Int.J.Curr.Microbiol.App.Sci (2018) *7*(8): 225-248

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.708.029

Morphological Characterization of *Bipolaris sorokiniana* Infecting Wheat

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Keywords

Bipolaris sorokiniana, Morphological characterization, Conidial structure, Septation, Spore morphology

Article Info

Accepted:
04 July 2018
Available Online:
10 August 2018

A B S T R A C T

Variability among isolates of *Bipolaris sorokiniana* was determined based on conidial morphology. The pathogen was isolated from wheat host from different agro-climatic zones of West Bengal and grown on four different media for investigation of conidial morphology of this pathogen. The size of colony (length and breadth) was increased with increasing incubation period. Different media produces different growth characteristics (Colony diameter) on different media and Carrot Agar media produces highest colony diameter whereas minimum in Potato Dextrose Agar media in every days after inoculation. Maximum growth was obtained from DWR isolate on CA medium from 7th day old culture. Among the four media, PDA media produced maximum length, breadth and septation of the conidia. Among the isolates Alipurduar isolate (I_1) produced maximum length, breadth and septation of the conidia irrespective of media used.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grain crops providing nearly 20% of the total world food requirement (Uddin *et al.*, 2006). It is considered as the second most staple food crop next to rice in India. In India, the contribution of wheat to total food grains production has been ranging between 35-37% in last 5 years. The contribution of wheat to total food grain is impressive. However, in the background of increasing population, there is a demand for more production of food grains from same piece of land. In order to meet the needs of growing population it will be necessary to produce about 110 m tons of wheat by 2020 (Swaminathan, 2000) and it is believed that India has the potential to become the largest wheat producer in the world by the end of the year 2020 provided the technological advances in rainfed/drylands are continued with evolution of improved genotypes. The production of wheat in India has improved tremendously with the expansion of high yielding dwarf varieties and better used of inputs.*Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus)* is the causal agent of common root rot, leaf spot disease like leaf blotch, seedling blight, head blight, and black point of wheat and barley. The fungus is one of the most important foliar disease constraints for both crops in warmer growing areas and causes significant yield losses. High

temperature and high relative humidity favour the outbreak of the disease, particularly in South Asia's intensive 'irrigated wheat–rice' production system. In West Bengal as well as all over Eastern India the main important fungal disease is foliar blight caused by *Bipolaris sorokiniana* and *Alternaria triticina* may attack singly or together and caused a loss of yield exceeding 60% (Prabhu and Singh, 1974). The importance of this foliar blight must be expressed in terms of yield losses but an estimate was widely varied according to variety (Nema and Joshi, 1971) assuming significant far and wide in the country. It is apparent from their development that foliar blight may pose a threat to wheat in near future. Considering high yield losses, breeding for resistance demands high priority. It is necessary to have ample genetic variability within the host population. Intensive efforts in many countries are now underway to identify the sources of resistance against foliar blight disease of wheat. As the cultivation of wheat in West Bengal is demanding for increasing food production and farmers are cultivated the crop without knowing the proper cultural practices which decrease the yield by increasing the important disease like foliar blight.

No information has been available regarding the nature of this disease, losses caused by them, epidemiology and management in these agro climatic zones of West Bengal. However, the information on this disease was reported from other parts of the country (Malik *et al.*, 2008, Singh *et al.*, 2003, 2001). But it needs to be constantly improved with regards to several aspects if any safeguard against this risk is to be developed in near future. Different researcher has carried their work on different locations and developed prediction equation for disease forecasting, management (Singh *et al.*, 2004), screening of varieties (Kumar *et al.*, 2010) and others. But in West Bengal condition no information has been available regarding the important pathogens and their variability, causing crop loss, viable and accurate prediction for disease severity and eco-friendly management.

Materials and Methods

The whole experimental work was carried out with the *Bipolaris sorokiniana* that were isolated from wheat (*Triticum aestivum*) and collected from different locations like Old Alluvial Zone (North Bengal), Trans-gangetic plain region, and New Alluvial Zone (Kalyani, Nadia). The morphological studies of the pathogen were conducted on different solid media *viz,* Potato dextrose agar, carrot agar, oat meal agar, carrot potato agar.

Isolation and identification of the pathogen

The infected plant parts (leaves) showing typical symptoms of the disease was collected from the field. The standard tissue isolation procedure was followed to isolate the pathogen. The infected tissue with some green portion was surface. sterilized with 0.1% mercuric chloride ($HgCl_2$) for 30 seconds and repeatedly washed separately in sterilized distilled water and then transferred to the sterilized petriplates containing Potato Dextrose Agar (PDA).The petriplates were incubated at room temperature (27±1 $^{\circ}$C) and observed periodically for the growth. Bit of fungal growth developed from the infected tissue was transferred to PDA slants. Then the mycelia tip or single spore isolation was done for purification of the pathogen. Then such pure culture was used for further studies. Identification was done by using microscopes and characters were studied on the basis of their morphological levels.

Maintenance of culture

All the fungal cultures were maintained in PDA slants and kept in a refrigerator at 5° C

and the cultures were sub-cultured at every 30 days interval regularly or as and when necessary.

Cleaning and sterilization of glass wares and preparation of different media

All the petriplates and another required glass wares were washed thoroughly with detergent powder and running tap water, air dried and wrapped together in a brown paper. Then the glass wares were sterilized in hot air oven at 161°C for 2 hours.

Inoculation

All the petriplates containing different media were inoculated separately with the test fungi (*Bipolaris sorokiniana)* with the help of inoculating needle aseptically under laminar airflow and kept in a B.O.D incubator at 27±1 °C for proper growth of the fungi.

Measurement of radial growth

After 24 hours of inoculation, radial growth of the fungus was measured by standard millimeter (mm) scale and it was continued every 24 hours interval upto a certain day (s) in each media.

Other morphological studies of the fungus

For this purpose, the fungus was allowed to grow in Carrot Agar media.

The slides of the selected cultures or colony were prepared in order to study the fungal morphology such as conidial length, breadth, number of septations. The prepared slides were observed under Phase-contrast microscope.

The photographs of the observed conidia are taken and the micrometer measurements of the conidia were done.

Results and Discussion

Isolation of pathogen and its pathogenicity test

Pathogen was isolated from the infected leaves of wheat from different locations and maintained on PDA through sub- culturing at 15 days intervals. For pathogenecity test, the seeds were sown in perforated aluminium tray containing sterilized potting media (loamy garden soil: compost: weed ash= 65kg: 20kg: 0.15kg) that was free from infection from any sources. All the pots were kept in glass house and spore suspension containing 5X105 conidia/ml were sprayed on the leaves in evening for observing symptoms. Observation after 10 days interval revealed that in all the sprayed plant produced symptoms like small and dark brown lesion on leaves. The experiment clearly confirms the fact that all the isolates of the fungus *Bipolaris sorokiniana* can produce the leaf spots (blotch) on wheat.

Morphological variability

Colony diameter (mm)

Morphological characters of five representative isolates of *Bipolaris sorokiniana* including colony size, number of septations of the conidia, length-breadth of conidia were measured on high power (40X) using calibrated filar micrometer and microscope from 10 days old culture on different media and observed that the conidiophores of the fungus were formed singly, straight or flexious, brown to olive brown, the conidia were solitary, straight or slightly flexious and ellipsoidal tapering, pale or olivaceous brown in colour. The different isolates of *Bipolaris sorokiniana* collected from different locations produces different morphological characters on different media. The results showed that the colony diameter

(length and Breadth) of different isolates were different and their difference were statistically significant. Different media produces different growth characteristics (Colony diameter) on different media. It was observed that Carrot Agar media produces highest colony diameter (i.e. 48.47mm length and 48.00mm breadth) whereas minimum in Potato Dextrose Agar media (i.e. 42.61mm length, 41.84 mm breadth) and their difference was statistically significant (Table 1). Here it was also observed that irrespective of the isolates and days after inoculation all the media produces different length and breadth of the colony growth and their difference was statistically significant. Different days after inoculation also produces different growth characteristics (Length-breadth) and with increasing day of inoculation there is significant increase in colony diameter (length-breadth of the isolates) irrespective of isolates and media used. Maximum growth was observed on 7days old culture (66.94mm length and 65.71mm breadth) and lowest growth was obtained on 1st days old culture (12.06mm length and 12.07mm breadth) (Table 1). Different isolates produces different growths (length and breadth) irrespective of different media and their days of inoculation. It was observed that DWR (I_4) isolate produces maximum growth (55.95mm length, 55.54mm breadth) and minimum was obtained on Kisanganj (I_2) isolate (i.e. 40.64mm length, 39.54mm breadth) and their difference was statistically significant irrespective of different media and days after inoculation. It was also observed that isolate Kisanganj and Pundibari showed no significant differences in between them in respect to length and breadth of colony diameter. Medium growth was observed (44.58mm length and 44.39mm breadth) was obtained from Kalyani isolate (Length and Breadth) (Table 1). The interaction between media and days after inoculation also showed significant difference among themselves in respect to length and breadth of colony diameter. In every media the colony diameter (length and breadth) is increased significantly with increasing age of growth of the isolates. On Potato Carrot Agar media maximum growth was obtained on 6th day old culture (60.97mm length, 60.25mm breadth) whereas in Carrot Agar media (75.04mm length, 74.07mm breadth) was also observed on 7th day old culture which was statistically at par with Oatmeal Agar media on same day old culture (i.e. 73.3mm length, 70.82mm breadth) and the minimum growth was obtained from PDA media (57.51mm length, 56.80mm breadth) irrespective of different isolates. It was observed that 4th old culture on CA media produces similar growth with that of 5th day old culture of PDA media (Table 1). Similarly, PCA media and PDA media produced same growth of colony diameter (length and breadth) on 1day old culture. It was also observed that 3rd day old culture produces similar type of growth on PCA, CA, OMA whereas PDA media produces highest growth on the same day old culture than the above mentioned media. Interaction between Isolate and different media also produced different growth characteristics and their differences were statistically significant. On PCA media DWR (I4) isolate produces maximum growth (55.74mm length, 54.64mm breadth) and minimum by Kisanganj (I_2) isolate (39.31mm length, 38.23mm breadth) and theirdifference was statistically significant, whereas Alipurduar (I_1) isolate and Pundibari (I_3) isolate produces similar type of growth, Pundibari isolate (42.91mm length, 43.24mm breadth) and Alipurduar isolate (43.3mm length, 43.41mm breadth) DWR isolate (I4) also produced maximum growth on CA media. (58.92mm length, 58.86mm breadth) and minimum produced by Kisanganj isolate (I_2) (44.1mm length, 42.80mm breadth) and their difference was statistically significant. Isolates Pundibari (I_3) and Kalyani (I_5) produced statistically at par growth

(Pundibari-47.49mm length; 44.11mm breadth) and (Kalyani-47.1mm length; 46.74mm breadth) on above mentioned media. Different isolates produced different growth on OMA also. Here Kalyani isolate produced maximum growth on this media. (50.55mm length; 50.30mm breadth) and minimum growth was observed on Alipurduar isolate (40.06mm length; 40.16mm breadth) and their difference was statistically significant.

On these media Kisanganj and DWR isolate produces similar type of length of colony diameter whereas the breadth of the colony diameter was different in between them (Table 1). Different isolates produced different type of growth on PDA medium also. DWR isolate produces maximum growth (62.26mm length; 61.80mm breadth) and minimum was obtained from Pundibari (31.21mm length; 30.98mm breadth), followed by Kisanganj isolate through their differences are statistically significant. Alipurduar isolate and Kalyani isolate produced similar length of colony growth (Alipurduar I1 42.11mm length; Kalyani 42.83mm length) though breadth of their growth was statistically different.

From this above interaction it was observed that DWR isolate produced maximum colony growth on PDA media (62.26mm length; 61.80mm breadth) followed by CA media by the same isolate (58.92mm length; 58.86mm breadth) and their difference was statistically significant. Minimum growth was obtained from Pundibari isolate on PDA followed by Kisanganj by same media through their difference was statistically significant. Similarly Alipurduar, Kisanganj and Pundibari isolate produced statistically significant. On 1day old culture produced maximum growth by Kalyani isolate (13.35mm length; 13.12mm breadth) and minimum by DWR (10.94mm length; 10.68mm breadth) though all the isolates showed no significant difference among themselves in respect to their growth on 1day old culture.2nd day old culture also produced different growth by different isolates. Maximum was obtained from DWR isolate (28.12mm length; 28.83mm breadth) and Minimum in (Kisanganj isolate 23.06mm length; 22.88mm breadth0 statistical at par with Pundibari (24.17mm length; 24.65mm breadth).

Though Alipurduar isolate and Kalyani isolate showed no significant difference in between them in respect to length and breadth of colony growth on 2nd day old culture. On 3rd day old culture maximum colony diameter was obtained from DWR isolate (51.33mm length; 51.22mm length) and minimum from Pundibari (34.31mm length; 34.36mm breadth) statistically at par with Kisanganj though Alipurduar isolate and Kalyani isolate showed no significant difference in between them in respect to their growth on 3 day old culture. DWR isolate also produced maximum growth on 4th day old culture and (66.40mm length; 65.8.mm breadth) whereas minimum growth was obtained from Kisanganj isolate (42.62mm length; 41.59mm breadth) followed by Pundibari (43.46mm length; 43.26mm breadth) though their difference was not statistically significant.

Alipurduar isolate and Kalyani isolate also produce similar type of growth pattern on 4th day culture also. On 5th day old culture maximum growth was also obtained by DWR isolate (75.22mm length; 73.40mm breadth) and minimum in Kalyani isolate (54.98mm length; 55.08mm breadth) and different was statistically significant. Though the isolates of Alipurduar, Pundibari and Kalyani produced statistically at par results in respect to growth pattern of 5th day old culture. Similar results was also produced on 6th day old culture that DWR isolate showed maximum length and breadth of the growth of the colony (76.94mm length; 76.56mm breadth). Here Alipurduar, Kisanganj, Pundibari showed no significance

among themselves in respect to their growth of colony of 6th day old culture. In 7th day old culture maximum growth was obtained from DWR isolate (80.75mm length; 79.90mm breadth) and minimum by Alipurduar isolate and their difference was statistically significant. Though Alipurduar isolate and Pundibari isolate showed no significant difference in between them in respect to their growth on 7th day old culture maximum growth was obtained from DWR isolate (80.75mm length; 79.90mm breadth) and minimum by Alipurduar isolate and their difference was statistically significant. Though Alipurduar isolate and Pundibari showed no significant difference in between them in respect to their growth on 7th day old culture also.

So, it was observed that with increase in the age of the growth of the colony. There is significance increase in length and breadth of the colony structure. Maximum growth was observed on 7th day growth culture irrespective of isolate and DWR isolate produce maximum growth above all isolates. Interaction between media, days and isolates produce different growth characteristics (length and breadth) and difference were statistically significant. All the isolates produced maximum growth in 7th old culture irrespective of media used. Maximum growth was obtained from DWR isolate on CA medium from 7th day old culture (85.10mm length; 84.73mm breadth) statistically at par with same isolate on 6th day old culture of same media and 7th day old culture from PDA media by the same isolate.

It was also observed that DWR is also produced statistically at par growth on 6th day old culture on PDA media and 5thday old culture on PDA media. Minimum growth was obtained from 1st day old culture of OMA media from Alipurduar, Pundibari and DWR isolates (7.10mm length; 9.40mmbreadth); (7.13mm length, 7.1\mm breadth) and (7.47mm length, 7.73mm breadth) respectively (Table 1; Fig. 1, 2 and 5). The analysis of the variances of growth on the basis of length and breadth of the colony of isolates of *Bipolaris sorokiniana* on different media after different days after inoculation were present in the Table 2 and 3.

Conidial structure (Length)

Different isolates produced different conidial structure (length and breadth) on different media and their different media and their difference were statistically significant. The length of conidia showed that media plays an significant role in respect to their growth. Maximum length of conidia was obtained on PDA media (71.33mm) followed by OMA (62.43mm) and difference were statistically significant and minimum length of conidia was obtained on CA media (38.08mm) followed by PCA media and difference were statistically significant. When isolates are considered maximum length of conidia was obtained from Alipurduar isolate (58.65mm) followed by DWR isolate (58.52mm) and Kisanganj isolate (57.89mm) and their differences were not statistically significant. Whereas minimum length was obtained from Kalyani (55.48mm) isolate statistically at par with Pundibari (56.45mm) isolate irrespective of different media. The interaction between Media and Isolate were also produced different conidial structure (length and difference were statistically significant. It was observed that all the isolates produced maximum length of conidia on PDA media and maximum was observed from Alipurduar isolate (72.19mm) on PDA media, statistically at par with the Kalyani isolate (71.88mm) followed by Pundibari isolate (71.96mm) and DWR isolate (71.21mm) and their difference were not statistically significant (Table 4; Fig. 3 and 4).

Table.1 Colony characters analysis of different isolates of *Bipolaris sorokiniana* in different media

Treatment	Length (mm)	Breadth (mm)
Media (M)		
M_1	43.82	43.53
M_2	48.47	48.00
M_3	45.07	44.59
M_4	42.61	41.84
SEm (±)	**0.330**	**0.425**
CD (P=0.05)	**0.919**	**1.183**
Days (D)		
D_1	12.06	12.07
D_2	26.77	26.75
D_3	39.56	39.88
D_4	49.43	49.45
D_5	57.46	56.58
D_6	62.75	60.99
D_7	66.94	65.71
SEm (□)	**0.436**	**0.563**
CD (P=0.05)	**1.214**	**1.567**
Isolates (I)		
I_1	42.80	43.00
I_2	40.64	39.53
I_3	41.00	40.19
I_4	55.95	55.34
I_5	44.58	44.39
SEm (±)	**0.369**	**0.475**
CD (P=0.05)	**1.027**	**1.322**

Interaction between media and days after inoculation

Treatment	Length (mm)	Breadth (mm)
Media (M)		
M_1	43.82	43.53
M_2	48.47	48.00
M_3	45.07	44.59
M_4	42.61	41.84
SEm (±)	**0.330**	**0.425**
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Days (D)		
D_1	12.06	12.07
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D_3	39.56	39.88
D_4	49.43	49.45
D_5	57.46	56.58
D_6	62.75	60.99
D_7	66.94	65.71
SEm (±)	**0.436**	**0.563**
CD (P=0.05)	**1.214**	**1.567**
Isolates (I)		
I_1	42.80	43.00
I_2	40.64	39.53
I_3	41.00	40.19
I_4	55.95	55.34
I_5	44.58	44.39
SEm (±)	**0.369**	**0.475**
CD (P=0.05)	**1.027**	**1.322**

Interaction between media and isolate

(M×I)		
M_1I_1	43.35	43.41
M_1I_2	39.31	38.23
M_1I_3	42.91	43.24
M_1I_4	55.74	54.64
M_1I_5	37.80	38.10
M_2I_1	44.69	47.50
M_2I_2	44.11	42.80
M_2I_3	47.49	44.11
M_2I_4	58.92	58.86
M_2I_5	47.15	46.74
M_3I_1	41.06	41.16
M_3I_2	44.49	43.01
M_3I_3	42.38	42.43
M_3I_4	46.88	46.04
M_3I_5	50.55	50.30
M_4I_1	42.11	39.94
M_4I_2	34.64	34.06
M_4I_3	31.21	30.98
M_4I_4	62.26	61.80
M_4I_5	42.83	42.41
SEm (±)	**0.737**	**0.591**
CD (P=0.05)	**2.052**	**1.645**

Interaction between days after inoculation and isolates

(D × I)		
D1I1		
	13.19	13.87
D_1I_2		
	11.17	10.93
D1I3	11.66	11.73
D_1I_4		
	10.94	10.68
D1I5	13.35	13.12
D_2I_1		
	28.61	28.83
D2I2	23.06	22.88
D_2I_3		
	24.17	24.65
D2I4	30.07	29.71
D_2I_5		
	27.93	27.67
D3I1	39.04	40.09
D_3I_2		
	34.43	33.93
D_3I_3	34.31	34.36
D_3I_4	51.33	51.22
D3I5	38.66	39.81
D_4I_1		
	47.14	48.63
D_4I_2	42.62	41.59
D_4I_3	43.46	43.62
D_4I_4	66.40	65.88
D_4I_5	47.52	47.54
D_5I_1	54.04	53.48
D_5I_2	49.68	48.43
D_5I_3	53.37	52.49
D_5I_4	75.22	73.40
D_5I_5	54.98	55.08
D_6I_1	57.15	57.24
D_6I_2	58.95	57.04
D_6I_3	58.24	53.12
D_6I_4	76.94	76.57
D_6I_5	62.48	60.97
D_7I_1	60.44	58.89
D_7I_2	64.55	61.86
D_7I_3	61.78	61.37
D_7I_4	80.75	79.90
D_7I_5	67.17	66.53
SEm (±)	**0.976**	**1.258**
CD (P=0.05)	**2.717**	**3.502**

Interaction between media, days after inoculation an isolates

(M × D × I)		
$M_1D_1I_1$	15.67	15.70
$M_1D_1I_2$	12.37	12.03
$M_1D_1I_3$	13.67	13.40
$M_1D_1I_4$	11.07	10.00
$M_1D_1I_5$	14.43	13.37
$M_1D_2I_1$	29.67	30.00
M1D2I2	21.37	22.00
$M_1D_2I_3$	26.47	26.70
$M_1D_2I_4$	29.17	28.37
$M_1D_2I_5$	27.47	25.70
$M_1D_3I_1$	38.40	39.37
$M_1D_3I_2$	31.43	32.53
M1D3I3	34.83	34.47
M1D3I4	48.73	48.77
$M_1D_3I_5$	34.80	37.73
$M_1D_4I_1$	47.10	49.10
M1D4I2	44.57	39.50
$M_1D_4I_3$	47.20	47.73
$M_1D_4I_4$	64.43	65.67
$M_1D_4I_5$	41.53	43.00
M1D5I1	53.10	53.03
$M_1D_5I_2$	45.13	47.10
$M_1D_5I_3$	57.10	56.33
$M_1D_5I_4$	83.07	77.60
$M_1D_5I_5$	46.83	47.33
M1D6I1	58.17	58.03
$M_1D_6I_2$	59.20	56.73
$M_1D_6I_3$	61.07	62.00
M1D6I4	76.53	74.67
$M_1D_6I_5$	49.90	49.83
$M_1D_7I_1$	61.33	58.67
M1D7I2	61.10	57.73
$M_1D_7I_3$	60.07	62.03
$M_1D_7I_4$	77.17	77.40
$M_1D_7I_5$	49.60	49.73
M2D1I1	13.10	13.73
$M_2D_1I_2$	10.70	10.03
$M_2D_1I_3$	13.40	13.00
$M_2D_1I_4$	13.43	13.00

Contd…

M2D1I5	13.40	14.03	
M2D2I1	31.07	33.33	
M2D2I2	23.20	22.50	
$M_2D_2I_3$	24.50	25.00	
$M_2D_2I_4$	32.57	32.37	
$M_2D_2I_5$	24.90	25.67	
$M_2D_3I_1$	44.83	49.00	
M2D3I2	32.90	34.53	
$M_2D_3I_3$	35.13	36.37	
M2D3I4	48.73	48.37	
$M_2D_3I_5$	35.30	36.67	
$M_2D_4I_1$	50.13	56.03	
$M_2D_4I_2$	45.13	43.67	
$M_2D_4I_3$	48.90	49.00	
M2D4I4	69.10	68.37	
M2D4I5	51.47	49.07	
M2D5I1	55.37	60.00	
$M_2D_5I_2$	53.67	52.90	
M2D5I3	61.23	60.47	
M2D5I4	80.43	79.83	
$M_2D_5I_5$	60.03	59.83	
$M_2D_6I_1$	57.23	60.03	
M2D6I2	66.83	63.03	
$M_2D_6I_3$	71.07	48.93	
M2D6I4	83.07	85.37	
$M_2D_6I_5$	70.43	65.57	
$M_2D_7I_1$	61.10	60.33	
M2D7I2	76.30	72.90	
$M_2D_7I_3$	78.20	76.03	
$M_2D_7I_4$	85.10	84.73	
$M_2D_7I_5$	74.50	76.33	
$M_3D_1I_1$	7.10	9.40	
M3D1I2	10.33	10.67	
$M_3D_1I_3$	7.13	7.17	
$M_3D_1I_4$	7.47	7.73	
$M_3D_1I_5$	14.17	14.03	
M3D2I1	24.47	23.37	
$M_3D_2I_2$	22.30	21.90	
M3D2I3	19.63	20.87	
$M_3D_2I_4$	21.60	21.67	

Contd….

M3D2I5	**30.10**	**30.67**
M3D3I1	34.67	34.53
M3D3I2	35.57	33.53
$M_3D_3I_3$	35.40	34.87
$M_3D_3I_4$	40.80	40.70
$M_3D_3I_5$	43.90	45.13
$M_3D_4I_1$	43.77	45.33
M3D4I2	42.20	44.33
$M_3D_4I_3$	42.33	43.00
M3D4I4	54.80	53.63
$M_3D_4I_5$	54.87	55.43
$M_3D_5I_1$	54.00	53.97
$M_3D_5I_2$	59.37	53.50
$M_3D_5I_3$	58.23	57.50
M3D5I4	58.17	57.37
M3D5I5	62.40	62.70
M3D6I1	58.57	58.50
$M_3D_6I_2$	66.17	65.00
M3D6I3	63.20	64.33
M3D6I4	68.10	66.20
$M_3D_6I_5$	69.97	69.47
$M_3D_7I_1$	64.87	63.03
M3D7I2	75.50	72.13
$M_3D_7I_3$	70.70	69.27
M3D7I4	77.23	75.00
$M_3D_7I_5$	78.47	74.67
$M_4D_1I_1$	16.90	16.63
M4D1I2	11.27	11.00
$M_4D_1I_3$	12.43	13.37
$M_4D_1I_4$	11.80	12.00
$M_4D_1I_5$	11.40	11.03
$M_4D_2I_1$	29.23	28.63
M4D2I2	25.37	25.13
$M_4D_2I_3$	26.07	26.03
$M_4D_2I_4$	36.93	36.43
$M_4D_2I_5$	29.23	28.63
M4D3I1	38.27	37.47
$M_4D_3I_2$	37.83	35.13
M4D3I3	31.87	31.73
$M_4D_3I_4$	67.07	67.03

Contd….

$M_4D_3I_5$	40.63	39.70	
$M_4D_4I_1$	47.57	44.03	
$M_4D_4I_2$	38.57	38.87	
$M_4D_4I_3$	35.40	34.73	
$M_4D_4I_4$	77.27	75.83	
$M_4D_4I_5$	42.20	42.67	
$M_4D_5I_1$	53.70	46.90	
$M_4D_5I_2$	40.53	40.23	
$M_4D_5I_3$	36.90	35.67	
$M_4D_5I_4$	79.20	78.80	
$M_4D_5I_5$	50.67	50.43	
$M_4D_6I_1$	54.63	52.40	
$M_4D_6I_2$	43.60	43.40	
$M_4D_6I_3$	37.63	37.20	
$M_4D_6I_4$	80.07	80.03	
$M_4D_6I_5$	59.60	59.00	
$M_4D_7I_1$	54.47	53.53	
$M_4D_7I_2$	45.30	44.67	
$M_4D_7I_3$	38.17	38.13	
$M_4D_7I_4$	83.50	82.47	
$M_4D_7I_5$	66.10	65.40	
SEm (±)	**1.591**	**2.516**	
CD (P=0.05)	**4.429**	**7.004**	

M_1	Potato Carrot Agar	D_1	day1	I_1	Alipurduar
M_2	Carrot Agar	D_2	day2	I 2	Kisanganj
M_3	Oatmeal Agar	D_3	day3	I_3	Pundibari
M_4	Potato Dextrose Agar	D_4	day4	I_4	DWR
		D_5	day5	I_5	Kalyani
		D_6	day6		
		D_7	day7		

Table.2 Analysis of variance of growth (length) of colony of isolates of *Bipolari sorokiniana* on different media after different days after inoculation

	Type III				
Source	Sum of Squares	df	Mean Square	F	Sig.
Media	2010.672	3	670.224	58.685	0.000
Days	145096.369	6	24182.728	$2.117E^{3}$	0.000
Media* Days	4778.207	18	265.456	22.243	0.000
Isolates	13437.688	4	3359.422	294.150	0.000
Media* Isolates	7183.695	12	598.641	52.417	0.000
Days* Isolates	5248.580	24	218.691	19.149	0.000
Media* Days* Isolates	4260.028	70	59.167	5.181	0.000
Error	3197.813	280	11.421	-	-
Total	1035470.070	420	-	-	-
Corrected total	185213.053	490	-	-	-

Table.3 Analysis of variance of growth (breadth) of colony of isolates of *Bipolaris sorokiniana* on different media after different days after inoculation

	Type III	df	Mean Square	F	Sig.
Source	Sum of Squares				
Corrected Model	173033.353[a]	139	1244.844	65.556	0.000
Intercept	831268.957	1	831268.957	$4.378E^{4}$	0.000
Media	2131.051	3	710.350	37.408	0.000
Days	136821.194	6	2283.532	$1.201E^{3}$	0.000
Media* Days	3820.893	18	212.272	11.179	0.000
Isolates	13690.886	4	3422.721	180.247	0.000
Media* Isolates	6835.008	12	569.584	29.995	0.000
Days* Isolates	5504.759	24	229.365	12.079	0.000
Media* Days* Isolates	4229.562	72	58.7744	3.094	0.000
Error	5316.940	280	18.989	-	-
Total	1009619.250	420	-	-	-
hujCorrected total	178350.293	490	-	-	-

Table.4 Morphological structure (length of conidia in µm) of different isolates of *Bipolaris sorokiniana* on different growth media

Media (M)										
Isolates	**M_1**		**M_2**		**M_3**		**M_4**		**Mean**
(I) AL									
I_1	61.23		61.76		39.42		72.19		**58.65**
I_2	66.82		62.69		33.36		71.21		**58.52**
I_3	57.41		62.42		34.01		71.96		**56.45**
I_4	59.92		61.82		40.42		69.41		**57.89**
I_5	43.42		63.47		43.17		71.88		**55.48**
Mean	**57.76**		**62.43**		**38.08**		**71.33**		
	Media (M)			**Isolates (I)**				**M × I**	
SEm (±)	**0.411**			**0.459**				**0.918**	
CD (P=0.05)	**1.144**			**1.278**				**2.556**	

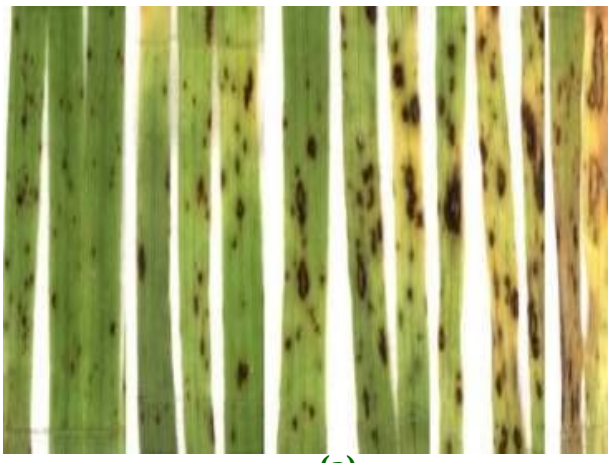
Table.5 Morphological structure (breadth of conidia in µm) of different isolates of *Bipolaris sorokiniana* on different growth media

Media (M) / Isolates (I)	M_1	M_2	M_3	M_4	Mean
I_1	22.80	22.64	21.88	21.20	**22.13**
I_2	18.28	17.80	21.75	22.05	**19.97**
I_3	18.71	19.97	18.65	21.66	**19.75**
I_4	20.11	21.67	21.21	21.18	**21.04**
I_5	14.64	21.81	20.17	20.69	**19.33**
Mean	**18.91**	**20.78**	**20.73**	**21.35**	
	Media (M)		**Isolates (I)**		**M × I**
SEm (±)	**0.253**		**0.282**		**0.565**
CD (P=0.05)	**0.704**		**0.785**		**1.573**

Table.6 Morphological structure (septations conidia) of different isolates of *Bipolaris sorokiniana* on different growth media

Media (M)					
Isolates	**M_1**	**M_2**	**M_3**	**M_4**	**Mean**
(I)					
I_1	5.87	4.27	4.07	6.27	**5.12**
I_2	6.60	3.53	3.07	6.80	**5.00**
I_3	5.33	4.47	3.73	5.93	**4.87**
I_4	5.47	4.67	3.73	7.07	**5.23**
I_5	4.60	4.20	4.00	7.67	**5.12**
Mean	**5.57**	**4.23**	**3.72**	**6.75**	
	Media (M)	**Isolates (I)**	**M × I**		
SEm (±)	**0.187**	**0.209**	**0.418**		
CD (P=0.05)	**0.521**	**NS**	**1.164**		

Fig.1 (a) *Cochliobolus sativus tritici* (b) Cochliobolus leaf spot caused by *Bipolaris sorokiniana.* (c) Typical symptoms of leaf blotch caused by *Bipolaris sorokiniana.* (d) Dark discolouration on infected subcrown internode of a matured plant



(a)

(b)

(c)

(d)

FIG:1: EFFECT OF MEDIA ON COLONY DIAMETER

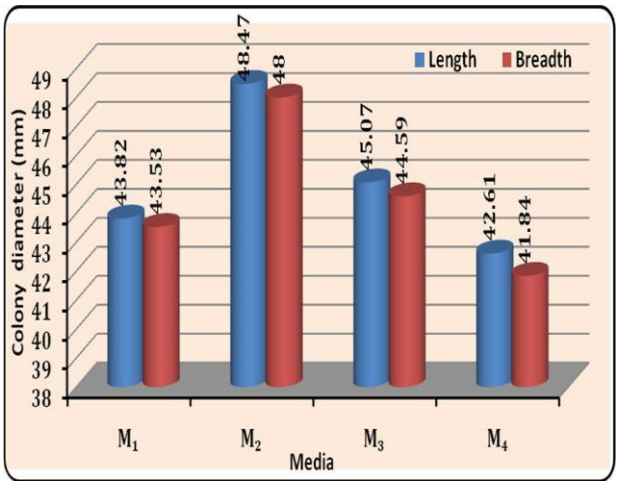


FIG:2: EFFECT OF DAYS ON COLONY

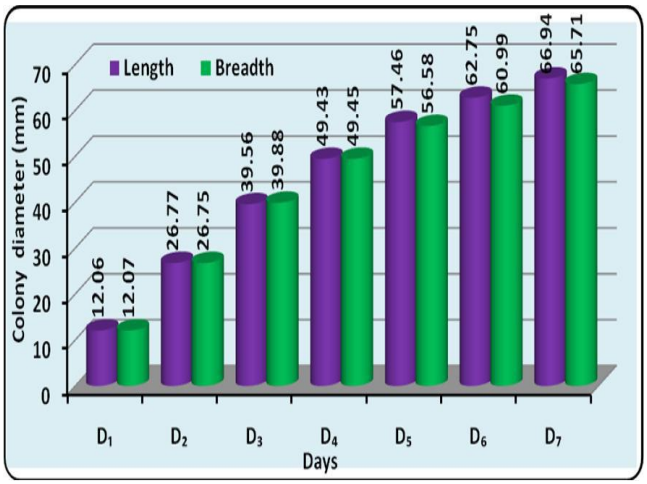


Fig.3 Effect media on spore morphology

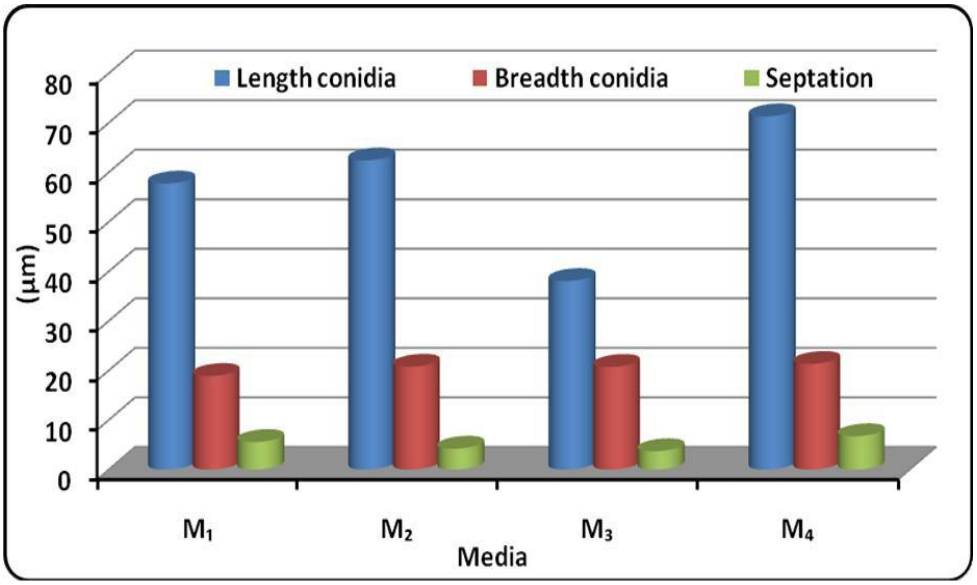


Fig.4 Effect of isolates on spore morphology

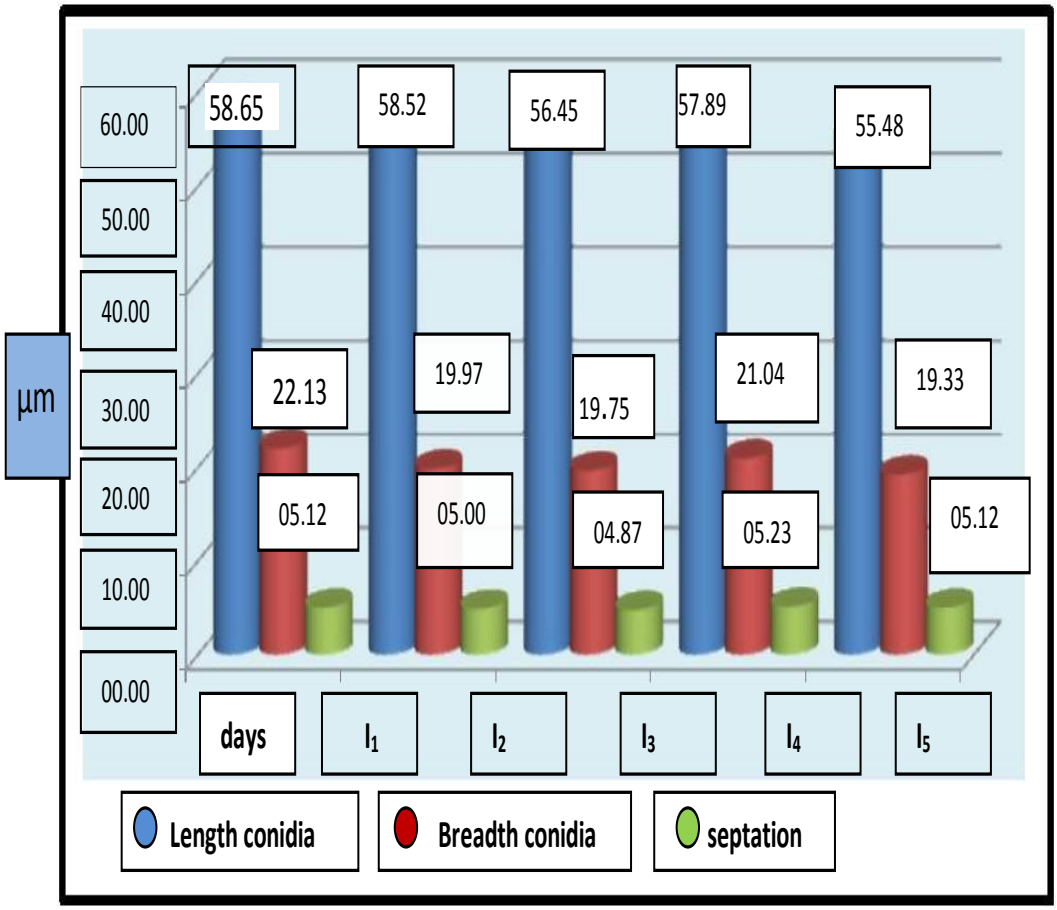


Plate.1 Morphological characteristics of spores (conidia) of different isolates of *Bipolaris sorokiniana* in PCA media. (A) I1 Alipurduar (B) I2DWR (C) I3 Pundibari (D) I4 Kisanganj (E) I5 Kalyani

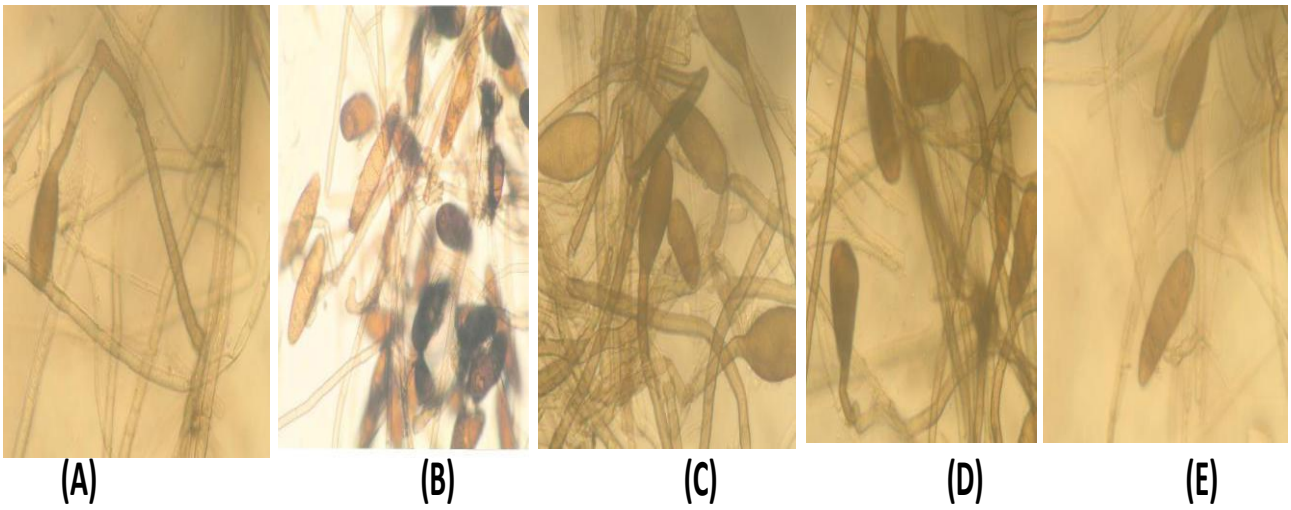
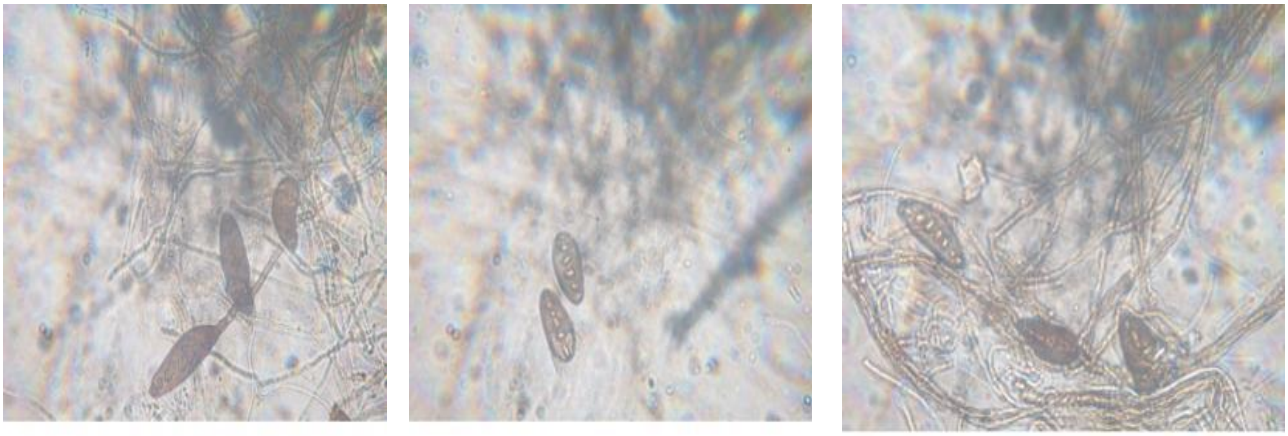
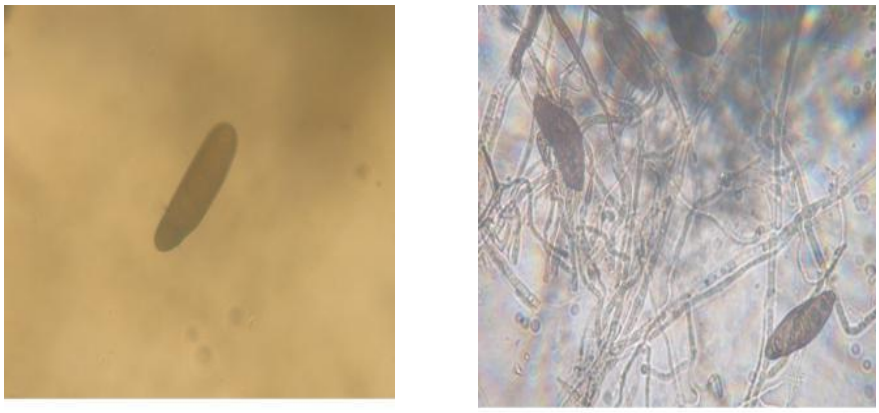


Plate.2 Morphological characterization of spores of different isolates of *Bipolaris sorokiniana* in OMA media. (A), (B) and (C) conidia of I1 isolate; (D) conidia of I2 isolate; (E) hypha and conidia of I3 isolate; (F) conidia of I4 isolate; (G) bipolar germination of the spore, I3 isolate; (H) spore of I5 isolate



(A) (B) (C)



(D) (E)

(F) (G) (H)

Plate.3 Morphological characterization of spores (conidia) of different isolates of Bipolaris sorokiniana in CA media (A) B1; (B) germtube emerging from the outerwall of conidia of B1 isolate; (C) conidiophores with conidia B1 isolate; (D) B2 isolate; (E) germtube emergence of B2 isolate; (F) conidiophores with conidia of B2 isolate; (G) B3 isolate with hypha; (H) and (I) B4 isolate (J), (K) and (L) conidia of B5 isolate

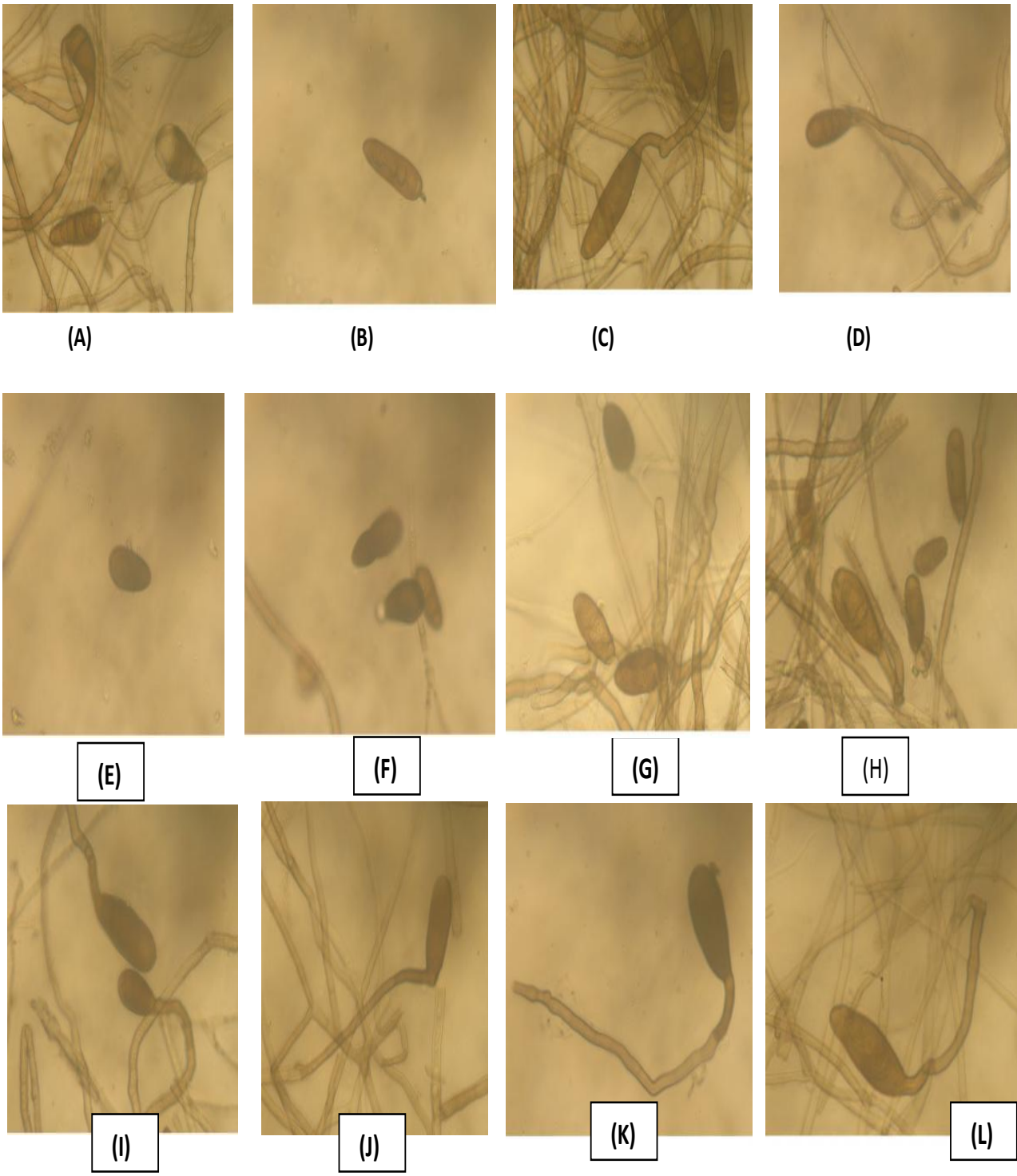
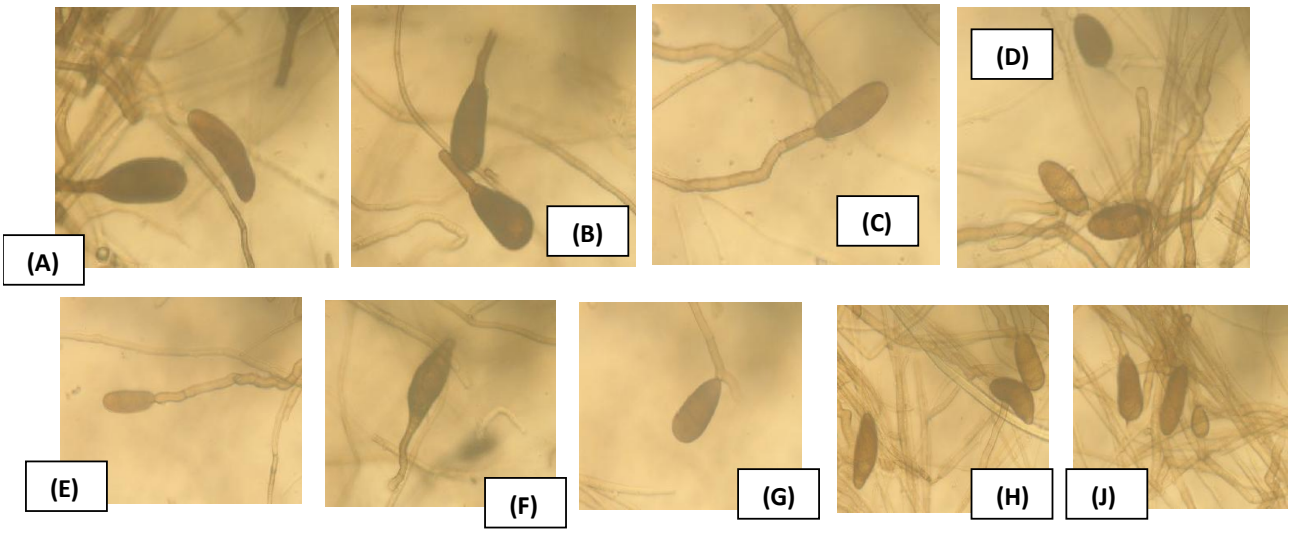


Plate.4 Conidial morphological characteristics in PDA media: (A) Alipurduar isolateI1 (B) DWR isolate I2 (C), (D) and (E) Pundibari isolate I3 (F) Kisanganj isolate I4 (G), (H) and (I) Kalyani isolate I5



Conidial structure (breadth)

The breadth of the conidia was also different on different media by different isolates and their difference was statistically significant. Different isolates produced different breadth of conidia irrespective of different media used and their difference was statistically significant. Maximum width of conidia was obtained on Alipurduar isolate (22.13mm) followed by Kisanganj isolate (21.04mm) and their difference was statistically significant. Whereas DWR, Pundibari and Kalyani isolate showed no significant difference among themselves in respect to width of conidia grown on different media. Among them 4 media maximum breadth of conidia was obtained on PDA media (21.35mm) statistically at par with OMA and CA medium irrespective of different isolates. Whereas minimum width of conidia was obtained on PCA media (8.91mm) The interaction between Media and Isolate in respect to the width of conidia also showed different results and their difference were statistically significantly. Maximum breadth of conidia was obtained on PCA media (22.80mm) from DWR isolate followed by OMA (22.64mm). Alipurduar isolate followed by PDA media (22.05mm) by DWR isolate and their difference was not statistically significant. Minimum breadth of conidia was obtained by Kalyani isolate on PCA media (17.80mm) and their difference was statistically different (Table 5; Fig. 3 and 4).

Conidial structure (Septation)

The septation of the conidia was also different on different media produced by different isolate and their differences were statistically significant. Among 5 isolates maximum septation was obtained from Kisanganj isolates (5.23mm) and minimum in Pundibari Isolate though the difference between septation among the isolates showed no statistical significant results. The septation of isolates among the 4 media were different and their difference was statistically significant. Maximum septation was obtained on PDA media (6.75mm) followed by PCA media (5.57mm) irrespective of isolates and their difference was statistically significant. Minimum septation was obtained from Carrot

Agar media (3.72mm) followed by OMA (4.23mm) and their difference were statistically significant. The interaction between Media and Isolate on septations were also statistically significant. Maximum septation was obtained from PDA media by Kalyani isolate (6.67mm) followed by Kisanganj isolate (7.07mm) on the same media. The isolate of Alipurduar and DWR also produce statistically at par septation on PDA media and also on PCA media. The minimum septation was located from CA media (3.73mm) by Kisanganj isolate and Pundibari isolate followed by the same isolate on OMA (Table 6; Fig. 3 and 4).

Variability among isolates of *Bipolaris sorokiniana* was determined based on morphological characters of conidia (Kumar *et al.*, 2002). The conidia of *Bipolaris sorokiniana* isolated from different agro-ecological regions of wheat and investigated their differences in conidial morphology and colony diameter on different media on different days after inoculation. The result showed that the isolates of *Bipolaris sorokiniana* produced similar type of symptoms when inoculated individually. The size of colony (length and breadth) was increased with increasing incubation period. Different media produces different growth characteristics (Colony diameter) on different media and Carrot Agar media produces highest colony diameter whereas minimum in Potato Dextrose Agar media in every days after inoculation among the four media used. It was also observed that all the isolates produced maximum growth in 7th old culture irrespective of media used. Maximum growth was obtained from DWR isolate on CA medium from 7th day old culture. Different isolates produced different type of conidial morphology on different media particularly length, breadth and septation of conidia. Among the four media PDA media produced maximum length, breadth and septation of the conidia (71.33×21.35 with 6.75 septation). Among the isolates Alipurduar isolate (I_1) produced maximum length, breadth and septation of the conidia (58.65µm, 22.13µm with 5.12 septation) irrespective of media used. So, it can be concluded from this experiment that *Bipolaris sorokiniana* isolates execute very few morphological variables among themselves. The most reliable technique is DNA technology but before using this technique the pathogenic aggressiveness of the isolates is most important criterion for future research work of this pathogen.

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How to cite this article:

Ankita Biswas and Srikanta Das. 2018. Morphological Characterization of *Bipolaris sorokiniana* Infecting Wheat. *Int.J.Curr.Microbiol.App.Sci.* 7(08): 225-248.
doi: https://doi.org/10.20546/ijcmas.2018.708.029