia (12.33 X 4.33 µm) was recorded in Cg-8, isolated from chilli and it was on par with Cg-1. While that of small sized conidia (10.00 X 3.00 µm) were recorded in Cg-2 and Cg-9, isolated from custard apple and ginger respectively. The maximum L X B ratio (3.33) was observed in Cg-2 and Cg-9, both these isolates were from custard apple and ginger. While it was minimum (2.75) in Cg-14 *i.e.* isolated from sweet orange.

The above findings with respect to conidial length and width are analogous with those reported by Gaikwad and Sawant (2005). They have found that isolate Cg 6413 produced large sized conidia measuring 12.26 µm in length, while conidia of isolate Cg 0818 had maximum width of 4.78 µm. Cg 0914 produced shortest conidia having the length of 9.96 µm.

Highest L : B ratio of 3.94 was observed in Cg 06413, while it was lowest (2.41) in Cg 0736. Shape of most of the conidia was reported to be cylindrical but sometimes oval to cylindrical forms were also observed (Table 7).

Table.1 Collection of disease samples collected from different crops in Western Maharashtra

Sr. No.	Name of crop	Host part	Location		
			Place/Village	Tahsil	District
01	Pomegranate	Fruit	Akolevasud	Sangola	Solapur
02	Papaya	Fruit	Akluj	Malshiras	Solapur
03	Guava	Fruit	Rahuri	Rahuri	A.nagar
04	Strawberry	Fruit	Bhilar	Mahableshwar	Satara
05	Custard apple	Fruit	Jejuri	Purandar	Pune
06	Papaya	Fruit	Shirbavi	Sangola	Solapur
07	Mango	Fruit	Kothali	Shirol	Kolhapur
08	Strawberry	Fruit	Panchgani	Mahableshwar	Satara
09	Acid lime	Fruit	Varvand	Daund	Pune
10	Pomegranate	Fruit	Mahud	Sangola	Solapur
11	Custard apple	Fruit	Lonarwadi	Pandharpur	Solapur
12	Chilli	Fruit	Tawadi	Phaltan	Satara
13	Guava	Fruit	Aasu	Phaltan	Satara
14	Ginger	Leaf	Pusegoan	Khatav	Satara
15	Papaya	Fruit	Rede	Malshiras	Solapur
16	Turmeric	Leaf	Sangawade	Karveer	Kolhapur
17	Mango	Fruit	Khanapur	Bhor	Pune
18	Garlic	Leaf	Vidani	Phaltan	Satara
19	Maize	Leaf	Paniv	Malshiras	Solapur
20	Jasmine	Leaf	Pandare	Baramati	Pune
21	Turmeric	Leaf	Bujgoan	Miraj	Sangli
22	Strawberry	Fruit	Mahableshwar	Mahableshwar	Satara
23	Sweet orange	Fruit	Kashti	Shrigonda	A. nagar
24	Papaya	Fruit	Rajale	Phaltan	Satara
25	Mango	Fruit	Untwadi	Jat	Sangli
26	Jasmine	Leaf	Sangavi	Phaltan	Satara
27	Ginger	Leaf	Amnapur	Palus	Sangli
28	Custard apple	Fruit	Saswad	Purandar	Pune
29	Acid lime	Fruit	Kashti	Shrigonda	A. nagar
30	Mango	Fruit	Rahuri	Rahuri	A. nagar
31	Chilli	Fruit	Songoan	Phaltan	Satara
32	Guava	Fruit	Nira-Wagaj	Baramati	Pune
33	Ginger	Leaf	K.Digras	Miraj	Sangli
34	Turmeric	Leaf	K.Digras	Miraj	Sangli
35	Onion	Leaf	Nandurshingote	Sinnar	Nashik
36	Turmeric	Leaf	Nandani	Shirol	Kolhapur
37	Garlic	Leaf	Mirgoan	Phaltan	Satara
38	Onion	Leaf	Dahiwadi	Man	Satara
39	Sweet orange	Fruit	Mirajgoan	Karjat	A. nagar
40	Maize	Leaf	Tavashi	Baramati	Pune
41	Pomegranate	Fruit	Sonake	Pandharpur	Solapur

Table.2 Isolates of *C. gloeosporioides* collected from different hosts from Western Maharashtra region

Isolate No.	Name of the crop	Host part	Location		
			Place/ Village	Tahasil	District
Cg-1	Pomegranate	Fruit	Akolevasud	Sangola	Solapur
Cg-2	Custard apple	Fruit	Saswad	Purandar	Pune
Cg-3	Papaya	Fruit	Akluj	Malshiras	Solapur
Cg-4	Guava	Fruit	Rahuri	Rahuri	A.nagar
Cg-5	Mango	Fruit	Rahuri	Rahuri	A.nagar
Cg-6	Strawberry	Fruit	Mahableshwar	Mahableshwar	Satara
Cg-7	Lime	Fruit	Kashti	Shrigonda	A.nagar
Cg-8	Chilli	Fruit	Tawadi	Phaltan	Satara
Cg-9	Ginger	Leaf	Amnapur	Palus	Sangli
Cg-10	Turmeric	Leaf	Sangawade	Karveer	Kolhapur
Cg-11	Garlic	Leaf	Vidani	Phaltan	Satara
Cg-12	Jasmine	Leaf	Pandare	Baramati	Pune
Cg-13	Onion	Leaf	Nandurshingote	Sinnar	Nashik
Cg-14	Sweet orange	Fruit	Kashti	Shrigonda	A.nagar

Table.4 Variability in mycelium growth rate among different isolates of *C. gloeosporioides* collected from different crops in Western Maharashtra

Sr. No.	Isolate	Mycelium (Septate/Aseptate)	Mycelial growth rate (mm) per day		
			3rd day	5th day	7th day
01	Cg-1	Septate	33.00	60.00	85.00
02	Cg-2	Septate	29.00	55.00	80.00
03	Cg-3	Septate	30.00	58.00	80.00
04	Cg-4	Septate	30.00	59.00	80.00
05	Cg-5	Septate	30.00	60.00	80.00
06	Cg-6	Septate	28.00	54.00	80.00
07	Cg-7	Septate	30.00	60.00	84.00
08	Cg-8	Septate	30.00	54.00	80.00
09	Cg-9	Septate	28.00	55.00	80.00
10	Cg-10	Septate	29.00	55.00	80.00
11	Cg-11	Septate	30.00	54.00	82.00
12	Cg-12	Septate	28.00	56.00	80.00
13	Cg-13	Septate	28.00	55.00	80.00
14	Cg-14	Septate	30.00	60.00	85.00
SE (±)			**0.53**	**0.49**	**0.39**
CD at 1 %			**2.09**	**1.90**	**1.56**

Table.3 Pathogenicity symptoms produced by *C. gloeosporioides* isolates collected from different crops in Western Maharashtra

Isolate code	Crop	Symptoms	No. of days required
Cg-1	Pomegranate	Rotting was noticed from fruit end portion resulting into dark brown to black in colour.	8 – 9
Cg-2	Custard apple	Large necrotic round to irregular lesions were formed on fruits showing brown colour.	7 – 8
Cg-3	Papaya	Lesions were observed, which began as dark brown, punctate, circular to irregular spots of < 1.5 mm in diameter, often with distinctly graycenters.	7 – 8
Cg-4	Guava	Lesions appeared as dark brown irregular and that become sunken on the rind tissues.	7 – 8
Cg-5	Mango	Dark brown to black coloured spots were observed, which later coalesce to form sunken patches.	7 – 8
Cg-6	Strawberry	Brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, spots coalesced to form necrotic rotten patches.	7 – 8
Cg-7	Acid lime	Spots may appear as dark brown irregular and that become sunken on the rind tissues.	8 – 9
Cg-8	Chilli	Observed typical anthracnose symptoms including sunken necrotic tissues, with concentric rings of acervuli on Chilli fruits.	7 – 8
Cg-9	Ginger	Brown spots were observed, which later turns ellipsoidal or spindle shaped with halo. The affected leaves eventually turn brown and results in dry rot. Also this leaf spot characterized with small round to oval, light yellow spots on leaves and leaf sheaths, which gradually increase in size and coalesce to form large discoloured areas. The infected areas often dry up at the center, forming shot holes.	7 – 8
Cg-10	Turmeric	Brown spots were noticed on the upper surface of the young leaves, spots are irregular in shape and white or grey in the centre. Later, two or more spots may coalesce and formed an irregular patch covering almost the whole leaf.	8 – 9
Cg-11	Garlic	Observed typical anthracnose symptoms including sunken necrotic tissues on the leaves.	9 – 10
Cg-12	Jasmine	Observed the anthracnose symptoms on leaves. The spots were dark gray and mostly irregular shaped.	9 – 10
Cg-13	Onion	Observed typical anthracnose symptoms including sunken necrotic tissues on the leaves.	9 – 10
Cg-14	Sweet orange	Spots may appeared as dark brown irregular and that become sunken on the rind tissues.	7 – 8

Table.5 Variability in acervuli among different isolates of *C. gloeosporioides* collected from different crops in Western Maharashtra

Sr. No.	Isolates	Acervuli (Present/ Absent)	Acervuli length (µm)	Acervuli width (µm)
01	Cg-1	Present	150.00	100.00
02	Cg-2	Present	175.00	112.67
03	Cg-3	Present	175.00	118.00
04	Cg-4	Present	137.67	87.67
05	Cg-5	Present	175.00	170.00
06	Cg-6	Present	170.00	100.00
07	Cg-7	Absent	00.00	00.00
08	Cg-8	Present	162.67	95.00
09	Cg-9	Absent	00.00	00.00
10	Cg-10	Absent	00.00	00.00
11	Cg-11	Absent	00.00	00.00
12	Cg-12	Absent	00.00	00.00
13	Cg-13	Absent	00.00	00.00
14	Cg-14	Present	140.00	90.00
SE (±)			**0.39**	**0.25**
CD at 1 %			**1.55**	**0.99**

Table.6 Variability in length of setae among different isolates of *C. gloeosporioides* collected from different crops in Western Maharashtra

Sr. No.	Isolates	Setae (Present/ Absent)	Length (µm)
01	Cg-1	Present	61.67
02	Cg-2	Present	60.00
03	Cg-3	Present	62.00
04	Cg-4	Present	62.67
05	Cg-5	Present	61.00
06	Cg-6	Present	60.00
07	Cg-7	Absent	00.00
08	Cg-8	Present	87.67
09	Cg-9	Absent	00.00
10	Cg-10	Absent	00.00
11	Cg-11	Absent	00.00
12	Cg-12	Absent	00.00
13	Cg-13	Absent	00.00
14	Cg-14	Present	64.00
SE (±)			**0.34**
CD at 1 %			**1.34**

Table.7 Variability in conidia among different isolates of *C. gloeosporioides* collected from different crops in Western Maharashtra

Sr. No.	Isolates	Shape of Conidia	Size of conidia	
			Length (µm)	Width (µm)
01	Cg-1	Hyaline, Unicellular, Cylindrical with an obtuse apex.	12.33	4.00
02	Cg-2		10.00	3.00
03	Cg-3		10.67	3.33
04	Cg-4		10.33	3.67
05	Cg-5		11.33	4.00
06	Cg-6		11.00	3.33
07	Cg-7		10.00	3.33
08	Cg-8		12.33	4.33
09	Cg-9		10.00	3.00
10	Cg-10		10.67	3.33
11	Cg-11		11.00	3.67
12	Cg-12		11.33	4.00
13	Cg-13		10.33	3.67
14	Cg-14		11.00	4.00
SE (±)			**0.36**	**0.33**
CD at 1 %			**1.44**	**1.31**

Table.8 Variability in colony characters among different isolates of *C. gloeosporioides* collected from different crops in Western Maharashtra

Sr. No.	Isolates	Colour	Colours at reverse	Distinct feature	Margin	Shape
01	Cg-1	Creamy white to Gray	Dark gray	Tight mycelium growth with compact colony.	Entire	Regular
02	Cg-2	Creamy white to Dark gray	Dark gray to black	Tight mycelium growth with compact colony and salmon concentric rings.	Entire	Regular
03	Cg-3	Pinkish gray to Dark gray	Dark gray to black	Fluffy mycelium, partially submerged growth.	Entire	Regular
04	Cg-4	Dark gray to black with white islands	Black with salmon patches	Fluffy mycelium growth with somewhat loose sub aerial hyphae.	Entire	Regular
05	Cg-5	Dark gray	Gray to black	Tight mycelium growth with compact colony and salmon concentric rings.	Entire	Regular
06	Cg-6	Creamy white	Creamy yellow	Fluffy mycelium with profusely growing dense aerial cottony growth.	Undulate/ Wavy	Irregular

07	Cg-7	White to light gray	Creamy yellow with salmon patches	Tight mycelium with flat growth.	Entire	Irregular
08	Cg-8	Dark gray	Black with salmon patches	Fluffy mycelium growth with dense aerial hyphae.	Entire	Irregular
09	Cg-9	Dark gray	Black gray	Fluffy mycelium with profusely aerial growth.	Undulate/ Wavy	Irregular
10	Cg-10	Whitish gray	Dark gray with creamy center	Fluffy mycelium with flat growth.	Entire	Regular
11	Cg-11	Creamy white	Creamy yellow	Tight mycelium with flat growth.	Entire	Regular
12	Cg-12	White to light gray	Creamy yellow	Tight mycelium with flat growth.	Entire	Regular
13	Cg-13	Creamy white to light gray	Black with salmon patches	Fluffy mycelium growth with somewhat loose sub aerial hyphae.	Undulate/ Wavy	Irregular
14	Cg-14	Pinkish gray	Creamy yellow	Fluffy mycelium with partially submerged growth.	Undulate/ Wavy	Irregular

Colony characters (colour, colour at reverse, distinct feature, margin and shape)

There was great deal of variation in the colony characters within *C. gloeosporioides* isolates. The colony colour was the most variable factor and none of the isolate was found to be identical with each other. The colonies of all isolates were initially white later gaining differential colour shades upon aging. The most predominant colour was creamy white to gray with various intermediate shades. While in case of colony colour at reverse, most of the isolates had dark gray to black colour with slightly different shades. Out of 14 isolates, eight isolates (*viz.*, Cg-3, Cg-4, Cg-6, Cg-8, Cg-9, Cg-10, Cg-13 and Cg-14) had fluffy mycelium growth, while that of six isolates (*viz.*, Cg-1, Cg-2, Cg-5, Cg-7, Cg-11, and Cg-12) had tight mycelium growth.

Isolates (*viz.*, Cg-1, Cg-2, Cg-3, Cg-4, Cg-5, Cg-10, Cg-11 and Cg-12) had entire margin with regular shape. Entire margin with irregular shape of colonies were noticed in the isolates Cg-7 and Cg-8 respectively. While that of Cg-6, Cg-9, Cg-13 and Cg-14 had undulate or wavy margin with irregular shaped colonies. These results show the similar trends as that reported by Fokunang *et al.*, (2000), Joshi (2008), Chowdappa *et al.*, (2012), Adhipathi *et al.*, (2013) and Kumar *et al.*, (2015) while studying the colony characters of *C. gloeosporioides* infecting different crops (Table 8).

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