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Dispersal of airborne pathogenic conidia of *Bipolaris oryzae* inciting brown spot disease of paddy in West Bengal, India

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Abstract

Brown spot (BS) is one of the most damaging diseases of paddy observed from seedling stage in nursery to milky stage in main field, caused by the pathogenic fungus *Bipolaris oryzae* (BO, Syn: *Drechslera/ Helminthosporium oryzae*; Sexual stage: *Cochliobolus miyabeyanus*). A two-year (2016 and 2017) aerobiological survey was carried out continuously in a paddy field of 24 Parganas (North) district in the lower Gangetic basin of West Bengal using Burkard 7-day volumetric sampler. The seasonal variation, diurnal periodic pattern and vertical profile (in peak period upto 5 m height using rotorod sampler) of airborne conidia of pathogenic conidia were recorded. The BS disease progress in the crop was recorded with time in field condition. Conidia of *Bipolaris oryzae* were found throughout the year in the air with two peaks, one during April-May (<24 conidia/d/m^3 air) and another in October-November (<28 conidia/d/m^3 air), just after the harvest of dry and wet season crops respectively. The occurrence of BO conidia was compared with airborne fungal conidia of *Nigrospora oryzae* (NO), which causes minor opportunistic infection in rice, producing minute leaf spots. Airborne BO conidia showed no correlation with meteorological parameters, whereas relative humidity had significant correlation with airborne NO conidia. Disease development analyses of both the dry and wet season crops revealed that both the leaf and grain infections had positive and significant correlation with airborne BO conidia ($p<0.1-0.001$), which could be expressed with fitted regression models. In case of NO conidia, no such regression models could be fitted. Comparative study with airborne small-sized NO conidia, with only <4 per cent leaf infection, clearly indicated that airborne BO conidia were potential threats to rice crop, which might also induce primary infection in field condition. Airborne BO conidia depicted a bimodal diurnal pattern with two maxima at morning (8.00 AM, 5.5 conidia/h/m^3 air) and afternoon (15.00 PM, 4.0 conidia/h/m^3 air). It had the maximum level of concentration at one metre height (2.5-10 conidia/h/m^3 air) over crop canopy.

Key words: Airborne *Bipolaris oryzae* conidia, Brown spot of rice, Disease development, *Nigrospora oryzae*.

Introduction

Rice (*Oryza sativa* L., Family: Poaceae) is one of the most important food crops of the world. India is the second largest rice producing country (104.32 million tonnes during 2015-16 period) in the world (Ministry of Agriculture & Farmers' Welfare, Government of India, 2016). More than 50 per cent of the global population is dependent on rice as their staple food. However, this crop is prone to many diseases, some of which causing sudden and widespread damage in the form of epidemics (Saha et al., 2020).

Bipolaris oryzae (Breda de Haan) Shoemaker [Synonym: *Helminthosporium oryzae* Breda de

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Haan; *Drechslera oryzae* (Ito & Kuribayashi) Drechsler], the anamorph of *Cochliobolus miyabeanus* (Ito & Kuribayashi) is the fungal pathogen of brown spot (BS) disease of paddy, which affects the crop with a relative yield reduction of 4-52 per cent every year (Barnwal et al., 2013). This disease was associated with two major epidemics (during 1918-19 in Krishna-Godavari delta and 1942 in undivided Bengal) in India (Chakrabarti, 2001). In lowlands of tropical and subtropical Asia, BS disease was reported to cause approximately 10 per cent loss of attainable yield (Savary et al., 2012). Hence, BS is considered as one of the yield reducers among rice crop diseases in present scenario.

Primary infection of BS is reported to be seed-borne (Sharma and Maheswari, 1982; Damicone et al., 2001), while secondary infection generates from wind-borne inocula from other infected plants and debris (Sato et al., 2008). Soil and some weed hosts also often serve as inocula reservoirs (Biswas et al., 2008). Overall aerobiological studies from rice crop field revealed that *Bipolaris/Drechslera oryzae* frequently contributes 10.63-14.58 per cent of the total airborne fungal spora (Chakraborty et al., 2003). Uddin (2004) conducted an aerobiological survey on aeromycoflora by culture plate exposure for dry season rice crop and recorded viable spore counts upto 444 CFU during harvesting. However, little specific information is available on the dispersal of pathogenic conidia of BS disease in the air of rice crop field and its relation with disease development.

The present study had the objective to survey the dissemination pattern of the pathogenic propagule of BS disease of rice plants. Keeping this in view, monitoring was done for a dry season (Boro: January - May) and a wet season (Aman: July - October) crop in each year (2016-17), along with the disease progress. The diurnal periodicity and vertical profile of the pathogenic propagule were also observed. The dynamics of the airborne pathogen was compared with a small sized, frequent and non-pathogenic airborne spore type, *Nigrospora ozyzae*, causing minor opportunistic infections (Pinciroli et al., 2013; Ravat and Basu, 2017).

Material and Methods

Aerobiological survey with Burkard 7-day volumetric sampler in paddy field

A continuous aerobiological survey was carried in a paddy field at Madhyamgram (22º53^{1}223 N, 88º28^{1}293 E), a suburban area, 19 km north to the city of Kolkata (Figure 1a-b) for two years (2016 and 2017) using Burkard 7-day automatic

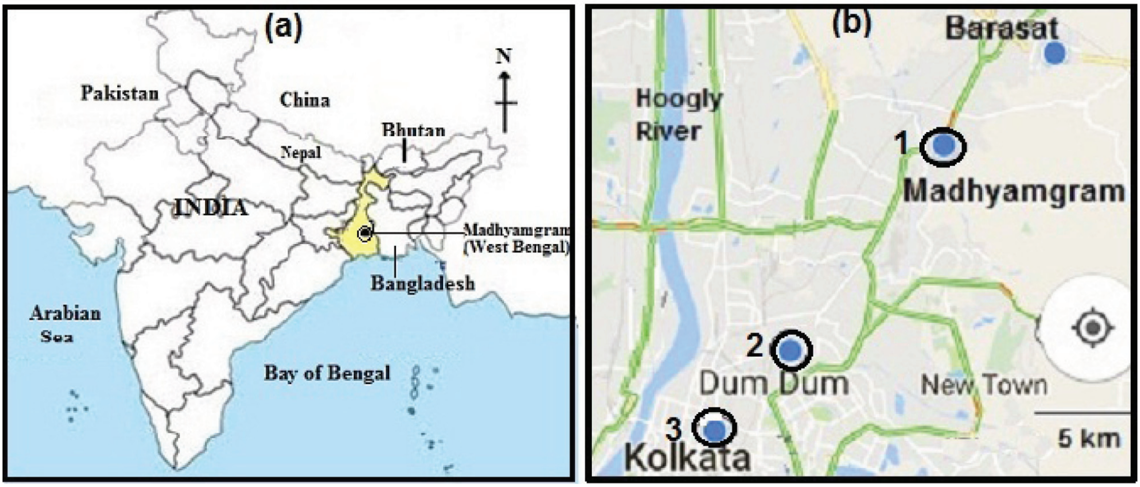

Figure 1(a-b). Location of aerobiological sampling in a paddy field at Madhyamgram, (1), meteorological station at Dumdum (2) and Kolkata city (3) of the state of West Bengal, India volumetric sampler (Burkard Manufacturing Co., U.K). The sampler was placed at 0.5 m height at the centre of a 20 X 20 m^2 rice crop field (Figure 2), in the agricultural area of Madhaymgram, North 24 Parganas district, West Bengal. There were four adjacent 20 X 20 m^2 plots of the same crop, adjacent to the study plot on its four sides. On the west of the rice plot, there was a plantation of *Cocos nucifera* L. (coconut) at 20 m distance from the plot. Moreover, there was a mixed vegetation of *Cynodon dactylon,Eleusine* sp.and *Imperata cylindrica* (Poaceae)*, Amaranthus spinosus* (Amaranthaceae)*,* and *Parthenium hysterophorus* (Asteraceae), in the space between the paddy and the coconut plantation (Figure 2).

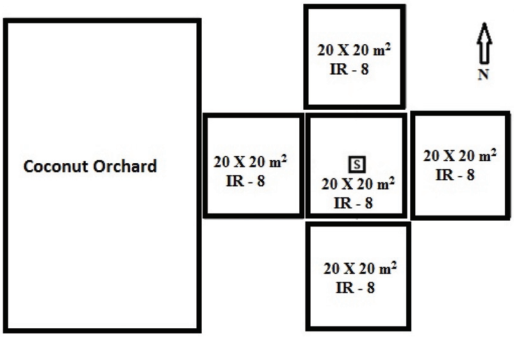

Figure 2. Schematic representation of the location of 7-day volumetric sampler (S) in the crop field planted with IR-8 variety of rice

The variety of rice planted was IR-8, a high yielding popular type, reported to be susceptible to BS disease (Vidyasekaran et al., 1990) for both dry (local name 'Aman') and wet seasons (Boro) crop (Table 1). Prior to the study, baseline aerobiological survey was conducted in the same field for the year 2015, when there was no crop. In the baseline study in the same field, presence of BO conidia was recorded in air in the concentration range of 0-1.9 conidia/day/cubic meter air with same spore trapping device, which may have arisen from soil and debris and paddy fields of locality. No fungicide or manure was used, but insecticides and herbicides were applied when required. In each crop, surface sterilized (0.1% $HgCl_2$ solution) healthy seeds were used for cultivation to rule out the chance of any other contamination from seed surface and to get the focus on airborne pathogen. After three weeks of germination, healthy seedlings were selected from seed bed for transfer to the field. After transfer (13-16 days for wet season and 78-79 days for dry season crop), the seedlings were found to be affected with BS. Dark brown, oval to sesame shaped spots appeared on the upper surface of the leaves (Figure 3). A fully developed lesion (4-5 mm size) appeared to have a greyish brown central zone surrounded by deep reddish brown margin. Dark brown/black oval spots were noticed on the grains, sometimes covering whole grain surface (Figure 3) as lesions at final stage of the crop.

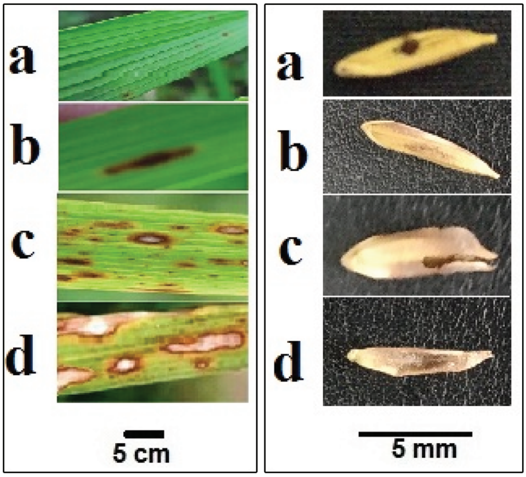

Figure 3. Brown spot disease manifestation on *Oryza sativa* leaves (left) and grains (right) from early (a) to late stage (d) before harvesting

Study of disease progress in the crop field

Disease progress in the crop was examined by the fraction of surface area of leaf, stem and when appropriate, the inflorescence and grains with BS disease symptoms. The growth stages of the plants were recorded weekly after transplanting following decimal code (Zadoks et al., 1974; Tottman, 1987) along with BS disease measurement over the plant surface area (Aluko, 1970; Nyval and Percich, 1999). In each case, infected plants (n = 5 X 5 = 25) were sampled from the four sides and centre of the crop field and the average was recorded.

Identification of pathogen

For pathogen identification, pieces of infected leaf with lesions (surface sterilized with 0.1% $HgCl_2$ solution) were placed on an autoclaved moist filter paper (Whatman No.1) in 9 cm diameter sterile Petridishes (in triplicates), at 25°C in a B.O.D. incubator and the growth was examined after 11-15 days for *Bipolaris oryzae*, using a compound microscope (Motlagh and Kaviani, 2008). Culture growth of the pathogen was carried out by placing the inocula in nutrient media [2% Potato Dextrose Agar (PDA) and Rice Leaf Extract (RLE) medium]. The identification was based on characteristics of the colony,mycelia, conidiophores, conidia and conidial germination (Kumar et al., 2016; Valamarthy and Ladhalakshmi, 2018). The morphological microscopic identification was confirmed by Agharkar Research Institute, Pune.

Study of seasonal periodicity of airborne Bipolaris conidia and Nigrospora spores

Daily and hourly occurrence of trapped airborne pathogenic conidia was microscopically recorded following standard guidelines (The British Aerobiology Federation, 1995; Lacey and West, 2006). The seasonal variation of pathogenic BO conidia [Fig. 4 (i&ii)] was compared with the occurrence of another comparatively small-sized, weakly-pathogenic and frequent spore of *Nigrospora oryzae* throughout the study period. Identification of *Nigrospora oryzae* was confirmed by the microscopic morphological examination of colony, mycelia, sporulation and spores in the exposed petriplates with PDA culture media, which was confirmed by Agharkar Research Institute, Pune, India.

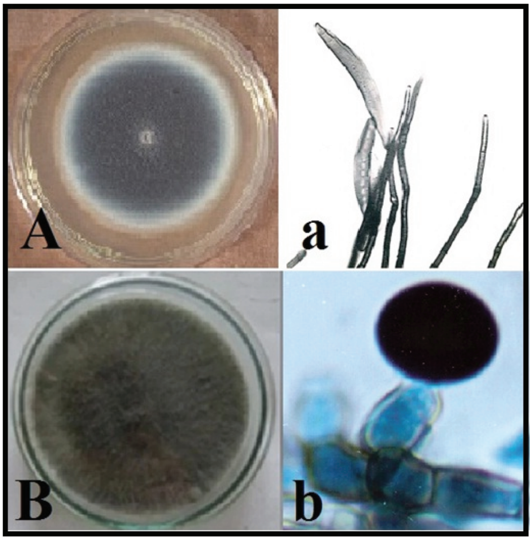

Figure 4 (i). Colony in culture plates (A & B) and photomicrographs (500 X) of conidia (a &b) grown in culture for *Bipolaris oryzae* and *Nigrospopra oryzae*

Study of vertical profile of airborne Bipolaris conidia

To observe the variation of the pathogenic conidial concentration along with height over crop field, monitoring was performed with rotorod sampler (2800 rpm) placed on a mast (metallic stand with adjustable attachment system for samplers at different points) at different heights (upto 5 m) over crop canopy, during the period of dry and wet season crops.

Meteorological data collection

Throughout the study period, meteorological data were collected from Netaji Subhas Chandra Bose International Airport, Dum Dum, North 24 Parganas district of West Bengal. It is the nearest authentic meteorological station (2.5 km southward) from the sampling site (Figure 1b).

Statistical analyses

To measure the effects of different meteorological factors on concentration levels of relevant airborne fungal propagule, a step down regression analysis was performed. For the leaf and grain infections, linear models were fitted for both the wet season and dry season crops and all calculations were done using RStudio (Version 1.2.1335) software.

Results and discussion

A wide variety of fungal pathogens are dispersed through the atmosphere, resulting in crop diseases when there is susceptible host and favourable environmental factors. To control crop diseases, detection and monitoring of the pathogenic fungal propagule in atmosphere provides important source of information. Further analyses of the collected data lead to successful development of disease control strategy. Present study highlights the dispersal of pathogen of brown spot of rice in a paddy field of West Bengal.

The aerobiological sampler was placed at the centre of a paddy field having sufficient area to trap airborne pathogenic propagules originating from the crop at all stages in downwind direction (Fig. 2). In the study, the pathogen of rice plant (Fig. 3) was identified as the conidial stage of fungi *Bipolaris oryzae* (BO, Syn: *Helminthosporium/Drechslera oryzae*). In RLE and PDA media, the mycelia were grayish in colour, forming colony with cottony texture. Upon maturity, the mycelia became septate forming conidiophores with single/numerous conidia at the tip. The conidia was 5-9 septate, (40-100 X 11- 16) µm^2, 4-11 septate, curved, initially hyaline and dark brown at maturity, dimension range 60-104 X 10-16) µm^2. Conidial germination took place from both the ends. *Bipolaris oryzae* (BO) conidia [Fig. 4(i)] were found in the air [Fig. 4(ii)] throughout the study period (Fig. 5).

Along with BO conidia, airborne propagules of *Nigrospora oryzae* (Berk & Broume) Petch (NO), were also recorded in the atmosphere of study area. *Nigrospora oryzae* (NO) is basically a soil-borne as well as saprophytic fungus colonizing debris of different living and non-living plant species and considered as weak parasite, producing minute grain spots (<0.5 mm diameter) of rice. It acts as an endophyte as well as a parasite of rice plant, depending upon environmental conditions. The teleomorph of NO is *Khuskia oryzae* H. Huds, a widespread tropical fungus.

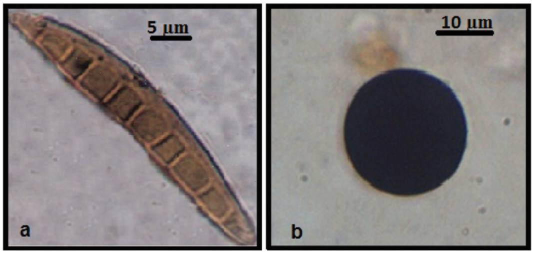

*Figure 4(ii).*Photomicrograph of airborne conidia of (a) *Bipolaris oryzae* (400X) and (b) *Nigrospora oryzae* (500X).

In the exposed PDA culture plate of the field, the colonies of NO were initially white, later dark brown to black at maturity. Conidiophore branched with solitary round/elliptical black, smooth and single conidium at the top [Fig. 4(i&ii)].

In the present study, there was very little NO induced grain spots observed in the sampled crop plants of the study field (<4% grain surface area). As NO is also reported to be associated with rice crop (Mew and Gonzales, 2002), the seasonality of this particular conidia, which is also smaller in size, was compared in terms of dispersal in the atmosphere among the other 28 fungal spore types (dominated with Aspergilli-Penicilli group members) recorded from the atmosphere of the crop field.

The BO conidia showed two major peaks (Figure 5), one during first week of May (23 conidia/d/m^3 air) and another in 2nd week of October (28 conidia/d/m^3 air), whereas NO showed the maxima during third week of October (700 conidia/d/m^3 air) with a minor peak during third week of September (434 conidia/d/m^3 air). Hence, NO was found to produce huge number of conidia, without producing specific disease manifestation on rice plants probably due to its latent pathogenic nature, inducing disease only

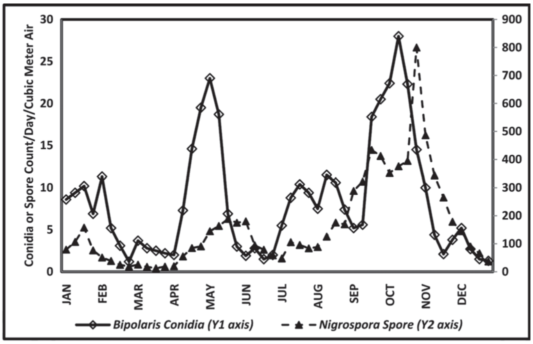

*Figure 5.*Seasonal periodicity patterns of *Bipolaris* conidia and *Nigrospora* spore over paddy field during 2016-17.

under specifically stressed condition (Mew and Gonzales, 2002).

The two maxima of BO were found to occur just after the harvesting of both dry and wet season rice crops respectively, which seemed to be the source of large number of pathogenic conidia. After these two peaks, the number of BO conidia gradually went down, and became lowest during the last week of December of both the years. In case of airborne NO conidia, the peak was noticed closely after the harvest of wet season crop and not after the dry season crop.

Sometimes, it was observed that rice crop disease outbreaks depended on weather and climatic conditions (Shahriar et al., 2010). When the occurrence of airborne conidia was compared with the meteorological factors, none of the meteorological parameters showed significant correlation with airborne BO conidia (Fig. 6a). However, relative humidity was significantly correlated ($r = 0.44$, $p<0.01$) with the occurrence of NO conidia (Fig. 6b). Airborne conidia could be also fitted with a regression model with a step down equation:

Concentration of NO conidia = 289.838 + 5.93* Average Relative Humidity (%)

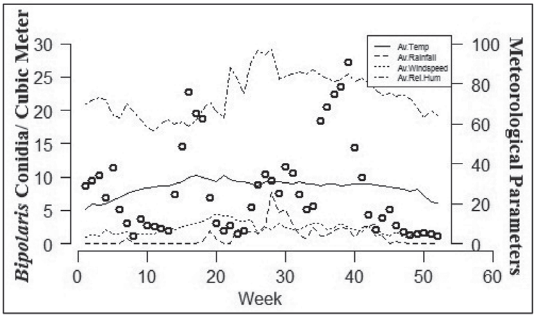

*Figure 6a.*Relationship of meteorological parameters with airborne conidia of *Bipolaris oryzae*

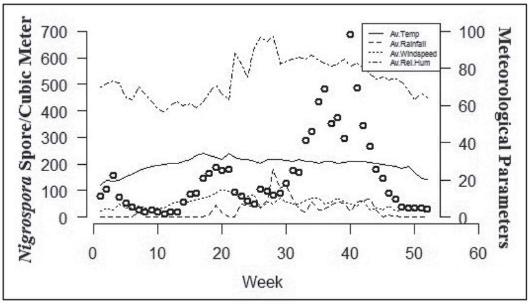

Figure 6b. Relationship of meteorological parameters with airborne conidia of *Nigrospora oryzae*

This result indicated the probable positive correlation of very weakly pathogenic airborne NO conidia with meteorological factors, whereas pathogenic BO conidia seemed to be totally independent of these factors. BO conidia were found in lower concentration during the infection period (Fig. 7) and attained the peak during harvest. Uddin (2004) and Chakraborty et al. (2003) also reported higher concentration of airborne *Helminthosporium/ Drechslera* during harvesting of rice crop. However, Pannu et al. (2005) reported negative correlation of temperature, relative humidity and rainfall on the development of brown leaf spot caused by

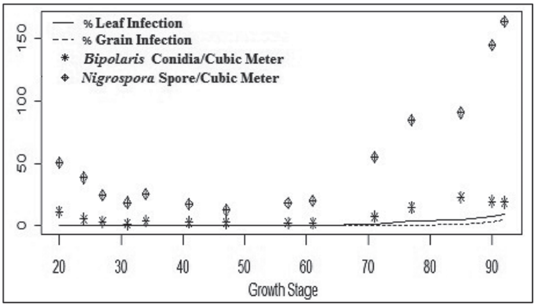

Figure 7a. Disease progress and dispersal of pathogenic and non-pathogenic fungal propagules over dry season crop

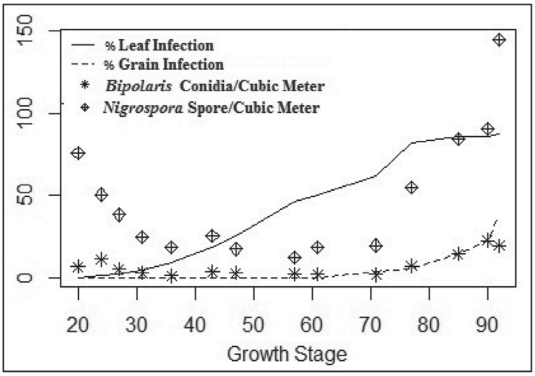

Figure 7b. Disease progress and dispersal of pathogenic and non-pathogenic fungal propagule over wet season crop

Helminthosporium oryzae in the rice crop from Punjab, India.

During monitoring of BS disease progress in rice crop (Fig. 7a&b), initial infection was recorded in 12th week of dry season crop and 2nd -3rd week of wet season crop after transplanting of rice seedlings (Table 1). As there was no chance of seed borne infection, plants may have been infected by windborne pathogen. There were some grasses like *Cynodon dactylon*, *Digitaria, Eleusine* sp., growing around the crop field, which might act as collateral host (Sreeramulu and Ramalingam, 1966) during the period (May-June and October-December), when there was no crop plant in the field. In previous report on aeromycoflora over a rice crop field during 1994-1999 (Chakraborty et al., 2003), seedlings of Ratna variety were planted, which was moderately resistant to BS (www.krishisewa.com). For the observation of disease progress without special inhibition from host plant and subsequent free type pathogen dispersion, IR-8, which was reported as susceptible variety to BS disease, was cultivated.

At the time of harvesting, 82-87.5 percent of the leaves and 37.5 – 39.5 percent of the grains were found to be infected for wet season crop. For dry season crop, the observed infection was in the range of 8.4-10.5 per cent (leaves) and 3.5 – 6.35 per cent (grains), respectively (Table 1).

In the infected portions, minute brown spots appeared first, later assuming oval/ rectangular shape, 2.5 cm (±0.14) long (average) with light brown centre. The infected glumes were dark brown to black in colour and finally found to be coated with velvety dark sporulating mass (Fig. 3).

Throughout the aerobiological survey period, the number of BO conidia was found always less than small-sized *Nigrospora* conidia. Both were present in air round the year (Figure 5).

For dry season crop, airborne BO conidia were positively correlated both for leaf ($r = 0.58$, p-value $= 0.03$) and grain ($r = 0.86$, $p<0.001$) and a regression correlation on the BO conidial concentration with leaf infection was fitted as a model as follows:
Percentage leaf infection = 18.020 + 2.940*BO conidia concentration in air

However, for grain infection, no such regression was found.
In case of wet season crop, leaf infection of BS was positively correlated with airborne BO conidia ($r = 0.87$, p-value<0.001), and the fitted model is:
Percentage leaf infection = 1.55036 + 0.0649*BO conidial concentration

Similar type of scenario was observed for grain infection. Airborne BO conidia were significantly correlated (p-value <0.001) with the fitted equation:
Percentage of grain infection = 0.6892 + 0.3587*BO conidial concentration

For airborne NO conidia, though there is positive correlation with leaf and grain infection for wet season crop infection ($p<0.1$), no regression equation could be fitted.

The diurnal periodic pattern of the airborne BO conidia showed two peak concentrations (Fig. 8) in the morning (8.00 A.M, 5.5 conidia/m^3/h) and afternoon (15.00 P.M., 4.0 conidia/m^3/h). The first

Table 1. Overview of the paddy field (2016 and 2017)

Year	Crop	Date of transplanting	No. of plants	Date of initial infection (after field transfer)		Harvesting (with respect to field transfer)	Percentage of incidence justbefore harvesting	
				On Leaf	On Grain		On Leaf	On Grain
2016	Dry Season	28.01.2016	3905	78 days	96 days	99 days	08.40	03.50
	Wet season	07.07.2016	4060	16 days	58 days	94 days	82.00	39.50
2017	Dry Season	19.01.2017	3725	79 days	95 days	96 days	10.50	6.35
	Wet Season	09.07.2017	3950	13 days	61 days	97 days	87.50	37.50

Rice cultivar: IR-8 Plot size: 20 x 20 square metres

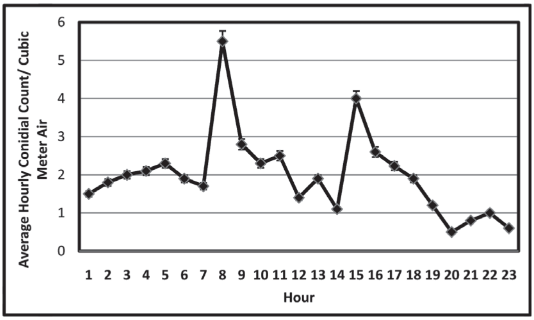

Figure 8. Diurnal periodicity pattern of airborne conidia of *Bipolaris oryzae* over rice crop field (2016-17), bars indicate standard error

report of diurnal periodicity of *Helminthosporium oryzae* indicated the peak time to be the afternoon, more than 50 years back (Sreeramulu and Ramalingam, 1966). Chakraborty et al. (2003) reported day-time double maxima for *Drechslera oryzae*, similar to present study, from the same area during 1994-1999, where the conidia attained the peak at 11.00 and 16.00 h.

When the vertical profile of BO conidia was recorded in the field using rotorod sampler (Fig. 9), maximum concentration (approximately 2.5-10 conidia/m^3/h) was recorded at 1.0 m height in dry season crop, wet season crop and in the intermediate period (when there was no crop in the field). There was a negative correlation with height of sampling ($p>0.05$) observed for the airborne BO conidia in the field. This was probably due to the larger size of the BO conidia, which could not disperse to greater heights due to the retardation by gravity. In addition to this, the crop canopy alsomight act as a rich source of airborne pathogenic BO propagules, which were released and dispersed due to turbulence from point sources of various levels of infected plant leaves and grains (Reddy and Ramakrishna, 1978; Isard and Chamecki, 2016).

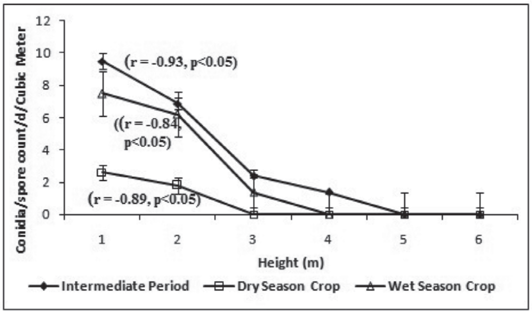

Figure 9. Vertical profile of airborne conidia of *Bipolaris oryzae* during dry and wet season crop, and intermediate

Airborne pathogenic fungal propagules are potential threats to crops growing in natural field condition (Levetin, 2016). Conidia of *Bipolaris oryzae*, the causal organism of BS disease of rice, were found to be airborne and present in the field throughout the year. The correlative study of airborne BO conidia and disease development in crop confirmed that it might be a cause of primary infection, as predicted by Chakrabarti and Chaudhuri (1992). Saha et al. (2020) also made similar prediction for false smut disease of rice in the crop field of West Bengal. The comparison of the atmospheric dispersal of BO conidia with the smaller, lighter and less harmful *Nigrospora* conidia made the base of the prediction stronger.

Detailed information about the air-dispersal of *Bipolaris oryzae*conidia over a rice field will be highly useful to control the BS disease by planned application to improve the result of traditional fungicide spray (Sharma and Maheswari, 1982), with correct prediction of the peak days of airborne BO pathogen propagule, at specific time and height, at appropriate growth stage of host plant. Hence, our result will be helpful in management of BS disease of paddy by improving the quality vis-à-vis quantity of yield in the study area.

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