

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

EFFECT OF INCUBATION TEMPERATURES ON MYCELIAL GROWTH, CONIDIAL FEATURES AND DENSITY OF Stemphylium botryosum Walr ISOLATES

Subash S.*, Saraswati N.

National Maize Research program, Rampur, Chitwan

*Corresponding author's e-mail: subedi.subash1@gmail.com Received 5 May, 2016; Revised 7 June, 2016

ABSTRACT

The cultural and morphological variability of Stemphylium botryosum Walr was studied with five isolates viz. isolate 1 (Stb-11), isolate 2 (Stb-I2), isolate 3 (Stb-I3), isolate 4 (Stb-I4) and isolate 5 (Stb-I5) collected from Parsa, Rautahat, Dang, Banke and Chitwan districts of Nepal respectively during 2011-2012 crop season with Completely Randomized Design (CRD) maintaining 3 replications at Plant Pathology Laboratory of Grain Legumes Research Program (GLRP), Rampur, Chitwan. Single spore isolation technique was followed to isolate the pathogen. The pathogen was grow on Potato Dextrose Agar (PDA) and incubated with different temperatures in BOD incubator. The calibrated ocular micrometer was used to measure the length and breadth of the conidia while conidial count with sporulation intensity was attempted with hemocytometer. The mycelium color of the different isolates was varied from white, grey, brown and brownish black in peripheral part while mostly black pigmentation was noticed in lateral part of the culture plate. The shape and texture was regular and velvety for most isolates and in some isolate, roughly irregular shape with cottony texture was found. The higher colony diameter of 7.66 cm and conidial dimension of 29.42×18.12 µm (L/B ratio-1.62) with profuse sporulation intensity (46.67 conidia /0.01 ml) was observed at 25 °C after 15 days of incubation on PDA. However, 2.84 cm diameter, 19.58×9.81 µm (L/B ratio-2.00) with poor conidial density (7.60 conidia /0.01 ml) was noticed at 10 °C. The observation at 10 and 35 °C was at par in case of mycelial growth, conidial features and sporulation intensity. Mostly conidia of all isolates were brown in color at lower incubation temperature but they became dark grey brown at 25 and 30 °C and grey color was noticed when incubated at 35°C on PDA after 15 days of incubation.

Keywords: Conidial features, Density, Growth, Stemphylium botryosum, Temperature.

INTRODUCTION

The global lentil yield level, at present, is far below than potential yield. The reason behind the low productivity of most of the crops is due to the attack of many plant diseases at different stages of crop. Lentil plants are affected by wide range of pathogen with fungal diseases being the most important. Stemphylium blight that associated with *S. botryosum* is the most important disease of lentil in Nepal [1,2] and estimated yield losses of about 60-90% and sometime total crop collapsed have been reported [3]. In Bangladesh and India estimated yield losses of 62% and total crop failure have been reported in some cases where the disease defoliated the crop in the early pod setting stage [4]. In Nepal, the disease was first reported in 1993 and widespread throughout major growing areas [5] and later observed increasingly in Banke, Bardia, Rupandehi, Chitwan, Nepalgunj, Makwanpur, Bara, Parsa and Rautahat districts. This disease has been also reported in Bangladesh, Egypt, Syria and the USA (Bayaa and



Erskine, 1998). *S. botryosum* causes leaf blight on lentil that can result in large scale defoliation of plants. The initiation and development of plant disease is affected by environmental factors through their influence on host susceptibility, pathogen infectivity and the host-pathogen interaction [7,8]. Except few negligible researches focusing on disease management part through the application of fungicides and host resistance, research activities focusing on the detailed study of morphological variability of the *S. botryosum* was scanty. The present in *vitro* study was organized to know the effect of incubation temperatures on mycelial growth, conidial features and sporulation intensity of 5 different Nepalese isolates of *S. botryosum*.

MATERIALS AND METHODS

A series of Laboratory experiments were carried out to study on effect of incubation temperatures on mycelial growth, conidial features and sporulation intensity of five isolates of S. botryosum at Grain Legumes Research Program, Rampur, Chitwan, Nepal during March to May 2012. Five isolates were collected from the main lentil growing areas of the country during winter legume disease monitoring period of 2011-2012 crop season. Isolate 1 (Stb-I1), isolate 2 (Stb-I2), isolate 3 (Stb- I3), isolate 4 (Stb-I4) and isolate 5 (Stb-I5) were collected from Parsa, Rautahat, Dang, Banke and Chitwan districts of Nepal respectively. Single spore isolation technique was followed to isolate the pathogen S. botryosum. Lentil plants showing characteristics stemphylium blight symptoms were collected from 5 different districts Parsa, Rautahat, Dang, Banke and Chitwan and brought under laboratory conditions at GLRP, Rampur, Chitwan during 2012-2013. They were cut into small pieces of 1.0 to 2.0 cm, surface sterilized with 0.1% sodium hypochloride (Naocl) solution for one minute and subsequent washing in distilled water for 2 minute and blot dried with filter paper. The sterilized samples were placed in petriplates containing filter paper and incubated at $25\pm0.5^{\circ}$ C for about 48 hours and observed for the fungal growth under sterio-binocular microscope. Effort was made to pick up the conidium of the concerned pathogen only with the sterilized needle from the profuse fungal growth with the help of sterio-binocular microscope. Picked up spores were put in the sterilized vials containing 1 ml distilled water and gently shacked by hand for 4-5 minute to dislodge the spores. Then after spores were poured in to the petriplates containing 20 ml water agar medium and uniformly spread by sterilized glass rod and finally sealed with parafilm. All these procedures were conducted under laminar flow-hood chamber. Sealed agar plates were incubated at $25\pm0.5^{\circ}$ C for 24-36 hour and observed under compound microscope (10 or 40X) when the spore just started to germinate. The agar slice containing single spore with just initiation of mycelium development was picked up carefully by cutting the slice with the sterilized needle and put in to the sterilized test tubes and petriplates containing PDA₂₅ medium (25% strength i.e. PDA-6.75 gm, Agar-8.44 gm, Distilled water- 750 ml) and sealed with parafilm tape. The concept for using the PDA₂₅ medium was to grow the pathogen fast under deficit nutritive medium. The sealed plates and tubes containing pure culture were incubated at $25\pm0.5^{\circ}$ C for about 2 weeks and maintained in refrigerator for in vitro study. Both mycelial & conidial features of the S. botryosum was observed on the potato dextrose agar (PDA) medium after 15 days of incubation at $25\pm0.5^{\circ}$ C. The mycelium color, shape, texture and the conidial features of S. botryosum isolates were observed both under compound microscope and naked eye view. The calibrated ocular micrometer was used to measure the length and breadth of the conidia. 4mm diameter of S. botryosum of 1-week old culture was cut by sterilized cork borer, picked up with the help of inoculating needle and placed onto the center of the PDA plate as upside down for better contact



of pathogen to the media and incubated at 10, 15, 20, 25, 30 and 35^oC for 15 days under BOD incubator. Three replications were maintained for each incubation temperatures in completely randomized design. The colony diameter (cm) of the pathogen in both the experiments was determined by measuring the average radial growth on different incubation temperatures after 15 days of incubation period. Average radial growth was recorded by using a measuring scale from the lower view of the petri-plates. Mycelial growth inhibition percent was calculated by following formula.

Mycelial growth inhibition (%) = $[(dc-dt) / dc] \times 100$

where, dc = average diameter of fungal colony in the control

dt = average diameter of fungal colony in the treatment group

Conidial count with sporulation intensity was attempted with hemocytometer. Sporulation intensity was observed with the following rating scale i.e.0-none, 1- poor, 2- moderate, 3- abundant & 4- profuse.

RESULTS AND DISCUSSION

Mycelial growth

The colony diameter (cm) of the *S. botryosum* was significantly varied among different incubation temperatures (0 C) and isolates both. The higher colony diameter was recorded at 25 0 C for all isolates. The higher average colony diameters of 7.42 cm, 6.85 cm, 8.38 cm, 8.68 cm & 6.98 cm were recorded for isolates 1, 2, 3, 4 & 5 respectively when incubated at 25 0 C on PDA after 15 days of incubation (Table 1). The trend of increasing colony diameter with the increase in incubation temperatures were same for all 5 isolates up to 25 0 C while diameter was noticed gradually decreasing at 30 and 35 0 C respectively (Table 1).

Incubation	Colony diameter (cm) of S. botryosum								
temperatures (⁰ C)	Isolate 1 (Stb-I1)	Isolate 2 (Stb-I2)	Isolate 3 (Stb-I3)	Isolate 4 (Stb-I4)	Isolate 5 (Stb-I5)				
10	2.80 ^{d†}	2.15 ^e	3.12 ^e	3.95 ^e	2.22 ^d				
15	3.42 ^{cd}	2.79 ^d	4.45 ^c	5.02 ^d	2.55 ^d				
20	3.98 ^c	3.25 ^c	5.78 ^b	5.95 ^c	3.52 ^c				
25	7.42 ^a	6.85 ^a	8.38 ^a	8.68 ^a	6.98 ^a				
30	6.28 ^b	4.98 ^b	6.02 ^b	7.18 ^b	5.02 ^b				
35	3.02 ^d	2.25 ^e	3.95 ^d	2.98 ^f	2.05 ^d				
F test	**	**	**	**	**				
LSD (≤0.01)	0.75	0.29	0.45	0.62	0.64				
CV%	6.74	3.16	3.40	4.37	6.79				

Table 1: Effect of incubation temperatures on mycelial growth of 5 different isolates of *S. botryosum* on PDA after 15 days of incubation.



[†] Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). ⁰C-degree centigrade, cm- centimeter, **- highly significant

Conidial dimension

Incubation temperatures had highly significant effect on conidial length and breadth of all 5 isolates. The longest and broadest conidia were produced at 25^oC on PDA after 15 days of incubation. The largest conidial length of 29.18 μ m (27.85-30.25), 25.97 μ m (24.35-27.32), 33.02 μ m (31.45-34.35), 32.82 μ m (32.35-33.25) & 26.12 μ m (24.95-27.65) were recorded for isolates 1, 2, 3, 4 &5 respectively when incubated at 25^oC on PDA after 15 days of incubation (Table 2,3&4). Similarly, the largest conidial breadth of 22.48 μ m (20.45-24.65), 15.42 μ m (14.25-16.65), 18.55 μ m (18.35-18.75), 18.48 μ m (17.65-19.25) & 15.65 μ m (14.35-16.65) were found for isolates 1, 2, 3, 4 & 5 respectively when incubated at 25^oC on PDA after 15 days of incubation (Table 2,3&4).

Table 2: Effect of incubation temperatures on conidial features of S. botryosum Isolates 1 &2 (Stb-I1 &Stb-I2) on PDA after 15 days of incubation

Incubation	Conidial dimension of S. botryosum									
temperatures	Isola	te 1 (Stb-I1)		Isolate 2 (Stb-I2)						
(⁰ C)	*Conidial *Conidial L/B		*Conidial *Conidial		L/B					
	length (µm)	breadth (µm)	ratio	length (µm)	breadth (µm)	ratio				
10	21.30 ^{c†}	13.75 ^{cd}	1.55	16.78 ^d	7.32 ^d	2.29				
	(20.24-22.32)	(11.35-14.65)		(15.25-18.65)	(6.37-8.35)					
15	24.62 ^b	16.62 ^{bcd}	1.48	17.60 ^{cd}	10.34 ^c	1.70				
	(23.45-25.75)	(15.75-17.65)		(16.25-19.21)	(9.34-11.24)					
20	26.02 ^b	19.08 ^{ab}	1.36	21.02 ^{bc}	11.38 ^{bc}	1.85				
	(24.65-27.65)	(17.95-19.85)		(19.35-22.45)	(10.35-12.45)					
25	29.18 ^a	22.48 ^a	1.30	25.97 ^a	15.42 ^a	1.68				
	(27.85-30.25)	(20.45-24.65)		(24.35-27.32)	(14.25-16.65)					
30	24.35 ^b	17.35 ^{bc}	1.40	21.59 ^b	13.74 ^{ab}	1.57				
	(23.25-25.45)	(16.25-18.35)		(20.25-23.21)	(13.42-14.35)					
35	21.02 ^c	13.45 ^d	1.56	15.68 ^d	9.21 ^{cd}	1.70				
	(20.25-21.35)	(12.75-14.35)		(14.25-17.35)	(8.25-10.24)					
F test	**	**		**	**					
LSD (≤0.01)	2.85	3.61		3.89	2.43					
CV%	4.69	8.44		7.89	8.68					

[†] Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). ⁰C-degree centigrade, μ m- micrometer, [•] Mean number of 10 times observations for each replication, L/B ratio- Length/breadth ratio, **- highly significant, value in the parenthesis indicate the range value.

Both conidial length and breadth of all 5 isolates increased as the temperature increased up to 25° C while they were gradually decreased when temperature was increased from 25° C. The shorter length with narrow breadth conidia were noticed at 10 & 35° C temperature. The higher L/B ratio was noticed at 35° C



for isolate 1 (1.56), isolate 4 (2.41) & isolate 5 (2.46) while isolate 2 and 3 had higher L/B ratio of 2.29 & 1.88 respectively when incubated at 10 0 C on PDA after 15 days of incubation. Likewise, the lower L/B ratio was noticed at 25 0 C for isolate 1 (1.30) & isolate 5 (1.66) while isolate 2 and 4 had lower L/B ratio of 1.57 & 1.55 respectively when incubated at 30 0 C on PDA after 15 days of incubation. In case of isolate 3, lower L/B ratio of 1.57 was noticed at 20 0 C (Table 2, 3 &4).

Table 3: Effect of incubation temperatures on conidial features of *S. botryosum* Isolates 3 &4 (Stb-I3 &Stb-I4) on PDA after 15 days of incubation

Incubation	Conidial dimension of S. botryosum								
temperatures	Isola	ate 3 (Stb-I3)		Isolate 4 (Stb-I4)					
(⁰ C)	*Conidial	*Conidial	L/B	*Conidial	*Conidial	L/B			
	length (µm)	breadth (µm)	ratio	length (µm)	breadth (µm)	ratio			
10	20.95 ^d	11.15 ^d	1.88	22.35 ^{cd}	9.72 ^{cd}	2.30			
	(19.25-22.35)	(10.75-11.75)		(21.25-23.45)	(8.75-10.95)				
15	22.68 ^{cd}	13.28 ^c	1.71	23.62 ^{bcd}	11.78 ^{bc}	2.01			
	(21.45-24.24)	(12.65-13.75)		(22.75-24.65)	(11.15-12.45)				
20	25.88 ^{bc}	16.45 ^b	1.57	26.85 ^b	13.82 ^b	1.94			
	(24.25-27.65)	(15.25-17.64)		(25.75-28.35)	(12.75-14.75)				
25	33.02 ^a	18.55 ^a	1.78	32.82 ^a	18.48 ^a	1.77			
	(31.45-34.35)	(18.35-18.75)		(32.35-33.25)	(17.65-19.25)				
30	26.98 ^b	16.35 ^b	1.65	25.22 ^{bc}	16.25 ^a	1.55			
	(25.75-28.35)	(15.45-17.35)		(23.65-27.65)	(15.65-17.45)				
35	19.82 ^d	11.35 ^{cd}	1.75	20.62 ^d	8.55 ^d	2.41			
	(18.35-21.37)	(10.45-12.34)		(19.25-22.45)	(7.85-9.45)				
F test	**	**		**	**				
LSD (≤0.01)	3.75	2.01		3.44	2.30				
CV%	6.03	5.54		5.47	7.03				

[†] Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). 0 C-degree centigrade, μ m- micrometer, [•] Mean number of 10 times observations for each replication, L/B ratio- Length/breadth ratio, **- highly significant, value in the parenthesis indicate the range value.



Table 4: Effect of incubation temperatures on conidial features of S. botryosum Isolate 5 (Stb-I5) on PDAafter 15 days of incubation

Incubation	Conidial dimension of S. botryosum							
temperatures	Isolate 5 (Stb-I5)							
(⁰ C)	*Conidial length (μm)	*Conidial breadth (µm)	L/B ratio					
10	16.52 ^c (15.65-17.05)	7.12 ^c (6.45-7.55)	2.32					
15	17.75 ^c (16.65-19.25)	7.58 ^c (6.95-8.35)	2.26					
20	22.02 ^b (21.75-22.35)	10.85 ^b (9.85-11.75)	2.03					
25	26.12 ^a (24.95-27.65)	15.65 ^a (14.35-16.65)	1.66					
30	23.58 ^{ab} (22.75-24.25)	14.08ª (13.85-14.45)	1.67					
35	16.25 ^c (14.15-18.35)	6.58 ^c (5.95-7.25)	2.46					
F test	**	**						
LSD (≤0.01)	3.12	1.95						
CV%	6.14	7.59						

[†] Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). ⁰C- degree centigrade, µm- micrometer, [•] Mean number of 10 times observations for each replication, L/B ratio- Length/breadth ratio, **- highly significant, value in the parenthesis indicate the range value.

Conidial color

The conidial color of 5 different isolates varies from brown to dark grey brown and grey at different incubation temperatures. Mostly conidia of all isolates were brown in color at lower incubation temperature i.e. at 10 or 15^oC but they became dark grey brown at 25 and 30 ^oC on PDA after 15 days of incubation. Grey color was noticed for the conidia of most of the isolates when incubated at 35^oC on PDA after 15 days of incubation (Table 5).

Table 5: Effect of incubation temperatures on conidial color of 5 isolates of S. botryosum on PDA after15 days of incubation

Incubation	Conidial color of S. botryosum							
temperatures (⁰ C)	es Isolate 1 Isolate 2 Isolate 3 (Stb-I1) (Stb-I2) (Stb-I3)		Isolate 4 (Stb-I4)	Isolate 5 (Stb-I5)				
10	BG	В	G	BG	В			
15	BG	В	G	GB	G			
20	GB	BG	BG	G	В			
25	DGB	DGB	DGB	DBG	DGB			
30	DGB	DGB	DGB	DGB	DGB			
35	G	G	GB	G	GB			

Note: BG-Brownish Grey, B- Brown, G-Grey, DGB- Dark Grey Brown, DBG- Dark Brownish Grey, GB- Grey Brown, ⁰C-degree centigrade, Stb- Stemphylium blight



Conidial density

The conidial density (conidia/0.01 ml) of 5 different isolates of *S. botryosum* were significantly varied among different incubation temperatures. The higher number of conidia per 0.01 ml of 38.33, 28, 57.67, 61.33 & 48 at 25° C while lower number of conidia per 0.01 ml of 7, 3.67, 8, 11.33 and 8 for isolate 1, 2, 3, 4 & 5 respectively were noticed at 10° C on PDA after 15 days of incubation period (Table 6). The numbers of conidia were recorded at an increasing order up to 25° C while they were decreasing when temperature was increased after 25° C.

Table 6: Effect of incubation temperatures on conidial sporulation of 5 isolates of S. botryosum on PDAafter 15 days of incubation

Incubation	Conidial density of S. botryosum									
Temp (⁰ C)	Isolate 1		Isolate 2		Isolate 3		Isolate 4		Isolate 5	
	(Stb-I1)		(Stb-I2)		(Stb-I3)		(Stb-I4)		(Stb-I5)	
	Conidia/	SI	Conidia/	SI	Conidia/	SI	Conidia/	SI	Conidia/	SI
	0.01 ml		0.01 ml		0.01 ml		0.01 ml		0.01 ml	
10	7.00 ^{e†}	1.00	3.67 ^e	1.00	8.00 ^e	1.00	11.33 ^{de}	1.33	8.00 ^c	1.00
15	14.00 ^d	1.33	6.33 ^{de}	1.00	17.67 ^d	2.00	16.67 ^d	2.00	14.67 ^c	2.00
20	25.33 ^c	3.00	15.67 ^c	2.00	29.00 ^c	3.00	31.67 ^c	3.00	26.67 ^b	2.33
25	38.33 ^a	4.00	28.00 ^a	3.00	57.67 ^a	4.00	61.33 ^a	4.00	48.00 ^a	4.00
30	31.66 ^b	4.00	21.00 ^b	2.67	38.33 ^b	4.00	41.33 ^b	3.67	35.00 ^b	3.33
35	16.33 ^d	1.67	10.33 ^d	1.67	7.67 ^e	1.00	8.00 ^e	1.00	7.00 ^c	1.00
F test	**		**		**		**		**	
LSD (≤0.01)	5.51		4.11		6.55		6.73		9.03	
CV%	10.00		11.65		9.95		9.50		15.59	

[†]Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). ⁰C- degree centigrade, Temp- Temperatures, ml- milliliter, SI- Sporulation intensity, **- highly significant

Interaction effect between temperature and isolates

The interaction effect between different temperatures and 5 isolates had significantly varied on colony diameter, conidial dimension and density of the pathogen. The gross average higher colony diameter of 7.66 cm was noted for all 5 isolates when incubated at 25° C after 15 days of incubation on PDA. Similarly, lower diameter was noticed at 35 and 10 $^{\circ}$ C i.e. 2.86 and 2.85 cm respectively, which were statistically at par (Table 7).

The higher average conidial length and breadth of 29.42 μ m (24.35-34.35) & 18.12 μ m (14.25-24.65) were noted for all 5 isolates when incubated at 25^oC after 15 days of incubation on PDA. Similarly, lower conidial length of 18.68 μ m (14.15-22.45) was noticed at 35^oC and breadth 9.81 μ m (6.37-14.65) was found for 10 ^oC which were statistically at par with each other.



The higher average L/B ratio (2.00) was obtained for 10 0 C while lower (1.57) was recorded at 30 0 C. The average higher number of conidia per 0.01 ml of 46.67 was noticed at 25 0 C while lower number of conidia per 0.01 ml of 7.60 was noticed at 10 0 C on PDA after 15 days of incubation period. The higher sporulation intensity of score near to 4 i.e. profuse was noticed at 25 and 30 0 C for all 5 isolates (Table 7).

Incubation	Colony		CS				
temperatures (⁰ C)	diamete	* Conidial	*Conidial	L/B	Color	Conidia	SI
	r (cm)	length (µm)	breadth (µm)	ratio		/0.01 ml	
10	2.84 ^{e†}	19.58 ^c	9.81 ^c	2.00	BG	7.60 ^e	1.07
		(15.25-23.45)	(6.37-14.65)				
15	3.64 ^d	21.25 ^{bc}	11.92 ^c	1.78	BG	13.87 ^d	1.67
		(16.25-25.75)	(6.95-17.65)				
20	4.50 ^c	24.36 ^b	14.32 ^b	1.70	GB	25.67 ^c	2.67
		(19.35-28.35)	(9.85-19.85)				
25	7.66 ^a	29.42 ^a	18.12 ^a	1.62	DGB	46.67 ^a	3.80
		(24.35-34.35)	(14.25-24.65)				
30	5.89 ^b	24.35 ^b	15.55 ^b	1.57	DGB	33.47 ^b	3.53
		(20.25-28.35)	(13.42-18.35)				
35	2.85 ^e	18.68 ^c	9.83°	1.90	G	9.87 ^{de}	1.27
		(14.15-22.45)	(5.95-14.35)				
Factor A (Temp)	**	**	**			**	
Factor B (Isolates)	**	**	**			**	
A*B	**	*	**			**	
LSD (≤0.01)	0.49	2.99	2.20			5.74	
CV%	5.01	6.00	7.66			11.56	

Table 7: Effect of incubation temperatures on mycelial growth, conidial features and sporulation intensity of 5 isolates of *S. botryosum* after 15 days of incubation on PDA

[†] Means of 3 replications. Means in column with same superscript is not significantly different by DMRT (P<0.01). ⁰C- degree centigrade, cm- centimeter, μm- micrometer, ml- milliliter, [•] Mean number of 10 times observations for each replication, L/B ratio- Length/breadth ratio, Temp- Temperature, BG- Brownish Grey, GB- Grey Brown, DGB- Dark Grey Brown, G- Grey, CS- Conidial Sporulation, SI-Sporulation intensity **- highly significant, * - significant, value in the parenthesis indicate the range value.

The study on cultural and morphological variability of disease causal agents lighted the detailed biology of pathogens which significantly useful for the understanding of pathogen behavior and its structural and morphological composition. The higher colony diameter along with higher conidial length, breadth and sporulation intensity of the most of the isolates of *S. botryosum* were recorded at 25 ^oC while lower diameter with lower length and breadth of conidia with minor conidial density was noticed at 35 and 10 ^oC after 15 days of incubation on PDA. Mostly conidia of all isolates were brown in color at lower incubation temperature i.e. at 10 or 15^oC but they became dark grey brown at 25 and 30 ^oC and grey color was noticed for the conidia of most of the isolates when incubated at 35^oC on PDA after 15 days of



Kathmandu University Journal of Science, Engineering and Technology

Subash & Saraswati, Vol. 12, No. I, June, 2016, pp 80-89.

incubation. This result is strongly correlated with the findings which described that incubation temperatures had significant effect on conidial dimension, L/B ratio, color and sporulation intensity of 12 studied isolates of S. botryosum from New Zealand [9]. This finding is also well supported by the observation that stated S. botryosum varied in their colony color, texture, margin, shape and also size of conidia on PDA medium while worked on 4 isolates and measured the conidia as $13.33-16.04 \times 6.46$ -9.17 µm [10]. The result is also in agreement with the report that the optimum temperature for radial mycelia growth of S. botryosum was 25 °C [11]. Temperature plays an important role in influencing the growth of fungi [12]. Normally, the growth temperature for the majority of fungi is between 25 °C to 30 ⁰C and above 40 ⁰C, the growth is poor [13]. Evidence of increased aggressiveness at optimum temperatures of some pathogens has been reported, suggesting that they can adapt to and benefit from the required temperature [14]. The role of temperature, high or low, operates by affecting the genetic machinery of the pathogen cell by favouring or inhibiting the expression of certain genes/protein involved in pathogen growth. The study on temperature effect on S. botryosum gave the basic idea of pathogen morphology and its response towards the climatic variables. The effect of other factors like relative humidity, pH and growth media could be the further scope for conducting new research activities on this pathogen. The genetic study of this pathogen in future will also explore more detailed and newer hidden scientific facts towards its structural composition.

ACKNOWLEDGEMENTS

Plant Pathologists of NARC are also acknowledged for their valuable suggestions. Authors are thankful to Nepal Agricultural Research Council for financial assistance and Grain Legume Coordinator for continuous support and research facilities.

REFERENCES

- [1] Joshi S, Review of important grain legume diseases and their management, in *Proceedings of a national workshop on Integrated Pest Management (IPM)*, Plant Protection Society, Kathmandu, Nepal, 25-26 August (2006), 100-116.
- [2] Gharti D B, Jha P, Darai R, Ghale D, Joshi S & Wagle B P, Studies on Management of Stemphylium Blight (*Stemphylium sarciniforme* Walr) of Lentil (*Lens culinaris* Medik.) at NGLRP, Rampur and RARS, Nepalgunj, in *Program and Abstract of a 27th National Winter Crops Workshop "Ensuring Food Security through Crop Diversification"*, Nepal Agricultural Research Council, Kathmandu, Nepal (2008), 35-36.
- [3] GLRP, *Annual Report 2068/69 (2011/12)*, Grain Legumes Research Program, NARC, Rampur, Chitwan, Nepal (2012).
- [4] Bakr M A, Plant protection of lentil in Bangladesh, in *Proceedings of the Seminar on Lentil in South Asia*, New Delhi, India, 11-15 March (1991), 7-12.
- [5] Bayaa B, Joshi S, Karki P B & Jha P, *Lentil disease survey report*, Kathmandu, Nepal, 23rd February (1998).



Kathmandu University Journal of Science, Engineering and Technology

Subash & Saraswati, Vol. 12, No. I, June, 2016, pp 80-89.

- [6] Bayaa B & Erskine W, *Lentil pathology*, In: Allen D & Lenné J. editors, Pathology of Food and Pasture Legumes, Commonwealth Agricultural Bureaux International, U.K in association with: International Crop Research Center for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India (1998), 423-472.
- [7] Agrios G N, *Plant Pathology*, Elsevier Academic Press, 30 Corporate Drive, Suite 400, Burlington, MA 01803, USA (2005), 5.
- [8] Campbell C L & Madden L V, *Introduction to Plant Disease Epidemiology*, John Wiley and Sons., New York, USA (1990).
- [9] Singh G, *Plant pathogenic species of Stemphylium Walr. in New Zealand*, Ph.D. Thesis, Massey University, New Zealand (1977), 141.
- [10] Hosen M I, Ahmed A U, Zaman J, Ghosh S, & Hossain K M K, Cultural and Physiological Variation between Isolates of *Stemphylium botryosum* the Causal of Stemphylium Blight Disease of Lentil (*Lens culinaris*), *World Journal of Agricultural Sciences*, (2009) 5(1): 94-98.
- [11] Rahman T, Ahmed A U, Islam M R & Hosen M I, Physiological study and both *in vitro* and *in vivo* antifungal activities against *Stemphylium botryosum* causing Stemphylium blight disease in lentil (*Lens culinaris*), *Plant Pathology Journal*, (2010) 9: 179-187.
- [12] Cohrane V W, *Physiology of fungi*, John Wiley & Sons Inc., New York, USA (1958).
- [13] Cooney D C & Emerson R, Thermophillic fungi, W.H. Freeman, San Francisco, USA (1964).
- [14] Mari M & Martini C, Possible effects of climate changes on plant diseases. In: *Proceedings of* 50th Croatian and 10th International Symposium on Agriculture, University of Zagreb, Faculty of Agriculture, Opatija, Croatia, 16-20 February (2015), 37-41.