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Evaluation of nutritional amendments and fungicides against brown leaf spot of rice

Fiza Mohsin[1], Safdar Ali[1], Muhammad Usman Ghani[2], Waqas Ashraf[3], Muhammad Ahmad Zeshan*[4], Rizwan Mahmood[5], Muhammad Muntazir Mehdi Khan[6]

[1]*Department of Plant Pathology, University of Agriculture Faisalabad. Pakistan.*
[2]*Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Pakistan.*
[3]*Department of Plant Pathology, Faculty of Agriculture and Environmental Sciences, The Islamia University Bahawalpur. Pakistan.*
[4]*Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha. Pakistan.*
[5]*Department of Horticulture, University of Sargodha, Sargodha. Pakistan.*
[6]*College of Plant Protection (Plant Pathology), China Agricultural University Beijing, China.*

Abstract

Helminthosporium oryzae, the causal organism of bacterial leaf spot (BLS) has brought a tremendous loss of rice crop in the field. Rice seedlings of four commercial varieties (Nayab basmati, Kiran 434, Super basmati, and Pak 386) were screened against BLS in the field by using a randomized complete block design (RCBD). The present experiment was encompassed the evaluation of fermented farmyard manure, leaf manure, Boron, and NPK solution against BLS. The effect of different fungicides (Score, Flumax, Melodyduo, and Polyram) was also checked on the mycelial growth of *Helminthosporium oryzae in-vitro* and *in-vivo*. The results showed that Pak 386 was highly resistant with 17.22% disease severity and Super Basmati was found moderately resistant with 22.31% disease severity; variety Kisan 434 was found moderately susceptible (24.15%) and Nayab Basmati (24.82%) was susceptible. Among all NPK solutions showed the best results in growth enhancement, plant vigor as well as reduction in disease severity followed by FFYM, boron and leaf manure. Under *in-vitro* conditions, all the fungicides performed best at 150 ppm in terms of colony growth reduction. The maximum growth inhibition was recorded in Score followed by Flumax, Melodyduo, and Polyram. Score gave a significant reduction in BLS disease severity and maximized the agronomic attributes. The fungicides performed best at 150 ppm in terms of colony growth reduction. The maximum growth inhibition was recorded in Score followed by Flumax, Melodyduo, and Polyram. Score gave a significant reduction in BLS disease severity and maximized the agronomic attributes.

Key words: HBV, HCV, ICT, PCR, Risk factors, Mansehra.

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**Corresponding Author:*
Muhammad.ahmad@uos.edu.pk

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1. INTRODUCTION

Biotic and abiotic factors decrease rice production to a considerable level. Monetary losses due to diseases may reach up to $5 billion per annum[1]. Rice diseases caused by microorganisms are classified as 36 fungal, 21 viral and 6 nematode diseases worldwide[2]. Brown leaf spot (BLS) disease caused by *Helminthosporium oryzae* (*Cochliobolus miyabeanus*), also known as *Dreschslera oryzae*, causes maximum yield loss in certain areas. In severe cases, the losses caused by BLS disease may reach up to 90%[3]. In addition to reducing the plant growth and yield, *H. oryzae* also cause crop discoloration at maturity and hence market value is lowered[4].

Brown leaf spot (BLS) can cause serious damage due to cool summer conditions and nitrogen deficiency. High humidity (> 92.5% and many drops of water on the leaves) and high temperatures (24°C to 30°C) are beneficial for the development of the disease[5]. Brown spot damage is particularly prominent when potassium levels in nutrients are low[6]. The pathogen causes brown to dark brown lesions (5-15 x 1-2 mm) in round stems at the intersection of flagpole leaves and stems. In severe cases, gray mycelial growth is observed between sheaths and stalks. Sometimes the fungus also produces a brown or gray-brown patch on the neck compared to the blackening of the neck[7].

One of the most significant management methods worldwide is the use of resistant varieties and chemical controls[8]. Resistant varieties help to sustain yield but approaches to manage brown spot for resistance is unusual and unstable when new or toxic pathogens occur[9]. Most varieties cultivated globally are vulnerable to *H. oryzae*. The management of this confusing illness requires the combination of various management approaches. The major goal of rice disease management strategy is to use integrated management strategies to prevent infectious diseases[10].

So, there is a suggestion as a management tool for the use of fungicides and the balance of plant nutrition[11] but the frequent application of fungicide in crops can cause plant pathogens and pathogens resistance to human health and beneficial microbial health. The use of various organic dietary supplements to increase the resistance of host plants is a suitable method of culture before transplanting the rice plants which may be a necessary nutrient factor for appropriate growth of the plant and is a method of resistance to pathogens and defense activator of rice plants that help increase tolerance.

The use of integrated disease management (IDM) consist of resistant or intermediate resistant varieties, good cultural practices and foliar fungicides is an economical and effective way to decrease harvesting losses due to these diseases. So, due to lack of acceptable resistance cultivars, fungicides remain an important option for controlling brown leaf spot.

2. MATERIALS AND METHODS

2.1. Collection of rice germplasm

The seedlings of four rice cultivars i.e. Super basmati, Nayab 2013, Pak-386 and Kisan 434 were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad (Pakistan).

2.1.1. Transplanting of germplasm

The seedlings of all the cultivars were transplanted in the research area Department of Plant Pathology, University of Agriculture Faisalabad (UAF-Pakistan). Plants were transplanted in a randomized complete block design (RCBD) by maintaining 6 inches plant to plant distance and 18 inches row to row distance.

2.1.2. Infected sample collection

The samples of rice plants with brown leaf spot (BLS) disease were collected from the research area of Plant Pathology, UAF (Pakistan). Leaf selection was done on the basis of signs and symptoms (brown spots with grey center.) of BLS of rice. Leaves were collected, preserved and labeled carefully in polythene bags with the name of disease, name of researcher, name of crop, location and date of collection.

2.2. Pathogenicity test

2.2.1. Isolation of *Helminthosporium oryzae* from diseased sample

Potato dextrose agar (PDA) was used for the isolation of fungus. Infected lesions were taken from leaf margins 0.5-1 cm section and after this 1.0% sodium hypochlorite was used for sterilization followed by washing with distilled water to remove the extraneous surface sterilant. Blotter paper was used to absorb moisture from sterilized samples. These pieces were placed on PDA plates in a laminar flow and incubation was done at 27 °C and purification was done after 5-7 days of incubation.

2.2.2. Identification

A compound microscope was used to identify *Helminthosporium oryzae* on the basis of physical appearance. Fungus growth on PDA was grey to dark greenish-grey. Sporulation generally sparse and cottony, aerial and fluffy mycelium was identified.

2.2.3. Inoculation of *Helminthosporium oryzae* on healthy plants

Distilled water was used for fungal spore suspension and adjusted at 10^6 spores/ml with the help of hemocytometer. Inoculated plants were sprayed for up to five days 3-4 times per day.

2.2.4. Re-isolation and identification

From inoculated plant nodes and leaves fungus was isolated and identified as described previously.

2.2.5. Purification and storage of pathogen

Greyish green colonies on PDA plates were taken and streaked on new PDA plates with the help of a wire loop. PDA slants were prepared (2% Potato dextrose, 2% glucose and 2% agar in test tubes) for long-time preservation of fungus (*Helminthosporium oryzae*). Slants were kept in refrigerator for further studies and in the freezer for a longer period storage.

2.3. Evaluation of different fungicides against the *Helminthosporium oryzae in-vitro*

2.3.1. Poisoned Food Technique

In-vitro evaluation of four various fungicides (Polyram, Melodyduo, Flumax, Score) with different concentrations (100 ppm and 150 ppm) were done to find out the most efficient fungicide. The concentration of 1000 ppm solution of every fungicide was made by dissolving 1g of fungicide in 1000 ml of water. The 100-ppm concentration was prepared by taking 1.0 ml from 1000 ppm stock solution and dissolving in 100 ml of PDA media. Similarly, a concentration of 150 ppm was made by adding 1.5 ml from 1000 ml fungicide solution into 100 ml of PDA media.

Agar disc of 5 mm of pathogen culture was put at the center of petri plates containing media having fungicides. Plates were wrapped with parafilm and incubated at optimum temperature. Data of mycelial sporulation was recorded after 48, 72 and 96 hours of incubation and compared with the control. The effective fungicides were further used for evaluation in the field.

2.4. Management

Three moderately resistant to moderately susceptible varieties were sown in RCBD for management trial. BLS disease was managed by nutritional amendments and fungicides.

2.4.1. Collection of organic waste materials

Organic matter i.e. fermented farmyard manure (FFYM) was collected from the cowshed, UAF. Farmyard manure and fallen leaf were fermented with sugarcane and yeast extracts. This waste organic matter was completely fermented and decomposed by keeping in a polythene bag under sunlight and used as nutritional amendments.

2.4.2. Incorporation of nutritional amendments in soil

Before transplantation of rice seedling, soil was properly pulverized and four blocks were made in a plot having 10ft wide and 30 ft long size. In each block, 1kg of fermented organic waste material like farmyard manure, leaves manure, nutrients, and boron in single and in different combinations with three replications were applied separately. Complete incorporation of soil followed by irrigation was done by deep ploughing.

2.4.3. Use of fungicides against *H. oryzae*

Defense activator viz. fermented organic materials were tested to reduce the prevalence of pathogens and disease preparing their concentrations at standard doses. Foliar application of different fungicides like score and moledyduo 5 ml/liter and boron 4 ml/liter was used with irrigation at 15 days intervals. Three sprays were applied; the first application was done after thirty days of transplanting, to increase plant strength by increasing resistance against BLS of rice.

2.5. Data recording:

At various stages of growth and disease cycle, data were recorded and calculated disease incidence by using formula.

$$\text{Disease severity \%} = \frac{\text{Number of diseased leaves}}{\text{Total number of leaves inspected}} \times 100$$

Disease assessments were done by using a standard disease rating scale developed by SES IRRI, 1996.

Disease Rating Scale:

Less than 1% of the leaf	Highly resistant
1-10%	Resistant
11-20%	Moderately resistant
21-50%	Susceptible
51% or more	Highly susceptible

2.5.1. Statistical analysis

For lab experiment, CRD design was used and data was recorded which is subjected to analysis of variance (ANOVA) at a 5% level of significance. This data was analyzed by using LSD test using "Statistics"[12].

3. RESULTS AND DISCUSSIONS

3.1. Response of rice germplasm against Brown Leaf Spot Disease

The results showed that among four varieties screened against brown leaf spot of rice, one variety Pak 386 was highly resistant with 17.22% disease severity and Super Basmati was found moderately resistant with 22.31% disease severity; variety Kisan 434 was found moderately susceptible (24.15%) and Nayab Basmati (24.82%) were susceptible. Nayab Basmati showed maximum disease severity while Pak-386 showed minimum disease severity (Fig. 1).

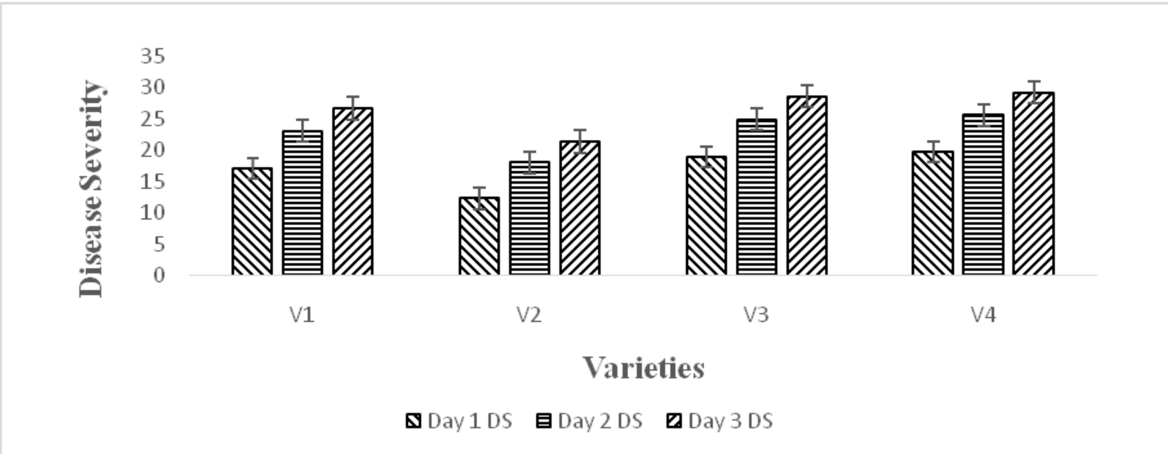

Figure. 1. Temporal assessments of disease severity on different rice cultivars (V_1= super basmati, V_2= Pak 386, V_3= Kisan 434, V_4= Nayab basmati).

Rice production in Pakistan is limited due to diseases such as brown leaf spot[13]. However, the most important method in the world is to use susceptible cultivars and chemical management. Susceptible cultivars contribute to the stabilization of yield, but methods to control brown spot for resistance are not stable due to the presence of more serious pathogenic strains[14, 9]. The cultivars which are used for screening against *Helminthosporium oryzae* exhibited variable response. Within 28 strains selected from the Nuclear Institute for Agriculture and Biology (NIAB) for 2 years, RSP-4 was resistant and PS-2 showed moderate resistance to *H. oryzae*. RSP-4 and PS-2 can be useful to develop a variety of rice for BLS disease[15].

Partial resistance to brown spots in five rice genotypes for three years under field conditions was investigated. BLS disease severity on leaves, panicles and NBLS disease was used to determine visible infectivity rate (r) and Area of disease progression curve (AUDPC). Resistance parameters such as incubation time, frequency of infection, latency and production of spores were determined by using six strains of *Magnaporthe grisea*. A significant correlation was found between the (r) and AUDPC values of panicle and neck[16].

Guilli[17] conducted a test to determine the resistance to brown spots of rice varieties used with Diner, Iram 6, Thaibonnet, Bhaja, Elio, Hayat, Kenz, Magherb, Match-97, Farah, Ariete and Maratelli. Eight species are vulnerable. Such as Thaibonnet, Dinar and Iram-6 had the slightest resistance gene for some isolates. Farah has completely resisted every Moroccan state. Elio and Thaibonnet showed incomplete resistance to the disease.

3.2. Effect of nutritional amendments on the severity of brown leaf spot disease and agronomic attributes of rice

The use of various fermented organic nutrient additives prior to transplanting rice to increase the resistance of host plants was a suitable method of cultivation that provided the essential nutrient elements for proper plant growth and was more resistant to pathogens. Fermentation additives play a vital role not only in increasing the rice yield but also in spraying rice deactivators, which increases the resistance of plants. The effect of nutritional amendments on BLS disease severity was assessed three times at fifteen days interval. The maximum disease severity was recorded in plants treated with leaves manure (28.76%) followed by boron application (27.26%), fermented farmyard manure (25.31%) and less disease severity was recorded in NPK (18.84%) compared with control (32.08%) (Table. 1). For the enhancement of resistance in rice varieties against BLS environment-friendly strategies were used as an application of organic nutrients just before the transplantation which enhanced the plant yield and vigor of the plant to provide the basic nutrients to the soil. The use of composite materials increases the concentration of organic materials and yields. The physical appearance of the soil can be enhanced through the use of compost materials such as fermented farmyard manure (FYM) etc. has improved efficiently[18].

Table. 1. Effect of nutritional amendments on BLS disease severity and agronomic attributes of rice

Treatment	Disease severity	Plant Height (cm)	Length of panicle	No. of panicle	Weight of panicle
NPK	18.84 e	44.55 a	8.91 a	3.33 a	1.52 a
FFYM	25.33 d	41.45 b	8.82 b	2.67 b	1.45 b
Boron	27.26 c	40.25 b	8.75 c	2.58 b	1.36 c
Leaf Manure	28.76 b	37.34 c	8.67 d	2.17 c	1.28 d
Control	32.08 a	36.49 c	7.94 e	2.00 c	1.25 d
HSD value	0.26	0.23	0.25	0.27	0.22

Symptoms of brown leaf spots appear at the plant tillering stage. It has been observed that stress interferes with the growth of the plant due to disease, which may or may not limit yield characteristics. The plant height was measured by measuring tape in the area of the stem of the plant at the surface of the soil with stems in the leaves above the rice. The plant height was compared with the control of all infected plants. A slight difference was observed between control and infected plant height.

The yield parameter of the plant growth is infected due to disease which may be limited by this factor. Stress or disease affects the vegetative growth of the plant. According to the data recorded at fifteen days interval of transplantation, plant height with NPK treatment was (44.55 cm) followed by fermented farmyard manure (FFYM) treatment was showing (41.45 cm), Boron treatment (40.25 cm), fermented leaves manure treatment showing (37.34 cm) as compared to control (36.49 cm).

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Panicle length was examined by the measuring tape from starting region of the panicle at the stem. Panicle length was compared in all treatments with a control. Some varieties were showing the short length of panicle as compared to control plants. The average length of panicle with NPK was (08.91cm) followed by FFYM (08.82 cm), Boron was (08.75 cm) leaf manure (08.67 cm) as compared to control (07.94 cm).

It was observed that stress disturbs the vegetative growth of the plant due to the disease which limits the yield traits or not. A number of panicles is important trait that affects the yield parameter of the plants. A number of panicles were measured by counting the panicles of each plant. Those were compared with the control. The average no. of panicles with NPK (3.33), Fermented farmyard manure (2.67), boron (2.58), and leaf manure (2.17) as compared to control (2.00). Average weight of panicles of Super basmati treated with NPK was (1.52 g) followed by FFYM (1.45 g), boron (1.36 g), leaf manure (1.28 g) as compared to control (1.25 g).

Sarker[19] observed that the physical condition of soil is improved due to microbial attack on organic source and then important nutrients are available to soil. Because of the improved condition of soil yield components of soil increased as height of plant, no of spikelet, panicles no, grain yield, weight of grain and tillers per plant. Salem[20] reported that by using nitrogen fertilizer with FYM higher no of panicles its length and panicle weight of rice was estimated.

Magar[21] *et al.,* (2015) evaluated rice cultivars against brown leaf spot resistance, thousand-grain weight and grain yield. HJ-G1 and HJ-G2 had the lowest severity of disease with a high yield.

In-vitro evaluation of fungicides against *H. oryzae*

Colony growth of *Helminthosporium oryzae* was recorded at different fungicides concentrations (Table 2). The results showed that various fungicides have different impact on colonial growth. Statistical analysis shows that maximum growth of colony was recorded in control and maximum growth inhibition was observed in Score, followed by Flumax, Melodydue and Polyram. Maximum growth of colony was found after 48 that gradually reduced after 72, 96 hours respectively.

Table 2. *In-vitro* evaluation of fungicides against *H. oryzae*

Treatment	After 48 Hours	After 72 Hours	After 96 Hours
FLUMAX@0PPM	3.5300c	2.1733c	1.4967e
FLUMAX@100PPM	3.1333de	1.4967d	1.3100e
FLUMAX@150PPM	2.9567e	1.3567d	1.1233e
MELODYDUO@0PPM	3.4933cd	2.7267b	1.6333de
MELODYDUO@100PPM	2.9500e	2.1333c	1.5233e
MELODYDUO@150PPM	2.5600f	2.0333c	1.4200e
POLYRAM@0ppm	5.2533a	3.6967a	3.2600a
POLYRAM@100PPM	4.8467b	2.8000b	1.9000cd
POLYRAM@150PPM	3.9867ab	2.4667a	1.7667b
SCORE@0PPM	3.2767cde	2.5533b	1.9833c
SCORE@100PPM	2.4400f	1.5733d	0.5767f
SCORE@150PPM	2.3067f	1.4167d	0.4267f
Alpha 0.05	HSD = 0.3463	HSD = 0.3376	HSD = 0.3766

The data revealed that when score was used at 150ppm concentration, (0.426mm) maximum inhibition of growth was recorded and on 100ppm, colony growth was (0.576mm) while in control maximum growth (1.983mm) was recorded. Flumax at 100ppm concentration depicted (1.31mm) and at 150ppm growth was (1.123mm) while control showed maximum growth (1.496mm) followed by Melodydue at 100ppm (1.523mm) and at 150ppm colony growth was (1.42mm) while control showed maximum growth

(1.633mm) and minimum growth inhibition was on Polyram on 100ppm (1.90mm) and on 150ppm (1.76mm) while maximum growth was observed on control (3.26mm).

Due to the reduced availability of resistant sources, the use of fungicides as a balanced method to reduce brown spot disease is also a successful treatment. The fungicides used for *Helminthosporium oryzae* showed reductions in the severity of the disease. All the fungicides at 150 ppm were evaluated in-vivo against BLS disease severity and growth parameters (Table 3).

Table. 3. Effect of fungicides on BLS disease severity and agronomic attributes of rice

Treatment	Disease severity	Plant Height (cm)	Length of panicle	No. of panicle	Weight of panicle
Flumax	14.85 d	45.41 b	8.15 b	2.06 b	1.78 b
Melodyduo	17.57 c	43.59 c	8.04 c	1.93 c	1.54 c
Polyram	21.62 b	41.72 d	7.12 d	1.48 d	1.27 d
Score	12.13 e	47.07 a	9.13 a	3.08 a	2.01 a
Control	48.23 a	31.43 e	6.52 e	1.34 e	1.12 e
HSD value	0.11	0.13	0.14	0.19	0.15

Gupta[22] performed experiments in lab to check seven fungicides (propiconazole, carbendazim, triadimefon, hexaconazole, triciclazole, mancozeb and azoxystrobin). Propiconazole exhibited the highest inhibition of 97%. Three varieties of rice, Basmati-370, Jaya and PC-19 were used to test fungicide from 2011 to 2012 under field, and the use of propiconazole increased the grain yield of all varieties compared to their respective controls. As a result, propiconazole has been shown to enhance rice yield by reducing the severity of the disease when properly sprayed.

Shabana[23] stated that resistance activators like benzoic acid, salicylic acid and hydroquinone at various concentrations decreased the disease severity with the benzoic acid at 20mM was found most efficient. Jatoi[24] sprayed four inhibitors, Mencozeb, thiophanate methyl, proprie iprovalicarb + and propineb, at four concentrations of 50, 100, 150 and 200 ppm in which growth of pathogenic fungi was inhibited *in-vitro*.

Pandey[25] evaluated the efficiency of fungicides against *H. oryzae* and it was found that Bavistin @ 1500 ppm was quite effective in inhibiting radial growth of mycelium and second treatment chitosan was found after 144 hours of incubation at the same concentration.

Kumar[26] *et al.*, (2017) tested four antifungal agents (Carbendazim (Bavistin) 50 WP, Carboxin (Vitavax) 50 WP, Propiconazole (Tilt) 25 EC and Hexaconazole (Contaf) 25 EC) against bacterial leaf spot disease. The analyzed substances at various concentrations were observed for the antifungal activity against *D. oryzae* in vitro and in vivo. Propiconazole (Tilt) inhibited the growth of *D. oryzae* up to (96.58%) at 500 ppm. Treatment of seeds with Bavistin @ 0.2 g ai / kg and Tilt @ 1 ml / L leaf spray (FS) under field conditions significantly reduced the disease severity (37.26%) and showed a significant increase in grain yield (55.49%) and its component.

4. CONCLUSIONS

It is concluded that the NPK found effective against BLS among nutrients and maximum growth parameters were recorded. Score at 150 ppm showed best results among all the fungicides and enhanced agronomic parameters were observed.

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CONFLICT OF INTEREST

There is no conflict of interest among authors.

REFERENCES

1. West J S, Bravo C, Oberit R, Lemaire D, Moshou D, Mcartney H A. The potential of optical canopy measurement for targeted control of field crop diseases. Annual Review of Phytopathology 2003; 41: 593-614.

3. Sarkar D, Mandal R, Roy P, Taradar J, Dasgupta B. 2014. Management of brown spot disease of rice by using safe fungicides and some bioagents. The Bioscan 2014; 437-441.

3. Srinivasachary S, Willocquet L, Savary S. Resistance to rice sheath blight (*Rhizoctonia solani* Kuhn) Teleomorph: *Thanatophorus cucumeris* (A.B Frank) Donk. disease: current status and perspectives Euphytica 2011; 178:1-22.

5. Kobayashi T, Kanda E, Kitada K, Ishiguro K, Torigoe Y. Detection of rice panicle blast with multispectral radiometer and the potential of using airborne multispectral scanners. Phytopathology 2001; 91: 316-323.

6. Iqbal M F, Hussain M, Waqar M Q. Evaluation of best fungicide for controlling brown leaf spot in transplanted rice. International Journal of Advance Research in Biological Sciences 2015; 2: 44-48.

7. Sunder S, Singh R, Dodan D S, Mehla D S. Effect of different nitrogen levels on brown spot (Drechslera oryzae) of rice and its management through host resistance and fungicides. Plant Disease Research 2005; 20: 111-114.

8. Kumar M K P, Gowda D K S, Moudgal R, Kumar N K, Gowda K T P, Vishwanath K. Impact of fungicides on rice production in India. Fungicides: Showcases of integrated plant disease management from around the world. 2013; 4: 77-98, Intech open Publisher.

9. Katasntonis G, Koutrovbas S, Ntanos D, Lupoho E. A comparison of three experimental designs for the field assessment of resistance to rice brown leaf spot of rice disease (*Bipolaris oryzae*), Journal of Phytopathology 2007; 155: 204-210.

10. Asghar A., Rashid H, Ashra M, Khan M H, Chaudry A Z. Improvement of Basmati rice against fungal infection through gene transfer technology. Pakistan Journal of Botany 2007;39: 1277-1283.

11. Razende D C, Rodrigues F A, Carre-Missio V, Schurt D A, Kawamura I K, Korndorfer G H. Effect of root and foliar applications of silicon on brown spot development in rice. Australasian Plant Pathology 2009; 38: 67-73.

12. Steel R G D, Torrie J H. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Ed. 1997. 336-352. McGraw Hill Book Co. Inc., New York, USA.

13. Fazlu S F I, Schroeder H W. Effect of kernel infection of rice by *Helminthosporium oryzae*. M. S. Thesis. 1996. College, Leguna, the Philippines, University of Philippines at Las Banos, Philippines. Pp. 10-17.

14. Lee F N. Brown spot. In compendium of rice disease. (Eds R. K. Webster, P. S. Gunnel) APS Press: St Paul, MN. 1992. pp. 14-17.

15. Arshad H M I, Hussain N, Ali S, Khan J A, Saleem K, Babar M M. Behaviour of *Biploris oryzae* at different tempratures, culture media, fungicides and rice germplasm for resistance. Pakistan Journal of Phytopathology 2013; 25: 84-90.

16. Malvolta V M A, Pettinelli J A, Fantin G M, Souza T M W. Assessment of partial resistance levels to brown leaf spot on five rice genotype. Phytopathology 1995; 21: 117-123.

17. Guilli M, Jarifi A, Milazzo J, Adreit H, Ismaili M, Lyamani A, Notteghem L J, Tharreau D. Assessment of resistance to *Biploris oryzae* in rice cultivars used in Morocco Al. Awania 2000; 102: 93-81.

18. Hussain N, Hassain G, Arshadullah M, Mujeeb F. Evaluation of amendments for the improvement of physical properties of sodic soil. International Journal of Agriculture and Biology 2001; 3: 219-322.

19. Sarker M A R, Pramanik M Y A, Faruk G M, Ali M Y. Effect of green manures and level of nitrogen on some growth attributes of transplant aman rice. Pakistan Journal of Biological Sciences 2004; 7: 739-742.

20. Salem M, Akram M, Akhtar M E, Ashraf M. A Production Handbook, Balanced Fertilization for Maximizing Economic Crop Yields. Pakistan Agricultural Research Council, Islamabad. 2000. pp. 1-8.

21. Magar P B. Screening of rice varieties against brown leaf spot disease at Jyotinagar, Chitwan, Nepal. International Journal of Applied Science and Biotechnology 2015; 3: 56-60.

22. Gupta S K, Rai A K, Kanwar S S, Chand D, Singh N K, Sharma T R. The single functional blast resistence gene Pi54 activates a complex defense mechanism in rice. Journal of Experimental Botany 2013; 63: 757-762.
23. Shabana Y M, Abdel-Fattah G M, Ismail A E, Rashad Y M. Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants. Brazilian Journal of Microbiology 2008; 39: 438-444.
24. Jatoi G H, Keerio A U, Abdulle Y A. Effect of selected fungicides and Bio-Pesticides on the mycelial colony growth of the *Helminthosporium oryzae*. brown spot of rice. Acta Ecology Sin 2018; 7: 11-24.
25. Pandey S. Efficacy of leaf extracts in controlling leaf BLAST And brown spot in rice (*Oryza sativa* L.). International Journal of Recent Scientific Research 2015; 6: 5476-5479.
26. Kumar H, Ahmad S, Zacharia S, Kumar S, Ali A. Impact of different fungicides combination against brown leaf spot (*Drechslera oryzae*) of rice under the in vitro and in vivo. Journal of Pharmacogn Phytochemistry 2017; 6: 341-344.