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Effects of fungicide application timings and genetic resistance of rice varieties on the reduction of percent severity of narrow brown leaf spot disease

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Narrow brown leaf spot disease of rice caused by fungus *Cercospora oryzae* Miyake emerged as potential threat to rice crop in previous few years worldwide and can cause more than 40% yield loss. The disease symptoms were noted on various commercially important rice varieties in the rice growing districts of Punjab province of Pakistan. Hence, the studies were planned to assess the effects of Propiconazole fungicide application timings and different rice varieties on the severity of narrow brown leaf spot (NBLS) disease of rice to develop an accurate management strategy for the disease. The Propiconazole fungicide was tested at three various growth phases (early booting, late booting, and soft dough) of four rice varieties against the percent severity of the NBLS disease The experiments were designed under strip-plot (crisscross) design with four replications at research area of Plant Pathology Department, University of Agriculture Faisalabad. Analysis of variance has demonstrated that the main and interactive effects of fungicide applications and rice varieties were found significant. The main effects of years were found non-significant but their interactions with fungicide application timings and rice varieties were significant. Fungicide applications at soft dough and late booting phases of rice variety B-515 were found least effective to reduce the disease severity, respectively. Fungicide application at early booting of rice variety PK-1121 was found most effective by reducing the maximum percent diseases severity (47.25%) compared to control. It is concluded that, to minimize hazardous effects of chemicals on the environment by practicing multiple sprays at inappropriate growth phases of rice crop, fungicides must be applied during the early and late booting phases of rice varieties on mildly resistance, susceptible and highly susceptible rice varieties to reduce NBLS disease severity and to enhance the rice production and quality.

Keywords: Propiconazole**,** Rice varieties, Growth phases, *Cercospora oryzae*, Narrow brown leaf spot, Percent disease severity.

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INTRODUCTION

The rice is an important food and cash crop of Pakistan. It is estimated that rice is cultivated approximately on an area of 2899 thousand hectares, which resulted in 7442 metric tons production. Its contribution in value addition is 1.3 % of the agriculture sector and 0.6 % in gross domestic production of Pakistan (Anonymous, 2018-19). The world's best rice (basmati) is produced in the country, which is highly demanded nationally and internationally due to its delicious taste and distinctive aroma (Zaman *et al.*, 2015; Iqbal *et al.*, 2022a). Pakistan earned approximately 500 million US $ per year from the export of rice and ranked as third biggest exporter (Mustafa *et al.*, 2013). However, per acre yield of rice crop in Pakistan is much less in comparison to other advanced countries due to poor disease management practices. Several diseases attack on rice crop among them fungal diseases (blast, brown leaf spot, narrow brown leaf spot, sheath blight and bakanae) are devastating. Among these diseases, narrow brown leaf spot disease caused by *Cercospora oryzae* Miyake (Iqbal *et al.*, 2022b) always remained overlooked due to its sporadic nature and no studies were carried out in the country earlier regarding its management. But it has become a serious threat to the rice industry in the rice growing districts of Pakistan and around the world over the last few decades. It has recently attracted a lot of attention due to several of its outbreaks that have significantly reduced the quality and quantity of the rice crop (Groth and Hollier, 2010; Groth, 2013; Groth *et al.*, 2014; Iqbal *et al.*, 2022a). More than 40% loss of production is reported due to some epidemics of the disease (Zhou and Jo, 2014; Mani, 2015; Soura *et al.*, 2020). In severe cases, more than 50% of the cultivated area can be damaged (Groth, 2013). Now it is considered to be one of the biggest and most

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serious rice diseases in some major rice-growing regions of the world (Sah and Rush, 1988; Uppala *et al.*, 2019), including Pakistan. The continuous evolution of new species of the *Cercospora oryzae* Miyake can easily overcome the genetic resistance of rice varieties and make them vulnerable to the pathogen attack (Groth, 2013). Mostly commercial rice varieties grown in the rice zone of the Punjab province rated as mildly resistance, susceptible and highly susceptible under controlled environmental conditions (Iqbal *et al.*, 2022a). In this respect, it is the need of time to determine a suitable management strategy to reduce the disease pressure well in time. It is documented that fungicidal management is a quick and time-saving strategy to manage or control different fungal diseases in different crops (Iqbal *et al.*, 2013; Mustafa *et al.*, 2013; Wamishe *et al.*, 2018; Barúa *et al.*, 2019; Chaulagain *et al.*, 2019; Haq and Ijaz, 2020; Persaud *et al.*, 2020). Different fungicides (Propiconazole, Azoxystrobin, Propiconazole plus Azoxystrobin, Propiconazole plus Arifloxystrobin, Flotolanil, Pyraclostrobin, Fluxapyroxad) have been tested for their efficacy against the infection of *Cercospora oryzae*, and among them, Fluxapyroxad and Propiconazole respectively were shown good results in the reduction of disease (Uppala *et al.*, 2016; Uppala and Zhou, 2018). The sprays during booting stages proved more effective in a multi-year studies (Uppala and Zhou, 2019). In a comparative study, Propiconazole was found quite effective as spray and soil drenching in the booting phase compared to Baviston and compost tea (Sultana *et al.*, 2017). Propiconazole significantly reduces fungal infections on rice crop (Gupta *et al.*, 2013). Hence, fungicides have adverse effects on physiological functions of plants and human health via food chain (Shahid and Khan, 2018). So, unnecessary use of chemicals should be avoided. Fungicides use at inappropriate growth stage should also be avoided because it may not be judicial due to their side effects. Hence, the judicial use of chemicals is an appropriate way for the timely management of plant diseases. The application timing of fungicides affects its efficacy against the progress of plant diseases. The significant variation in the efficacy of Propiconazole against the severity of NBLS disease has been noted due to changing its application timing on different rice varieties (Mani, 2015; Mani *et al.*, 2016; Uppala and Zhou, 2019). However, assessing accurate application time of fungicides at appropriate growth phase of rice varieties can minimize the side effects of chemicals on plants, human and environment and maximize the output of rice crop. Because, the management of plant diseases with fungicides has remained essential and unavoidable due to the gradual decline in the genetic resistance of plants to the new pathogenic species and resistant rice varieties become susceptible over time (Uppala *et al.*, 2016; Uppala and Zhou, 2018). Therefore, keeping in view the importance and side effects of the chemical management of the disease, the studies were planned with the following null hypothesis that Propiconazole applications at different timings on different rice varieties have same effects against the severity of NBLS disease. The objective of study was to assess the accurate timing of fungicide application on different rice varieties against the severity of NBLS disease.

MATERIALS AND METHODS

Selection of rice varieties: The four commercially important rice varieties i.e., Basmati-Super (B-Spr), PK-1121, Basmati-515 (B-515), and Basmati-Punjab (B-Pb), having different levels of resistance/susceptibility were collected from Rice Research Institute, Kala Shah Kaku (Sheikhupura) for the studies.

Inoculation of seeds: The disease is seed (Sah, 1986; Sah and Rush, 1988; Zhou and Jo, 2014; Uppala and Zhou, 2018; Wamishe *et al.*, 2018) and soil borne. The target pathogen *Cercospora oryzae* was isolated on potato dextrose agar (PDA) media from previous year preserved infected rice leaf samples obtained from Rice Research Institute, Kala Shah Kaku (Sheikhupura). The inoculum of *Cercospora oryzae* was prepared in V-8 agar media in the Mycology Laboratory of Plant Pathology Department, University of Agriculture Faisalabad. The seeds of four rice varieties were inoculated separately by soaking in the spore solutions of *Cercospora oryzae*, concentrated 10^5 conidial spores/ml (Uppala *et al.*, 2019) for 24h at room temperature and then dried by placing on blotter papers before sowing.

Experimental layout: The studies were arranged in strip plot/split block/crisscross design (Montgomery, 2005) with four replications at research farm (31.44° N and 73.07° E) of Department of Plant Pathology, University of Agriculture Faisalabad during the month of June-November of the year 2018 and were repeated in 2019. The soil was silt loam (pH 6, clay 18 %, silt 66%, and sand 16 %). The four rice varieties were sown with drill horizontally on four strips named as whole plots in each replication. Each whole plot was vertically divided in to four sub-plots for fungicide applications. The fungicide (Propiconazole) was applied in the sub-plots at different growth phases (early booting, late booting and soft dough) of rice varieties. No application (NA) of fungicide to a sub-plot of each whole plot for control purpose. The dimensions of each whole plot were 12 m long horizontally and 3 m vertical in the form of a strip, and the size of each sub-plot was 3 × 3 m in each whole plot. Recommended agronomic and insect pest practices were applied.

Fungicide application timings: The recommended dose of Propiconazole (Tilt 250 EC, Syngenta) fungicide (0.15 kg a.i. ha^{-1}) was sprayed at early booting (EB), late booting (LB), and soft dough (SD) phases of four rice varieties. One experimental unit (sub-plot) in each whole plot carried no application (NA) of Propiconazole for comparison with other treatments and named as control. At the EB phase, the spray was done when panicle size was reached 8 cm in the boot. At the LB phase, the spray was applied just before the emergence of heads. The third application of Propiconazole was sprayed on the SD growth phase of rice varieties.

Assessment of NBLS disease: The disease development data was recorded after two months of germination of rice seedlings on two weeks interval just before harvesting. In each experimental unit (sub-plot), ten plants were tagged arbitrarily to observe the final NBLS percent disease severity (PDS). The PDS was measured from the lower middle, central, and flag leaves by visual observations according to a slightly self-modified 0-9 disease rating scale (Mani *et al.*, 2016; Iqbal *et al.*, 2022a).

Statistical analysis of the disease data: The disease data of each experiment was analyzed by computer software GenStat (10^{th} edition) to interpret the results accurately with relevant analysis of variance (George and Mallery, 2019). The rice varieties (RV), fungicide applications (FA), and Years (Yr) were taken as treatments to analyze their main and interactive effects. The RV was placed in rows as whole plots and FA in columns as sub-plots. The replications (R) were used as block during the statistical procedure. The normality of percent disease severity (PDS) data of narrow brown leaf spot (NBLS) was tested by box and whisker plots. The main and interactive means of PDS were used to assess the effects of treatments by Fisher's protected least significant difference (LSD) test at a 5 % significance level.

RESULTS

ANOVA (Table 1) has shown that NBLS percent disease severity (PDS) is significantly affected due to fungicide applications (FA) at different growth phases, genetic resistance/susceptibility of rice varieties (RV), and their interactions as their F. Pr. values were ≤ 0.01. Fungicide application at various growth phases of all rice varieties was the critical tool that significantly reduced PDS over control. The difference in genetic level of resistance/susceptibility of rice varieties (RV) was the second source, and their interaction with fungicide applications (RV × FA) were found third source that significantly affected the percent disease severity of NBLS disease. Box and whisker plot showing the five number summaries of the PDS data on four rice varieties affected by fungicide applications at their three growth phases in figure 1. Box and whisker plots show that there is no outlier exists in the data set of PDS distribution on all rice varieties. The test value of skewness (1.28) and kurtosis (1.39) shows that the distribution of PDS data is normal (George and Mallery, 2019). Table 1 has exhibited that the interactions (FA × RV) of fungicide applications rice varieties were significant. So, interactive means (FA × RV) were used to draw inference beyond analysis of variance. Table 2 is showing the main and interactive means of PDS due to fungicide application timings in the different growth phases of rice varieties. The effects of fungicide applications at SD and LB phases of rice variety B-515 was at par in the reduction of PDS and found least effective to reduce the NBLS disease severities, respectively. The fungicide applications on EB and LB growth phases of rice varieties B-Pb and B-Spr have statistically similar effects on the progress of PDS. The fungicide application at EB phase of rice variety PK-1121 was recorded most effective by reducing the maximum PDS (47.25%) compared to control. The sprays of Propiconazole were found more effective at EB and LB growth phases, respectively, of four rice varieties in reducing the NBLS severity, compared to control. Means having the same letters did not significantly differ. Table 1 also depicted that the main effects of years (Yr) were non-significant while the interactions of years with fungicide application timings (FA × Yr) have significant effects on the development of PDS. During the year 2019, more PDS was noted in control and fungicide treated EB phase of rice varieties respectively as compared to 2018 as depicted in table 3. During the year 2018, more PDS was observed at fungicide treated SD and LB phases as compared to the year 2019. Figure 2 also depicted the trend of PDS on rice varieties affected by fungicide applications at various growth phases during the both years of study. Other interactions of years (RV × Yr, and RV × FA × Yr) were not significant regarding posing the effects on PDS development. Figure 4 depicting the error bars with standard error (SE) on the development of PDS due to rice varieties and fungicide applications interactive effects (RV × FA). Error bars also reflecting the estimated PDS between samples and populations. The percent coefficient of variation (CV) values of all PDS data strata was < 10 which exhibited variability and relative standard deviation among datasets as shown below table 1. The percent severity of NBLS disease on four rice varieties noted in the control plots (no application of fungicide) is shown in the fig. 3.

Table 1. Analysis of variance (crisscross) for final PDS affected by rice varieties and fungicide applications at various growth phases in 2018 and 2019

S.O.V.	D.F.	M.S.	V.R.	F. Pr.
RV	3	7127.71	965.56	<0.001
FA	3	3377.31	2672.16	<0.001
RV×FA	9	525.45	208.25	<0.001
Yr	1	0.03	0.06	0.816
RV×Yr	3	0.20	0.35	0.790
FA×Yr	3	38.84	68.42	<.001
RV×FA×Yr	9	0.95	1.68	0.120

Stratum	Coefficient of variation (CV %)
R × RV	04.10
R × FA	01.70
R × RV × FA	04.80
R × RV × FA (Units)	03.20

LSD for RV = 1.54, FA = 0.64, RV×FA = 1.90, FA×Yr = 0.71, Yr = 0.37

Table 2. Comparison among main and interactive (RV×FA) means of final percent disease severity (PDS) affected by fungicide applications (FA) at various growth phases of four RV for the years 2018-19

Variety	Fungicide applications (FA) at growth phases				
	EB	LB	SD	NA (control)	Means
B-515	05.38^{l}	07.38^{k}	07.50^{k}	10.00^{j}	07.56^{D}
B-Pb	14.63^{i}	22.50^{g}	26.75^{e}	43.38^{b}	26.81^{B}
B-Spr	9.25^{jk}	14.50^{i}	20.38^{h}	25.38^{ef}	17.38^{C}
PK-1121	24.38^{f}	33.63^{d}	40.88^{c}	71.63^{a}	42.63^{A}
Means	13.41^{D}	19.50^{C}	23.88^{B}	37.59^{A}	23.59

Table 3. Comparative main and interactive (FA×Yr) means of final PDS affected by fungicide applications (FA) at various growth phases of four rice varieties for the years 2018-19

		Years (Yr)		Means
		2018	2019	
Fungicide applications (FA) at growth phases	EB	12.44^{h}	14.38^{g}	13.41^{D}
	LB	20.38^{e}	18.63^{f}	19.50^{C}
	HP	24.88^{c}	22.88^{d}	23.88^{B}
	NA (Control)	36.63^{b}	38.56^{a}	37.59^{A}
	Means	23.58^{A}	23.61^{A}	23.59

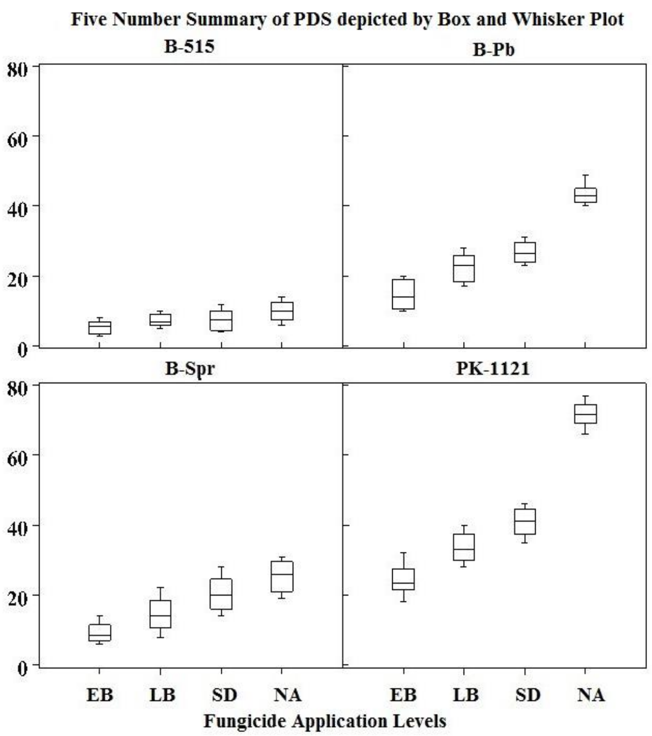

Figure 1. Box and whisker plot depicting the five number summary of final NBLS percent disease severity affected by fungicide applications (FA) in three growth phases (NA is control) of four rice varieties (RV). It is also depicted that there is no outlier in the NBLS disease data sets.

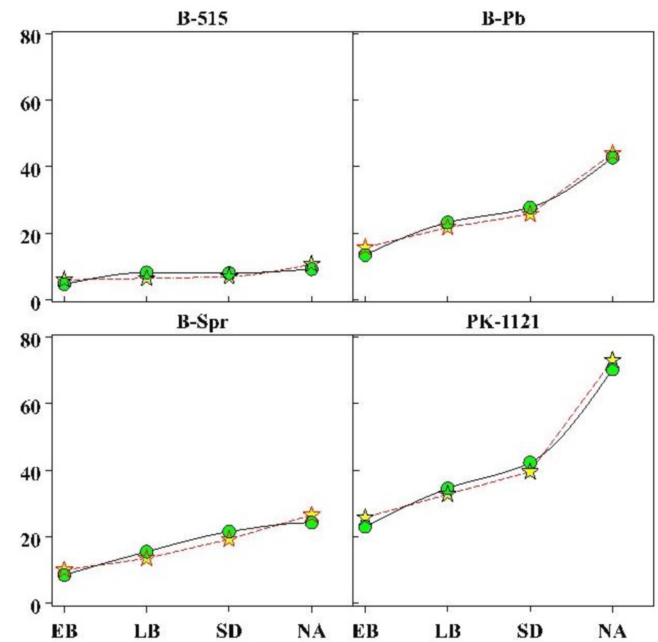

Figure 2. The trend of final PDS affected by interactions of fungicide applications (FA) at three growth phases (NA is control) of four rice varieties (RV) for the years 2018 (circles with solid line) and 2019 (stars with dot line)

Figure 3. Pictures a-d, showing the percent severity of NBLS disease on rice varieties B-515, B-Pb, B.Spr and PK-1121 respectively in the control plots (No application of fungicide)

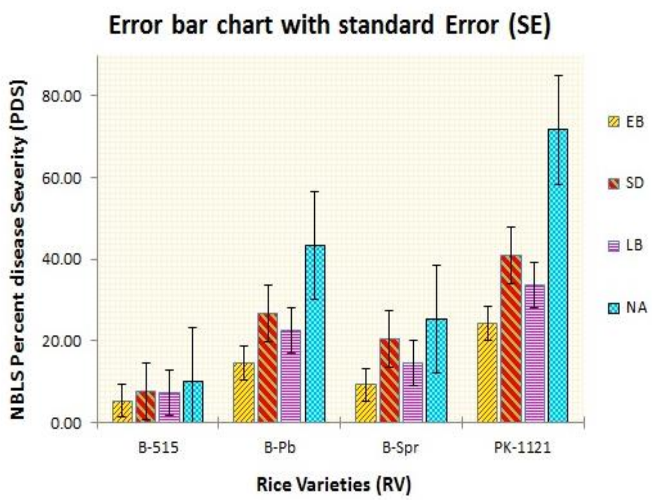

Figure 4. NBLS percent disease severity influenced by fungicide applications (FA) in different growth phases (NA is control) of four rice varieties (RV). Error bars depicted the estimated standard errors among the PDS means of samples from the actual population.

DISCUSSION

The results exhibited that fungicide application at any crop growth phase reduced PDS in all rice varieties compared to control. The results of present studies are likewise with the findings of various documented other studies which exhibited that Propiconazole significantly suppresses the infection of *Cercospora oryzae* Miyake (Groth *et al.*, 1990; Zhou and Jo, 2014; Lema, 2015; Mani, 2015; Razu and Hossain, 2015; Mani *et al.*, 2016; Uppala *et al.*, 2016; Tuli *et al.*, 2017; Uppala and Zhou, 2018; Uppala and Zhou, 2019). The Propiconazole was selected keeping in view its efficacy against the infection of *Cercospora oryzae* compared to other fungicides mentioned in literature which is demonstrated by the results of present studies. In a an experiment, the efficacy of Propiconazole was proved to be the most effective in restricting the NBLS disease severity (Uppala *et al.*, 2016; Uppala and Zhou, 2018) which justified the results of present studies. The *in-vitro* efficacy of Potent 250 EC (Propiconazole) was assessed at two levels (0.1% and 0.05% concentrations), and Potent 250 EC (Propiconazole) at 0.1 % concentration resulted in a maximum reduction in NBLS severity (Mahmud and Hossain, 2017). In another *in-vitro* study, five different groups of fungicides were tested via the relative percent germination technique of fungal spores to evaluate their efficacy against the production of different isolates of *Cercospora oryzae* on PDA media. The Propiconazole was found effective in suppression of conidial spore's production of different isolates of *Cercospora oryzae* Miyake and NBLS disease severity which favoring the findings of the present studies. In an *in-vivo* study, six fungicides were evaluated separate and in combination to assess their efficacy against NBLS severity during the years 2012 and 2014. Among them, Propiconazole solely and combinedly with other fungicides were highly effective in reducing the severity of narrow brown leaf spot disease (Lema, 2015). In an *in-vivo* comparative study, Tilt (0.1%) was sprayed after 80 days of transplantation of rice cultivar BRRI Dhan-49 to minimize the incidence of NBLS disease that was found to be most effective in suppressing the NBLS incidence and severity than others (Razu and Hossain, 2015). In a six years study, the efficacy of different fungicides against the NBLS severity was not the same. But the application of Propiconazole solo and combinedly with other fungicides remained consistent in significantly reducing the severity of NBLS disease in comparison with controls throughout the years (Uppala and Zhou, 2018). The mycelium development of *Cercospora oryzae* showed a significant reduction in Petri plates contained Propiconazole amended media (Uppala *et al.*, 2016). These studies argued that NBLS disease severity can be quickly managed with Propiconazole fungicide which justified the conduction of present studies. Several studies also advocated that timing of fungicides is very important and cost effective in reducing the disease spread. The spray of Propiconazole at panicle initiation and early booting phases has been shown to be more effective than late booting against NBLS disease. This fungicide is a sterol biosynthesis inhibitor or demethylation that restricts the production of the fungal cell wall and spores germination (Mani, 2015; Mani *et al.*, 2016). The results supported the finding of present studies that NBLS disease severity significantly reduced with Propiconazole spray at early booting phase. In another study, the single and multiple sprays of Propiconazole at booting and heading phases of main and ratoon crops of rice were proved effective against the NBLS disease severity compared to control. Multiple sprays of Propiconzole on ratoon crops have shown greater efficiency against the NBLS disease severity. The sprays during booting stages proved more effective in a multi-year study (Uppala and Zhou, 2019). In case of severe infection of NBLS disease, spraying of Tilt (Propiconazole) was helpful to avoid great loss of yield when applied in the booting phases (Zhou and Jo, 2014) which are proved by the results of current studies that sprays at early and late booting growth phases are more effective than spray at soft dough or heading phase. In a comparative study, Propiconazole was found quite effective as spray and soil drenching in the booting phase (Sultana *et al.*, 2017). The fungicide Proud (Propiconazole) 25 EC 0.1% exhibited minimal disease severity in dough and maturity stages, respectively (Tuli *et al.*, 2017) which are also proved in the present studies that spray at soft dough phase was proved less effective against the severity of NBLS disease. However, assessing accurate application timing in the appropriate development phases of the plants can maximize the output and minimize the side effects of chemicals. Because the management of plant diseases with fungicides has remained essential and unavoidable due to the gradual decline in the genetic resistance of plants to the new pathogenic species and resistant varieties become more vulnerable to pathogenic attacks over time (Uppala *et al.*, 2016; Uppala and Zhou, 2018). These results also endorsed the findings of other studies conducted on the sheath blight of rice (Groth and Bond, 2007; Groth, 2008), which demonstrated more severity on the susceptible or moderately resistant rice varieties and less severity on resistant rice varieties at all growth phases. The application of fungicides at inappropriate times may lead adverse effects on plants health. Because, fungicides application disturbed normal physiological functions of plants and have negative role on plant health (Wu and von Tiedemann, 2002; Petit *et al.*, 2012; Shahid and Khan, 2018). That's why no need of fungicides application at heading phases of rice varieties to manage the NBLS disease. The fungicide application is not necessary on resistant rice varieties, because it may have adverse effects on plants health and may not be cost effective. Several other studies exhibited that judicial use of fungicides at appropriate timings is considered cost effective, easy and quick way to manage the fungal diseases of plants (Wamishe *et al.*, 2018; Barúa *et al.*, 2019; Chaulagain *et al.*, 2019; Haq and Ijaz, 2020; Persaud *et al.*, 2020)

***Conclusion*:** It is concluded that the spray of Propiconazole fungicide must be done at early and late booting phases of mildly resistant, susceptible and highly susceptible rice varieties for timely management of NBLS disease. The fungicidal spray should be carried out on the appearance of initial few disease symptoms. There is no need of any fungicidal spray at resistant/mildly susceptible rice varieties because it may not be cost effective and have adverse effects on the physiology of rice plants.

***Conflict of interest*:** I have no business interest from this research work. All authors are agreed to publish this research work in the "Pakistan Journal of Agricultural Sciences" and there is no conflict of interest among all authors.

***Authors' Contribution Statements*:** MI conceived the idea, executed the field research work, data analysis and wrote the manuscript, NJ supervised the research work, MS improved the manuscript, SA facilitated in execution of study, MA facilitated in data analysis.

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