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Effect of Temperature on Growth, Sporulation and Sclerotial Formation of the Fungus *Botrytis Gladiolorum* Timm. in Different Culture Media and Standardization of Inoculum Load of the Fungus for Generation of Disease

P.K. Sehajpal and P.J. Singh
Department of Floriculture and Landscaping,
Punjab Agricultural University, Ludhiana – 141004, India

ABSTRACT

Gladiolus has been cultivated extensively the world over as a popular cut flower. Botrytis blight caused by Botrytis gladiolorum Timm. is the most potential disease of gladiolus. Colony morphology and growth of the fungus B. gladiolorum is known to be influenced by nutrient availability and the temperature conditions. Therefore, the effect of temperature on mycelial growth, sporulation and sclerotial production of B. gladiolorum was investigated in seven different culture media. The maximum radial growth was found in peptone agar, however, profuse growth was observed in gladiolus tepal decoction dextrose agar. The best temperature for mycelial growth was found to be 20 ±1 ^{0}C followed by 15, 25, 10 and 30±1 ^{0}C, respectively. An excellent degree of conidial and sclerotial production took place on gladiolus corm decoction dextrose agar and potato dextrose agar media. Conidial and sclerotial formation occurred at temperatures of 15, 20 and 25±1 ^{0}C only. The severity of botrytis blight is known to be influenced by inoculum load of the fungus. Therefore, inoculum concentration for optimum development of the disease was standardized for leaf and floral tissues. The optimum spore concentration for development of disease on the leaf tissue was found to be 4x10^4 conidia/ml of water, whereas 1x10^4 conidia/ml of water were adequate for infection of the floral tissue, as recorded after 8 days of inoculation.

Key Words: *Botrytis gladiolorum*, culture media, temperature, inoculum load

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is an ornamental bulbous plant that belongs to the family *Iridadceae*. It has long magnificent flower spikes of attractive colours. In India, it is cultivated on an area of around 6000 ha (Sharma *et al* 2012). The area under this crop in Punjab state is 180.6 ha (Singh *et al* 2014). The major gladiolus growing districts are Jalandhar, Patiala and Ludhiana. Since gladiolus is a very popular cut flower and is extensively used in bouquet making and decorations, there is lot of scope for increasing the area under this crop.

Gladiolus is attacked by number of diseases caused by fungi, bacteria and viruses, among which Botrytis blight caused by *Botrytis gladiolorum* Timm. causes significant economic losses. The pathogen can attack at all the stages of crop growth. The nutritional requirement of pathogens differs from one another and there is no single medium which can be universally suitable for growth of all the pathogens. The selection of an appropriate medium is necessary for mass production of inoculum and other epidemiological studies. The temperature also has profound effect on the biological processes. Therefore, the effect of temperature on mycelial growth, sporulation and sclerotial production of the fungus *B. gladiolorum* was studied in different culture media.

The number of pathogen propagules is considered to be important for successful creation of any disease. The conidia are the main source of infection in gladiolus (Magie

1958). Therefore, studies on determination of optimum spore (conidial) load of the fungus were undertaken using different levels of inoculum. Since, the floral tissue is more prone to infection by *B. gladiolorum* than the foliar tissue (Singh *et al* 2008), spore concentration of the fungus was standardized separately for the foliar and floral tissues of gladiolus.

MATERIALS AND METHODS

Effect of temperature on the fungus in different culture media

Seven culture media, namely potato dextrose agar, gladiolus leaf decoction dextrose agar, gladiolus corm decoction dextrose agar, gladiolus tepal decoction dextrose agar, peptone agar, malt extract agar and botrytis selective medium were prepared for the studying the effect of media on growth and sporulation of the fungus. Potato dextrose agar, malt extract agar and botrytis selective medium were prepared as given by Ainsworth, (1963), Galloway and Burgess (1952) and Edwards and Seddon, (2001) respectively. The composition of the other media was as follows:- Gladiolus leaf decoction dextrose agar medium: gladiolus leaves- 200 g, dextrose- 20 g, agar- 20 g, water- 1 litre to make; Gladiolus corm decoction dextrose agar: gladiolus corms- 200 g, dextrose- 20 g, agar- 20 g; water- 1 litre to make; Gladiolus tepal decoction dextrose agar medium: gladiolus tepals- 200 g, dextrose- 20 g and agar- 20 g, water- 1 litre to make; Peptone agar: peptone- 10 g, sodium chloride- 5 g, agar- 14 g, water- 1 litre to make.

All the media were sterilized at 15 psi (121^0C) for 15 minutes. The pre-sterilized media were poured in Petri plates and inoculated in the middle with 0.5 mm discs cut out with a cork borer from 10-days old culture of the fungus *B. gladiolorum* grown on PDA medium. The number of replications was four in each medium. The inoculated Petri plates were incubated at temperature of 10, 15, 20, 25 and 30 ± 1^0C in B.O.D. incubators for 20 days and observed for growth, sporulation and sclerotial production. The growth was measured in terms of colony diameter (cm) after 2, 4, 6, 8 and 10 days of incubation. The extent of sporulation (conidial/sclerotial formation) was recorded at 2 days intervals up to 20 days, using the following indices: – = No, + = Poor; ++ = Fair; +++ = Good; and ++++ =Excellent sporulation.

Standardization of inoculum load for development of Botrytis blight

Corms of gladiolus variety 'Sancerre' were planted in the field at 30 x 20 cm spacing and recommended package of practices followed for crop cultivation (Kumar and Sidhu, 2011). The culture of the fungus was raised on gladiolus tepal decoction dextrose agar which turned out to be the best medium. The spore suspension was prepared from 14 days old culture of the fungus. The concentration of spores was determined using Neubauer Hemacytometer and standardized at $1x10^4$, $2x10^4$, $3x10^4$, $4x10^4$, $5x10^4$, $6x10^4$, $7x10^4$, $8x10^4$, $9x10^4$ and $10x10^4$ conidia/ml of water. The concentrations thus prepared were used for artificial inoculation of apparently healthy leaves and flower spikes which were brought from field to the laboratory in the early morning hours. There were four replications in each treatment, with twelve leaves/spikes in each of the replicates. The observations on per cent severity of the disease were recorded after 2, 4, 6 and 8 days of inoculation on a 0-4 rating scale as mentioned by Sehajpal and Singh (2014).

The data were analyzed by using Statistical Package for Social Science ver.16 (SPSS 16). The factorial two way analysis of variance (ANOVA) was performed at one and five percent levels of significance.

RESULTS AND DISCUSSION

Effect of temperature on the fungus in different culture media

Different culture media had profound influence on cultural and morphological characters of the fungus *B. gladiolorum* (Table 1). The highest colony diameter (8.50 cm) was observed on peptone agar and gladiolus tepal decoction dextrose agar media, followed by gladiolus leaf decoction dextrose

agar (6.45 cm), potato dextrose agar (6.30 cm), gladiolus corm decoction dextrose agar (5.87 cm), malt extract (4.14 cm) and Botrytis selective medium (4.07cm), after 6 days of incubation, at 20±1^{0}C. A similar trend of colony growth was observed at all other temperatures. Temperature of 20±1^{0}C was found to be the best for mycelial growth of the fungus, followed by 15, 25, 10 and 30±1^{0}C. Although, the radial growth of the fungus was maximum in peptone agar but the colony growth was sparse and no conidial and sclerotial formation occurred in the medium. It may be inferred that gladiolus tepal decoction dextrose agar was the best medium for colony growth of the fungus.

An excellent degree (++++) of conidial and sclerotial production took place on gladiolus corm decoction dextrose agar and potato dextrose agar media, which were ranked as the best media for development of conidia and sclerotia. Gladiolus tepal decoction dextrose agar and gladiolus leaf decoction dextrose agar supported good conidial and sclerotial production (+++). Malt extract agar medium showed fair (++) degree of conidial and sclerotial formation, whereas nil (-) production of conidia and sclerotia was recorded in peptone agar and botrytis selective medium. The conidial and sclerotial formation occurred at temperatures of 15, 20 and 25±1^{0}C only. No conidial and sclerotial production was recorded at 10 and 30±1^{0}C.

The results of the present study are in agreement with the findings of other workers who found that temperature of 20±1^{0}C was optimum for growth of *B. cinerea* in some other hosts also (Ahmed *et al* 2007, Hosen 2010 and Hosen 2011). The best growth and conidial and sclerotial formation of the fungus were recorded in media comprising host tissue, which may be attributed to the correct proportion of nutrients present in the host tissue. The floral tissue of gladiolus has been reported to be more prone to infection than the leaves (Singh *et al* 2008), which may be the reason why there was more colony growth, spore formation and sclerotial production in the medium to which floral tissue was added.

Potato dextrose agar medium supported good colony growth and excellent sporulation of the fungus *B. gladiolorum*. It has been reported to be the best medium for *B. cinerea and B. alii* by several workers (Bryk 1985, Choi *et al* 1990, Tian and Bertolini 1995, Martínez *et al* 2009).

Malt extract agar medium was not suitable for growth of *B. gladiolorum.* The conidial and sclerotial formation was found to be ‘fair ’in malt extract agar medium while no conidial and sclerotial production was recorded in peptone agar medium. It was observed by Stewart (1986) that malt agar medium showed significant difference in sporulation and sclerotial formation than that of peptone medium. Botrytis selective medium was found to have least mycelial growth and no conidial and sclerotial production was recorded in this medium

Effect of inoculum load for development of Botrytis blight of gladiolus

The data show that when the concentration of spores was increased from 1 x10^4 to 10 x10^4, by an increment of 10,000 spores each time, the disease severity showed a progressive increase with each higher level of concentration (Table 2, 3). The disease severity on the *foliar tissue* was observed to be 5.21, 7.28, 13.54, 21.87, 23.96, 25.00, 27.08, 29.16, 31.24 and 33.32 per cent, respectively, at these concentrations, after 2 days of incubation. The severity of the disease increased on prolonged incubation in all the spore concentrations, and it was not practically feasible to record disease severity after day 8 in any of the concentrations due to setting in of senescence or drying of leaves. It was observed that 50 per cent disease severity was observed at an inoculum load of 5x10^4 after 4 days of inoculation.

There are several reports of effect of spore concentration on infectivity of *Botrytis* in various crops such as tulips, onion, broadbean, etc. The infection has generally been reported to high at higher spore concentrations by many workers (Segall and

Newhall 1960, Last and Hamley 1956, Price 1970, Mansfield and Hutson 1980, Stewart and Mansfield 1984).

It was observed that disease severity on the *floral tissue* was higher as compared to foliar tissue. Additionally, the disease developed faster on flower tissue than the leaf tissue, as is evident from the comparative data. Maximum disease severity (100 %) was recorded in the lowest spore concentration, i.e. 1x 10^4 conidia/ml of water after 8 days of inoculation. The obvious reason is that the flowers are more sensitive and easily prone to infection by *B. gladiolorum*. Several workers have also reported similar findings on higher susceptibility of floral tissue over foliar tissue in geranium (Sirjusingh *et al* 1996, Michael *et al* 1999). The results of the present study also show that there is a need to test still lower concentrations of spores for floral tissue.

CONCLUSIONS

It may be concluded that the 20 ±1^{0}C was the best temperature for the growth and sporulation of the fungus in all the culture media. The best medium for growth of the fungus was found to be gladiolus tepal decoction dextrose agar, whereas gladiolus corm decoction dextrose agar and potato dextrose agar medium proved to be the best for conidial and sclerotial production. Conidial and sclerotial production took place at temperatures of 15, 20 and 25±1^{0}C only. Studies on standardization of spore load show that the floral tissue was more prone to infection by the fungus than the leaf tissue,

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Table 1. Effect of different temperature levels on colony growth, sporulation and sclerotial formation of *Botrytis gladiolorum* in different culture media (Pooled analysis 2011-12, 2012-13)

Temperature (±1^{0}C)	Culture medium	Colony growth (cm) after different time periods (days)					Conidial production*	Sclerotial formation*
		2	4	6	8	10		
10	PDA	0.94	1.87	3.09	4.15	5.54	-	-
	GLDA	1.06	2.10	3.35	4.43	5.80	-	-
	GCDA	0.92	1.49	2.64	3.84	5.42	-	-
	GTDA	1.20	2.23	3.97	5.25	6.32	-	-
	PA	1.74	2.87	4.67	5.92	7.17	-	-
	MEA	0.61	1.30	2.17	3.65	4.77	-	-
	BSM	0.60	0.98	1.75	2.74	3.75	-	-
LSD (P < 0.05)	Time period (A) = 0.13, Culture media (B) = 0.14, A x B = 0.08							
15	PDA	1.09	2.23	3.65	5.78	7.12	++++	++++
	GLDA	1.16	2.29	4.02	5.94	7.42	+++	+++
	GCDA	1.02	2.07	3.41	5.54	6.66	++++	++++
	GTDA	1.21	2.37	4.33	6.76	8.50	+++	+++
	PA	1.63	4.18	6.58	8.38	8.50	-	-
	MEA	0.69	1.39	2.78	3.80	4.94	++	++
	BSM	0.64	1.27	2.21	3.17	4.44	-	-
LSD (P < 0.05)	Time period (A) = 0.27, Culture media (B) = 0.30, A x B = 0.17							
20	PDA	1.67	3.85	6.30	7.53	8.50	++++	++++
	GLDA	1.73	3.25	6.45	7.71	8.50	+++	+++
	GCDA	1.64	3.22	5.87	6.94	8.50	++++	++++
	GTDA	2.35	5.32	8.50	8.50	8.50	+++	+++
	PA	3.09	6.25	8.50	8.50	8.50	-	-
	MEA	1.31	2.70	4.14	5.40	6.96	++	++
	BSM	1.09	2.50	4.07	5.30	6.60	-	-
LSD (P < 0.05)	Time period (A) = 0.09, Culture media (B) = 0.10, A x B = 0.06							
25	PDA	0.99	2.17	3.51	5.35	6.82	++++	++++
	GLDA	1.10	2.25	3.97	5.68	7.35	+++	+++
	GCDA	0.99	2.07	3.37	5.25	6.36	++++	++++
	GTDA	1.24	2.34	4.26	6.41	8.50	+++	+++
	PA	1.64	3.92	6.05	7.70	8.50	-	-
	MEA	0.62	1.33	2.22	3.86	4.98	++	++
	BSM	0.62	1.09	2.01	3.19	4.41	-	-
LSD (P < 0.05)	Time period (A) = 0.08, Culture media (B) = 0.08, A x B = 0.05							
30	PDA	0.74	1.57	2.14	3.66	4.67	-	-
	GLDA	0.94	1.89	2.58	3.75	4.87	-	-
	GCDA	0.69	1.32	2.26	3.43	4.49	-	-
	GTDA	1.21	2.11	2.76	3.91	4.90	-	-
	PA	2.08	2.41	3.66	4.76	5.20	-	-
	MEA	0.60	1.00	1.95	2.96	3.91	-	-
	BSM	0.60	0.90	1.72	2.74	3.10	-	-
LSD (P < 0.05)	Time period (A) = 0.06, Culture media (B) = 0.07, A x B = 0.04							

*= As recorded after 20 days; Abbrs. used: PDA - Potato Dextrose Agar; GLDA - Gladiolus Leaf Decoction Dextrose Agar; GCDA - Gladiolus Corm Decoction Dextrose Agar; GTDA - Gladiolus Tepal Decoction Dextrose

Agar; PA- Peptone Agar; MEA - Malt Extract Agar; BSM - Botrytis Selective Medium Indices used: - - No, + - Poor, ++ - Fair, +++ - Good, ++++ - Excellent conidial/ sclerotial production

Table 2. Effect of inoculum load of *Botrytis gladiolorum* on severity of botrytis blight on leaf tissue of gladiolus cv. Sancerre (Pooled analysis 2011-12, 2012-13)

Sr. No.	Spore concentration (Conidia/ml water)	Disease Severity (%) (Days)			
		2	4	6	8
1.	$1x10^4$	5.21	27.08	52.08	78.12
2.	$2x10^4$	7.28	30.21	55.20	84.37
3.	$3x10^4$	13.54	37.50	60.41	92.70
4.	$4x10^4$	21.87	46.87	75.00	100.00
5.	$5x10^4$	23.96	50.00	75.00	100.00
6.	$6x10^4$	25.00	53.12	78.12	100.00
7.	$7x10^4$	27.08	57.28	80.20	100.00
8.	$8x10^4$	29.16	59.36	82.28	100.00
9.	$9x10^4$	31.24	63.53	84.36	100.00
10.	$10x10^4$	33.32	66.66	85.40	100.00
	LSD (P < 0.05)	Days (A) = 1.32 , Spore concentration (B) = 1.67, A x B = 0.82			

Table 3. Effect of inoculum load of *Botrytis gladiolorum* on severity of botrytis blight on floral tissue of gladiolus cv. Sancerre (Pooled analysis 2011-12, 2012-13)

Sr. No.	Spore concentration (Conidia/ml water)	Disease Severity (%) (Days)			
		2	4	6	8
1.	$1x10^4$	13.54	40.62	81.25	100.00
2.	$2x10^4$	19.79	46.87	89.58	100.00
3.	$3x10^4$	23.96	51.04	100.00	100.00
4.	$4x10^4$	27.08	55.21	100.00	100.00
5.	$5x10^4$	29.17	58.33	100.00	100.00
6.	$6x10^4$	33.33	61.46	100.00	100.00
7.	$7x10^4$	36.45	66.66	100.00	100.00
8.	$8x10^4$	38.54	70.83	100.00	100.00
9.	$9x10^4$	40.62	73.96	100.00	100.00
10.	$10x10^4$	42.71	76.04	100.00	100.00
	LSD (P < 0.05)	Days (A) = 1.75, Spore concentration(B) = 2.14 A x B = 1.04			

Plate1. Comparative growth of *Botrytis gladiolorum* at different temperatures after 6 days of incubation (For abbrs. refer to Table 1)