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Physiological and Pathological Variability of *Botrytis cinerea* Causing Botrytis Grey Mould of Himachal Pradesh in India

Gurvinder Kaur[1*], Hoshiyar Singh Negi[2] and Pallavi Ghosh[1]

[1]*School of Agricultural Sciences, RIMT University, Sirhind-140 406, India*
[2]*Department of Plant Pathology, College of Agriculture*
Banda University of Agriculture and Technology, Banda-210 001, India
**E-mail: kaur.plantpatho@gmail.com*

***Abstract*:** Botrytis grey mould (BGM) is observed in the fields and one of the most important plant pathogen because of the detrimental ramification on the ornamental flowers and vegetables every year in Himachal Pradesh, India. The focus of this work is to study the physiological and pathological variability was studies in fifteen isolates of *Botrytis cinerea* causing botrytis grey mould of gladiolus collected from different agro climatic area of Himachal Pradesh. This research aimed to explore the effect of different factors which affects the growth of *Botrytis cinerea*. Moreover, to find out the pathological variability among the isolates. The optimum temperature for the best mycelial growth of *B. cinerea* were 20 ºC and thereafter it decreased gradually up to 30ºC. The production of sclerotia was affected by the distinct temperature regimes and was maximum at temperature 10°C and 15°C. Furthermore, also observed that the glucose (fructose) and nitrogen (asparagines) sources were to enhance the mycelial growth of *B. cinerea.* Maximum disease severity was shown by KBC-16 (58.78%) on Jester cultivar of gladiolus whereas, minimum severity was given by KBC-5 (34.47%). Under in vitro conditions, it is observed that on fifth day the flowers were completely deteriorated with the fungal growth.

***Keywords*:** Botrytis grey mould, *Botrytis cinerea* and Himachal Pradesh

Gladiolus *(Gladiolus grandiflorus* L.) belonging to the family Iridaceae and is most commercial cultivated cut flower in India (Bika et al 2020, Bi et al 2022). Botrytis grey mould (BGM) *Botrytis cinerea* is one of the major and destructive disease of gladiolus and possess a major constraint to production of flower, cormels and corms of gladiolus. In mostly, the approximately 30 recognized *Botrytis* species with various trophic lifestyles and is placed among the top ten most dominant fungal pathogens (Hurtado-Bautista et al 2021). It is also known as soft corm rot, core rot, grey mould and leaf spot (Schuster 2004, Tesfaye and Kapoor 2004, Sultana et al 2019).Botrytis grey mould is found worldwide and causes diseases in different flowers, vegetables crops and fruits (Boff 2001, Hosen 2011, Carisse 2016, Wang et al 2022). Variability in fungi is noticed in morphology (Chardonnet et al 2000) and pathogenicity (Aboelghar et al 2019). The spores of the *B. cinerea* is found highly in numbers and comes out from the infected tissues (Jarvis 1962, Dewey and Grant- Downton 2016). *B. cinerea* mainly enters the host via wounds or natural openings (Holz et al 2007). It can actively promote susceptibility in the host by inducing different virulence factors (Choquer et al 2007, Nakajima and Akutsu 2014, Petrasch et al 2019). *B. cinerea* causes lysis of plant cell and facilitates penetration by loosens host wall (Blanco-Ulate et al 2016). The grey mould agents are one of the most studied models (Veloso and Van Kan 2018, Fekete et al 2011). *B. cinerea* is not specific and its virulence varies from host to host (Derckel et al 1999, Mirzaei et al 2009, Romanazzi and Feliziani 2014). *B. cinerea* rated as second most important fungal pathogen widely adopted as molecular model organism (Dean et al 2012). Strains of *B. cinerea* may start accumulate mutations in its genetic material that allow it to survive in different environment, resulting in damages to various crops worldwide (Harper et al 2022). In this study, our objective was to characterize the physiological and pathological variability in between fifteen *B. cinerea* isolates that were isolated from gladiolus on the radial colony growth on different temperature regimes and colony morphology, sporulation and sclerotia production of *B. cinerea* isolates grown on liquid growth media.

MATERIAL AND METHODS

Collection, isolation and preservation of *B. cinerea*: The fifteen isolates of *Botrytis cinerea* were isolated from the different major gladiolus growing districts of Himachal Pradesh namely Solan, Shimla, Sirmour, Mandi and Kullu. These *B. cinerea* isolates were isolated using single spore isolation technique and inoculated on potato dextrose agar (PDA) medium at 20± 0.5ºC (Mian 1995). Fifteen single spore isolates were produced from the diseased gladiolus

sample and designated as BC-1. B C-6, BC-8, KBC-10, KBC-1, KBC-14, KBC-13, KBC-16, KBC-5, KBC-15, KBC-9, BC-5, KBC-2, KBC-3 and KBC-11. The pure cultures of *B. cinerea* isolates were maintained on PDA slants and stored in 15% glycerol stocks at -20°C.

Physiological variability: Radial colony growth *B. cinerea* isolates were determined by inoculation of a 5 mm diameter portion of agar colonized with mycelium from 2-day-old cultures placed on center of a PDA plate and grown in the dark at room temperature (20°C). The inoculated Petri plates were incubated at different temperature ranging between 10, 15, 20, 25 and 30°C following completely randomized design (CRD) comprising of three replications. The observation on growth character, sporulation and other characters were recorded after 7 days of inoculation.

Effect of liquid nutrient media (carbon and nitrogen sources) on growth of isolates: Czapeck's Dox broth medium was used as basal medium for identifying the best carbon and nitrogen sources for growth characters, sporulation and sclerotial formation of different isolates of *Botrytis cinerea*. The carbon and nitrogen sources already present in the basal medium was substituted with galactose, dextrose, fructose, raffinose, ammonium nitrate, glutamine, potassium nitrate and asparagine with equal amount as per liter as obtained from sucrose and sodium nitrate. No carbon and nitrogen sources were added to control. Dry mycelia weight (mg), sporulation, and spore size were measured after 20 days of inoculation. The mycelial mats of isolates were filtered through pre-weighed Watman No.1 filter paper, and washed thoroughly with distilled water, and dried in a hot air oven at 60°C for 24 hour, cooled in a desiccator and weighed. The statistical analyses were performed to accomplish the best sources of carbon and nitrogen for the test pathogen. The sclerotial distribution of all the isolates were recorded on the 20th day after inoculation in a PDA medium, and classified based on the types of distribution suggested by Tanovic et al (2009), with some modifications (Table 1).

Pathological variability: The virulence of the *Botrytis cinerea* isolates were tested on the most demanding and commercial cultivars of gladiolus viz., Jester, Peter Pears, Rose Prosperity and White Prosperity were selected for artificial inoculation. Isolates considered for pathogenicity study were collected from different locations of gladiolus growing areas of different districts of Himachal Pradesh. The test on aggressiveness was conducted according to Abdel Wahab (2015). Healthy spikes of gladiolus used in the pathogenicity assessment were collected from three months old plants and inoculated uniformly with fungus spore suspension at the concentration 10^6 CFU/ml. Ten ml of the spore suspension was applied for each treatment using a hand atomizer. After inoculation, plants were covered with polyethylene cover for 48 h to provide high moisture, then polyethylene opened partially, after 72 h the polyethylene was removed. Plants were kept under normal conditions until the appearance of the symptoms. Disease severity was noticed up to five days from inoculation (Fig. 3). Uninoculated spikes of each cultivars served as control treatment. The disease appearance was also noticed up to five days to assess the virulence among the isolates. The per cent disease index (PDI) was assessed using the following rating scale and was calculated as per Mc Kinney (1923).

Table 1. Disease rating scale for calculating per cent disease incidence for Botrytis grey mould

Ratings	Infected spikes parts
0	No infection
1	< 1/4 area of flower infected
2	>¼ to ½ area of flower infected
3	>½ to ¾ area of flower infected
4	>3/4 area of flower infected

RESULTS AND DISCUSSION

Physiological variability: The radial mycelial growth at 10 °C was reduced as compared to 15 and 20°C and gradually decreased up to 30°C (Table 2). At 20°C highest colony diameter was shown by all the isolates except KBC-3 (82.0 mm) followed by KBC-2 (84.0 mm). The maximum sporulation given by all *B.cinerea* isolates at 25°C followed by 20 °C. The size of conidia was increased with increase in temperature (Table 4). The maximum spore size was observed in KBC-16 (9.00 x 6.30 µm) followed by BC-1 (9.10 x 4.48 µm) at 25°C. At 20°C maximum spore size was recorded again in KBC-16 (8.79 x 5.66 µm) followed by KBC-14 and BC-1. The effects of five temperature regimes were observed same on colony colour and texture in all the different *Botrytis* isolates except KBC-9 which showed dark grey color and cottony texture at 10, 15 and 20°C, while the same isolate observed off white colour and fluffy texture at 25°C and 30°C (Table 3). The production of sclerotia was maximum at temperature 10°C and 15°C, respectively whereas minimum production of sclerotia occurred at 25°C temperature. No sclerotia were formed at 30°C in all the isolates tested (Table 4). Isolates of *B. cinerea* showed good colony growth at the wide range of temperature (15-25°C). The least growth was recorded at 10 and 25°C. The optimum temperature for the growth of *B. cinerea* isolates was 20°C (Pande et al 2010; Fernandez et al 2014, Sehajpal and Singh 2014). The distribution of sclerotia also varied among *B.*

cinerea isolates which included centrally placed large sclerotia, arranged in concentric rings, towards the periphery and sclerotia arranged irregularly (Kuzmanovska et al 2012, Sehajpal and Singh 2014, Kumari et al 2014, Mang et al 2020).

Effect of liquid nutrient media (carbon and nitrogen sources) on growth of isolates: The effect of carbon (galactose, dextrose, fructose and raffinose) and nitrogen (ammonium nitrate, glutamine, potassium nitrate and asparagine) sources on mycelial growth of *Botrytis cinerea* was studied under *in vitro* conditions (Table 5). Maximum dry weight (660 and 560 mg) was shown by KBC-10 with respect to fructose and raffinose (carbon sources) followed by KBC-9 (640 and 580 mg). Though maximum conidial size in KBC-16 was recorded in fructose (11.66 x 6.01 µm) followed by raffinose (11.33 x 5.99 µm) while minimum conidial size (4.99 x 2.02 µm) was obtained by BC-6 in raffinose. However, excellent sporulation was observed in fructose, raffinose and galactose by BC-1, KBC-10, KBC-16 and KBC-9 isolates collected from Jhiri (Kullu), Rajgarh (Sirmour), Sundernagar (Mandi) and Namhole (Bilaspur).

During nitrogen sources estimation maximum dry weight i.e. 610 mg (asparaine) and 540mg (potassium nitrate) was shown by BC-8. While no mycelial growth was observed in ammonium nitrate and glutamine by KBC-1 isolate. Maximum spore size (12.11 x5.66 µm) was observed in KBC-9 supplemented with asparagine followed by KBC-10 (10.45 x 4.99 µm) in same nitrogen source. However the excellent sporulation was found in all nitrogen sources by four isolates such as BC-1, KBC-10 and KBC-9. Hence the fructose and asparagines were reported to enhance the mycelial growth of *B. cinerea.* During the estimation of carbon and nitrogen sources, carbon (fructose and raffinose) in comparison to nitrogen (asparagine) sources was found to be best as it increased the dry mycelial weight (660 and 560 mg), conidial size (11.66 x 6.01 and 11.33 x 5.99 µm), excellent sporulation and sclerotia production in isolates BC-1, KBC-10, KBC-16 and KBC-9. However all the isolates showed more growth in carbon sources than nitrogen (Table 6). The maximal increment in dry weight (610 mg) was observed in BC-8, spore size (12.11 x 5.66 µm) in KBC-9, excellent sporulation and sclerotia production in medium supplemented with asparagine. Hence, the fructose and asparagine were reported to enhance the mycelial growth, conidial size, sporulation and sclerotia production of *B. cinerea.* The isolates of *B. cinerea* showed variation in mycelial growth on

Table 2. Effect of temperature on mycelial growth and spore size of different *Botrytis cinerea* isolates

Isolates	Mycelial growth (mm)						Conidia size (µm)*				
	Temperature (°C)						Temperature (°C)				
	10	15	20	25	30	Mean	10	15	20	25	30
BC-1	71.0	88.0	90.0	84.0	77.0	82.0	-	6.54 x 2.66	8.12 x 3.44	9.10 x 4.48	-
BC-6	70.0	88.0	89.0	83.0	76.0	81.0	-	5.44 x 2.22	6.77 x 3.33	7.80 x 4.46	-
BC-8	69.0	88.0	89.0	83.0	75.0	81.0	-	5.66 x 2.33	6.34 x 4.12	7.39 x 5.04	-
KBC-10	63.0	87.0	90.0	80.0	71.0	78.0	6.99 x 3.88	7.11 x 4.09	7.22 X 4.55	7.46 x 4.84	-
KBC-1	57.0	84.0	87.0	75.0	68.0	75.0	NS	NS	NS	NS	-
KBC-14	62.0	86.0	90.0	81.0	71.0	78.0	-	7.23 x 4.33	8.11 x 5.00	8.28 x 5.09	-
KBC-13	60.0	86.0	90.0	80.0	70.0	77.0	-	6.33 x 2.11	7.66 x 3.66	8.82 x 4.66	-
KBC-16	61.0	87.0	90.0	82.0	72.0	78.0	-	6.99 x 4.22	8.79 x 5.66	9.00 x 6.30	-
KBC-5	51.0	81.0	86.0	70.0	60.0	70.0	NS	NS	NS	NS	-
KBC-15	71.0	87.0	90.0	78.0	68.0	79.0	-	6.33 x 2.22	7.88 x 3.88	8.09 x 4.96	-
KBC-9	68.0	89.0	90.0	84.0	78.0	82.0	6.55 x 2.70	5.99 x 2.88	7.44 x 3.23	8.16 x 4.29	-
BC-5	59.0	86.0	90.0	78.0	65.0	76.0	-	5.22 x 2.01	6.33 x 3.55	7.30 x 4.50	-
KBC-2	18.0	56.0	84.0	48.0	20.0	46.0	NS	NS	NS	NS	-
KBC-3	14.0	45.0	82.0	23.0	13.0	37.0	NS	NS	NS	NS	-
KBC-11	59.0	86.0	90.0	77.0	63.0	75.0	-	5.11 x 2.09	6.22 x 3.34	7.44 x 4.15	-
Mean	57.0	82.0	90.0	74.0	63.0	-	-	-	-	-	-
CD (p=0.05)	Isolates = 0.07 Temperature = 0.04 Isolates x Temperature = 0.61										

***** NS= Non sporulating, Absent= -

Table 3. Effect of temperature on colony color and texture size of different *Botrytis cinerea* isolates

Isolates	Morphology characteristics and Texture									
	10		15		20		25		30	
	Color	Texture	Color	Texture	Color	Texture	Color	Texture	Color	Texture
BC-1	Off white	Velvety with radial growth	Off white	Velvety with radial growth	Off white	Velvety with radial growth	Off white	Velvety with radial growth	Off white	Velvety with radial growth
BC-6	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety
BC-8	White	velvety	White	Velvety	White	Velvety	White	Velvety	White	Velvety
KBC-10	Light grey	Fluffy	Light grey	Fluffy	Light grey	Fluffy	Light grey	Fluffy	Of white	Fluffy
KBC-1	Off white	Cottony	Off white	Cottony	Off white	Cottony	Off white	Cottony	Off white	Cottony
KBC-14	Ashy off white	Cottony	Ashy off white	Cottony	Ashy off white	Cottony	Ashy off white	Cottony	Off white	Cottony
KBC-13	Off white	Cottony	Off white	Cottony	Ashy off white	Cottony	Ashy off white	Cottony	Off white	Cottony
KBC-16	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety
KBC-5	Off white	Cottony	Off white	Cottony	Off white	Cottony	Off white	Cottony	Off white	Cottony
KBC-15	Dark grey	Fluffy	Fluffy	Fluffy	Dark grey	Fluffy	Dark grey	Fluffy	Dark grey	Fluffy
KBC-9	Dark grey	Cottony	Dark grey	Cottony	Dark grey	Fluffy	Off white	Fluffy	Off white	Fluffy
BC-5	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety
KBC-2	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety
KBC-3	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety	Off white	Velvety
KBC-11	Off white	Fluffy	Off white	Fluffy	Off white	Fluffy	Off white	Fluffy	Off white	Fluffy

Table 4. Sporulation and production of sclerotia of isolates of *Botrytis cinerea* at different temperatures

Isolates	Degree of Sporulation*					Production of Sclerotia**				
	Temperature (℃)									
	10	15	20	25	30	10	15	20	25	30
BC-1	+	+++++	++++	+++	-	++	++	+	-	-
BC-6	++	+++++	++++	+++	-	++	++	+	-	-
BC-8	++	+++++	++++	+++	-	++	++	+	+	-
KBC-10	++	+++++	++++	+++	+	++	++	+	-	-
KBC-1	NS	NS	NS	NS	NS	NP	NP	NP	NP	NP
KBC-14	++	+++++	++++	+++	+	++	++	+	-	-
KBC-13	++	+++++	++++	+++	-	++	++	+	-	-
KBC-16	++	+++++	++++	+++	+	++	++	+	+	-
KBC-5	NS	NS	NS	NS	NS	NP	NP	NP	NP	NP
KBC-15	++	+++++	+++	+++	+	++	++	+	+	-
KBC-9	++	++++	+++	+++	+	++	++	+	+	-
BC-5	++	++++	+++	++	+	++	++	+	-	-
KBC-2	NS	NS	NS	NS	NS	NP	NP	NP	NP	NP
KBC-3	NS	NS	NS	NS	NS	NP	NP	NP	NP	NP
KBC-11	+	+++	++	++	+	++	++	+	-	-

*+ + + += Excellent, + + += Very Good, + + = Good, + = Poor and NS= Non sporulating
**Sclerotia Non- producing = NP, Good= +, Excellent= ++, Absent = -

Table 5. Effect of different carbon sources *in vitro* on the growth and sporulation of *Botrytis* isolates

Isolates	Dry weight (mg)						Conidial size (µm)*					Sporulation*				
	Control	Galactose	Dextrose	Fructose	Raffinose	Mean	Control	Galactose	Dextrose	Fructose	Raffinose	Control	Galactose	Dextrose	Fructose	Raffinose
BC-1	80	250	430	460	330	310	8.22 x 4.33	10.56 x 4.87	11.22 x 4.99	9.88 x 4.77	9.22 x 4.66	+	+ + + +	+ + +	+ + + +	+ + + +
BC-6	110	450	420	390	540	380	-	5.22 x 2.09	4.99 x 2.01	5.77 x 2.11	4.66 x 2.02	-	+	+	+	+
BC-8	100	220	390	410	400	310	6.90 x 4.02	7.89 x 5.04	8.01 x 4.90	8.66 x 5.44	8.22 x 5.01	+	+ + +	+ + +	+ + +	+ + +
KBC-10	150	450	380	660	560	440	7.66 x 3.55	10.11 x 3.99	9.98 x 3.66	10.45 x 4.80	10.33 x 4.11	+ +	+ + + +	+ +	+ + + +	+ + + +
KBC-1	080	320	280	360	270	260	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-14	100	410	440	100	380	290	-	9.66 x 5.11	9.87 x 5.10	-	9.22 x 5.01	-	+	+ + +	-	+
KCB-13	120	390	290	300	240	270	7.45 x 2.99	8.99 x 4.78	8.22 x 4.22	8.77 x 4.55	8.01 x 3.99	+	+ +	+	+	+
KBC-16	200	490	460	540	510	440	9.11 x 5.99	11.89 x 6.33	11.22 x 5.99	11.66 x 6.01	11.33 x 5.99	+ +	+ + + +	+ + +	+ + +	+ + + +
KBC-5	120	470	380	510	400	380	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-15	110	420	400	460	330	350	6.22 x 3.55	7.11 x 3.99	7.22 x 4.00	7.88 x 4.11	6.99 x 3.22	-	+	+	+	+
KBC-9	170	500	480	640	580	470	10.01 x 4.22	11.01 x 5.01	10.88 x 4.22	11.22 x 5.22	11.09 x 5.04	+ +	+ + + +	+ + + +	+ + + +	+ + + +
BC-5	150	380	340	410	300	320	7.77 x 4.11	8.22 x 4.23	8.01 x 4.09	8.44 x 4.99	8.02 x 3.99	+	+ + +	+	+ + +	+ +
KBC-2	90	300	280	320	250	250	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-3	0	0	0	0	100	20	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-11	100	400	120	440	360	280	-	5.88 x 2.99	-	6.55 x 3.10	5.33 x2.01	-	+	-	+	+
Mean	110	360	340	370	400	-	-	-	-	-	-		-	-	-	-
CD (p=0.05)	Isolates = 0.007															
	Carbon sources = 0.004															
	Isolates x Carbon sources = 0.01															

** + + + += Excellent, + + += Very Good, + + = Good, + = Poor, NS= Non sporulating and Absent = -

Table 6. Effect of different nitrogen sources *in vitro* on growth and sporulation of *Botrytis* isolates

Isolates	Dry weight						Spore size(µm)*					Sporulation*				
	Control	Ammonium nitrate	Glutamine	Potassium nitrate	Asparagine	Mean	Control	Ammonium nitrate	Glutamine	Potassium nitrate	Asparagine	Control	Ammonium nitrate	Glutamine	Potassium nitrate	Asparagine
BC-1	140	400	420	490	570	400	8.99 x 3.22	11.22 x 4.77	11.66 x 4.90	11.99 x 5.02	12.10 x 5.99	+ +	+ + + +	+ + + +	+ + + +	+ + + +
BC-6	0	380	320	450	520	330	-	5.99 x 4.01	5.33 x 3.99	6.22 x 4.22	6.55 x 4.22	-	+	+	+	+ +
BC-8	120	480	500	540	610	450	6.55 x 3.99	7.99 x 4.98	8.22 x 5.01	8.55 x 5.11	8.77 x 5.44	+	+ + +	+ + +	+ + +	+ + +
KBC-10	90	460	510	370	540	390	7.88 x 3.66	10.12 x 3.99	10.22 x 4.33	9.99 x 3.55	10.45 x 4.99	+ +	+ + + +	+ + + +	+ + + +	+ + + +
KBC-1	70	000	0	320	140	110	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-14	100	400	370	430	480	360	5.88 x 3.55	7.88 x 4.98	8.66 x 5.01	8.77 x 5.77	8.99 x 5.99	+	+ +	+ +	+ +	+ +
KCB-13	100	370	350	480	500	360	5.99 x 2.55	7.11 x 3.01	7.01 x 3.02	7.44 x 3.22	7.99 X 3.99	+	+ +	+ +	+ +	+ +
KBC-16	120	420	290	370	300	300	6.88 x 3.88	10.88 x 5.99	9.66 x 4.22	10.11 x 4.99	9.99 x 4.88	+	+ +	+ +	+ +	+ +
KBC-5	0	450	270	380	490	320	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-15	100	420	320	390	480	340	-	7.11 X 3.99	6.99 X 3.22	7.22 X 4.11	7.88 X 4.22	-	+ +	+ +	+ +	+ +
KBC-9	110	460	41	500	520	400	9.88 x 4.33	11.11 x 5.01	10.99 x 4.89	11.33 x 5.22	12.11 x 5.66	+ +	+ + + +	+ + + +	+ + + +	+ + + +
BC-5	000	420	370	480	500	350	-	8.11 x 3.92	7.99 x 3.77	8.44 x 4.11	8.99 x 4.33	-	+ +	+ +	+ +	+ +
KBC-2	090	460	340	220	380	300	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-3	110	410	380	27	450	320	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KBC-11	120	280	490	380	340	320	-	6.55 x 3.01	7.88 x4.11	7.22 x 4.01	6.77 x 3.99	+	+ +	+ +	+ +	+ +
Mean	80	390	360	410	450											
CD (p=0.05)			Isolates=0.006													
			Nitrogen sources=0.003													
			Isolates x Nitrogen sources=0.013													

** + + + += Excellent, + + += Very Good, + + = Good, + = Poor, NS= Non sporulating and Absent = -

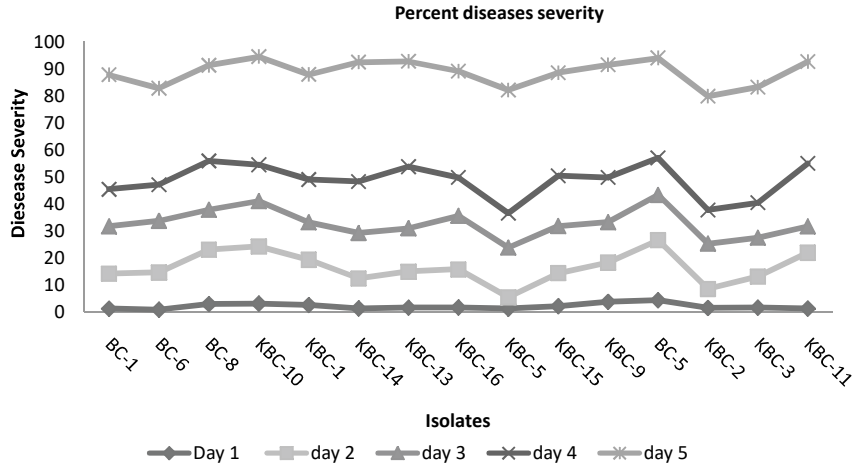

Fig. 1. Per cent disease severity of different commercial varieties of gladiolus against *Botrytis* isolates recorded for five days (*in vitro*)

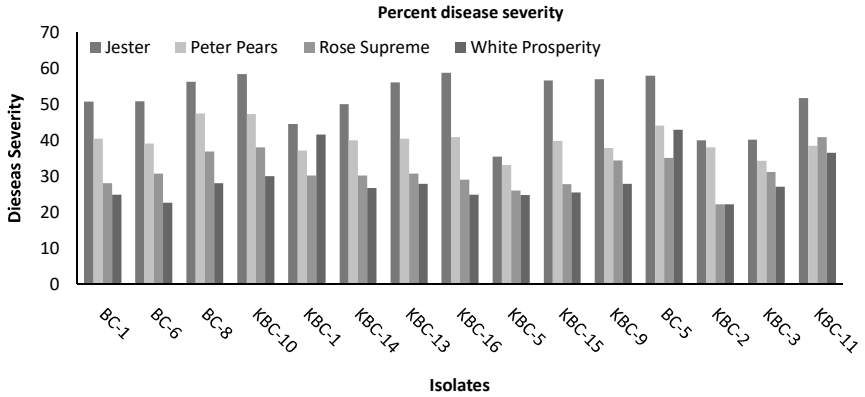

Fig. 1. Per cent disease severity of different commercial varieties of gladiolus against *Botrytis* isolates recorded for five days (*in vitro*)

liquid nutrient media supplemented with different carbon and nitrogen sources. Among different carbon sources the *B. cinerea* isolates showed maximum mycelial growth and sporulation in fructose at micromolar concentration than glucose (Doehlemann et al 2005, Waghmare et al 2011) concluded that the fructose and asparagine were the best carbon and nitrogen sources for the growth of pathogen. This result is supported with the findings of earliest researcher (Kaur et al 2016, Sultana et al 2017).

Pathological variability: The maximum disease severity (Fig. 1-2) developed on florets of gladiolus was shown by BC-5 (57.00%) and minimum severity was recorded by KBC-5 (36.58%). Most of the isolates didn't produced disease on cultivars Rose Supreme and White Prosperity even after 48 hours of inoculation followed by KBC-1, KBC-3, KBC-9, KBC-10 and KBC-11 showed disease severity on Rose Supreme and KBC-1 and BC-5 on White Prosperity. Maximum disease severity was shown by KBC-16 (58.78%) on Jester cultivar of gladiolus whereas, minimum severity was given by KBC-5 (34.47%). However in fifth day, the flowers were completely covered with the fungal growth and get rotted completely. All the isolates reported to be virulent on all the four cultivars studied. These results are in consonance with Riaz (2010) and Petrasch et al 2019. According to the latest taxonomical analysis, over 35 *Botrytis* species was found, out of which *Botrytis cinerea* is the most popular and accomplished (Richard 2021).

CONCLUSION

The *Botrytis* spp. belongs to the family *Sclerotiniaceae*, which contain fungal species all over the world, it causing huge damage to agricultural farms. The *B. cinerea* species is the renowned member of this genus, which shows a facultative secretive endophytic behaviour ('hide and seek'). Usually, when a pathogen, whether virulent or non- virulent, contains this level of plasticity in their genome that favors it to traverse

distinct niches and it changes to different epidemiological conditions, because of this its presence detected easily in various geographical and climatic regions of the India. However, numerous details about the variability of *B. cinerea* is still unknown. Here, we recapitulate, different carbon and nitrogen sources, high humidity and optimum temperature that favors the growth and infection strategies under *in vitro* conditions. Furthermore, the research on the variability of isolates will continue to be resourceful that should be scrutinize with substantial efforts to have improve and acceptable agricultural practices for the well- being of our planet.

REFERENCES

Abdel Wahab H 2015. Characterization of Egyptian *Botrytis cinerea* isolates from different host plants. *Advances in Microbiology* **5**: 177-189.

Aboelghar M, Moustafa MS, Ali AM and Abdel Wahab H 2019. Hyperspectral analysis of *Botrytis cinerea* infected lettuce. *EPH-International Journal of Agriculture and Environmental Research* **5**: 26-42.

Bi K, Liang Y, Mengiste T and Sharon A 2022. Killing softly: A roadmap of *Botrytis cinerea* pathogenicity. *Trends Plant Sciences* **28**: 211-222.

Bika R, Baysal-Gurel F, and Jennings C 2020. *Botrytis cinerea* management in ornamental production: A continuous battle. *Canadian Journal of Plant Pathology* **43**(3): 1-21.

Blanco Ulate B, Labavitch JM, Vincenti E, Powell ALT and Cantu D 2016. Hitting the wall: Plant cell walls during *Botrytis cinerea* infections In: *Botrytis – The fungus, the pathogen and its management in agricultural systems. In* Fillinger S. and Elad Y. eds., Cham: Springer international publishing. pp. 361-386.

Boff P 2001. *Epidemiology and biological control of grey moulds in Annual strawberry crops*. Wageningen, Wageningen University.

Carisse O 2016. *Epidemiology and aerobiology of Botrytis spp. In: Botrytis – The fungus, the pathogen and its management in Agricultural systems*. In Fillinger, S. and Elad, Y. eds, Cham: Springer Intentional Publishing. pp. 127-148.

Chardonnet CO, Sams CE, Trigiano RN and Conway WS 2000. Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. *Phytopathology.* **90**: 769-774.

Choquer M, Fournier E, Kunz C, Levis C and Pradier JM 2007. *Botrytis cinerea* virulence factors: New insights into a neurotropic and polyphagous pathogen. *FEMS Microbiology Letter* **277** (1): 1-10.

Dean R, Van KJ, Pretorius ZA, Hammond KKE and Pirtro AD 2012. The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Patholology* **13**: 414-430.

Derckel JP, Baillieul F, Manteau S, Audran JC and Haye B 1999. Differential induction of grapevine defenses by two strains of *Botrytis cinerea*. *Phytopathology* **89**: 197-203.

Dewey FM and Grant- Downton R 2016. *Botrytis- Biology, detection and quantification In: Botrytis – The fungus, the pathogen and its management in Agricultural systems*. In Fillinger, S. and Elad, Y. eds, Cham: Springer Intentional Publishing. pp. 17-34.

Doehlemann G, Molitor F and Hahn M 2005. Molecular and functional characterization of a fructose specific transporter from the gray mold fungus *Botrytis cinerea*. *Fungal Genetics and Biology* **42:** 601-610.

Egidi E, Delgado-Baquerizo M Plett JM, Wang J, Eldridge DJ, Bardgett RD, Maestre FT, Singh BK 2019. A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communication* **10:** 2369.

Fekete E, Irinyi L, Karaffa L, Árnyasi M and Asadollahi M 2011. Genetic diversity of a *Botrytis cinerea* cryptic species complex in Hungary. *Microbiology Research* **167**: 283-291.

Fernández JG, Fernández-Baldo MA, Sansone G, Calvente V and Benuzzi1 D 2014. Effect of temperature on the morphological characteristics of *Botrytis cinerea* and correlated with the genetic variability. *Journal of Coastal Life Medicine* **2**(7): 543-548.

Harper LA, Paton S, Hall B, McKay S, Oliver RP and Lopez-Ruiz FJ 2022. Fungicide resistance characterized across seven modes of action in *Botrytis cinerea* isolated from Australian vineyards. *Pest Management Science* **78:** 1326-1340.

Holz G, Coertze S and Williamson B 2007. The ecology of botrytis on plant surface In: *Botrytis: Biology, pathology and control,* edited by Iad Yigal, Williamson Brian, Tudzynski Paul and D Elen Nafiz, Dordrecht: Springer, Netherlands. pp. 9-27.

Hosen MI 2011. Cultural, physiological comparison and fungicidal sensitivity between two isolates of *Botrytis cinerea* and *Stemphylium botryosum. Emirates Journal of Food Agriculture* **23**(2): 120-129.

Hurtado-Bautista E, Pérez-Sánchez LF, Islas-Robles Á, Santoyo G, Olmedo-Álvarez G 2021. Phenotypic plasticity and evolution of thermal tolerance in two lineages of bacteria from temperate and hot environments. *Peer J*. 9. e11734.

Jarvis WR 1962. The infection of strawberry and raspberry fruits by *Botrytis cinerea*. *Annuals of Applied Biology* **50**(3): 569-575.

Kaur G and Chandel S 2016. First report of *Botrytis cinerea* causing grey mould of gladiolus in India. *Journal of Plant Pathology* **98**(1): 171-185.

Kumari S, Tayal P, Sharma E, Kapoor R 2014. Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. *Microbiology Research* **169**(11): 862-872.

Kuzmanovska B, Rusevski R, Jankuloski L, Jankulovska M, Ivic D, Bandzo 2012. Phenotypic and genetic characterization of *Botrytis cinerea* isolates from tomato. *Genetika* **44**(3): 633-647.

Mc Kinney HH 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum. Journal of Agriculture Research* **26**: 195-197.

Meng L, Mestdagh H, Ameye M, Audenaert K, Höfte M and Van Labeke MC 2020. Phenotypic Variation of *Botrytis cinerea* Isolates Is Influenced by Spectral Light Quality. *Frontiers in Plant Sciences* **11**: 1233.

Mian, I.H 1995. *Methods in Plant Pathology.* Institute of Post Graduate Studies in Agriculture, Salna, Gazipur, Bangladesh, pp: 51-53.

Mirzaei S, Goltapeh EM, Shamsbakhsh M, Safaie N and Chaichi M 2009. Genetic and phenotypic diversity among *Botrytis cinerea isolates* in Iran. *Journal of Phytopathology* **157:** 474-482.

Nakajimma M and Akutsu K 2014. Virulence factors of *Botrytis cinerea*. *Journal of General Plant Pathology* **80**(1): 15-23.

Pande S, Sharma M, Kishore GK, Shivram L and Mangala UN 2010. Characterization of *Botrytis cinerea* isolates from chickpea: DNA polymorphisms, cultural, morphological and virulence characteristics. *African Journal of Biotech* **9**(46): 7961-7967.

Petrasch S, Knapp SJ, van Kan JAL and Blanco-Ulate B 2019. Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen Botrytis cinerea. *Molecular Plant Pathology* **20** (6): 877-892.

Riaz T, Khan SN and Javaid A 2010. Management of Fusarium corm rot of gladiolus (*Gladiolus grandiflorus*) by using leaves of allelopathic plants. *African Journal of Biotechnology* **9** (30): 4681-4686.

Richards JK 2021. *Botrytis* spp.: A contemporary perspective and synthesis of recent scientific developments of a widespread genus that threatens global food security. *Phytopathology* **111**: 432-436.

Romanazzi G and Feliziani E 2014. *Botrytis Cinerea (Gray Mold)* Elsevier; Waltham, MA, USA: 2014.

Schuster J 2004. *Focus on plant disease. Grey mould, Botrytis*. http://www.urbanext.uiuc.edu/focus/graymold.html

Sehajpal PK and Singh PJ 2014. Effect of temperature, leaf wetness period, light and darkness on development of Botrytis Blight (*Botrytis gladiolorum* Timm.) of gladiolus (*Gladiolus grandiflorus* L.) *International Journal of Applied Research* 211-218.

Sultana N, Yeasmin FH, Islam MH, Robert L and Hossain MD 2019. Botyrtis blight of gladiolus in Mymensingh and its management. *Bangladesh Journal of Plant Pathology* **33**(1-2): 65-70.

Tanovic B, Delibasicg Milivojevic J and Nikolic M 2009. Characterization of *Botrytis cinerea* isolates from small fruits and grapevine in Serbia. *Archives of Biological Sciences*, Serbia **61**(3): 419-429.

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Tesfaye A and Kapoor IJ 2004. *In vitro* evaluation of *Trichoderma* and *Gliocladium* spp. against Botrytis corm rot (*Botrytis gladiolorum*) of gladiolus. *Pest Management Journal of Ethiopia* **8:** 97-103.

Veloso J and Van Kan JAL 2018. Many shades of grey in Botrytis-host plant interactions. *Trends in Plant Sciences* **23**(7): 613-622.

Wahmare MB, Wahmare RM and Kamble SS 2011. Bio efficiency of plant extracts on growth of *Botrytis cinerea* causing leaf blight of rose. *The Bioscan* **6**(4): 643-645.

Wang R, Liu K, Chen B, Ding W and Li Y 2022. Genetic and pathogenic variation of *Botrytis cinerea*, the causal agent of grey mould on Panax ginseng in China. *Canadian Journal of Plant Pathology* **44**: 744-753.