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## EPIDEMIOLOGY OF RICE BLAST (*PYRICULARIA ORYZAE*) DISEASE IN CENTRAL PUNJAB, PAKISTAN

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### ABSTRACT

Rice blast disease (RBD) is mostly controlled by fungicides by the farmers of central Punjab, Pakistan. However, the use of fungicides by the farmers is excessive and ill-advised, resulting in the emergence of new resistant strains of *Pyricularia oryzae*. The ill-advised employment of fungicides can be timed exploring the role of environmental factors favorable for this disease. The objective of current study was to determine the most favorable weather conditions for RBD in central Punjab, Pakistan, where this crop is mostly cultivated. Environmental factors including maximum and minimum temperatures (max and min temp), rainfall (Rf), relative humidity (Rh) and wind speed (Ws) conducive for RBD were characterized during this study. For this purpose, eight years (2009-2016) RBD severity data of susceptible to highly susceptible genotypes together with environmental data (max and min temp, Rf, Rh and Ws) was collected from Kala Shah Kako (KSK), Rice Research Institute (RRI), Punjab, Pakistan. The genotypes were cultivated for eight years in randomized complete block design (RCBD), and data was kept on recording during the months of high disease pressure. Data was collected after a ten days interval using disease scoring scale developed by International Rice Research Institute (IRRI) during 1996. Simple linear regression models were used to determine the relationship of environmental factors with RBD severity. The variation in RBD severity due to environmental factors was determined using the coefficient of determination ( $R^2$ ). In the present study, the relationship of max temp, Rf, Rh and Ws with RBD severity was positive, significant and linear, however, the relationship of min temp with RBD severity was negative. Max temp 40-42°C, min temp 21-23°C, Rf 2-3mm, Rh 50-70% and Ws 9-11 Km/h were found to be most favorable environmental conditions for RBD severity. The current research disclosed the significant role of all five environmental factors in the spread of RBD. Thus, future predictive models could be established using these five environmental factors for more accurate prediction of this disease in rice belt of Punjab, Pakistan, to time the application of fungicides.

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### INTRODUCTION

Rice (*Oryza sativa*) is the main kharif crop of Pakistan. It

is the second staple food of Pakistani people, and also the second major exportable commodity of Pakistan. In

Pakistan, its contribution to GDP is 0.6 percent, while it contributes 3.1 percent in value addition of agriculture (GoP, 2022). Rice crop is affected by different diseases which cause enormous losses worldwide. Rice blast disease (RBD) caused by *Pyricularia oryzae* is one of the most threatening diseases of rice crop. This disease may cause heavy yield losses in rice and even under severe conditions complete failure of crop has also been reported (Xiao *et al.*, 2020). This disease is causing about 15% yield losses in China every year (Feng *et al.*, 2022). Decrease in rice yield due to RBD is the alarming threat for world food security, because this crop is fulfilling the calories requirements of most parts of the world. Hence, the control of RBD is vital to ensure world food security. Different management approaches are being used to control this disease effectively, still there is need to develop further many strategies to control new virulent races of this pathogenic fungus (Simkhada and Thapa, 2022). The main reason for emergence of new races is the imprudent use of fungicides which can be made judicious with the knowledge of epidemiology of this disease.

The occurrence, development and spread of RBD depends greatly on the environmental factors that include maximum and minimum temperatures (max and min temp), relative humidity (Rh), rainfall (Rf) and wind speed (Ws) (Shahriar *et al.*, 2020; Ahmad *et al.*, 2022). In both tropical and sub-tropical regions, the fungal mycelia can survive on plant residues for more than one season. Temperature ranging from 18 to 20°C favors the survival of mycelia on rice straws for more than three years and high moisture conditions lead to development of conidia. The availability of favorable environment including Rh and strong winds allow the spread of conidia up to 230 meters (Agbowuro *et al.*, 2020). These airborne conidia can survive the whole year and are responsible for severe epidemics (Raveloson *et al.*, 2018). Spore growth and development of lesions is favoured by wetness of leaves, 92-96% Rh and 25-28°C air temperature (Kankanala *et al.*, 2007; Agbowuro *et al.*, 2020). The severity of RBD is always high during mild daytime temperature with regular and long duration of dew drops (Liu *et al.*, 2004).

Sporulation of the pathogen of RBD is favored by increased periods of leaf dampness due to deposition of dew and temperatures ranging from 17 to 23°C (Katsantonis *et al.*, 2017), as water is necessary for the discharge of conidia, and furthermore water on the leaf

surface favors their discharge at high rate. Research showed that blast lesions on seedlings developed at the temperature ranging from 25 to 30°C but were unable to develop at 15-20°C (Manandhar *et al.*, 1998). Thus, development of blast lesions in rice seedlings is highly dependent on temperature conditions (Katsantonis *et al.*, 2017). It is observed that incidence of RBD is correlated positively with Rh and Rf, and negatively with temperature, this means that disease incidence increases when Rf and Rh increase and it decreases when temperature increases above the favorable limits (Shafaullah *et al.*, 2011). So, it is evident that epidemiological factors play a crucial role in the development of RBD. But research is still lacking in this regard, as the pathogen is continuously evolving, and climate change is causing changes in environmental factors (Lee *et al.*, 2022). Thus, comprehensive new research is required to explore the effect of environmental conditions on the RBD development, overwintering and spread. This will help to find out the novel domains of environmental impact on RBD and will be beneficial in developing a disease predictive model for RBD. The following study was aimed at to characterize the most favorable weather conditions (max and min temps, Rf, Rh and Ws) for RBD to develop future decision support system for forecasting RBD and accordingly timing the application of fungicides in central Punjab, Pakistan. This will help curtailing the number of fungicides applications and subsequently conserving environment and avoiding the emergence of new resistant strains of *P. oryzae* in this region of Punjab, Pakistan.

## MATERIALS AND METHODS

To study the epidemiology of RBD, the historical RBD severity data from 2009 to 2016 was collected from Kala Shah Kako (KSK), Rice Research Institute (RRI), central Punjab, Pakistan (74.2677° E, 31.7250° N and 204 m above sea level). This data was recorded on susceptible to highly susceptible varieties. The varieties were cultivated for eight years in randomized complete block design (RCBD) in a plot of 2×10 meter. Three repeats were maintained in RCBD during the period of eight years (2009-2016). Each variety was being sown in three- meter row length during the period of eight years. The distance between the two plants and two rows were maintained at 9 inches, respectively. The soil of research trials was sandy-clay-loam and was being used

previously for screening trials of rice genotypes against RBD; hence, being served as sick plot during eight years research period. Further, the soil had 19% silt, 25% clay, 26% sand, 5% free lime, TSS 30.9 mmol/L, pH 7.39 and EC 3.08  $\text{dsm}^{-1}$ . Fertilizers in the research plots were applied on recommended rates, while recommended agronomic practices were performed to maintain the good soil conditions (Sarwar *et al.*, 2022). Weeds from the research area were being removed manually using labour. For the infection of RBD, artificial and natural inoculum were relied upon. The detail of culture preparation of RBD has already been described in detail

in our recently published paper (Saneela *et al.*, 2022). For artificial inoculation, spore suspension of  $10^6$  spores/mL was measured using hemocytometer from the prepared culture of *P. oryzae* and sprayed twice a day (morning and evening) on the research trials unless clear symptoms of RBD appeared on the varieties. The RBD severity data was recorded for two months (August to September 2009-2016) after ten days interval using zero to nine scoring scale (IRRI, 1996; Table 1). The reason for recording RBD severity in these two months was that in previous extension surveys disease was reported high during these two months.

Table 1. Disease scoring scale for RBD.

Grade/Rating	Disease Intensity/Severity
0	Zero Lesion
1	Pinpoint size brown specks or bigger brown spots containing no spores
2	Round, small, 1-2mm gray spots having brown margins. These spots are more evident on the leaves present on lower side of the plant
3	Number of lesions (same as in grading 2) on leaves present on upper side of the plant
4	Three-mm or even longer characteristic blast lesions covering <4% leaf area
5	Three-mm or even longer characteristic blast lesions covering 4-10% leaf area
6	Three-mm or even longer characteristic blast lesions covering 11-25% leaf area
7	Three-mm or even longer characteristic blast lesions covering 26-50% leaf area
8	Three-mm or even longer characteristic blast lesions covering 51-75% leaf area
9	Three-mm or even longer characteristic blast lesions covering >75% leaf area of infected plant

### Statistical analysis

The weather data of max and min temps ( $^{\circ}\text{C}$ ), Rh (%), Rf (mm) and Ws (km/h) of the years 2009-2016 was collected from the weather observatory, RRI, KSK. The effect of each weather parameter (max and min temps, Rh, Rf and Ws) on RBD severity was determined by plotting scatter graphs and using simple linear regression analysis (Steel *et al.*, 1997). In regression analysis, RBD severity served as dependent variable while weather parameters served as independent variable. Coefficient of determination ( $R^2$ ) was used to determine the variability in RBD severity due to environmental factors (Chattefuee and Hadi, 2006).

### RESULTS

There was a positive and linear relationship between max temp and RBD severity during all eight years (Figure 1). Linear regression models showed an average 86% variability in RBD severity as a result of max temp (Figure 1). The average eight years scatter plot showed maximum RBD severity at the max temp of 40-42 $^{\circ}\text{C}$ . Min temp

exhibited negative and linear relationship with RBD severity during eight years (Figure 2). Linear regression models explained an average 67% variability in RBD severity as a result of min temp (Figure 2). The average eight years scatter plot of min temp showed that RBD severity was maximum at the min temp of 21-23 $^{\circ}\text{C}$ . The relationship of Rf with RBD severity was also positive and linear. The average eight years scatter plot of Rf showed maximum RBD severity at 2-3 mm Rf. Linear regression models explained average 75% variability in RBD severity as a result of Rf (Figure 3). The relationship of Rh with RBD severity during the period of eight years was also positive and linear. Linear regression models explained average 78% variability as a result of Rh (Figure 4). The average eight years scatter plot of Rh showed maximum RBD severity at 50-70% Rh. Similarly, the relationship of Ws with RBD severity was also positive and linear. Linear regression models explained an average 87% variability in RBD severity as a result of Ws (Figure 5). The average eight years scatter plot of Ws showed maximum RBD severity at the Ws of 9-11 Km/h (Figure 5).

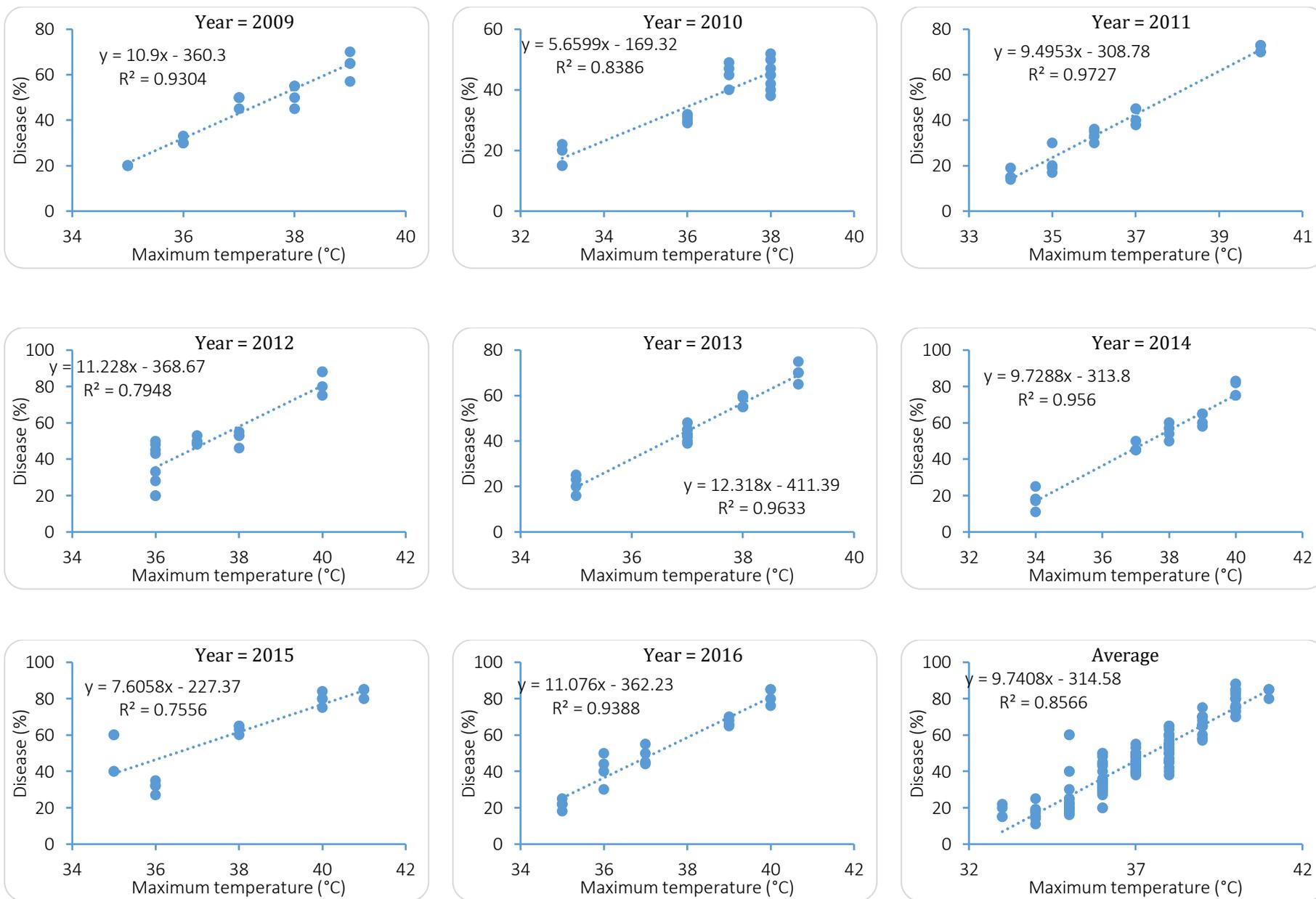


Figure 1. Relationship of max temp (°C) with RBD severity during 2009-2016.

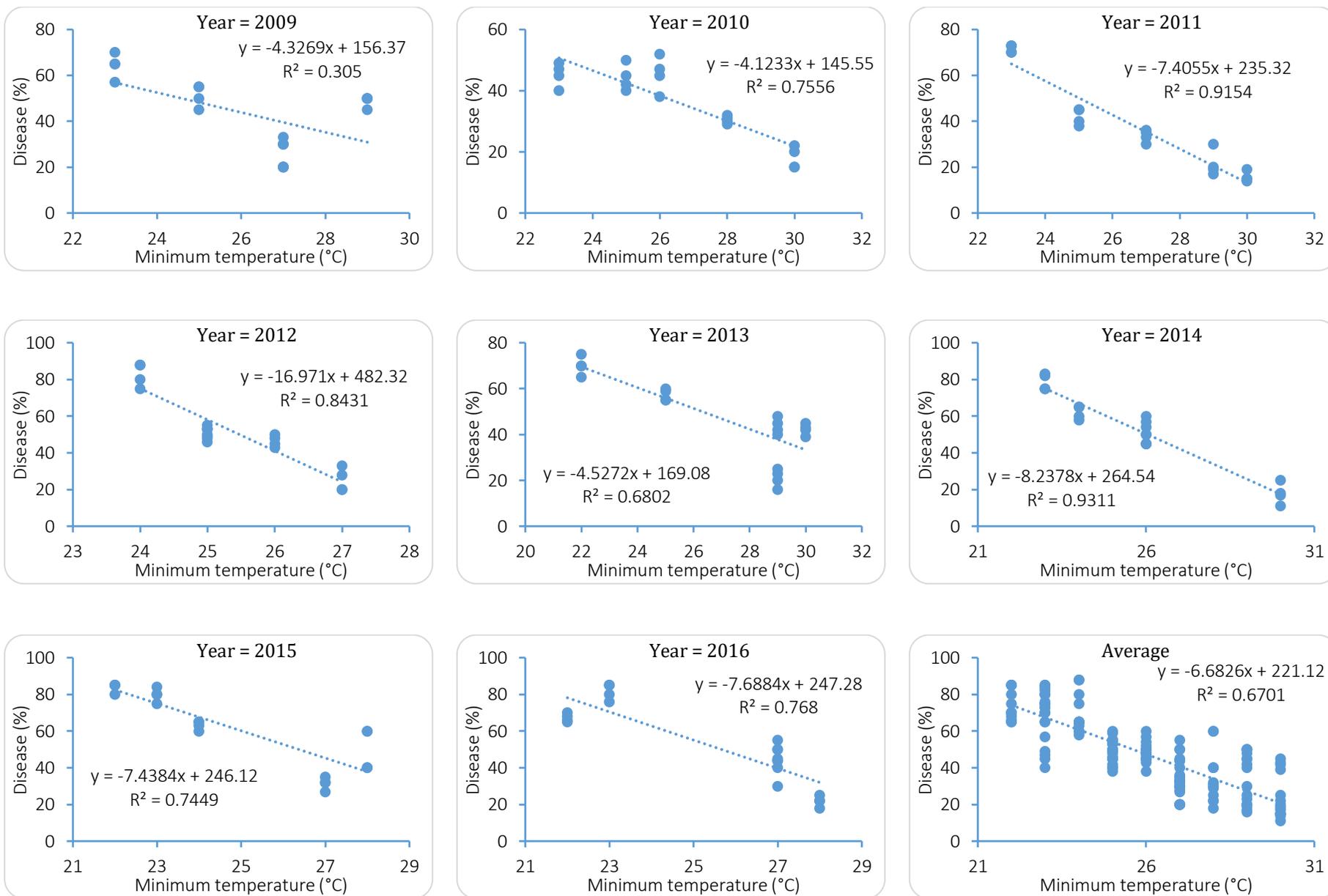


Figure 2. Relationship of min temp (°C) with RBD severity during 2009-2016.

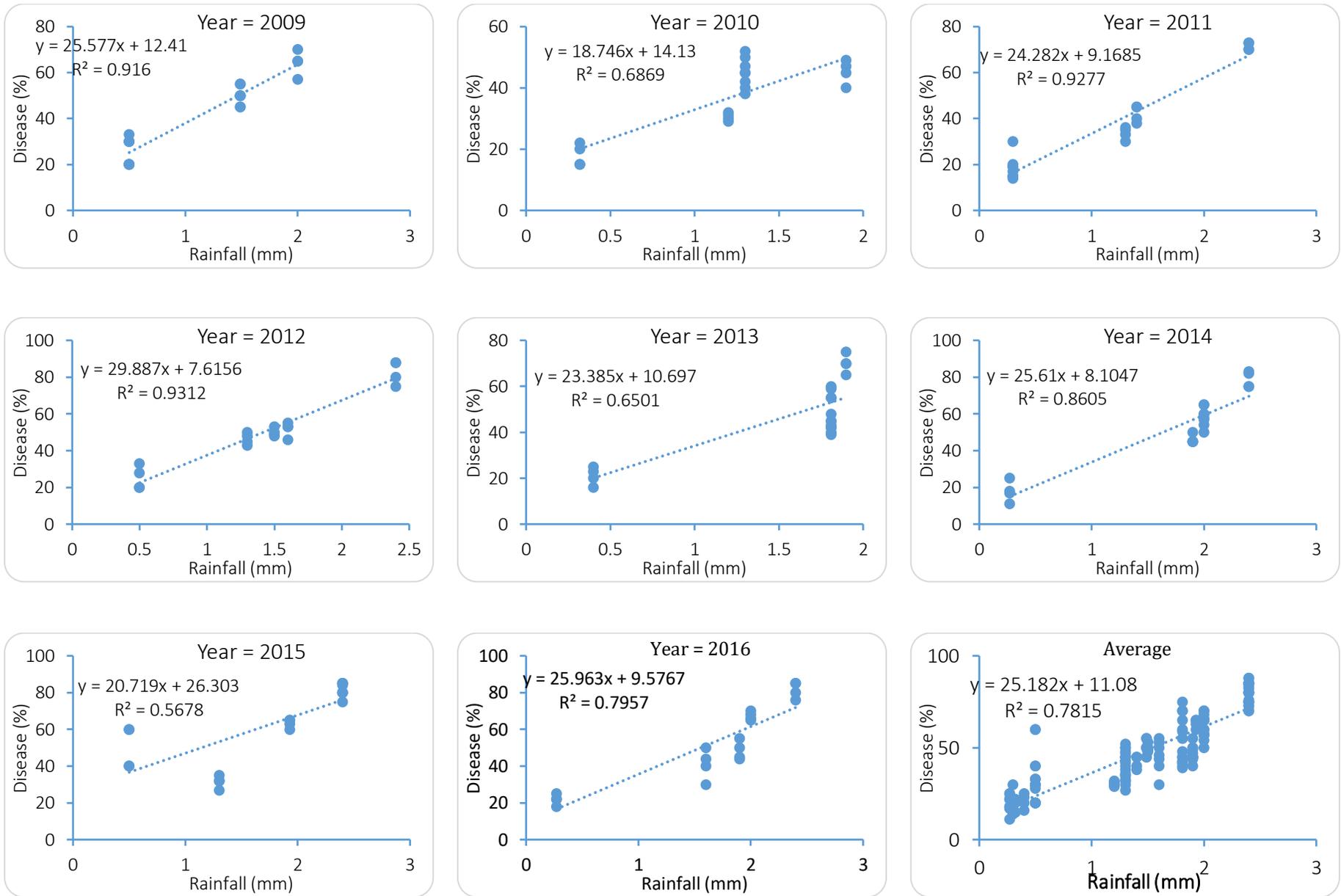


Figure 3. Relationship of Rf (mm) with RBD severity during 2009-2016.

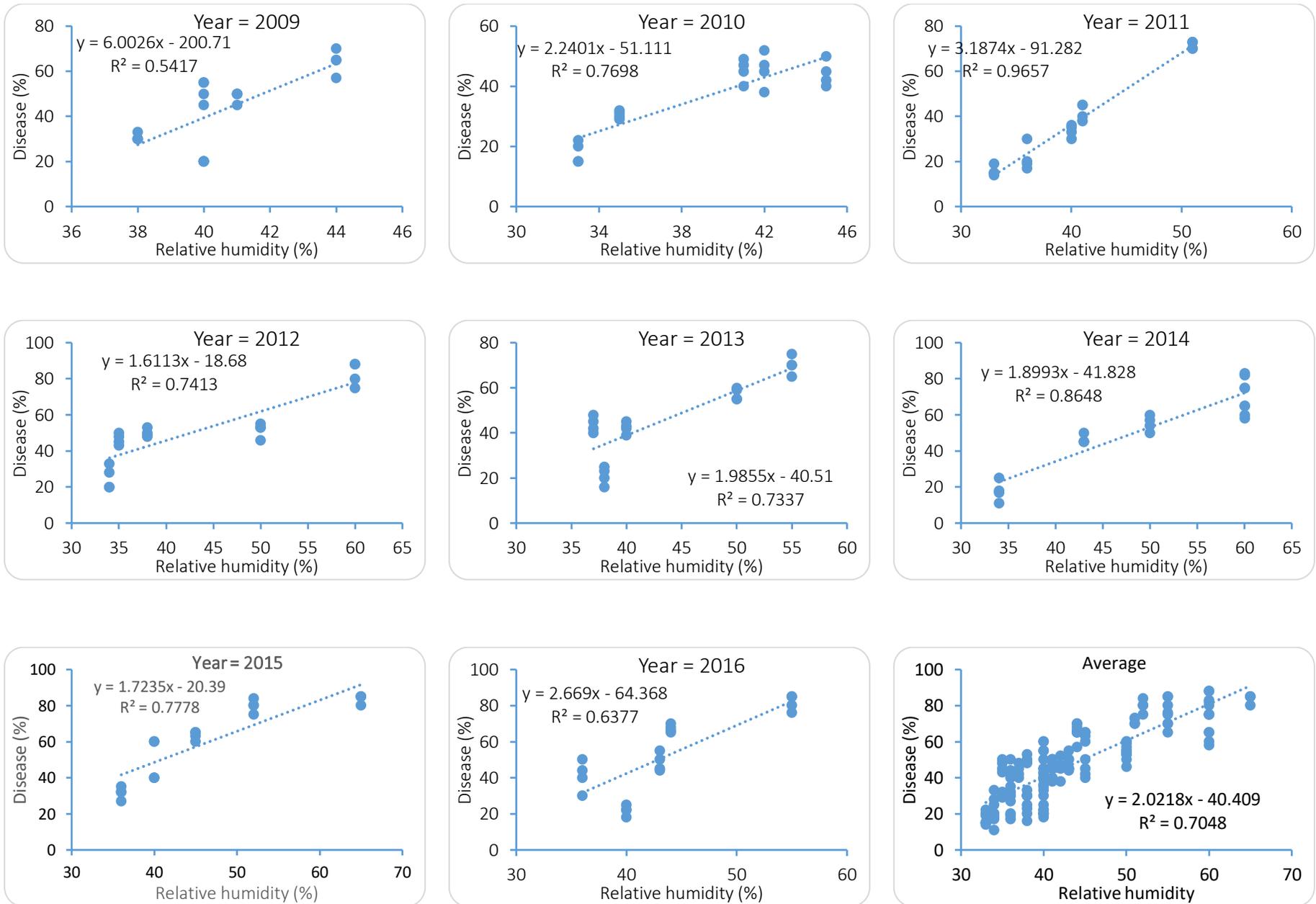


Figure 4. Relationship of Rh (%) with RBD severity during 2009-2016.

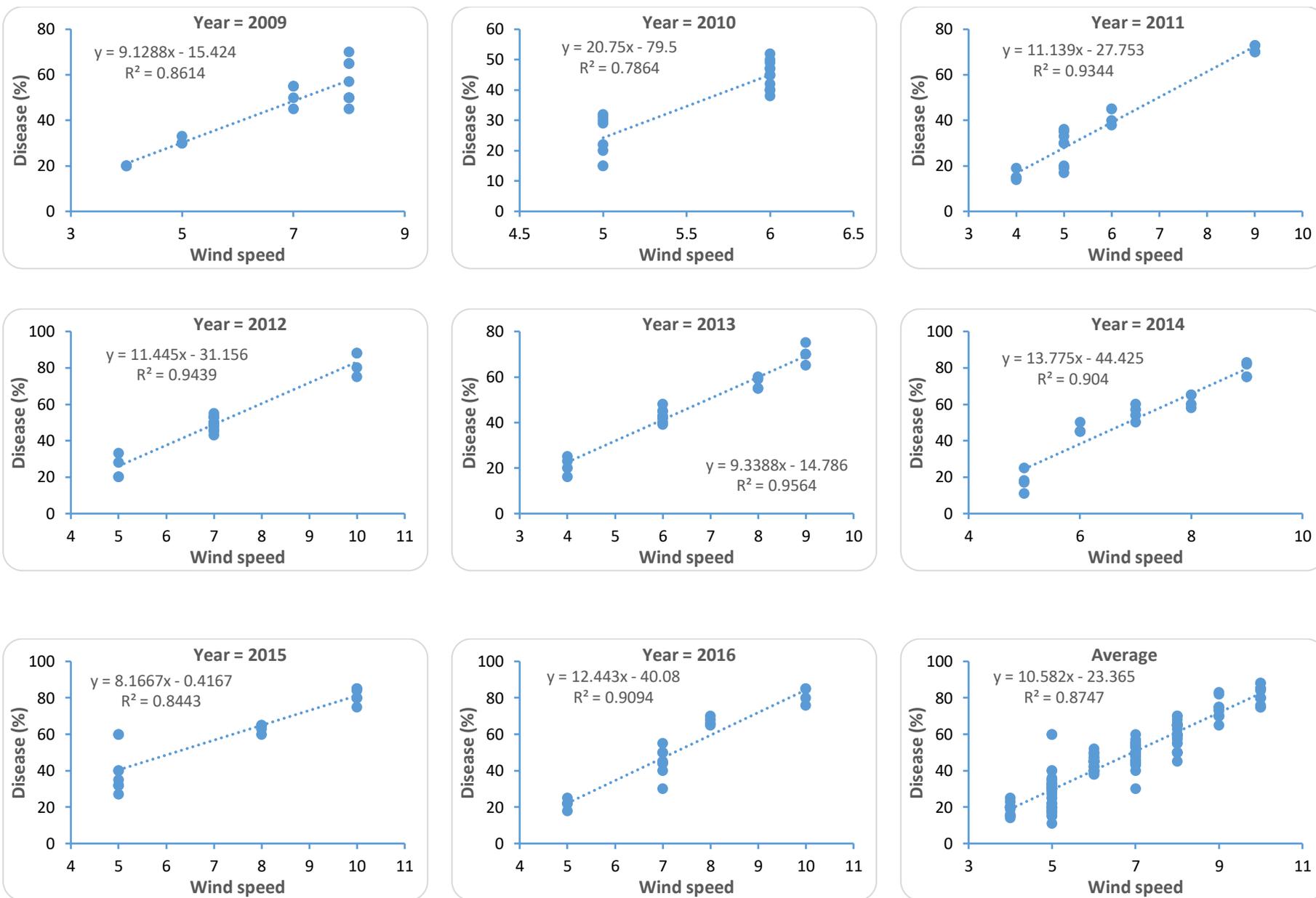


Figure 5. Relationship of Ws (km/h) with RBD severity during 2009-2016.

## DISCUSSION

The current study revealed that all five environmental factors, viz., max and min temps, Rf, Rh and Ws significantly affected RBD severity. It was observed that fluctuation in environmental factors also significantly changed RBD during the period of eight years. These findings are in line with Katsantonis *et al.* (2017). Weekly max temp had a significant effect on the RBD severity during all eight years (2009-2016). The relationship of max temp with RBD severity was linear and positive which means RBD severity increased with the increasing max temp. In our study, the max temp in the range of 40-42°C was found conducive for RBD severity. In the present study, min temp also significantly affected RBD, however, its effect was negative and RBD severity decreased with the increasing min temp. The negative relationship of min temp has also been reported by Shafaullah *et al.* (2011). They also reported the main temp ranges 20-22°C favorable for RBD. The significant effect of temperature on RBD may be due to different reasons. For example, temperature has a significant role in the survival of conidia. It has been observed that change in temperature ranges significantly affect survival of the conidia (Greer and Webster, 2001). In present study, the conducive ranges of min temp we found for RBD are not in agreement with the Rajput *et al.* (2017). They concluded in their study that temperature 22-32°C were conducive for RBD. This may be due to the reason that the area in which they conducted the trial might have a different climate. The second reason may be the virulence behavior of the pathogen present in that research area where the experiment was conducted. The temperature has also significant role in the sporulation and lesion development of *P. oryzae*. The infection model explains that with change in each degree of temperature (average temperature), lesion development and sporulation change 0.20 units (Rajput *et al.*, 2017). In our study, RBD severity increased with increasing max temp which is in agreement with Rajput *et al.* (2017). They reported that with the increase of max temp lesion development also increases owing to increase in sporulation. In our study, the role of Rf was also significant and linear. This is in line with Refaei (1977). In our research, the maximum RBD severity was observed at weekly average Rf of 2-3 mm. Similar findings have been reported by Katsantonis *et al.* (2017). The significant role of Rf in the spread of RBD may be due to its role in conidial discharge. It has

been reported that more water on the surface of leaves favours the discharge of conidia (Manandhar *et al.*, 1998). In our study, Rf caused variability in RBD severity near 80%. This is in line with Kim *et al.* (2017). The role of Rh in our study was also significant and linear and caused variability in RBD severity up to 70%. This is in agreement with Liu *et al.* (2021). In their study, the variability in RBD due to Rh was 60% which is almost close to our findings. Rh has also been found significant in the prediction models for blast disease (Donatelli *et al.*, 2017). Our findings also correlate with the findings of Donatelli *et al.* (2017) that with the increase of Rh blast severity also increases. It has been reported that RBD does not appear on susceptible cultivars unless suitable Rh prevails (Kim and Jung, 2020). In our findings, the role of Ws was also significant and linear for RBD. Our findings are in agreement with several researchers (Ashizawa *et al.*, 2005; Pandit *et al.*, 2023). Ws significantly affects the temporal dynamics of RBD (Katsantonis *et al.*, 2017). The significant role of Ws on RBD severity may be due to its role in dispersal and deposition of conidia (Katsantonis *et al.*, 2017). Ws also plays key role in liberation of conidia from conidiophore. Conidium production and concentration in the air also depend on Ws (Koizumi and Kato, 1991).

## CONCLUSION

The current research disclosed the significant role of all five environmental factors in the spread of RBD. Thus, future predictive models could be established using these five environmental factors for more accurate prediction of this disease in rice belt of Punjab, Pakistan.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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