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Cultural and physiological studies on *Colletotrichum gloeosporioides* and *Fusarium oxysporum* causing twister diseases in onion

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Abstract
Cultural and physiological studies on *Colletotrichum gloeosporioides* and *Fusarium oxysporum* were studied at the Laboratory of Department of Plant Pathology, College of Horticulture, Bagalkot during 2021-2022. Onion is one of the important oldest and widely grown bulbous vegetable crop among the genus *Allium* in India. Onion productivity is becoming quite low because of many fungal and bacterial diseases mainly twister disease which effect the yield of the crop. The Potato Dextrose Agar (PDA) medium was used to maintain the pure cultures isolated from twister disease infected onion plants and identified as *C. gloeosporioides* and *F. oxysporum*. The cultural studies were conducted to know the effect of growth and sporulation in different media among them both the pathogens gave maximum mycelial growth (87.92 and 88.00 respectively) on the 12th day after incubation in a PDA medium with better sporulation. Among liquid media, potato dextrose broth was observed most supportive for its growth. The various response of *C. gloeosporioides* and *F. oxysporum* to different levels of temperature, pH and light showed that temperature of 25 °C, pH 6 and alternate cycles of 12 hours of light and 12 hours of darkness was found congenial under *in vitro* conditions.

Keywords: Cultural, physiological, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*

Introduction
Onion (*Allium cepa* L.) is one of the important oldest and most widely grown bulbous vegetable crop among the genus *Allium* in India. It is called as "queen of kitchen" which belongs to the family Alliaceae having a chromosome number of 2n=16 (Firbas and Amon, 2014) [11]. Central Asia lies as a primary center of origin, whereas Mediterranean regions are the secondary centers of origin of onion (Vavilov, 1951) [34].

Onion productivity is becoming quite low because of many biotic and abiotic stresses which are restricting the successful production of onion in India. Disease is one of the major factors among the biotic stresses for the cultivation of onions. These diseases are *viz*., purple blotch, leaf rot, downy mildew, *Stemphylium* blight, basal rot, smuts, twister disease, sclerotial rot, bacterial soft rot, onion yellow dwarf virus, stem and bulb nematode, root-knot nematode and stubby root nematode (Mishra *et al*., 2014) [19]. Among them, onion twister disease is becoming an epidemic in many onion growing areas.

Onion twister disease was first reported near Zaria, northern Nigeria, in 1969 during the rainy season with 50-100 per cent yield loss (Ebenebe, 1980) [10]. In India, the twister disease was first reported in Bihar caused by the *Glomerella cingulate* during the *kharif* season (Sinha and Singh, 1994) [30]. In Karnataka, a report on twister disease complex severity (7-52%) has been submitted to the government from KVK, Hagari, Bellary. Which focuses on the investigations on bulb rotting and twisting of onion leaves and its management (Anon., 2005) [1]. In the coastal tract of Karnataka, Nargund *et al*. (2013) [22] recorded 30-40 per cent yield loss with twisting of leaves, stems and bulbs of onion. In different parts of Karnataka 9-26 per cent disease severity was reported during *kharif* and *rabi*/summer of 2011-12 and 2012-13, respectively (Patil, 2013) [23].

Colletotrichum gloeosporioides cause anthracnose/ twister disease (Mishra *et al*., 2014) [19]. Initially, water soaked pale yellow lesions, further developed into orange masses in concentric rings and later into a black acervulus. In the *Fusarium fujikuroi*, infected plants observed a yellow green discoloration of leaves with slight twisting from the neck of the plant (Perez and Alberto, 2020) [24].

This pathogen will produce three different asexual spores *viz*., microconidia, macroconidia and chlamydospores (Cramer, 2000) [6]. It can attack crops at any stage of the plant under highly humid conditions and conidia can disperse via air.

Due to the destructive nature of the pathogen and the importance of the crop, this study was conducted to deeply understand the cultural as well as physiological characters of the pathogen. Similar kinds of studies were conducted in different crops infected by *Colletotrichum* sp. and *Fusarium* sp. (Gupta *et al*., 2010; Thangamani *et al*., 2011; Singh and Kumar, 2016; Chaudhari *et al*., 2017; Kammar, 2019; Dharbale *et al*., 2019; Chaithra *et al*., 2020 and Nandhini *et al*., 2021) [13, 33, 29, 4, 15, 9, 3, 21].

Material and Methods

Cultural studies

Solid media

The growth characters of *C. gloeosporioides* and *F. oxysporum* were studied on twelve different selective solid and liquid broth media *viz.*, Asthan and Hawker's agar media, corn meal agar, Czapek's dox agar, glucose asparagine agar, malt extract agar, oat meal agar, potato dextrose agar, Richard's agar, rose bengal agar, Sabouraud's agar, soyabean casein digest agar and V8 juice agar. For all media pH was adjusted to 6.0 before autoclave. All the media were autoclaved at 121.6 ºC, and 15 lb pressure for 15 min. Twenty ml of each of the autoclaved media are poured into each Petri plate with three replications. Such plates were aseptically inoculated with a 5 mm bit taken from the periphery of the 12 days old culture and incubated at 25±2 °C for twelve days. Observations like colony diameter (12th days after inoculation), mycelia color, margin and topography of the colony, pigmentation, and sporulation were recorded.

Liquid media

C. gloeosporioides and *F. oxysporum* growth were also studied on twelve liquid media. The components of the media were similar to solid media except for agar. The pH of all the broth was adjusted to 6.0 before the autoclave. The broth was autoclaved at 121.6 °C at 15 lb for 15 minutes. The 5 mm disc of the pathogen was inoculated into a 100 ml flask containing 40 ml broths and incubated at 25±2 °C. For each of the treatments, three replications were kept. After twelve days of inoculation (DAI), the mycelia are collected by filtering through filter paper. Filter paper along with a mycelial mat was dried at 60 °C for 24 hours and weighed on an analytical balance. Dry mycelial weight was calculated by using the formula given by Dev *et al*. (2017) [8].

Dry mycelial weight (mg) = Total weight of filter paper along with mycelia – Initial weight of filter paper

Growth phase

For this study, 40 ml of PDB was prepared in 100 ml conical flasks and autoclaved. To these flasks after cooling, a 5 mm mycelial bit from 12 day old pure culture was inoculated and kept at 25±2 °C. For each treatment, three replications were kept. From the day of inoculation, once in every two days, three flasks were harvested till the 20th day of inoculation. The mycelial mat was harvested by passing through filter paper. Filter paper along with mycelia mat was dried at 60 °C and then weighed on a balance (Kammar, 2019) [15]. The difference between the final weight of filter paper with dried mycelium and the initial weight of filter paper was taken as the weight of the mycelial mat.

Physiological studies

Effect of different temperatures

The six different temperatures were selected *viz.*, 15, 20, 25, 30, 35, and 40 °C to understand the effect of the growth of the pathogens. About 40 ml of PDB was prepared in a 100 ml conical flask, autoclaved, and aseptically inoculated with a 5 mm disc of the fungus from a 12 days old culture. The inoculated flasks were placed at different temperatures with three replications. Finally, dried mycelium weight was recorded after 12 days of inoculation, and data were analyzed statistically (Kommula *et al*., 2017) [16].

Effect of hydrogen ion concentration (pH)

Six different levels of pH *viz*., 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were selected to know the effect of pH on the growth of the pathogens. The pH was adjusted by using 0.1N NaOH or 0.1N HCl and PDB was used as a basal medium. The 5 mm culture bit was inoculated to every 40 ml of PDB medium, which was previously autoclaved. For each pH, three replications were maintained. The dry mycelial weight of the fungus was recorded after 12 DAI and data were analysed statistically (Bhavya *et al*., 2019) [2].

Effect of light on the growth and sporulation of pathogens

The effect of light on growth and sporulation of the pathogen was studied on PDA media by exposing the pure culture to continuous light, continuous dark, alternating with 12 hours of complete light and 12 hours of complete darkness. The inoculation of culture to Petri dishes containing PDA media was done with five replication per treatment. The plates were incubated at 25±2 °C for 12 days. Observation on colony diameter and sporulation were recorded (Somu, 2017) [31].

Results and Discussion

Cultural studies

On solid media

Out of twelve solid media evaluated against the growth of *C. gloeosporioides* PDA (87.92 mm) recorded the highest colony diameter with excellent sporulation (++++) after 12 days of inoculation, which was followed by Richard's agar (86.67 mm) with moderate sporulation (++) (Table 1). The least growth was observed on rose bengal agar (31.93 mm). Table 1 shows that the growth of *C. gloeosporioides* on PDA media showed creamish white pigmentation, with regular margins and aerial mycelia. Whereas, on Richard's agar white aerial mycelium, irregular colony margins with creamish white pigmentation were observed. These results similar to the findings of Chaudhari *et al*. (2017) [4]; Dharbale *et al*. (2019) [9]; and Lokare and Fatima, (2021) [18] recorded the maximum growth and excellent sporulation of *C. gloeosporioides* on PDA (90 mm).

Table 2 showed that there was a significant difference concerning the growth of *F. oxysporum* on different solid media used for cultural studies. Results showed that PDA (88.00 mm) recorded the maximum colony diameter after 12 days of incubation with greyish white colony color, aerial mycelia with sectoring and light pinkish white pigmentation, with excellent sporulation. Asthana and Hawkers agar (86.33 mm) and Richard's agar (86.10 mm) medium were on par with each other with the white colony and white pigmentation. The least growth was observed on rose bengal agar (35.08 mm). These results are in agreement with the

findings of Chittem and Kulkarni (2008) [5] recorded the maximum growth of *F. oxysporum* f. sp. *dianthi* on PDA (90.00 mm) followed by Richard's agar (86.00 mm) and minimum radial growth was observed on rose bengal agar (10.66 mm). It is concluded that PDA has a simple formulation and more nutrient content, supporting the best mycelial growth of the pathogen. Similar findings are reported by Gupta *et al*. (2010); Singh and Kumar, (2016); and Chaithra *et al*. (2020) [29, 3].

Table 1: Morphological characteristics of *Colletotrichum gloeosporioides* on different solid media

Sl. No.	Media	Colony diameter (mm)	Colony character			Pigment production	Sporulation
			Colony color	Colony margin	Topography		
1.	Asthana and Hawkers medium	80.36	Creamy white	Regular	Submerged mycelia	Light white	++
2.	Corn meal agar	79.33	Creamy white	Regular	Submerged mycelia	Light white	+
3.	Czapek's dox agar	67.23	White	Regular	Flat mycelia	Greyish white	+
4.	Glucose asparagine agar	54.30	Greyish White	Regular	Greyish white mycelia with black ring	Greyish white	+
5.	Malt extract agar	82.77	Greyish white	Regular	Aerial mycelia	Brownish grey	++
6.	Oat meal agar	71.53	White	Irregular	Fluffy mycelia	White	+
7.	Potato dextrose agar	87.92	Greyish white	Regular	Aerial mycelia	Creamish white	++++
8.	Richard's agar	86.67	White	Irregular	Aerial mycelia	Creamish White	++
9.	Rose bengal agar	31.93	Pinkish grey white	Regular	Fluffy mycelia	Greyish white	+
10.	Sabouraud's agar	62.87	White	Irregular	Fluffy mycelia	White	+++
11.	Soyabean casein digest agar	77.08	White	Regular	Aerial mycelia	White	+
12.	V8 juice agar	43.23	White	Irregular	Fluffy mycelia	White	+
	S. Em ±	0.25					
	CD (P=0.01)	0.99					

- No sporulation; + Poor (1-25 spores/microscopic field 400 X); ++ Moderate (25-50); +++ Good (50-75); ++++ Excellent (>75)

Table 2: Morphological characteristics of *Fusarium oxysporum* on different solid media

Sl. No.	Media	Colony diameter (mm)	Colony character			Pigment production	Sporulation
			Colony color	Colony margin	Topography		
1.	Asthana and Hawkers medium	86.33	White	Regular	Aerial mycelia	White	++
2.	Corn meal agar	55.03	White	Irregular	Sparse mycelia	White	+
3.	Czapek's dox agar	70.67	White	Regular	Sparse mycelia	White	+
4.	Glucose asparagine agar	79.87	White	Regular	Aerial mycelia	White	+++
5.	Malt extract agar	82.71	Greyish white	Regular	Submerged mycelia	Creamish white	++++
6.	Oat meal agar	73.07	White	Irregular	Fluffy mycelia	Yellowish white	++
7.	Potato dextrose agar	88.00	Greyish white	Regular	Aerial mycelia with sectoring	Light pinkish white	++++
8.	Richard's agar	86.10	White	Regular	Aerial mycelia	Brownish white	+++
9.	Rose bengal agar	35.08	White	Regular	Fluffy mycelia	White	+
10.	Sabouraud's agar	52.37	Creamy white	Regular	Aerial mycelia	Yellowish white	+
11.	Soyabean casein digest agar	75.11	Brownish grey white	Regular	Flat mycelia with sectoring	Purplish white	++++
12.	V8 juice agar	64.84	White	Irregular	Aerial mycelia	White	+++
	S. Em ±	0.25					
	CD (P=0.01)	0.97					

- No sporulation; + Poor (1-25 spores/microscopic field 400 X); ++ Moderate (25-50); +++ Good (50-75); ++++ Excellent (>75)

On liquid media

Different liquid media have shown significant differences in the growth of *C. gloeosporioides*. The growth of pathogens was a maximum on the 12th day of incubation on Potato dextrose broth (PDB) (335.03 mg) followed by malt extract broth (293.87 mg), and Asthana and Hawkers broth (243.87 mg). The least dry mycelium weight was observed in rose bengal broth (107.80 mg) (Table 3). The observations are in accordance with the results of Kammar (2019) [15] with the highest dry mycelial weight of *C. lagenarium* (316.33 mg) in PDB which was isolated from anthracnose of bottle gourd.

The *F. oxysporum* was also tested for different liquid media for growth and which is presented in Table 3. It indicates that the growth was maximum at the 12th day of incubation on PDB (345.70 mg) followed by Asthana and Hawkers broth (292.27 mg) and malt extract broth (203.00 mg). The dry mycelial weight of the pathogens was the least in rose bengal broth (43.00 mg). Similarly, Nandhini *et al*. (2021) [21] recorded 330 mg of the dry mycelial weight of *F. oxysporum* (bitter gourd wilt) in PDB.

Growth phase

This study helps to know the progress of pathogen growth on liquid media over time. The growth of *C. gloeosporioides* has shown a significant difference among the different days of mycelial harvest. The maximum mycelial growth was observed on the 12th day after incubation (DAI) (337.23 mg), followed by the 10th day (316.03 mg). The least dry mycelial weight of 112.27 mg was observed after two days of incubation (Table 4). Similarly, Dev *et al*. (2017) [8] also got the higher dry mycelial weight of *C. gloeosporioides* (451.9 mg) on the 12th day of inoculation on PDB.

In the case of *F. oxysporum*, the growth of the pathogen was maximum on the 12th DAI (311.52 mg), followed by the 10th day (286.13 mg) as depicted in Table 4. The least dry mycelial weight of 93.04 mg was observed after two days of incubation. Similarly, Srivastava *et al*. (2011) [32] got higher dry mycelial weight on the 12th day of inoculation (350.00 mg). This study indicated that reduction in the dry mycelial weight of the fungus with the increase in the incubation period. This may be due to the accumulation of toxins and exhaustion of nutrients in the medium leading to autolysis of the mycelium, after incubation for an optimum number of days (Sayipratap *et al*., 2018) [25].

The mycelial growth of the pathogens increased with an increase in the number of days of incubation and reached its highest on the 12th day and subsequently decreased drastically. Therefore, the 12 DAI is considered the maximum growth period for both pathogens.

Table 3: Effect of liquid media on growth of *Colletotrichum gloeosporioides* and *Fusarium oxysporum*

Sl. No.	Media	Mean mycelial dry weight (mg)	
		C. gloeosporioides	***F. oxysporum***
1.	Asthana and Hawkers broth	243.87	292.27
2.	Corn meal broth	170.13	115.93
3.	Czapek's dox broth	110.70	177.17
4.	Glucose asparagine broth	108.83	33.07
5.	Malt extract broth	293.87	203.00
6.	Oat meal broth	177.97	84.30
7.	Potato dextrose broth	335.03	345.70
8.	Richard's broth	191.93	160.63
9.	Rose bengal broth	107.80	43.00
10.	Sabouraud's broth	195.00	189.83
11.	Soyabean casein digest broth	191.83	121.53
12.	V8 juice broth	182.70	91.90
	S.Em ±	0.22	0.23
	CD (P=0.01)	0.87	0.89

Table 4: Growth phase of *Colletotrichum gloeosporioides* and *Fusarium oxysporum* on Potato dextrose broth

Sl. No.	Days of incubation	Mean mycelial dry weight (mg)	
		C. gloeosporioides	***F. oxysporum***
1.	02	112.27	93.04
2.	04	159.27	131.93
3.	06	212.73	156.23
4.	08	295.33	219.30
5.	10	316.03	286.13
6.	12	337.23	311.52
7.	14	263.93	276.23
8.	16	257.27	151.40
9.	18	243.20	144.73
10.	20	171.03	136.13
	S. Em ±	0.29	0.31
	CD (P=0.01)	1.16	1.24

Physiological studies

Effect of temperature

Temperature is also an important physical environmental factor for regulating fungal growth and development. This study helps to know the optimal temperature for the growth of *C. gloeosporioides* and *F. oxysporum*. Table 5 tells that *C. gloeosporioides* showed the highest mycelial growth (241.87 mg) at 25 ˚C. The next best temperature was 30 ˚C (203.23 mg). Significantly least mycelial growth was observed at 40 ˚C (102.07 mg). The results are in agreement with the findings of Kommula *et al.* (2017) [16] got the maximum mycelial growth of *C. capsici* (72.4 mm) at 25 ˚C.

F. oxysporum grows well at 25 ˚C (284.23 mg) and it was found higher than all other tested temperatures. The next best temperature was 30 ˚C (176.07 mg). The temperature at 40 ˚C (105.30 mg) recorded significantly least mycelial growth (Table 5). Similar results were obtained by Sharma *et al.* (2011) [26]; Mohsen *et al*. (2016) [20]; and Chaithra *et al.* (2020) [3] obtained higher dry mycelial weight of *F. oxysporum* at 25 ˚C.

Thus, a temperature range of 25 to 30 ˚C was supportive for the growth of the *C. gloeosporioides* and *F. oxysporum.*

Effect of hydrogen ion concentration (pH)

This experiment was conducted to know the favorable pH for the growth of the

C. gloeosporioides and *F. oxysporum*. The results in Table 6describe that among the different pH tested, maximum dry mycelial growth of *C. gloeosporioides* and *F. oxysporum* were observed at the pH of 6 (281.95 and 212.75 mg, respectively) followed by 7 (208.00 and 194.33 mg, respectively). The pathogen showed the least mycelial weight at pH 9 (176.07 and 134.73 mg, respectively). Similarly, the results of Sharma and Kulshreshta (2015) [27]; Chaudhari *et al*. (2017) [4] and Dev and Somasekhara (2018) [7] reported maximum growth of *C. gloeosporioides* at pH 6.0. Maitlo *et al*. (2017) observed the higher dry mycelial weight of *F. oxysporum* f. sp. *ciceris* at pH 6 (1000 mg) followed by 7 (950 mg). Groenewald *et al*. (2006) [12]; and Kumari *et al*. (2021) [17] also obtained the same results.

Effect of light on the growth and sporulation

In the fungal kingdom, light is used for growth regulation, the direction of growth (phototropism), asexual and sexual reproduction and pigment production (Idnurm and Heitman 2005) [14]. This experiment helps to know the effect of light and darkness on the growth and sporulation of *C. gloeosporioides* and *F. oxysporum*. After the 12th day of incubation, the *C. gloeosporioides* and *F. oxysporum* had shown maximum growth of the fungus was recorded in alternate cycles of 12 hours of light and 12 hours of darkness (88.93 and 89.83 mm, respectively) with excellent sporulation followed by continuous light (68.67 and 83.00 mm, respectively) and continuous dark (57.87 and 72.63 mm, respectively) (Table 7). This agrees with the findings of Dev and Somasekhara, (2018) [7] recorded maximum radial growth of *C. gloeosporioides* 85.60 mm for 12 hrs light and 12 hrs dark compared to the continuous light (61.3 mm) and continuous dark (54.3 mm) exposure. This might be due to the induction of certain metabolic processes necessary for the growth and sporulation of pathogens, which usually do not occur in continuous light. Sharma *et al*. (2005) [28] observed excellent growth and sporulation of *F. oxysporum* f. sp. *lini* at alternate cycles of 12 hrs of light and 12 hrs of darkness (89.23 mm).

Table 5: Effect of temperature on growth of *Colletotrichum gloeosporioides* and *Fusarium oxysporum*

Sl. No.	Temperature (˚C)	Mean mycelial dry weight (mg)	
		C. gloeosporioides	***F. oxysporum***
1.	15	113.90	118.24
2.	20	146.43	140.87
3.	25	241.87	284.23
4.	30	203.23	176.07
5.	35	114.77	139.23
6.	40	102.07	105.30
	S. Em ±	0.29	0.25
	CD (P=0.01)	1.27	1.07

Table 6: Effect of different pH on growth of *Colletotrichum gloeosporioides* and *Fusarium oxysporum*

Sl. No.	pH levels	Mean mycelial dry weight (mg)	
		C. gloeosporioides	***F. oxysporum***
1.	4	196.23	153.42
2.	5	201.60	172.43
3.	6	281.95	212.75
4.	7	208.00	194.33
5.	8	185.97	145.88
6.	9	176.07	134.73
	S. Em ±	0.36	0.41
	CD (P=0.01)	1.57	1.78

Table 7: Effect of light and darkness on the growth and sporulation of *Colletotrichum gloeosporioides* and *Fusarium oxysporum*

Treatment	Colletotrichum colony diameter (mm)				Sporulation on 12th day	Fusarium colony diameter (mm)				Sporulation at 12th day
	5th day	7th day	10th day	12th day		5th day	7th day	10th day	12th day	
Continuous light	32.67	42.00	56.53	68.67	++	56.17	67.83	79.93	83.00	++
Continuous dark	23.03	30.63	50.40	57.87	+++	53.00	63.17	66.13	72.63	++++
12 hours light and 12 hours dark	42.73	57.87	86.07	89.93	++++	58.33	73.94	83.80	89.83	++++
S.Em±	0.49	0.37	0.31	0.28		0.34	0.26	0.34	0.27	
CD (P=0.01)	2.56	1.91	1.63	1.47		1.80	1.36	1.79	1.42	

- No sporulation; + Poor (1-25 spores/microscopic field 400 X); ++ Moderate (25-50); +++ Good (50-75); ++++ Excellent (>75)

Conclusion

In the present study, *C. gloeosporioides* and *F. oxysporum* was isolated from the twister diseased plants. Twelve different media were used to observe the growth and sporulation. Potato dextrose media was found best for growth and sporulation. Maximum growth was found at 6.0 pH of media and temperature of 25 °C was found congenial for the pathogen growth. The present study will be helpful in the research being carried out by different workers studying this pathogen to understand its congenial condition for host-pathogen interaction.

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