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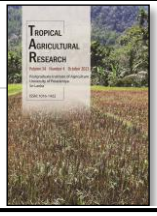

RESEARCH

Dynamics of Rice Brown Leaf Spot Disease (*Bipolaris oryzae*) Incidences Due to Seasonal Weather Differences in the Dry Zone of Sri Lanka

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ABSTRACT

Weather factors are key determinants in ecological disease management in sustainable agriculture, while judicious crop management systems deliver better control over rice diseases in tropical conditions. This study was designed to explore the effect of weather factors under different crop management systems and seasons on Rice Brown Leaf Spot (RBLS) disease incidences caused by *Bipolaris oryzae* in the tropical dry zone of Sri Lanka. The incidence of RBLS was measured under *Conventional*, *Reduced*, and *Organic* crop management systems commencing from the first occurrence of disease symptoms, at three-day sampling intervals in the tropical dry zone during wet (*Maha*) 2018/19 and 2019/20, and dry (*Yala)* 2019 and 2020 seasons. Secondary data on weather parameters were collected from the regional weather station. The RBLS incidences were highest in the wet season and were most abundant at the reproductive stage. The disease incidence dynamics over time were found to be similar among all the crop management systems in three seasons. The cumulative amount of rainfall seven days before the disease observation (RF7), the day-RH (DRH), and the maximum (TMAX48) and average temperature (TAVG48) that were recorded 48 h before the disease observations were found to be significantly correlated with the disease incidence of crop management systems in the wet season. DRH and minimum temperature (TMIN72) of 72 h before the disease observed in the wet season resulted in higher disease incidences. The RBLS disease can be managed concerning the crop management systems under high DRH and TMIN (20-25 ℃) in the wet season.

INTRODUCTION

Rice diseases are the most serious constraints that affect rice grain yield, straw yield, and the quality of production (Meng and Li, 2010). Rice crops are affected by 70 different diseases throughout the globe (Saha *et al*., 2015). Fungal diseases are the most crucial challenge to rice cultivation (Charles *et al*., 2016). Rice blast, brown spot, narrow brown leaf spot, and rice leaf scald are some of the important fungal diseases widely distributed in almost all the rice-growing regions of the world.

Rice Brown Leaf Spot (RBLS) disease caused by necrotrophic leaf fungus *Cochliobolus miyabeanus* (also called *Bipolaris oryzae*) is an epidemic plant disease that has been identified around the world as a threat to the quantity and the quality of rice harvest. Thus, it ranked among the most important rice diseases (Webster and Gunnell, 1992). The rice brown spot infects all parts of the rice crop and symptoms mainly appeared on the coleoptile, leaf sheath, leaf blade, glumes, and spikelet leading to a 5-45 % decrease in rice yield (IRRI, 2022). RBLS is commonly identified with the diagnostic symptoms of minute spots of brown to reddish-brown, circular to oval, and older spots are light reddish-brown or grey center along with dark to reddish-brown margins (Quintana *et al*., 2017).

Mineral nutrition is an important component for the growth and development of crops in all crop management systems. Supply of optimally balanced nutrition is a critical factor that allows crops to deliver their full yield potential (Roberts, 2008) and maintain proper health. Further, macro and micronutrients have long been recognized as associated with the quality and quantity of yield of any crop and with changes in disease incidence levels (Palti, 1998; Rush *et al*., 1997; Dordas, 2008). Soil nutrient level influences the plant nutrient content and plant health affects the occurrence of plant diseases (Agarwal *et al*., 1989; Mia and Safeeulla, 1998; Zadoks, 2003). According to IRRI (2018), the management of soil fertility which includes the timely supply of adequate amount of required fertilizers and regular monitoring of soil nutrients is the first step in managing RBLS. Owing to the ever-increasing fertilizer cost, farmers presently tend to adopt more economical and eco-friendly alternative crop management systems.

Alternate crop management systems such as organic farming or low-input systems have gained popularity in recent years as a replacement for conventional systems due to sustainability concerns (Reganold and Wachter, 2016). These alternate crop management systems differ in nutrient management by means of quantity supplied as well as types of nutrient sources which can alter nutrient dynamics in the soil and thus in the plant. The impact of these changes on soil nutrient management in disease dynamics particularly in rice is not well studied.

Environmental factors are also having a significant contribution to disease development (Keane and Kerr, 1997). Environmental factors such as temperature and relative humidity play a significant role in RBLS disease development (Dasgupta and Chattopadhyay, 1977). The high humidity (more than 90%) with a temperature of 24-30 °C favors the development of RBLS disease. Wind speed and rainfall are two other factors that contribute to the spread of inoculum from infected to healthy areas. If the environmental conditions are favorable to the pathogen, the losses may be more severe, and disease can prevail throughout the growing period (Liu *et al*., 2008). According to the forecasts of global warming and climate change, the earth's average surface temperature is expected to increase by 1.4-5.8 °C, in addition to the rainfall (both the precipitation and snowfall) and relative humidity (Guan, 2009). As a result, awareness and understanding of weather variation and disease occurrences are of timely importance for the management of diseases such as RBLS.

We hypothesized that the interaction of rainfall, temperature, and relative humidity in different crop management systems significantly influences the development of RBLS disease incidence. Therefore, this research was conducted to understand the impact of seasonal differences in weather parameters on RBLS disease incidence under different crop management systems in the dry zone of Sri Lanka.

METHODOLOGY

The experiment was conducted in the farm premises of the Faculty of Agriculture, Rajarata University of Sri Lanka during the wet (*Maha*) 2018/19 and 2019/20 seasons (from November to March) and Dry (*Yala*) 2019 and 2020 seasons (from May to September) as part of a long-term research project. The experimental site was located at Puliyankulama in the Anuradhapura district which belongs to the agroecological region of DL1b (8.0433° N, 80.5300° E). The study area was on imperfectly drained Reddish-Brown Earth soils (Wickramasinghe *et al*., 2021). Mean seasonal rainfall, maximum and minimum temperature and day and night relative humidity in the area were presented in Table 1.

Filed experimental design and treatments

The experiment consisted of three main crop management systems which were, T_1: *Conventional system – i.e*. with inorganic fertilizer application as recommended by the Department of Agriculture (DOA) 2013, T_2: *Reduced system – i.e*. 50% of the nutrient requirement provided with inorganic and organic fertilizer application, respectively, as of the *conventional* system, and T_3: *Organic system* - no inorganic fertilizers were added where the supply of nutrients was matched through pre-determined organic manure application at a rate equivalent to N applied in the *Conventional system*. These rates were decided considering the losses of N from urea and organic matter while aiming to provide adequate N for crop growth. Buffalo manure, cattle manure, sunn hemp crop, gliricidia leaves and other crop residues were used to prepare organic manure. The organic manure rate was determined according to the measured inherent N content of the organic manure, and 50% amount of organic manure from the organic nutrient management was added to the reduced nutrient management system. The three treatments were established as a randomized complete block design with six replicates. The crop management systems were defined based only on the elemental N supply and the sources. Therefore, the phosphorus and potassium rates were not standardized (Table 2). The amount of these two elements depended on the quality of the materials used to supply N in both *Reduced* and *Organic* crop management systems.

Table 1. Mean rainfall (mm), minimum and maximum temperature (^{0}C) and relative humidity (day and night) recorded in dry (2019 and 2020 season) and wet (2018/19 and 2019/2020) seasons

	Season			
	Wet 2018/19	**Dry 2019**	**Wet 2019/20**	**Dry 2020**
Mean rainfall (mm)	72.9	36.3	140.7	94.7
Minimum Temp (^{o}C)	23.4	25.4	23.4	25.1
Maximum Temp (^{o}C)	32.4	34.1	31.6	32.7
RH (%) – Day	67.6	64.6	71.2	70.4
RH (%) – Night	90.6	87.4	91.8	89.2

Table 2. Three crop management systems and their respective nutrient contents

Crop management system	Mineral nutrient (kg/ha)	Nutrients from alternative sources (kg/ha)
Conventional	N - 103.5 (Urea 46%)	N – 0
	P - 3.9 (P_2O_5 43.7%)	P - 0
	K - 30.0 (K_2O 60%)	K – 0
Reduced	N - 51.8 (Urea 46%)	N - 25.9
	P - 1.9 (P_2O_5 43.7%)	P - 0.65
	K - 15 (K_2O 60%)	K - 52.5
Organic	N – 0	N - 51.8
	P - 0	P- 1.3
	K – 0	K - 104.9

Crop establishment and management

Pre-germinated Bg300 rice seeds (the most common variety grown in the dry zone of Sri Lanka), were broadcasted at a rate of 120 kg/ha in both WS and DS. The application of inorganic fertilizer was carried out as per the DOA recommendation in 2013. Organic fertilizer was applied as the basal dressing and as the third top dressing of DOA inorganic fertilizer recommendation. Irrigation was carried out one week after the seed sowing and the water depth was maintained above the soil level to retain sufficient moisture throughout the cultivation period.

Insect pest and weed management

No chemical application was made to control pests, diseases, or weeds in organic systems. Pest and weed management of conventional and reduced systems were conducted following the DOA recommendation.

Data collection

The incidence of RBLS was recorded commencing from the time of the 1st appearance of the disease symptoms and continued at three days sampling intervals up to the harvesting stage. A 50 cm x 50 cm quadrat was used to collect samples in a plot with an area of 90 m^2. Two random quadrat samples were selected from each plot. The number of infected plants in each quadrat sample was counted. The disease incidence was calculated using Equation 1 (Groth et al., 1999).

$$Disease\ incidence = \frac{Number\ of\ infected\ plants\ \ per\ quadrat}{Total\ number\ of\ plants\ per\ quadrat} \quad \text{..... Equation 1}$$

Secondary data on climatic parameters, namely, temperature, rainfall, and relative humidity were collected from the regional weather station in Anuradhapura, Sri Lanka for the wet seasons 2018/19 and 2019/20, and dry seasons 2019 and 2020. The maximum and minimum temperatures were measured using an alcohol thermometer. Daily rainfall was measured using a tipping bucket rain gauge and relative humidity using a wet-dry bulb hydrometer.

Isolation and identification of the pathogen

Isolation and purification of the pathogen responsible for brown spots were done at the Plant Pathology Laboratory, Faculty of Agriculture, Rajarata University of Sri Lanka. First, the infected leaves were surface sterilized using 1% sodium hypochlorite solution and 70% ethanol for 1 min, followed by a washing step with sterilized distilled water. A Potato Dextrose Agar (PDA) medium was used for culturing the microorganisms. Surface sterilized samples were dried by placing them between two sterile filter papers. Then the samples were placed on PDA medium under aseptic conditions. Subsequently, the samples were incubated at 28 ± 1 °C for 72 h. Identification of fungi associated with brown spot lesions was done based on spore morphology, microscopic observations, and molecular biological techniques (Alsohaili and Bani-Hasan, 2018).

Extraction of DNA for molecular identification of isolates

Fungal DNA extraction was done from 7-8 days old fungal cultures according to McGravey and Kaper (1991) method with some modifications to the initial grinding of the sample. Here, 2 mL of the extraction buffer (7% CTAB, 1% Polyvinylpyrrolidone, 1.4 M NaCl, 20 mM EDTA (pH 8.0)) was used instead of liquid nitrogen, and the resulting pulp of the mycelium was taken for DNA extraction. Extracted DNA was visualized under gel electrophoresis (1% agarose) and later used in the Polymerase Chain Reaction (PCR).

Phylogenetic relationships of the isolated species

The resulting DNA was subjected to subsequent PCR assays (BIO-RAD My Cycler) with primers targeting the Internal Transcribed Spacer Region (ITS) of fungi. Accordingly, ITS1 (3' GCC GTA GGT GAA CCT GCG G 5') and ITS4 (3' GCC TCC GCT TAT TGA TAT GC 5') universal primer pairs were used in the PCR assay (White *et al*., 1990). During the PCR reaction, there were 40 cycles of

denaturation at 95 °C, 1 min, Annealing at 52 °C, 1 min, and Extension at 72 °C, 1 min. The PCR products were then separated and visualized on 1.5% agarose gel electroporation and sequence characterized (Macrogen Inc. Korea)

Data analysis

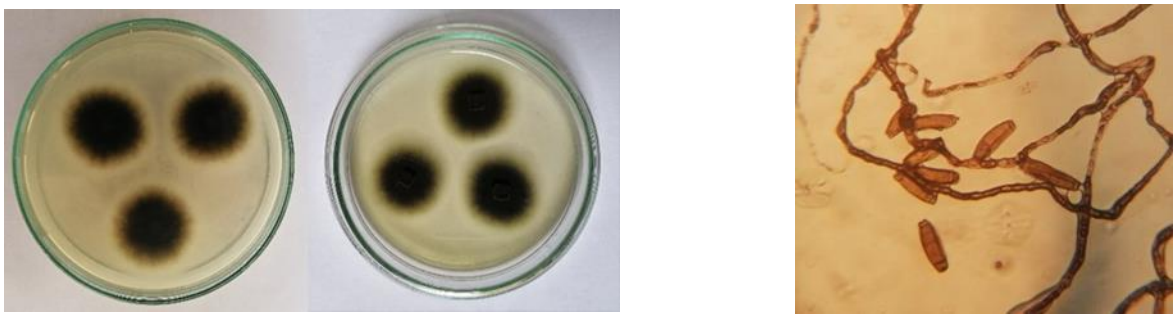
The PCR product sequences were aligned with three ex-type strain sequences from the National Center for Biotechnology Information (NCBI) gene bank and the established fungal taxonomy for identification. The DNA sequence obtained from a separate study by the research team and some reference DNA sequences of *B. oryzae* retrieved from the NCBI gene bank were also incorporated in the analysis. The aligned sequence file was used for constructing a phylogenetic tree by the Maximum Likelihood method based on the Jukes-Cantor model with 1000 bootstrap replications (Jukes and Cantor, 1969). The DNA sequence data of *Bipolaris oryzae* generated from this study were deposited in the NCBI gene bank under the accession number of MZ618926.

Disease incidence data were tested for normality and heteroscedasticity and then the data were transformed to square roots. Disease incidence was statistically analyzed using the SAS computer program version 9.0. Repeated measures analysis of variance (ANOVA) was carried out using the MIXED model to determine the significant differences in crop management systems and seasons on mean disease incidence. Crop management systems and the seasons were considered as fixed factors and the replicate (block) was considered as a random factor. All means were separated using Tukey's LSD at a 5% probability level. Time, crop management systems, and seasonal effects on disease incidence were plotted using the original software. The spearmen correlation coefficient was used to identify the correlation between the disease incidence and weather parameters; *i.e*. the cumulative amount of rainfall seven days before the disease observation (RF7) and three days before the disease observation (RF3), daytime relative humidity (DRH), nighttime relative humidity (NRH) and minimum (MIN), maximum (MAX) and average temperatures (TAVG) at the 24, 48, and 72 h before the disease observation (TMIN24, TMAX24, TAVG24, TMIN48, TMAX48, TAVG48, TMIN72, TMAX72, and TAVG72 respectively) during wet and dry seasons with a probability of 0.1%.

RESULTS AND DISCUSSION

Isolation and identification of brown spot (*Bipolaris oryzae*) pathogen using morphology, and microscopic observations

After 72 h of incubation, *B. oryzae* colony appeared whitish initially. As the colony matured, it gradually turned grey to dark grey with a whitish margin. The fully matured culture was black due to septation (Figure 1 a). Similar observations have been made by Monisha *et al*. (2019) and Nayak and Hiremath (2019).



(a) **(b)**

Figure 1. Macroscopic and microscopic features of *Bipolaris oryzae* (a) culture on PDA media after 7 days of incubation (b) spore morphology (x10x10x1)

Furthermore, the pathogen was microscopically confirmed by the presence of characteristic conidiophore that arises singly or as a group, multi-septate, and brown. Conidia were curved or slightly curved; initially hyaline and later maturity turn brown, and fusiform with hilum at the base (Figure 1 b). Similar observations have been made by Monisha *et al*. (2019).

The amplified PCR products of the DNA of some common fungal plant pathogens when amplified by ITS1 and ITS4 primers were shown in Figure 2. The Internal Transcribed Spare (ITS) region includes the ITS1 and ITS2 regions separated by the 5.8S gene and is situated between the 18S (Small Sub Unit-SSU) and 28S (Large Sub Unit-LSU) genes in the nrDNA repeat unit. The number of copies of ITS regions per cell (up to 250) makes the region an appealing target for sequencing studies.

The nucleotide sequence of these spacer regions is often much more polymorphic between species than within species. Therefore, polymorphisms in ribosomal DNA (rDNA) and sequence data of the internal transcribed spacer 1 and 2 regions have been widely used for the differentiation of fungal pathogens. The molecular size of the resultant amplicon was 490 – 500 bp.

Molecular validation of RBLS

With the sequence characterization results, RUSL_20 fungal isolate was identified using molecular techniques. The *B. oryzae* and the details of the NCBI homology search are given in Table 3. The study of rDNA allows phylogenetic studies of the species, which enables the differentiation of isolates inside of each species. The phylogenetic tree constructed for the isolates (Figure 3) indicates the evolutionary history of an organism, supposing relations of ancestry for a set of species (Crouch *et al*., 2009). The DNA molecular markers can be used to assist in the estimation of the genetic diversity between different isolates and degrees of relationship (Hamada and ben Ahmed, 2005). Phylogenetic separation showed three monophyletic clades. However, the bootstrap value did not support clade separation and the low quality of the DNA sequence obtained would be a reason for that. By studying the nucleotide sequences used in this study, it was evident that the clade separation was solely due to some single nucleotide polymorphisms observed among them.

Figure 2. PCR products of some fungal pathogens amplified using ITS1 and ITS4 primer pair, Lane 5 (RUSL_20) PCR product obtained from *Bipolaris oryzae*

Impact of crop management systems on RBLS disease incidence

The mean disease incidence was higher in the *Organic* crop management system (30.5) and lower in the *Conventional* system (27.8) signifying the availability of mineral nutrient elements in the latter (Table 2), especially nitrogen, phosphorus, manganese, iron, and calcium, contributing to the control of RBLS incidence (Imran *et al*., 2020). The results suggested that the supply of macro and micronutrients as per the DOA recommendation in the *Conventional* system may have enhanced the tolerance capacity of rice plants to the pathogen, reducing the damage by pathogens. This result confirms with that of Sun *et al*. (2020).

Impact of the weather factors of cropping seasons on RBLS disease incidence

A significantly higher disease incidence was reported during the 2018/2019 wet season whereas a significantly lower incidence was reported in the 2019 and 2020 dry seasons (Table 4 and Figure 4). Previous studies also showed that dry environmental conditions have a negative impact on disease incidence (Jain *et al*., 2019). Furthermore, prolonged leaf wetness and moisture retention in canopy surroundings might lead to increased lesion density by providing favorable conditions for pathogen development (Percich *et al*., 1997).

Table 3. Homology search results of the isolate RUSL_20

Sample	Description	Percentage Identity	Query cover	e-value	Accession No.
RUSL_20	*Bipolaris oryzae* strain ZMXR10 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	100.00	100	0.0	MT446115.1

Figure 3. Phylogenetic tree constructed with the DNA sequence of the isolate RUSL_20 and three reference sequences of *Bipolaris oryzae* extracted from NCBI gene bank using MEGA 6.06 software with 1000 bootstrap values

Table 4. Associated probability values for the effect of the crop management system, and cropping season on the mean disease incidence in the 2018/2019 Wet, 2019 Dry, 2019/2020 Wet, and 2018 Dry season

Treatment	P-value
Crop management system (CS)	**
Season (S)	**
CS × S	*ns*

** - significant at $P \leq 0.05$ level, ns – not significant at $P \leq 0.05$ level

RBLS disease incidence in different seasons

The disease incidence gradually increased with time within the growing season. Yet, the pattern of disease progression of three crop management systems during the 2018/2019 wet season (Figure 5 (a)) was different compared to other three seasons (Figures 5 (b), (c) and (d)). The increasing trend of disease incidence in both dry seasons started 75 days after sowing (DAS) (near dough stage) (Figures 5 (b) and (d)) with low pathogenic activity during the initial growth phase with sufficient nutrient content (or availability). However, as the crop is senesced, the nutrient content of the plant decreases, which in turn creates favorable conditions for the growth of pathogens due to the reduction of tolerance capacity (Ou, 1985). The increasing trend of the disease was reported with 64 DAS (initial ripening stage) in the 2019/2020 wet season with all three nutrient management systems (Figure 5d). The crop was completely infected with 64 DAS in the 2018/2019 wet season (Figure 5a). The pattern of the disease development curve confirms that the incidence of RBLS has increased along with the commencement of the reproductive stage of the rice crop (Figure 5). Based on Kohls *et al*. (1987), epidemics of RBLS disease were initiated from the reproductive stage of the rice crop. Jha (2001), Ou (1985) and Sunder *et a*l. (2014) also reported rice plants are more susceptible during panicle formation and up to dough and mature stages and the same observations were reported in the present study too. The inherent disease-resistant mechanisms of the plant may be comparatively strong during the vegetative phase which reduces towards the reproductive stage owing to the biochemical and physiological changes of the crop due to the transition to reproductive. Further, the remobilization of mineral elements and concentration of K for phloem translocation might have increased the vulnerability of leaves to RBLS.

Relationship of weather parameters and RBLS disease incidence

Changes in disease incidences were caused by fluctuations in various weather factors and their different effects, especially the monsoonal rainfall of wet and dry seasons. The correlations revealed certain significances between weather parameters and disease incidences in the wet season than dry season (Table 5). The literature revealed that temperature, relative humidity, and rainfall affect the fungal disease progression (Foister, 1946). The RH7 was positively and significantly correlated to disease incidence in three crop management systems during the wet season. The RH3 parameter was only significantly correlated with disease incidence of the *Conventional* system in the wet season. The DRH parameter was significantly correlated with all other systems in both wet and dry seasons except the *Organic* system. The TMIN at 24, 48, and 72 show significant positive correlations with all crop management systems in the wet season. Except for the organic system, the TMAX at 24 was found to be significantly correlated to the wet season's *reduced* and *organic* systems. The TMAX and TAVG at 48 were significantly correlated with all systems in both seasons. The TMAX at 72 and TAVG at 24 were significantly correlated with all systems of the wet season. The TAVG at 24, 48, and 72 were significantly correlated with the three crop management systems of the wet season (Table 5). According to the literature, generally, necrotic foliar pathogens require a short period of around one day for incubation (Klomp, 1977; Sarkar and Sen Gupta, 1978), around three to four days to infect (Van Ba and Sangchote, 2006), and sporulation peaks about six days after infection (Sarkar and Sen Gupta, 1978). Further, there were less amount of disease incidences during the dry season. Therefore, the wet season was only considered to understand the influential pattern of disease incidences with different weather parameters concerning the significance of correlation values.

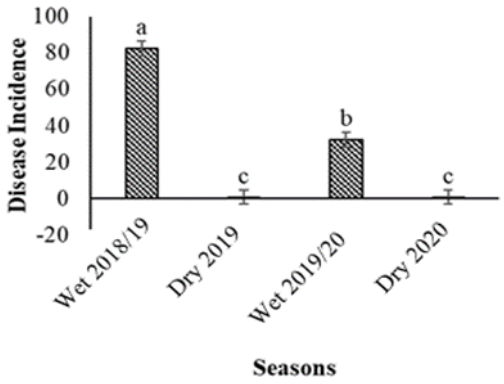


Figure 4. RBLS disease incidence under different cropping seasons (Data points with similar letters indicate no significant difference at *P≤0.05* (LSD), bars are SE)

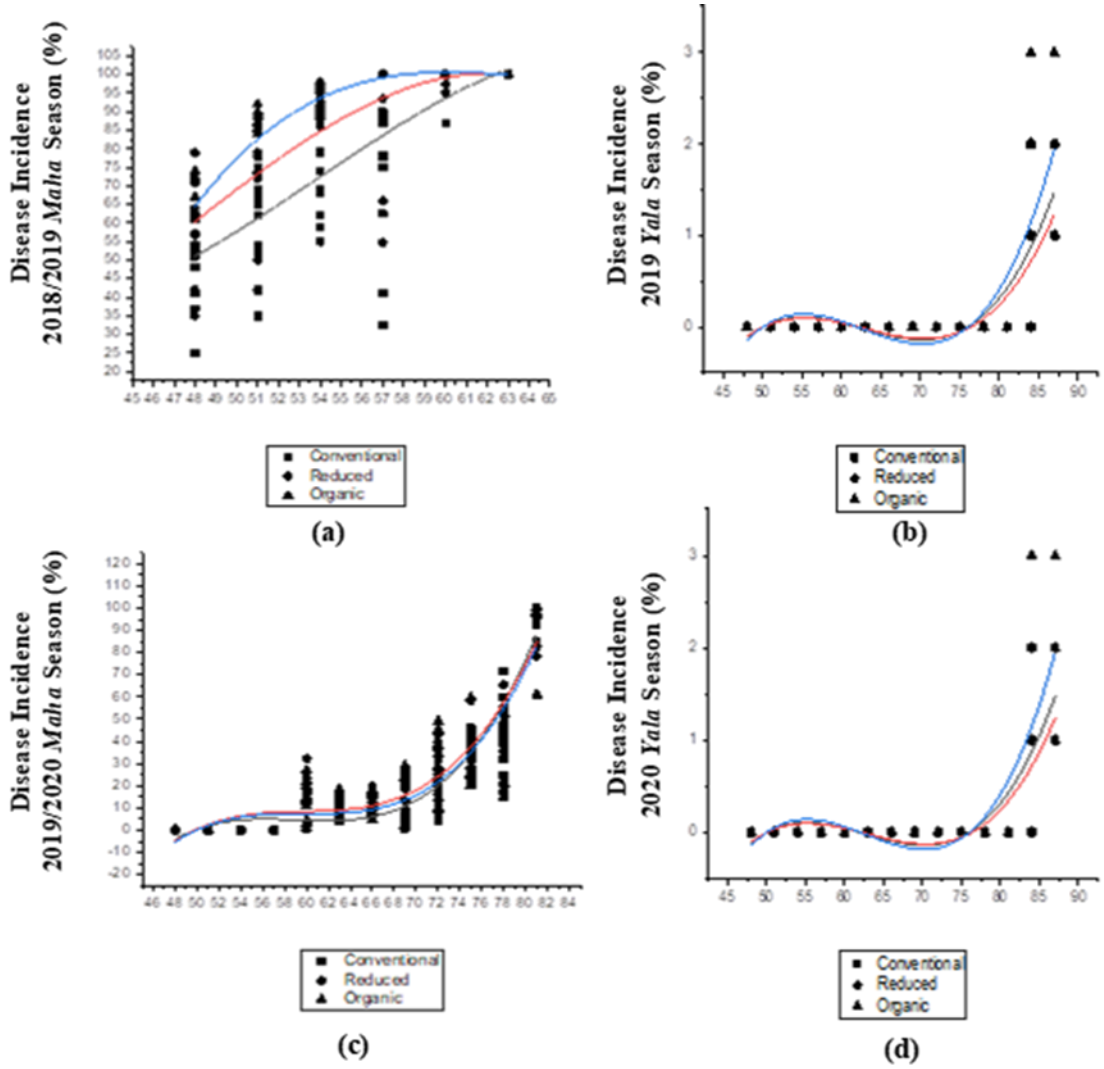


Figure 5. Time, cropping system and seasonal effect on disease incidence in (a) 2018/2019 wet season, (b) 2019 dry season, (c) 2019/2020 wet season, and (d) 2020 dry season

Table 5. Correlation of the disease incidence with weather parameters in both dry and wet seasons under three crop management systems

	Wet Season			Dry Season		
	Conventional	***Reduced***	***Organic***	***Conventional***	***Reduced***	***Organic***
RF7	0.265[#]	0.213	0.175	0.397	0.332	0.183
	**	**	**	**	**	*ns*
RF3	0.177	0.157	0.125	0.233	0.164	0.095
	**	*ns*	*ns*	*Ns*	*ns*	*ns*
DRH	0.214	0.262	0.273	0.377	0.325	0.188
	**	**	**	**	**	*ns*
NRH	0.030	0.063	0.062	0.260	0.246	0.176
	ns	*ns*	*ns*	**	**	*ns*
MIN24	0.412	0.381	0.360	0.099	0.145	0.290
	**	**	**	*Ns*	*ns*	**
MAX24	0.170	0.187	0.193	-0.365	-0.324	-0.170
	ns	**	**	**	**	*ns*
AVG24	0.287	0.272	0.258	-0.265	-0.212	-0.035
	**	**	**	**	*ns*	*ns*
MIN48	0.513	0.471	0.432	-0.018	0.032	0.188
	**	**	**	*Ns*	*ns*	*ns*
MAX48	0.303	0.297	0.287	-0.356	-0.307	-0.146
	**	**	**	**	**	*ns*
AVG48	0.395	0.361	0.336	-0.325	-0.267	-0.076
	**	**	**	**	**	*ns*
MIN72	0.439	0.389	0.346	0.054	0.105	0.271
	**	**	**	*Ns*	*ns*	**
MAX72	0.260	0.233	0.217	-0.272	-0.225	-0.039
	**	**	**	**	*ns*	*ns*
AVG72	0.410	0.362	0.336	-0.243	-0.187	0.014
	**	**	**	*Ns*	*ns*	*ns*

[#]The value with the mark is the Spearman correlation coefficient and the italic value below it is the probability value.
** - significant at $P \leq 0.001$ level, ns – not significant at $P \leq 0.001$ level
RF7- The cumulative amount of rainfall seven days before the disease observation; RF3- The cumulative amount of rainfall three days before the disease observation, DRH- Daytime relative humidity; NRH- Nighttime relative humidity; MIN- Minimum temperature; MAX- Maximum temperature; TAVG- Average temperatures at the 24, 48, and 72 h before the disease observation (TMIN24, TMAX24, TAVG24, TMIN48, TMAX48, TAVG48, TMIN72, TMAX72, and TAVG72 respectively)

No specific disease pattern was observed with the prevalence of rainfall during the 2018/2019 wet season (Figure 6 a). The intensity of previous rainfall may have contributed to the development and spread of the disease, while the effect of scattered rainfall during the season was pronounced (Figures 7 and 8) with stronger epidemics (Ou, 1985, Singh *et al*., 2000). Pannu *et al*. (2005) reported a significantly lower disease severity during high-rainfall years than in lower rainfall years. The disease incidence and yield losses were higher under water shortage/rainfed conditions (Hegde *et al*., 1999). It is well known that the pathogens can produce new races, which may overcome the resistance in a particular variety or may adapt to warmer temperatures to cause severe disease in previously unfavorable environments (Sato *et al*., 2008). However, Singh *et al*. (2000) recorded that rainfall did not play a major role in developing the disease. Despite a slight increasing trend of the disease incidence with DRH in the wet season (Figure

6 b), Dallagnol *et al*. (2011) reported that under high relative humidity (RH), the rice plant was more susceptible, or the pathogen became more aggressive. The conidial production by *B. oryzae* has shown that RH of 92% and above was associated with higher production of conidia with a maximum at 100% RH (Chattopadhyay and Gupta, 1965), however, such a high level of DRH was not reported during this study.

As illustrated in Figure 6 (d), disease incidences were increased under MIN72 temperature (20-25 ℃) during the wet season. Studies on conidial production by *B. oryzae* have shown that, a temperature range of 21-26 ℃ was optimum and higher production of conidia (Chattopadhyay and Gupta, 1965). The incidence of the disease was reported to decrease from 28–30 ℃, and the rate increased to 33 ℃ (Figure 6 d). The temperature range of 25-35 ℃ is congenial for the growth of the fungus, while 25-30 ℃ is optimal for disease development. Thus, during the wet season, the disease incidences were accelerated with an alternation between optimum conidial growth and disease development (Chakrabarti, 2001). Among the five temperatures studied by Arshad *et al*., 2013, 28 ℃ was the best temperature for growth followed by 25 ℃ and 30 ℃. The minimum temperature was at 20 ℃ and 35 ℃. These results are in line with Percich *et al*., (1997) who showed that the lesion density was increased at optimum temperature (25-30 ℃).

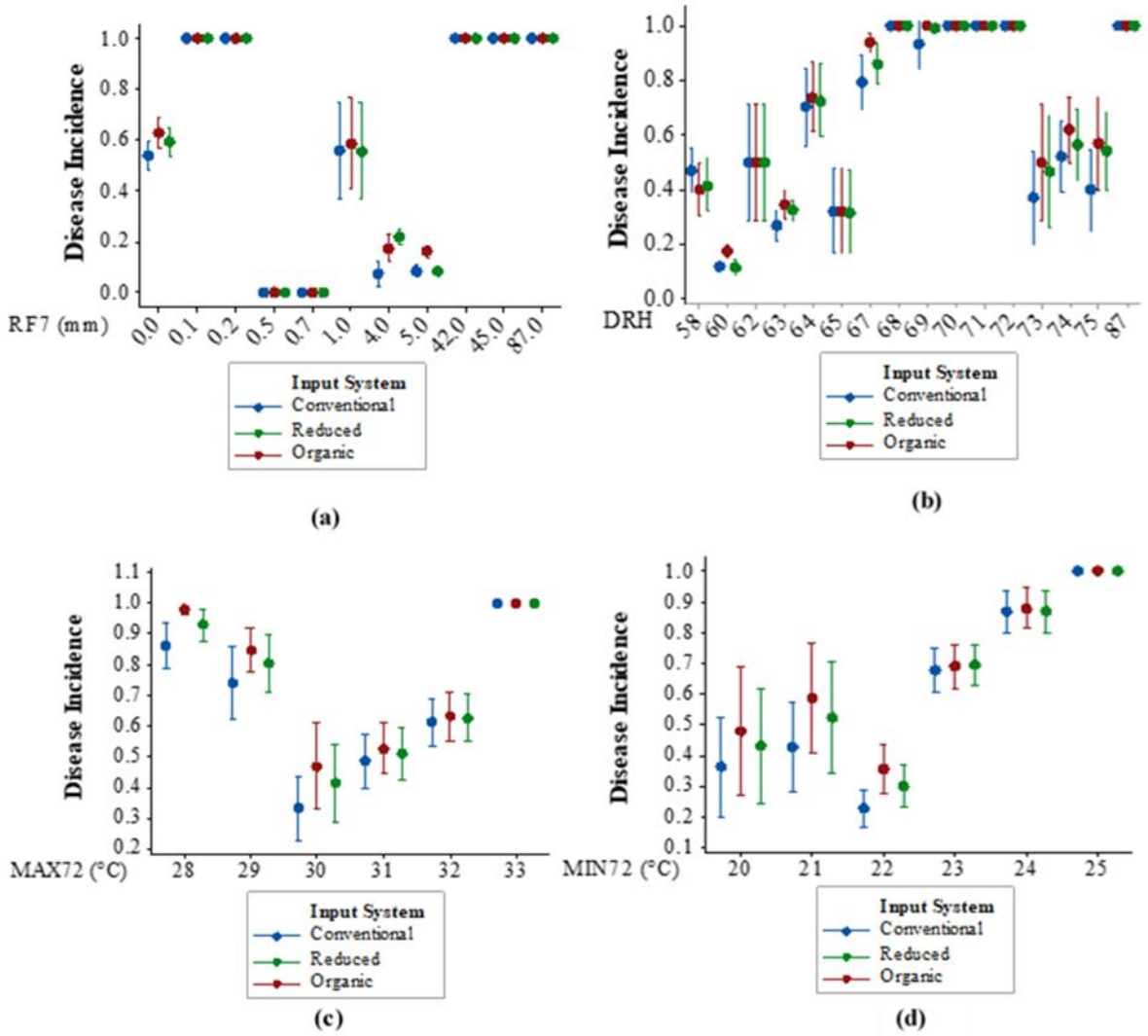


Figure 6. Effect of (a) RF7, (b) Day RH, (c) MAX 72 and (d) MIN 72 on wet season disease incidence with three different cropping systems

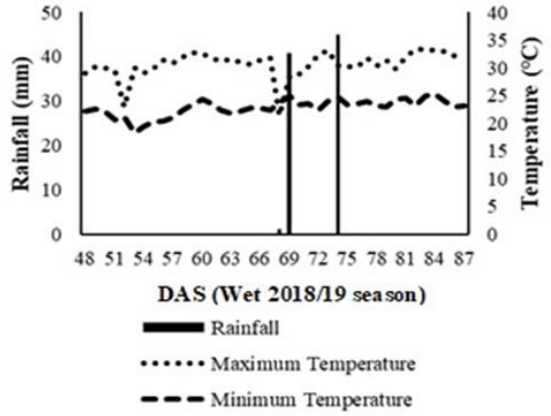


Figure 7. Daily rainfall, maximum and minimum temperatures in the wet 2018/19 season.

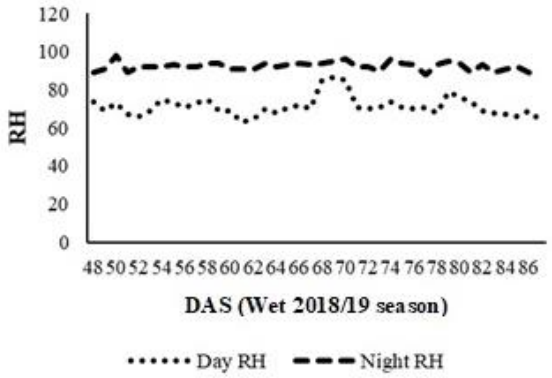


Figure 8. Day and Night RH of the wet 2018/2019 season.

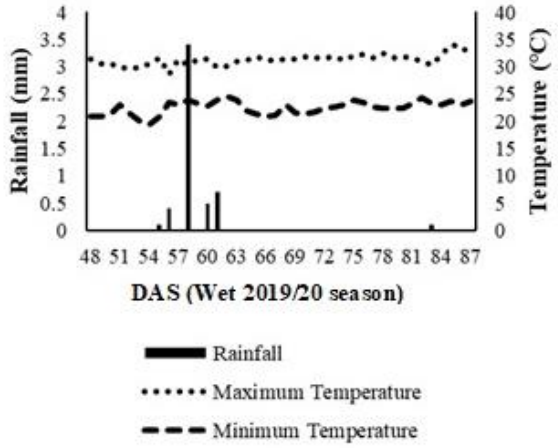


Figure 9. Daily rainfall, maximum and minimum temperatures in the wet 2019/2020 season.

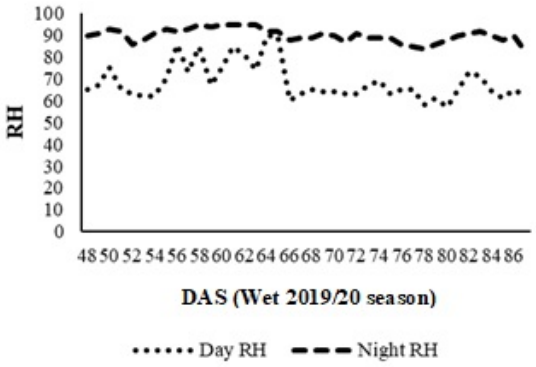


Figure 10. Day and Night RH of the wet 2019/2020 season.

CONCLUSIONS

The pathogen was microscopically and molecularly confirmed as *Bipolaris oryzae*. Phylogenetic separation showed three monophyletic clades, but with the sequence information, the locally isolated *B. oryzae* isolate only has some single nucleotide polymorphism from the other *B. oryzae* isolates. The *Organic* crop management system which was supplied with comparatively low N content was more prone to RBLS disease, whereas the *Conventional* crop management system (with a recommended dose of N) reported a lower incidence in both wet and dry seasons. The disease incidences were higher in wet seasons. The highest disease incidence was reported during the reproductive stage of the rice crop under all three crop management systems and seasons. The daytime relative humidity, and minimum temperature obtained 72 h of disease observation correlated with higher disease incidences, yet cumulative rainfall 7 days before was not correlated. Based on these findings, RBLS disease can be managed concerning different crop management systems as the best disease control remedy during the wet season under high daytime relative humidity and minimum temperature ranging from 20-25 ℃.

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