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Cultural characterization and cellulase activity of *Bipolaris sorokiniana* infecting wheat

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Abstract
Variability among isolates of *Bipolaris sorokiniana* was determined based on cultural characteristics. The pathogen was isolated from wheat host from different agro-climatic zones of West Bengal and grown on four different media for investigation of cultural chararacteristics and cellulase activity of this pathogen. The cultural variability showed different characters among the five isolates grown on different media like Potato Dextrose Agar, Carrot Agar, Oatmeal Agar and Potato Carrot Agar. The colour of the colony showed similar type of results on different type of media particularly whitish, greenish which was changed into dull green or dark green with increasing age of the fungal culture in every isolate with a few exception. Five isolates also produced thin to thick cottony growth in every media with a few exception that I_2 and I_5 produced fluffy growth particularly on PDA media. All the five isolates produced no zonation within the media with a few exception that Alipurduar (I_1) and Kisanganj (I_2) isolates produced concentric zonation particularly on PDA media and OMA after 6th days after inoculation. The cellulose activity was also different in isolates and maximum being observed in Alipurduar isolate (I_1) and minimum in Kalyani isolate (I_5).

Keywords: *bipolaris sorokiniana*, cultural characterization, colony colour, zonation, cellulase activity

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) [7] 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) [5]. 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) [4] 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) [5, 6]. 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 and management (Singh *et al*., 2007) [4], screening of varieties (Kumar *et al*., 2010) [2] 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.

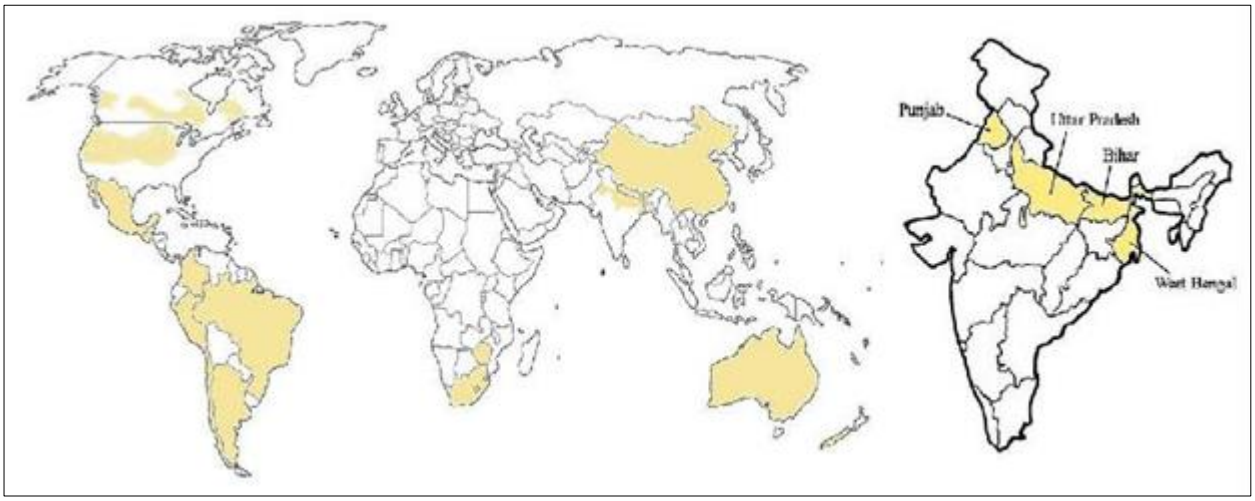

Fig 1

Fig 2

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 cultural 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 cultural and enzymatic levels.

Maintainance 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.

Most important extra-cellular enzymatic activity by *Bipolaris sorokiniana*: Cellulase activity

Bipolaris sorokiniana isolates were measured by culturing them with Carboxy Methyl Cellulase (CMC) medium. CMC was taken as the only source of carbon. Mycelial discs (5 mm) from the margin of actively growing 4-days old cultures were aseptically transferred to the centre of the petri-dish with culture media. It was then incubated for 3 days at 24°C. Each isolate was replicated thrice. After 3 days of incubation, the culture plates were flooded with 1% w/v of congo-red for 1 hr at room temperature and excess stain was discarded and the agar plate was thereafter, de-stained with 1 M of NaCl solution. Plates were kept overnight at 4°C and then examined for clear zone in substrate around the point of inoculations was compared with the control plate. The diameter of the clear zone was measured, recorded and documented. Cellulase activity was expressed as the ratio between clear zone to mycelial growth (Echandi,E and Walker J,1957).

Result and Discussion

Cultural variability

The variability of culture characters of 5 isolates of *Bipolaris sorokiniana* collected from different agro-climatic zones were grown on different media like PDA, CA, OMA, PCA and recorded their colony colour, mycellial growth, type of margin and zonation. The diversity in growth was studied on different days after inoculation after 7days of incubation. The result showed that the isolates of *Bipolaris sorokiniana* showed some different colony characters on different media. The colour of the colony showed differences on different media which was changed to white to dull white and green to dark green with the increasing days of fungal culture. (Table.7-10) In PDA media the 5 isolates produced white coloured colony on 1st day after inoculation. On 2nd day after inoculation the isolates I_1,$I_2$and I_3 produced dull green coloured whereas I_4 and I_5 produced dull green coloured except I_5 produces whitish dot like structures. I_4 and I_5 produced green coloured with white margin with some exception that I_5 isolate produced whitish dot like structure within the colony. 3rd day after inoculation I_1,I_3 and I_4 produced dark green coloured whereas I_2 produced dull green,I_5 produced dull white coloured colony. After 5th, 6th and 7th day after inoculation all the isolates have changed their colour to some extent from dark-green to dull-green changed whereas I_2 changed to dull green to dull white colour. (Table.10) Different types of the mycellial growth was also observed and it was observed that the mycellial growth was changed to thin to thick thread like structure with some exception that I_2 produces fluffy from 3rd day after inoculation to 7th day after inoculation. With the increasing age of the growth maximum isolate produced thick cottony growth like fungal structure except I_3 which are thin structure upto 7th day after inoculation. All the isolates produced circular margin upto 4th day of inoculation whereas after 5th day of inoculation I_1 produced irregular margin and other isolate produced circular one with a few exceptions. No zonation was observed on PDA media upto 7th day after inoculation except I_1 which produces concentric zonation after 7th day after inoculation. (Table10.) On OMA, all the isolates produced white-coloured colony upto 1st day after inoculation with the increasing days of culture.It was observed that with the increasing age of the white coloured colony changed into white to light green and dark green in colour with the increasing age of the culture, except I_5 produces dull white coloured colony from 2nd day after inoculation to 7th day after inoculation.(Table.9) The mycellial growth also was observed thin to thick in growth with increasing age of culture media except I_5 which are thin in growth upto 6th day of inoculation. The margin of the colony of each isolate showed circular margin from 1st day to 7th day after inoculation with a few exception like PDA no zonation was observed upto 7th day after each isolate except I_2 where concentric zonation was observed after 3rd day after inoculation. In CA media the colony colour of different isolates also showed the change in colony from white to dull white, light green to dark green in colour with increasing age of the culture growth. In every cases after 5th day all the isolates produced dull green to dark green coloured with few exceptions. On 7th day after inoculation it was also observed that the isolates I_2 and I_5 produced whitish dot with the colony. Mycellial growth also produced thin to thick cottony growth with increasing age of fungal culture observed in every isolates except I_2 where thick fluffy growth was observed. The margin of the colony were circular in all isolates from 1st day to 7th day except on the 6th day where maximum showed irregular margin except I_5, no zonation was observed upto 7th day after inoculation with a few exception (Table.8) The 5 isolates also produced different cultural characteristics on CA media also and it was observed that with increasing age of growth of culture media the colony colour was changed to dull-white to dull-green to dark-green colour upto 7th day after inoculation. Mycellial growth produces thin to thick cottony growth with a few exception that some isolate remain thin cottony growth upto upto 7th days after inoculation. The margin of the colony produced circular to irregular upto 2nd days after inoculation and after that all the isolates are circular in their margin except I_2 which showed irregular margin. It was also observed that on 6th after inoculation all the isolates produced irregular margin except I_5. No zonation was observed upto 6th days after inoculation whereas on 7th days after inoculation I_3 and I_5 produced concentric zonation. Therefore the results indicate that all the isolates were to some extent similar in their culture characteristics and their growth was thin to thick, white to dark green in colour, circular margin with no zonation. It was observed on all media tested for 5 isolates.

Table 7: Colony characteristics of different isolates of *Bipolaris sorokiniana* in pca media (m_1)

Day	Isolate	Colony colour	Mycelial growth	Margin	Zonation
1st	Alipurduar(I_1)	Dull white	Cottony	Circular	No
	Kisanganj(I_2)	Dull white	Thin thread like	Irregular	No
	Pundibari(I_3)	Dull white	Thin	Irregular	No
	DWR(I_4)	Dull white	Thin	Irregular	No
	Kalyani(I_5)	Dull white	Thin	Circular	No
2nd	Alipurduar(I_1)	Dull green	Thick	Circular	No
	Kisanganj(I_2)	Dull green	Thin cottony	Circular	No

	Pundibari(I_3)	Dull white	Thin cottony	Irregular	No
	DWR(I_4)	Dull green	Thick	Irregular	No
	Kalyani(I_5)	Dull white	Thin thread	Circular	No
3rd	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dark green	Thick cottony	Circular	No
	Pundibari(I_3)	Dull green	Thick cottony	Circular	No
	DWR(I_4)	Dull green	Thick	Circular	No
	Kalyani(I_5)	Dull green	Thin thread like	Circular	No
4th	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dark green	Fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thin	Circular	No
	DWR(I_4)	Dark green	Thick	Circular	No
	Kalyani(I_5)	Dull green	Thin cottony	Circular	No
5th	Alipurduar(I_1)	Dark green	Thick cottony	Circular	No
	Kisanganj(I_2)	Dull white	Fluffy with white margin	Circular	No
	Pundibari(I_3)	Dull green	Thin cottony	Circular	No
	DWR(I_4)	Dark green	Thick	Circular	No
	Kalyani(I_5)	Dull green	Thick	Circular	No
6th	Alipurduar(I_1)	Dark green	Thick	Irregular	No
	Kisanganj(I_2)	Dark green	Thin thread	Irregular	No
	Pundibari(I_3)	Dark green	Thin cottony	Irregular	No
	DWR(I_4)	Dark green	Thick	Irregular	No
	Kalyani(I_5)	Dark green	Thin thread	Circular	No
7th	Alipurduar(I_1)	Dark green	Thick	Irregular	No
	Kisanganj(I_2)	Dull white	Thick fluffy	Irregular	No
	Pundibari(I_3)	Dark green with white dots	Thin cottony	Circular	Concentric zonation
	DWR(I_4)	Dark green with white margin	Thin cottony	Circular	No
	Kalyani(I_5)	Dull green with white dots	Thin cottony	Circular	Concentric zonation

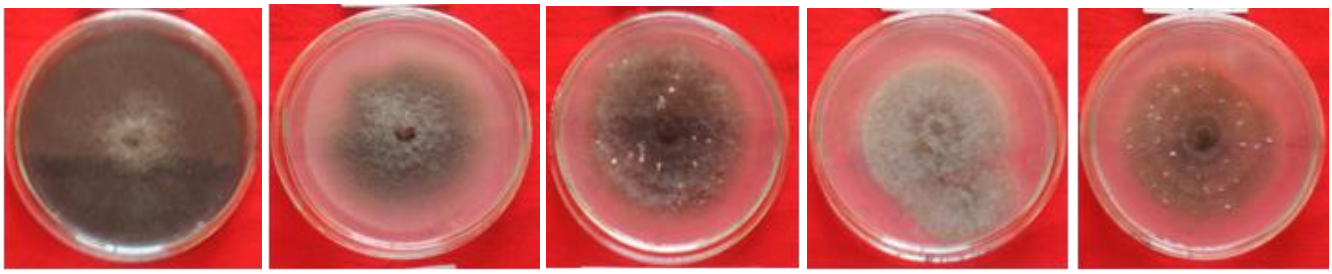

Fig 3

Table 8: Colony characteristics of different isolates of *Bipolaris sorokiniana* in ca media (M_2)

Day	Isolate	Colony colour	Mycelial growth	Margin	Zonation
1st	Alipurduar(I_1)	white	Thin	Circular	No
	Kisanganj(I_2)	Dull white	Thin	Irregular	No
	Pundibari(I_3)	Dull white	Thin	Circular	No
	DWR(I_4)	White	Thin	Circular	No
	Kalyani(I_5)	white	Thin	Circular	No
2nd	Alipurduar(I_1)	Light green	Thin	Circular	No
	Kisanganj(I_2)	Dull white	Thin thread like	Circular	No
	Pundibari(I_3)	Light green	Thin	Circular	No
	DWR(I_4)	Dull white	Thin	Circular	No
	Kalyani(I_5)	Light green	Thin	Circular	No
3rd	Alipurduar(I_1)	Light green	Thick	Circular	No
	Kisanganj(I_2)	Dull white	Thin cottony	Circular	No
	Pundibari(I_3)	Dark green	Thick cottony	Circular	No
	DWR(I_4)	Dull green	Thin cottony	Circular	No
	Kalyani(I_5)	Light green	Thin thread like	Circular	No
4th	Alipurduar(I_1)	Light green	Thin thread	Circular	No
	Kisanganj(I_2)	Dull white	Cottony	Circular	No
	Pundibari(I_3)	Greenish	Thin thread	Circular	No
	DWR(I_4)	Dull green	Thin thread	Circular	No
	Kalyani(I_5)	Dull green	Thin thread	Circular	No
5th	Alipurduar(I_1)	Dark green	Fluffy	Circular	No
	Kisanganj(I_2)	Dull white	Fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thick	Circular	No
	DWR(I_4)	Dull green	Thick	Circular	No
	Kalyani(I_5)	Dull green	Thin thread	Circular	No
6th	Alipurduar(I_1)	Dark green	Thin	Irregular	Concentric zonation

	Kisanganj(I_2)	Dull green	Thick	Irregular	No
	Pundibari(I_3)	Dull green with whitish dot	Thick fluffy	Circular	No
	DWR(I_4)	Dull green	Thick fluffy	Circular	No
	Kalyani(I_5)	Dull green with whitish dot	Thick	Circular	No
7th	Alipurduar(I_1)	Dark green	Thin	Irregular	No
	Kisanganj(I_2)	Dull green	Thick fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thick	Circular	No
	DWR(I_4)	Dark green	Thick cottony	Circular	No
	Kalyani(I_5)	Dull green	Thin	Circular	No

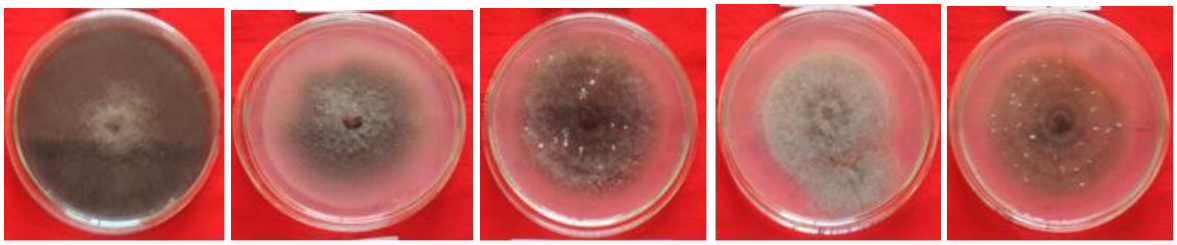

Fig 4

Table 9: colony characteristics of different isolates of *Bipolaris sorokiniana* in oma media (M_3)

Day	Isolate	Colony colour	Mycelial growth	Margin	Zonation
1st	Alipurduar(I_1)	white	Thin	Circular	No
	Kisanganj(I_2)	white	Thin	Irregular	No
	Pundibari(I_3)	white	Thin	Circular	No
	DWR(I_4)	white	Thin	Circular	No
	Kalyani(I_5)	white	Thin	Circular	No
2nd	Alipurduar(I_1)	green	Cottony Thin	Circular	No
	Kisanganj(I_2)	Light green	Thin	Circular	No
	Pundibari(I_3)	Light green	Thin	Circular	No
	DWR(I_4)	Light green	Thin	Circular	No
	Kalyani(I_5)	Light green	Thin thread like	Circular	No
3rd	Alipurduar(I_1)	greenish	Thin thread	Circular	No
	Kisanganj(I_2)	Dark green	Thin cottony	Circular	Concentric zonation
	Pundibari(I_3)	white	Thin	Circular	No
	DWR(I_4)	White	Thin	Circular	No
	Kalyani(I_5)	Dull white	Thin cottony	Circular	No
4th	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dull green	Thick	Circular	No
	Pundibari(I_3)	Dark green	Thin thread	irregular	No
	DWR(I_4)	Dark green	Thin	Circular	No
	Kalyani(I_5)	Dull white	Thin cottony	Circular	No
5th	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dark green	Thick fluffy	Circular	Concentric zonation
	Pundibari(I_3)	Light green	Thin	Circular	No
	DWR(I_4)	Dark green	Thin	Circular	No
	Kalyani(I_5)	Dull white	Thin	Circular	No
6th	Alipurduar(I_1)	Dark green	Thick	Irregular	Concentric zonation
	Kisanganj(I_2)	Dark green	Thick cottony	circular	Concentric zonation
	Pundibari(I_3)	Dark green	Thick fluffy	Circular	No
	DWR(I_4)	Dark green	Thin	Circular	No
	Kalyani(I_5)	Dull white	Thick	Circular	No
7th	Alipurduar(I_1)	Dark green	Thick	Irregular	No
	Kisanganj(I_2)	Dull green with white dots	Thick cottony	Circular	Concentric zonation
	Pundibari(I_3)	Dark green	Thin with white margin	irregular	No
	DWR(I_4)	Dark green	Thin	Circular	No
	Kalyani(I_5)	Dull green	Thick	Circular	No

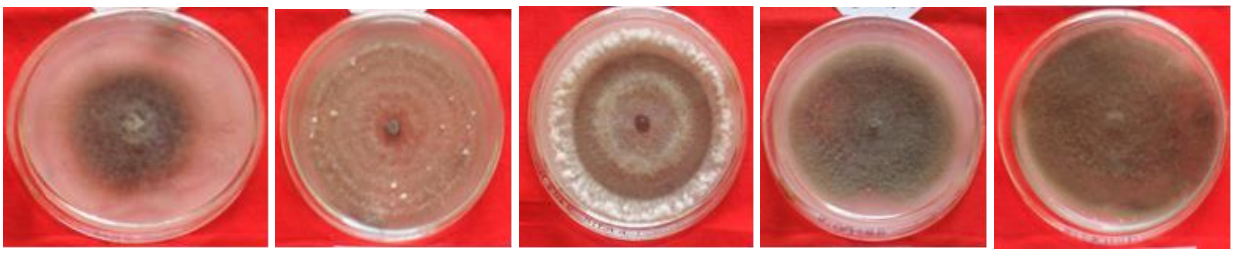

Fig 5

Table: 10: Colony characteristics of different isolates of *Bipolaris sorokiniana* in PDA media (M_4)

Day	Isolate	Colony colour	Mycelial growth	Margin	Zonation
1st	Alipurduar(I_1)	white	Thin	Circular	No
	Kisanganj(I_2)	white	Thin thread	Circular	No
	Pundibari(I_3)	white	Thin	Circular	No
	DWR(I_4)	white	Thin	Circular	No
	Kalyani(I_5)	white	Thin	Circular	No
2nd	Alipurduar(I_1)	Dull green	Thick	Circular	No
	Kisanganj(I_2)	Dull green	Thick cottony	Circular	No
	Pundibari(I_3)	Dull green	Thin	Circular	No
	DWR(I_4)	Dark green with white margin	Thin thread	Circular	No
	Kalyani(I_5)	Green with white margin	Thin thread like	Circular	No
3rd	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dull green	Fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thick	Circular	No
	DWR(I_4)	Dark green	Thick	Circular	No
	Kalyani(I_5)	Dull white	Cottony	Circular	No
4th	Alipurduar(I_1)	Dark green	Thick	Circular	No
	Kisanganj(I_2)	Dark green	Fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thick	Circular	No
	DWR(I_4)	Dark green	Thick cottony	Circular	No
	Kalyani(I_5)	Dull white	Fluffy	Circular	No
5th	Alipurduar(I_1)	Greenish with white margin	Fluffy	Irregular	No
	Kisanganj(I_2)	Dull white	Fluffy	Circular	No
	Pundibari(I_3)	Dark green	Thin	Circular	No
	DWR(I_4)	Dark green	Thick	Circular	No
	Kalyani(I_5)	Dark green	Thick	Circular	No
6th	Alipurduar(I_1)	Dull green	Cottony	Irregular	Concentric zonation
	Kisanganj(I_2)	Dull white	Fluffy	Circular	No
	Pundibari(I_3)	Dull green	Thin	Circular	No
	DWR(I_4)	Dark green	Fluffy	Circular	No
	Kalyani(I_5)	Dull green	Thick cottony	Circular	No
7th	Alipurduar(I_1)	Dull green	Thick cottony	Irregular	Concentric zonation
	Kisanganj(I_2)	Dull white	Fluffy	Circular	No
	Pundibari(I_3)	Dull green with less amount of whitish dots.	Thin	Irregular	Concentric zonation
	DWR(I_4)	Dark green	Thick	Circular	No
	Kalyani(I_5)	Dark green with whitish dots	Thick	Circular	No

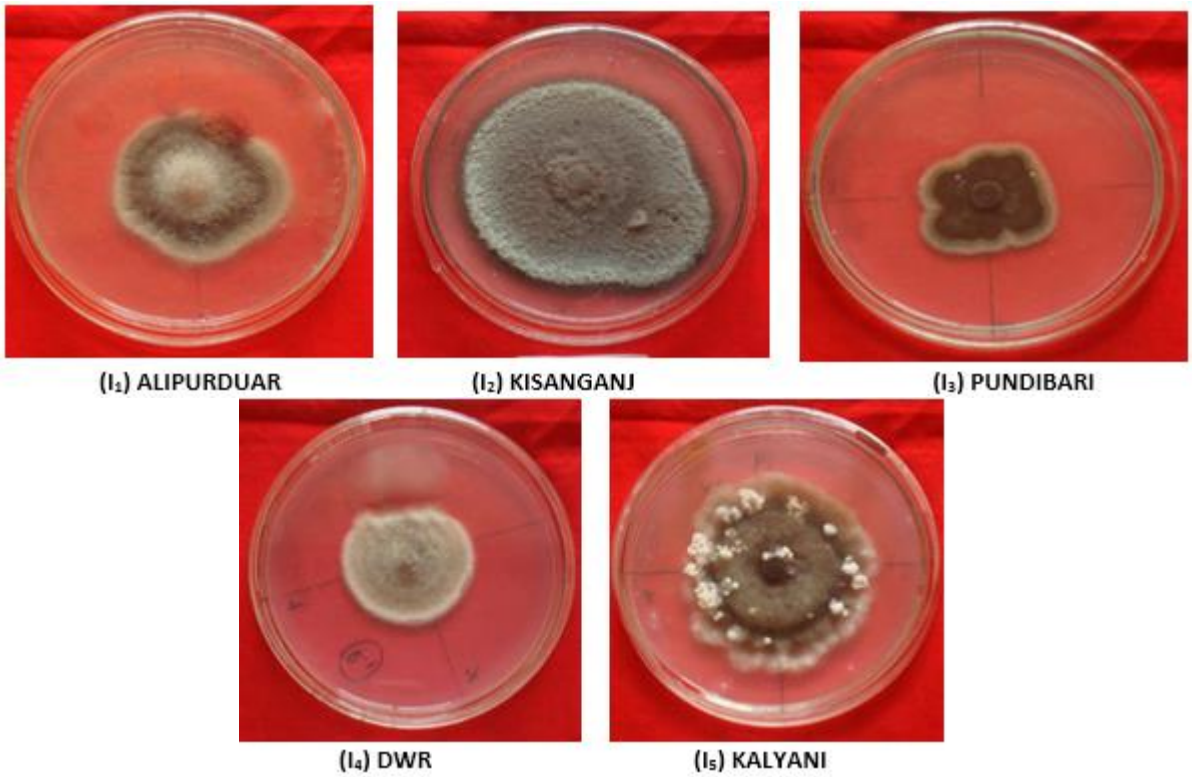

Plate 1: Colony characteristics of different isolates Of *Bipolaris sorokiniana* in pda media (M4)

Cellulase activity

The pathogen *Bipolaris sorokiniana* also functioned several enzymatic activities to cause disease to the host. According to the speed of the enzymatic activity the aggressiveness of the pathogen can be determined. Here only cellulose activity of 5 isolates were investigated and it was observed that Alipurduar isolate showed maximum cellulase activity (1.55) and minimum in Kalyani isolate(I_5)(0.1).This cellulose production was variable according to the carbon source.(Table.11) (Fig. 6).

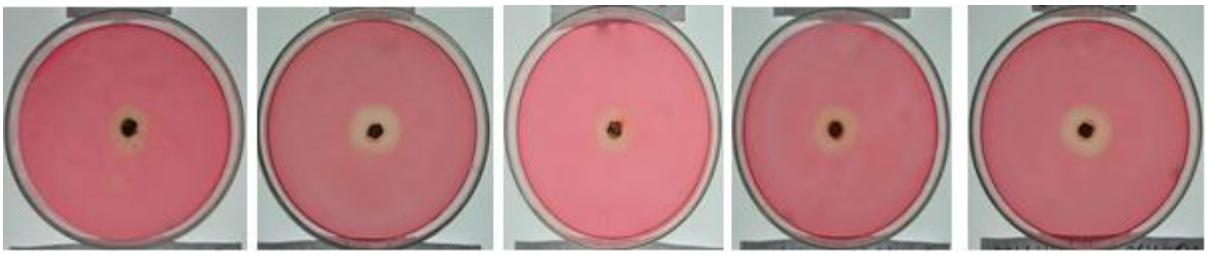

Plate 2: Cellulase activity of different isolates of *Bipolaris sorokiniana* in PDA media

Table 11: Cellulose activity of different isolates of *Bipolaris sorokiniana* in PDA media

Isolate	Measurement of mycelial disc (mm) Length/breadth	Clear zone(mm) Length/breadth	Cellulose activity (measurement of mycelia disc/measurement of clear zone)
Alipurduar(I_1)	7.0/8.0	20.0/18.0	1.55
DWR(I_2)	7.0 /8.0	20.1/24.1	0.12
Pundibari(I_3)	7.0/ 8.0	20.1/22.1	0.13
Kisanganj(I_4)	7.0 / 8.0	22.1/20.0	0.126
Kalyani(I_5)	7.0/8.0	23.1/24.1	0.10

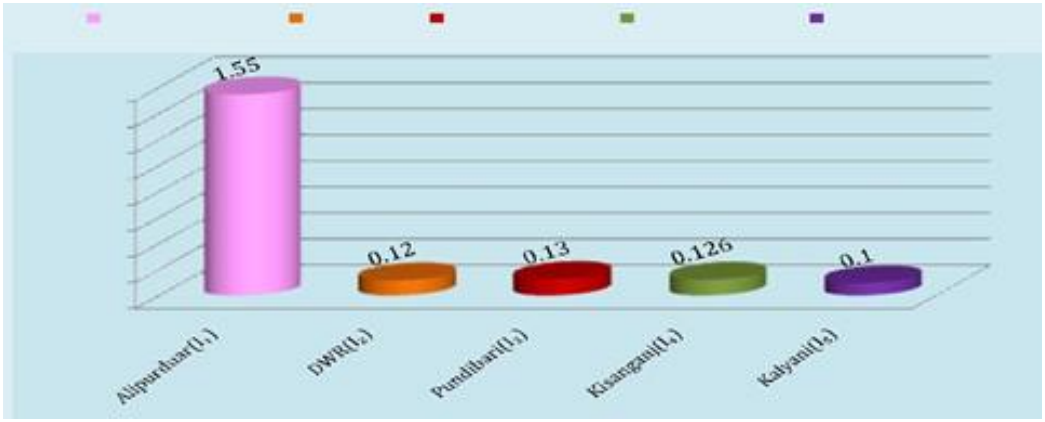

Fig 6: Effect of cellulase activities on different isolates in PDA media

Conclusion

Variability among isolates of *Bipolaris sorokiniana* was determined based on cultural characteristics (Kumar *et al*, 2002) [2] and their cellulase activity. The pathogen was isolated from different agro-ecological regions of wheat and investigated their differences in their cultural characteristics like colony colour, type of margin and zonation 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 cultural variability showed different characters among the five isolates grown on different media like Potato Dextrose Agar, Carrot Agar, Oatmeal Agar and Potato Carrot Agar. The colour of the colony showed similar type of results on different type of media particularly whitish, greenish which was changed into dull green or dark green with increasing age of the fungal culture in every isolate with a few exception. Five isolates also produced thin to thick cottony growth in every media with a few exception that I_2 and I_5 produced fluffy growth particularly on PDA media. All the five isolates produced no zonation within the media with a few exception that Alipurduar (I_1) and Kisanganj (I_2) isolates produced concentric zonation particularly on PDA media and OMA after 6th days after inoculation. The cellulase activity was also different in different isolates and maximum being observed in Alipurduar isolate (I_1) and minimum in Kalyani isolate (I_5). The cellulase activity was also different in different isolates and maximum being observed in Alipurduar isolate (I_1) and minimum in Kalyani isolate (I_5). So, It can be concluded from this experiment that *Bipolaris sorokiniana* isolates execute very few morphological and cultural variability 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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