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RESEARCH ARTICLE

Effect of temperature, light on germination and morphological characteristics of *Bipolaris sorokiniana*

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Abstract

Wheat is one of the most nutritious, proteinaceous and basic stable food crop is attacked by several factors. Among the biotic factors, the most notorious disease of wheat is spot blotch caused by *Bipolaris sorokiniana.* The behaviour of the pathogen with different combinations of weather regimes were studied *in-vitro*, in the dark at different temperatures. Maximum conidial germination (92.04%) was observed at 30 °C followed by 91.69% at 25 °C temperature after 4 h of incubation. Lowest germination was recorded at 10 °C. Maximum length of unipolar germ tube (26.28 µm) was recorded at 15 °C which was statistically superior than other treatments. Whereas, maximum width of unipolar germinated germ tube (4.49 µm) was observed at 25 °C followed by 15 °C. The length and width of germ tubes of the two poles of bipolar germinated *B. sorokiniana* spores were 19.74, 22.61 and 3.92, 4.11 µm, respectively at 15 °C followed by 20 °C temperature. Significantly lowest time required for secondary conidiophore formation (25 h) and conidia formation (39 h) was recorded at 25 °C. Highest time required for formation of secondary conidiophore (35 h) at 20 °C, conidia at 30 °C (80 h) but at 10 and 35 °C temperature secondary conidiophore and conidia formation was not observed within 80 h of incubation. Higher mycelial growth and no sporulation were observed under continuous light condition.

Keywords Unipolar and bipolar germination · Secondary conidiophore · Sporulation · Temperature

Introduction

Wheat is one of the most important cereal crops in the world. During 2013–2014, India recorded all time high 95.80 million tons of wheat production from an area of 31.30 million ha (Wheat Annual Report from Directorate of Economics and Statistics; Department of Agriculture and Cooperation 2013). The wheat production suppress by several biotic and abiotic factors, among the biotic factors, the most notorious, widely prevalent shifty enemy on wheat is spot blotch disease. Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum* Pamm., King & Bakke), teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsl. ex Dastur is an economically important fungal pathogen worldwide (Knight et al. 2010; Acharya et al. 2011). During past two decades incidence of spot blotch disease has caused substantial economic loss in wheat production, which affecting the livelihood of millions of small scale farmers (Krishnendu et al. 2011). It is estimated that globally 25 million ha^{-1} of wheat cultivated land affected by this notorious disease (Ginkel and Rajaram 1998). Of these roughly 9 million ha^{-1} are in India alone which mostly prevails under the rice wheat cropping system (Nagarajan and Kumar 1998). Epidemic has been observed in India, Punjab with the dominant pathogen *B. sorokiniana* followed by *Fusarium* spp. (Ansari 2015; Mahmood et al. 2011). This pathogen significantly occurs in Eastern Gangetic Plains (EGP) of South Asia, which includes India, Nepal and Bangladesh (Joshi et al. 2007). The yield loss due to the disease is very significant especially in North Eastern Plains Zone (NEPZ) of India, Nepal Terai and North

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Western Bangladesh, where it has assumes an epidemic proportion (Chowdhury et al. 2013).

The hyphae and conidia of this fungus are dark coloured due to presence of melanin pigment like other dematiaceous fungi (Bashyal et al. 2010). It produces both inter- and intracellularly septate mycelium in to the host tissue. Asad et al. (2009) have noticed that on artificial media the average size of conidia ranged from 38.3–65.8 µm × 12.3–25 µm, brown to olivaceous brown slightly curved, with 2–13 septa. Singh et al. (2013), who reported that the average length and width of conidia of Kalyani, West Bengal isolates was 20.35–90.30 and 11.95–23.43 µm. The average number of septa and spore size in control was 7 and 57 × 17.5 µm (l × b) respectively (Ansari 2015). Acharya et al. (2011) also concluded that the conidium size of *B. sorokiniana* ranging 40–120 and 15–28 µm. According to Bandyopadhyay et al. (2016) the highest average length of the conidia was 72.43 ± 23.70 µm in WB10 isolate, which was followed by WB 7 (65.01 ± 12.55 µm) and the highest average breadth was 23.68 ± 1.53 µm found in WB 6 followed by WB 8 (22.81 ± 1.85 µm). The most important factor, temperature plays a key role coupled with high humidity and Moderate to warm temperature range (18–32 °C) favours the growth of *B. sorokiniana* (Chowdhury et al. 2013). According to Hodges (1975) revealed that conidia germinated in large numbers, at a faster rate and produced more and longer germ tubes as temperature was increased from 10–22 °C and above 22 °C, percent germination and growth rate of germ tubes declined. However, Singh et al. (1998) reported that for rapidly infection favourable temperature is 28 °C. It is consequently quite close to real field environment prevailing in north eastern plains zone which is a spot blotch prone area having temperature range of 12–30 °C in the month of February and 16–35 °C in the month of March. The similar observation reported by Aggarwal et al. (2009) and Bashyal et al. (2010). For that reason, the temperature prevailing in this zone during February and March is quite favourable for the development of spot blotch. Fungi exhibit varying response to light, depending on the light intensity and duration of exposure and temperature. Exposure to light is needed by some fungi for sporulation, whereas other fungi sporulate better in dark and with the decrease in germination of conidia as the period of darkness increase (Rewal and Grwal 1989). Realizing the above facts, the present investigation on effect of different temperature and light/ darkness on germination and morphological characteristics of *Bipolaris sorokiniana* was studied to identify the optimum light and temperature combination required for germination, sporulation incubation, latent and infectious period of the pathogen.

Materials and methods

Pathogen was isolated from diseased wheat plants. Those were infected by the mother culture of most aggressive isolate of *Bipolaris sorokiniana* in the research fields of Uttar Banga Krishi Viswavidyala Pundibari, Coochbehar, West Bengal, India and maintained on Potato dextrose agar (PDA) growth medium until used.

The fungal mycelia mat of 7 days old pure fungal culture were cut into 4 mm diameter small pieces by cork borer and put into vials containing 10 ml of sterilized distilled water. The vials were vortexed with a spinmix tube shaker at full speed to liberate the conidia and after that the concentration of conidia in suspension was measured by using haemocytometer. Conidia from *Bipolaris* isolate were collected and the concentration was adjusted to 10^5 conidia ml^{-1}. An aliquot of this suspension (100 µL) was loaded onto slides containing 2% water agar block and incubated in Petri dishes on distilled water-saturated filter paper in the dark at temperature of 10, 15, 20, 25, 30 and 35 °C. Spore suspension on 2% water agar block in moist Petri plates were placed in a dark growth chamber maintained at a high relative humidity and different temperature. Each point is an average of three replicates. Evaluation of germination was recorded after 0.5, 1, 2, 4, 6 and 8 h incubation. Conidia were considered germinated if their germ tubes break open the spore cell wall. Germination percentage was then expressed as (number of germinated conidia/total number of conidia assessed) × 100, using a light microscope. Morphometric measurements (length and width) of grem tubes was also recorded. To determine the incubation period of complete growth stage of the pathogen the seven days old fresh fungal culture were used and spore suspension was prepared as mentioned earlier. An aliquot of this suspension (100 µL) was loaded onto slides containing 2% water agar block and incubated in Petri dishes on distilled water-saturated filter paper in the dark at temperature of 10, 15, 20, 25, 30 and 35 °C. Spore suspension on 2% water agar block in moist petri plates were placed in a dark growth chamber maintained at a high relative humidity and different temperature. Each point is an average of three replicates. Thereafter, different growth stage recorded at varying temperature regimes till the secondary conidia formation was completed. Morphometric measurements (length and width) of mother conidia and newly borne conidia and conidiophore was also recorded.

The effect of the weather parameters (temperature, light/darkness) on sporulation and infection of the pathogen was studied under controlled environmental condition (growth chamber) on PDA media. Disc of 4 mm fungal mycelia mat of 7 days old fungal culture were inoculated

into the Petri plates containing PDA and the plates were kept for incubation at 25 ± 1 °C under both fully light and dark in the growth chamber. In each condition five replications are maintained. After that, colony growth and characteristics was recorded at 24 h intervals, up to full plate colony growth was completed. We have also studied sporulation of *Bipolaris* spore under dark and light condition at 25 °C.

Results and discussion

Effect of temperature variables on spore germination

Spore germination was recorded from unipolar germinated (UG) and bipolar germinated (BG) *Bipolaris sorokiniana* spore. In case of unipolar, maximum conidial germination recorded at 20 °C temperature followed by 25 °C after incubation of 2 h. and lowest germination observed at 10 °C. But highest germination was recorded after 2–4 h of incubation as temperature increased from 15 to 30 °C and after 8 h of incubation, peak germination was recorded at 10 and 35 °C temperature.

Whereas, bipolar spore germination maximum recorded at 30 °C temperature followed by 25 °C. However, least germination was recorded 10 °C followed by 35 °C. The results reported are somewhat dissimilar with the work done by Hodges (1975) who reported that no conidia germinated at 34 °C. The length and width of germ tubes of the two poles of BG *B. sorokiniana* spores were 19.74, 22.61 and 3.92, 4.11 µm, respectively at 15 °C followed by 20 °C temperature. Findings of the present study clearly demonstrated that, both unipole and bipole germinated spore, maximum conidial germination were recorded up to 8 h of incubation (Table 1). Lowest germination was recorded at 10 °C temperature in both unipole and bipole germination. The conidial germination percentage increases with the increases of temperature from 10 to 30 °C and decline in germination percentage was recorded beyond 30 °C. Such variation of conidial germination was also reported by Hodges (1975) in species of *Bipolaris sorokiniana*.

The temperature and duration of temperature had a significant effect on unipolar and bipolar germination. Beyond 4 h of exposure from 20 °C and onwards had significant effect on decrease in the ratio of UG and BG pattern germination, whatever at the same condition there was significant increment on bipolar germination (BG) of *Bipolaris sorokiniana.* Thus, 20 °C onward temperature with greater than 4 h exposure duration induced bipolar germination of *Bipolaris sorokiniana* resulting in decreasing in percentage of unipolar germination. Louise et al. (2015) observed the consistence decrease in the ratio of unipolar germination vs. bipolar germination pattern of *Cochliobolius lunatus*. Sharma and Duveiller (2003) reported that under field condition the higher values of AUDPC/day or AUDPC/degree day under late-sown conditions are most likely caused by heat stress, which enhanced HLB development.

The consistent decrease in unipolar germination vs. bipolar germination ratio (Table 2) of germination pattern of *Bipolaris sorokiniana* was recorded from 15 °C onward at temperature range of 20–30 °C, whereas highest unipolar and bipolar germination ratio was recorded at 35 °C. Moreover, the least unipolar germination and bipolar germination ratio was recorded at temperature of 25 °C followed by 30 °C. Highest bipolar germination of *Bipolaris sorokiniana* was recorded at 25 °C followed by 30 °C (Table 1), thus the present findings indicated that more nos. of infection foci and colonization behavior of *Bipolaris sorokiniana* was observed at 25 °C followed by 30 °C. Both sigmoid and exponential models have been developed to the data set with the observed values of conidial germination under dark

Table 1 Effect of temperature on unipolar and bipolar germination percentage of *Bipolaris sorokiniana* under control dark condition

Temperature (°C)	Duration of exposure											
	0.5 h		1 h		2 h		4 h		6 h		8 h	
	Unipolar	Bipolar	Unipolar	Bipolar	Unipolar	Bipolar	Unipolar	Bipolar	Unipolar	Bipolar	Unipolar	Bipolar
10	0.00^{c}	0.0	0.00^{c}	0.00^{b}	3.67^{c}	0.00^{d}	35.18^{d}	9.69^{c}	50.22^{bc}	12.60^{d}	70.67^{a}	16.89^{d}
15	1.57^{c}	0.0	10.95^{b}	0.00^{b}	41.92^{b}	7.93^{cd}	61.51^{ab}	24.73^{b}	58.91^{b}	30.63^{bc}	53.10^{bc}	41.19^{bc}
20	5.86^{b}	0.0	19.14^{b}	1.01^{b}	57.95^{a}	20.22^{bc}	64.22^{a}	22.67^{b}	53.25^{b}	41.48^{b}	48.58^{cd}	48.84^{b}
25	8.71^{b}	0.0	18.89^{b}	3.25^{b}	55.61^{ab}	26.59^{b}	50.13^{bc}	43.52^{a}	39.72^{c}	55.94^{a}	35.91^{e}	62.23^{a}
30	13.07^{a}	0.0	28.83^{a}	7.19^{a}	43.34^{b}	40.55^{a}	41.95^{cd}	50.11^{a}	40.31^{c}	57.45^{a}	36.71^{de}	62.56^{a}
35	1.59^{c}	0.0	17.76^{b}	0.00^{b}	$45.35A^{b}$	8.26^{cd}	70.31^{a}	18.80^{bc}	74.95^{a}	19.75^{cd}	64.26^{ab}	31.43^{c}
SEM±	1.12	0.0	3.08	1.11	4.71	4.32	4.33	3.32	3.53	3.62	3.89	3.78
CD≥0.05	3.45	0.0	9.49	3.42	14.51	13.31	13.34	10.23	10.88	11.15	11.99	11.65

Mean values bearing different superscript letters are significantly different at 5% level of significance

Table 2 Effect of temperature on Unipolar vs. Bipolar germination of *Bipolaris sorokiniana*

Temperature (°C)	Duration of exposure					
	0.5 h	1 h	2 h	4 h	6 h	8 h
10	–	–	–	3.63	3.99	4.18
15	–	–	5.29	2.49	1.92	1.29
20	–	18.95	2.87	2.83	1.28	0.99
25	–	5.81	2.09	1.15	0.71	0.58
30	–	4.01	1.07	0.84	0.70	0.59
35	–	–	5.49	3.74	3.79	2.04

Table 3 Estimated parameters of fitted curve for *Bipolaris sorokiniana* germination under dark condition

Temperature (^{O}C)	Model fitted	Sig.	R^2	Fitted curve
10	Y = e** (5.98 − (4.90/t))	0.01	0.66	Sigmoid
	Y = 188.41 (e** (−373.79/t))	0.01	0.99	Exponential
15	Y = e** (7.97 − (185.39/t))	0.01	0.86	Sigmoid
	Y = 126.46 (e** (−117.05/t))	0.01	0.98	Exponential
20	Y = e** (11.74 − (546.21/t))	0.01	0.96	Sigmoid
	Y = 120.38 (e** (−79.53/t))	0.01	0.94	Exponential
25	Y = e** (14.40 − (811.24/t))	0.01	0.96	Sigmoid
	Y = 121.11 (e** (−74.11/t))	0.01	0.93	Exponential
30	Y = e** (13.98 − (781.49/t))	0.01	0.96	Sigmoid
	Y = 117.58 (e** (−60.35/t))	0.01	0.96	Exponential
35	Y = e** (7.91 − (186.85 /t))	0.01	0.85	Sigmoid
	Y = 126.37 (e** (−104.97/t))	0.01	0.98	Exponential

Sigmoid model: Y = e** (b0 + (b1/t)) or ln(Y) = b0 + (b1/t); exponential model: Y = b0 (e** (b1/t)) or Y = b0 (exp (b1/t))

conditions (Table 3). The three observations at each duration correspond to the three replicates performed. Comparison of the predicted values of the model fitted to the reduced data set with the observed values of conidial germination in dark conditions (Table 3). These models outputs could be used as an estimate of sporangial germination at varied level of temperatures under dark condition.

Morphometric characters of germinated *Bipolaris* spores under controlled condition

In order to study the optimum, maximum and minimum temperature required for one pole germ tube formation of mother conidia and length and width of germ tube of *B. sorokiniana,* tests were conducted at different temperature regimes ranging from 15 to 30 °C and observation were recorded after clear visible of septation. Spore suspension on 2% water agar block in moist petri plates were placed in growth chamber maintained at high relative humidity and different temperatures. Perusal of the data presented in Table 4 indicated that maximum length of unipolar germ tube (26.28 µm) was recorded at 15 °C which was statistically superior to other treatments followed by 20, 25 and 30 °C in descending order. Whereas, maximum width of germ tube (4.49 µm) was observed at 25 °C temperature followed by 15 °C temperature.

The length and width of germ tube of bipole germinated *Bipolaris* spores at different incubation temperatures (Table 4). The results showed that, maximum length of germ tube of the two poles of BG *B. sorokiniana* spores were 19.74 µm, 22.61 µm respectively was observed at 15 °C temperature followed by 20, 25, and 30 °C temperature. Whereas, maximum width of germ tube 3.92 and 4.11 µm was observed at 15 °C followed by 25 °C temperature. Table 4 showed that, the length of germ tube of

Table 4 Morphometric characters of unipolar and bipolar germinated *Bipolaris* spores under different temperature regimes

Temperature (°C)	Length of conidia (µm)		Width of conidia (µm)		Length of germ tube (µm)			Width of germ tube (µm)		
	Unipolar	Bipolar	Unipolar	Bipolar	Unipolar	Bipolar		Unipolar	Bipolar	
						Pole-I	Pole-II		Pole-I	Pole-II
15	53.81^{a}	57.36^{a}	21.96^{a}	22.66^{a}	26.28^{a}	19.74^{a}	22.61^{a}	4.01^{b}	3.92^{a}	4.11^{a}
20	44.91^{b}	57.70^{a}	20.02^{b}	20.26^{b}	19.12^{b}	19.18^{a}	17.17^{b}	3.64^{b}	3.36^{b}	3.28^{b}
25	47.07^{b}	45.74^{b}	19.34^{b}	18.30^{c}	17.34^{b}	14.72^{b}	15.38^{b}	4.49^{a}	3.76^{ab}	3.88^{a}
30	45.84^{b}	53.30^{a}	22.04^{a}	20.23^{b}	16.37^{b}	13.79^{b}	13.55^{b}	3.68^{b}	3.40^{b}	3.23^{b}
SEM±	1.98	2.27	0.62	0.56	1.61	1.13	1.76	0.15	0.28	0.14
CD ≥ 0.05	5.58	6.39	1.75	1.58	4.53	3.18	4.96	0.42	0.79	0.39

Mean values bearing different superscript letters are significantly different at 5% level of significance

BG *Bipolaris* spores have significant difference among incubation temperatures after clear visibility of germ tube septation, but in case of width of germ tube no significant difference was observed under varying temperature. The germ tube length and width followed a decreasing trend with increasing incubation temperature from 15 to 30 °C. Theses result somewhat accordance with the observation of Hodges (1975), who reported that germ tube length increased as temperature increased from 10 to 22 °C, above 22 °C germ tube length declined.

Effect of different temperature on secondary conidia and conidiophore formation of *Bipolaris sorokiniana*

In order to study the time required for the start of secondary conidiophores and conidia formation of *B. sorokiniana,* tests were conducted at different temperature regimes ranging from 10 to 35 °C and observation were recorded when secondary conidiophores and conidia formed. Significantly lowest time required for secondary conidiophore formation (25 h) and conidia formation (39 h) was recorded at 25 °C which was statistically superior to other treatments followed by 20, 15 and 30 °C in descending order (Fig. 1). Highest time required for secondary conidiophore and conidia formation ware recorded at 20 °C (35 h) and 30 °C (80 h) temperature respectively. No sporangial germination was observed at 10 and 35 °C temperature within 80 h of incubation. There were significant differences between time required for secondary conidiophores and conidia formation in 20 and 25 °C temperature.

This study has indicated differences in the effect of temperatures on secondary conidiophores and conidial morphology. The length and width of secondary conidia and conidiophores (Fig. 2). The microscopic studies revealed that the secondary conidia of *Bipolaris sorokiniana* produced at various temperature regimes on water agar were 60–77 × 20–27 µm in size, tapered at both ends, dark brown to olivaceous, distoseptate and irregular margin. The maximum length of secondary conidia was observed 76.97 µm under 15 °C temperatures followed by 72.49 µm at 20 °C temperatures. Whereas, maximum width (21.46 µm) of conidia was recorded at 20 °C followed by 21.29 µm under 15 °C temperatures. The length and width of conidia recorded at 25 °C was 71.48 and 21.11 µm respectively. The findings of the present study corroborates with the findings of Singh et al. (2013), who reported that the average length and width of conidia of Kalyani, West Bengal isolates was 20.35–90.30 and 11.95–23.43 µm. Asad et al. (2009) concluded that, the length and width of conidia vary from 38.3–65.8 and 12–25 µm respectively. Acharya et al. (2011) also concluded that the conidium size of *B. sorokiniana* ranging 40–120 and 15–28 µm. In addition to the Conidia, morphology of secondary conidiophores also studied and dark greyish olivaceous colour was recorded and had solitary emergence. The microscopic studies revealed that the average length and width of conidiophores was 83.36–172.60 and 5.52–6.76 µm. Such variations of characters in conidiophore were also reported by Bashyal et al. (2010) in species of *Helminthosporium*.

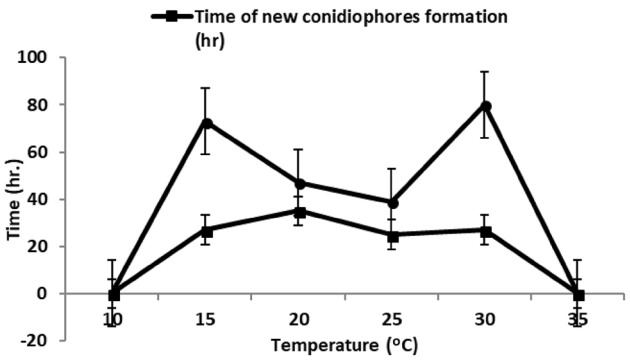

Fig. 1 Effect of different temperature on *Bipolaris sorokiniana* secondary conidia and conidiophore formation after 80 h incubation in growth chamber

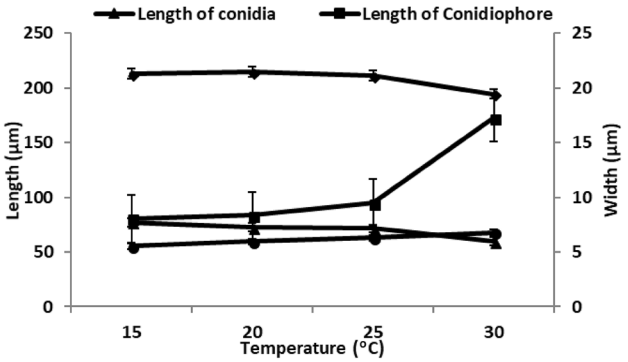

Fig. 2 Effect of different temperature regimes on length and width of new borne conidia and conidiophores

However, highest length and width of secondary conidiophores was observed 172.60 and 6.76 µm respectively at 30 °C temperature followed by 25 °C, where length of conidiophores 94.81 and width 6.29 µm was observed. Whereas, lowest length and width of the secondary conidiophores was 80.52 and 5.52 µm respectively at 15 °C temperature. The results present study, somewhat corroborates with observation of Acharya et al. (2011). The results reported are somewhat similar with the work done by Singh et al. (2013).

Effect of light and dark condition on colony growth of *Bipolaris sorokiniana* at 25 °C

The cultural characters exhibited by *B. sorokiniana* at different temperature regimes were recorded by visual observations. The study showed differences between light and dark

condition in respect of colony growth. Colonies of the studied pathogen grew faster in light condition than dark condition at 25 °C temperature on Potato Dextrose Agar media.

Under dark condition, on PDA media the *Bipolaris* pathogen formed wavy with scanty aerial mycelium, olivaceous brown colour and irregular margin and becoming dark brown to black colour, irregular shaped mycelia growth.

Similar results have also been discussed by Aggarwal et al. (2009) and Bashyal et al. (2010). The colony growth rate was slower in dark condition at 25 °C and the reverse of colonies was dark black. Whereas, in light condition whitish colour, fluffy type, round shaped mycelia growth formed, with a white grey reverse and growth rate was higher than the dark condition.

After 4 days (96 h) of growth at 25 °C, under dark condition average formed colonies of 4.36 cm in diameter on PDA and 6.23 cm under light condition. Subsequently, 9.0 cm and 6.26 cm average colonies diameter was observed after 144 h (6 days) under dark and light conditions respectively. Results of the observation are in accordance with the description by Morejon et al. (2006), who reported the colony becomes mature within 5–7 days at 25 °C. Similar results also reported by Yadav (2014). On the basis of macroscopic features of colonies of *Bipolaris* pathogen after six days (144 h) of incubation at 25 °C temperature on PDA, revealed that under light condition no sporulation initiation was observed but under dark condition (without light) numerous sporulation observed. The results reported is somewhat similar with the work done by Aggarwal et al. (2009), Bashyal et al. (2010) and Singh et al. (2013) who reported marked differences in sporulation of the pathogen *B. sorokiniana*. These findings are also supported Naresh et al. (2015).

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