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Epidemiological Studies of Bacterial Leaf Blight of Rice and its Management

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Abstract: Rice is one of the most important crops in Pakistan, grown all over the world. Rice production has decreased due to susceptibility to a number of biological and abiotic factors. One of the oldest diseases is bacterial leaf blight of rice. The causative agent of the disease is *Xanthomonas oryzae* pv. *oryzae*. In this study, seedlings of four commercially grown rice varieties were sown by following the randomized complete block design (RCBD). Four treatments were used to manage the disease and to promote the plant growth. Correlation and regression analysis was used to study the relation of BLB disease and environmental factors. It was observed that plant height increased by the application of NPK followed by Farm yard manure, boron and leaf manure. Panicle length and weight was maximum in boron treated plants while minimum in FYM. Minimum disease severity was recorded in NPK treatment followed by boron FYM and leaf manure. All the environmental variables showed significantly negative relationship with the development BLB disease. These results would be beneficial for the farming community to devise management options against BLB disease.

Keywords: Rice yield, Nutritional management, Environmental variables, Epidemiology.

Introduction

Rice (*Oryza sativa* L.) is the most important crop in the world that is consumed by almost half of the world's population (Wang & Li, 2005). If per capita consumption of rice is maintained at current levels, demand of rice will need to increase up to 20% by 2030 to fulfill its requirement (Peng *et al*., 2009). Rice plants are vulnerable to some bacterial pathogens, one of which is bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv *oryzae* (Xoo), that incites heavy losses in rice cultivation areas (Xu *et al*., 2010). BLB hinders the appearance of cones and grain fillings that reduce production by 20% (Khan *et al*., 2014). Xoo is distributed around the world and has 50-90% impact on the productivity (Ellur *et al*., 2016). The disease is widespread in Asia, United States, Latin America and Australia. In Pakistan, the disease is increasing year after year as the famous Basmati varieties are vulnerable to BLB disease (Khan *et al*., 2000). Crop losses are dependent on crop stage, level of susceptibility, and the available environment (Rangarajan *et al*., 1999). Pathogens move vertically through the leaves by primary vein. A few days later, bacterial cells and extracellular polysaccharides (EPS) fills the vessels and filter out from the hydathodes. As a result, 43 beads or exudates chains are formed on the surface of the leaves, forming a characteristic sign of disease (Nino-Liu *et al*., 2006). This bacterium has a type III protein secretion system that directly injects toxic factors into the host (Furutani *et al*., 2009).

BLB is spreading in Pakistan during the most recent years primarily in Kallar belt which is recognized as high quality rice producing areas (Khan *et al*., 2015). Pathogen (Xoo) is very diverse and difficult to control; the most efficient way of administering BLB is only the introduction of host resistance and its development (Yan *et al*., 2016). The integration of micronutrients with *Psedumonas fluorescens* enhanced its aggressiveness as a bioagent against Xoo (Sharma *et al*., 2017). The combination of seed treatment, sprinkling of the soil and leaves found a minimal incidence of bacterial blight of the leaf with a maximum yield compared to the untreated plants (Jeung *et al*., 2006). The most reasonable and popular approach to control BLB is resistance to rice varieties that is more effective than chemical controls (Lussewa *et al*., 2016). The value of diversity of resistance depends on the availability of resistant genetic resources and inequalities within the pathogen population (Webb *et al*., 2005). Some Xoo bacterial antagonists have been tried as biological agents and have not been commercialized (Yasmin *et al*., 2017). On the other hand, the use of cultural practices to control the disease by improving or changing cultural practices is only partially effective in limiting the spread of pathogens (Nino-Liu *et al*., 2006). However, the durability of resistance depends on the spread of pathogen competition over time and space (Jagjeet *et al*., 2010). This is because the Xoo pathogen is very diverse and there are more than 30 species of bacteria worldwide (Noda *et al*., 2001). Salim *et al*., (2003) stated that treatment of diseases such as bacterial leaf rot, including resistant breed breeding and chemical treatment, has been extensively pursued.

During the last decade, climate change has been adversely affected by Asian monsoons and flooding in Pakistan's rice-producing regions (Gumma *et al*., 2011). In future, there will be little chance of increasing the area of rice cultivation due to enlarged urbanization and the installation of the industrial in the main rice producing countries of Asia and the competition of water and farmland (Kueneman, 2006). Climate change presents significant challenge for the management of plant diseases. (Garrett *et al*., 2006). Elevated temperatures are expected to speed up the destruction of resistance to plant diseases through higher disease pressures and the efficacy of altered genes in most of the host pathogen systems. Keeping in view the described facts, the present study was aimed to check efficiency of chemicals and fermented nutritional amendments in managing bacterial leaf blight of rice and to check the effect of environment in relation to BLB disease of rice.

Materials & Methods

Collection of rice germplasm and transplanting

Seedlings of four commercially grown rice varieties as Chenab Basmati, Pak Basmati, Basmati 385 and Kisan were collected from research area of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (UAF), Pakistan. Rice nursery was transplanted in experimental area of Department of Plant Pathology, UAF by following augmented design for screening purposes. For management of the disease, four varieties were grown by following Randomized Complete Block Design (RCBD) with plant to plant distance 15.24cm and row to row 45.72cm.

Collection of infected leaf samples

Leaf samples of rice infected with disease bacterial blight of rice were collected. Collected samples were sealed in polythene bags and labeled carefully with name of disease, with researcher name, farmer name, location, with exact date when sample were collected.

Management of BLB disease and preparation of fermented material

Four treatments were used as fermented farmyard manure (FFYM), fermented leaves, nutrients (Boron, NPK) to manage bacterial leaf blight of rice. Waste organic material and fallen leaves were collected from the cow shed as organic material was fermented farm yard manure. Fallen leaves and farm yard manure were fermented with sugarcane, and with yeast extract. Then waste material were fermented and decomposed properly by placing them under sunlight in polythene sheet. As nutritional amendments these fermented material were used.

One kg of fallen leaves with one litter sugar cane juice and one gram of yeast powder for the preparation of fermented leaves organic matter and for the preparation of fermented farm yard manure one kg farm yard with one litter sugar cane juice and one gram of yeast powder. Soil was properly pulverized before transplantation of rice seedling and three blocks were made in plot having 10ft wide and 30ft long size. Fermented organic waste like Farm Yard manure leaves manure, nutrients and boron in single. With 1kg in each block with three replication were separately applied. Followed by irrigation complete assimilation was done with deep plugging. The effect of waste material was examined on number of grains per panicle, panicle length, and grain yield and grain weight per plant.

To reduce the occurrence of pathogen defense activator as fermented organic material were tested and concentrations at their average doses were prepared for the decreasing disease. Foliar application of Bordeaux mixture and boron 4ml/liter were used after 15 days interval. Two sprays were applied, after thirty days of transplantation, to increase vigor of plant by enhancing resistance against bacterial blight disease of rice.

Data recording

Data were recorded at different stages of disease and at various growth stages to calculate the percentage of disease. Data of disease incidence were collected by using formula.

$$\text{Disease incidence} = \frac{\text{Number of diseased plant}}{\text{Total number of plants inspected}} \times 100$$

Disease incidence percentage over control can be determined by using following formula.

%age Disease Decrease over control=

(Disease Incidence in Control-Disease Incidence in Treatment)/ (Disease Incidence in Control)

Disease severity

$$\text{Disease Severity} = \frac{Number\ of\ infected\ leaves}{Total\ number\ of\ leaves} \times 100$$

Disease severity index (%)= $\sum P \times Q/(M \times N) \times 100$

Where P = severity score, Q = number of infected plants having the same score; M = Total number of plants observed, N = Maximum rating scale number

Disease assessments were examined by using standard disease rating scale developed by SES IRRI, (1996).

Disease rating scale

Disease incidence (%)	AUDPC	Disease ratings
51% 7	551---	Highly susceptible
31-50% 5	451-550	Susceptible
11-30% 3	300-450	Moderately resistant
1-10% 1	101-300	Resistant
Less than 1% 0	0-100	Highly resistant

Area under disease progress curve (AUDPC)

The AUDPC was calculated by the trapezoidal integration of the disease incidence over time, considering the whole period evaluated, as follows

$$AUDPC = \sum_{i=1}^{n-1} [xi + xi + t/2](ti+1-ti)$$

where n is number of assessments; X is disease incidence (%);(ti+1-ti) time interval between two consecutive assessments.

Effect of BLB on agronomic attributes of rice

Plant height (cm)

Plant height with the help of measuring tape in centimeters all the four varieties was evaluated and compared with the control.

Length of Panicle (cm)

Length of the panicle was measured with the help of measuring tape of 5-6 panicles of each selected variety of plant and then compared with control.

Weight of panicles (g)

Panicles were plucked carefully and then kept in polythene bags and after all brought to the laboratory. To measure the weight of panicles weighing apparatus was used and calculated and compared with the control.

Statistical analysis

The recorded data was subjected to statistical analysis by using LSD test and correlation and regression analysis was done to measure the effects of environmental factors on the plant growth and disease (Steel *et al*., 1997).

Recording of climatic factors data

Environmental data (maximum and minimum air temperature, relative humidity and rainfall) were collected from Meteorological Station of Department of Crop Physiology, University of Agriculture, Faisalabad situated adjacent (50 meters) to Research Area of Plant Pathology Department on daily basis during the cropping season and weekly averages were calculated. The environmental factors were correlated with rice blast disease incidence through correlation analysis (Steel *et al*., 1997). The specific devices were installed at the Meteorological station i.e. Thermometer for temperature; hygrometer for relative humidity and rain gauge for measuring rainfall.

Table (1). Temporal assessments mean incidence and severity index of BLB disease in rice cultivars.

Varieties	Assessment 1		Assessment 2		Assessment 3	
	Incidence	Severity index	Incidence	Severity index	Incidence	Severity index
Basmati 385	16.35 a	0.35 a	21.95 a	0.47 a	25.55b	0.59 b
Chenab Basmati	15.86 a	0.33 a	19.69 b	0.42 b	23.53 c	0.55 c
Kisan Basmati	16.79 a	0.36 a	22.28 a	0.48 a	27.95 a	0.61 a
Pak Basmati	13.29 b	0.28 b	18.76 b	0.41 b	21.02d	0.49 d
LSD 0.05	1.5	0.03	1.7	0.27	1.2	0.03

Means with different letters are significantly different from each other according to LSD test at 5% probability level.

Results

Evaluation of rice germplasm against Bacterial

Leaf Blight disease

In first assessment, minimum BLB disease incidence was recorded as very few plants were infected by the pathogen and exhibited characteristic disease symptoms. Out of four rice cultivars only Pak Basmati depicted significant differences with rest in terms of disease incidence and severity index. BLB disease incidence ranged from 13.29% to 16.79% while severity index ranged from 0.28 to 0.36. All varieties except Pak Basmati showed more than 15% disease incidence (Table 2).

BLB disease incidence increased during second assessment. The number of diseased plants also increased. Basmati 385 and Kisan Basmati were significantly different from Chenab Basmati and Pak Basmati in terms of disease incidence and severity index. BLB disease incidence ranged from 18.76% to 21.95% while disease severity index ranged from 0.41 to 0.48. Basmati 385 and Kisan Basmati showed more than 20% disease incidence (Table 1).

During third assessment, BLB disease incidence was higher than previous two assessments. Total number of infected plants significantly increased as compared with first and second assessment. All the cultivars gave significantly different disease incidence and severity. The range of BLB disease incidence was 21.02% to 27.95% and of severity index was 0.49 to 0.61.Kisan Basmati depicted maximum disease incidence while Pak Basmati gave lowest (Table 1).

Based upon screening assessments for evaluating the rice cultivars against BLB disease incidence, all the cultivars (Basmati 385, Chenab Basmati, Kisan Basmati and Pak Basmati) were regarded as moderately resistant (Table 2). Paired t test results indicated the average disease incidence for all cultivars during first assessment was 15.57% which increased to 19.92% in second assessment and 24.51% in third assessment. The mean difference between first and second difference was 4.35 that was significantly different from each other at (P =0.0001). Similarly, there was a significant difference between second and third assessment (4.59). There was a significant difference between first and third assessments (8.94).

Efficacy of different treatments against BLB disease severity

Among all the treatments, NPK solution gave maximum reduction (67.37%) in disease incidence during first week of evaluation. Boron was the second most effective treatment with 64.88% reduction in disease incidence during first week followed by FYM (48.49%) and leaf manure (48.17%). There was significant reduction in disease incidence during second and third weeks as compared to first week. As far as the overall efficacy of the treatments is concerned; NPK solution was 79.71% efficient in reducing BLB disease incidence. Boron was 77.07% efficient followed by FYM (63.74%) and leaf manure (63.26%) (Table 3).

Effect of BLB disease on agronomic attributes of rice

There was significant difference in plant height, panicle length and weight of panicle in all the varieties before and after the application of treatments. Maximum increment of plant height was recorded in Kisan Basmati and minimum in case of Pak Basmati. Post application panicle length was maximum in Pak Basmati and minimum in Chenab Basmati. The weight of panicle was maximum 7.21 in Chenab Basmati followed by Pak Basmati 6.93, Basmati 385 (6.13) and Kissan Basmati 4.93 (Table 4).

Effect of different treatments on agronomic attributes of rice

Among different treatments, NPK gave maximum plant height (70.27 cm) as compared with control. FYM proved the second best in increasing plant height followed by boron and leaf manure. Maximum panicle length (24.79 cm) was measured in boron treated plants followed by NPK, leaf manure and FYM. There was significant difference of boron from NPK and leaf manure in increasing weight of panicle (Table 5).

Table (2): Rice cultivars sensitivity rankings based upon BLB disease incidence values summed up for all three assessments.

Varieties	Incidence*	Sensitivity	AUDPC
Basmati 385	20.28	Moderately resistant	381.92
Chenab Basmati	18.69	Moderately resistant	359.66
Kisan Basmati	22.34	Moderately resistant	410.76
Pak Basmati	17.69	Moderately resistant	345.66

*Mean disease incidence was calculated by averaging the disease incidence from three assessments

Table (3): Efficacy of nutrient solutions and organic material against BLB disease severity.

Treatments	**Disease incidence before spray**	**Week1**	**Week2**	**Week3**	**%Efficacy**
		DI/Reduction*	DI/Reduction	DI/Reduction	
Boron	58.14	20.42 c (64.88)	14.92c (74.34)	9.32 d (83.97)	77.07
Leaf manure	53.89	27.93 b (48.17)	23.24 b (56.88)	20.42 b (62.11)	63.26
FYM**	56.27	28.98b (48.49)	22.94 b (59.23)	18.72 c (66.73)	63.74
NPK***	58.16	18.98 d (67.37)	13.28 d (77.17)	7.27 e (87.5)	79.71
Control	62.63	63.74 a	68.95 a	62.14 a	64.94

LSD0.05 = 1.7

*Percent reduction is indicated in parenthesis calculated by subtracting pre and post application, DI = Disease incidence, **FYM = Farm yard manure, ***NPK= Nitrogen, phosphorus, potassium solution

Table (4): Comparison of different agronomic attributes in rice cultivars.

Varieties	Time of data	Plant Height (cm)	Panicle Length (cm)	Weight of Panicle (g)
Basmati 385	Pre treatment^	36.18 a	08.92 a	4.39 b
	Post treatment	62.41b	23.77 a	6.13 b
Chenab Basmati	Pre treatment	36.26 a	09.09 b	5.83 a

	Post treatment	59.34c	23.64 a	7.21 a
Kissan Basmati	Pre treatment	36.11a	07.35 b	3.73 b
	Post treatment	68.69a	23.73 a	4.93 c
Pak Basmati	Pre treatment	36.33 a	09.29 a	5.55 a
	Post treatment	55.27d	24.56 a	6.93 b
LSD 0.05	-------	2.4	2.6	1.2

^Similar letters in upper and lower rows against each variety in all columns are not significantly different

Table (5). Effect of different treatments on agronomic attributes of rice.

Treatments	Plant Height (cm)	Panicle Length (cm)	Weight of Panicle (g)
NPK	70.27 a	23.97 b	6.05 b
FYM	66.49 b	21.85 d	5.88 c
Boron	62.66 c	24.79 a	7.71 a
Leaf manure	56.17 d	22.69 c	6.58 b
Control	47.53 e	11.09 e	3.54 d
LSD 0.05	1.3	1.4	1.2

Overall correlation of environmental conditions with bacterial blight disease of rice for four different rice varieties

Bacterial growth was found best from 35°C to 39°C. The variety Chenab basmati has significant correlation on disease incidence with environmental conditions and variety Pak basmati, Basmati 385 and Kisan basmati have highly significant correlation on disease incidence with environmental conditions (Fig. 1). Maximum temperature showed negative effect on disease incidence of Chenab basmati and variety Pak basmati showed significant result on disease incidence with maximum temperature then variety Basmati 385 showed highly significant effect on disease incidence with maximum temperature and the variety Kisan basmati showed significant effect on disease incidence with maximum temperature.

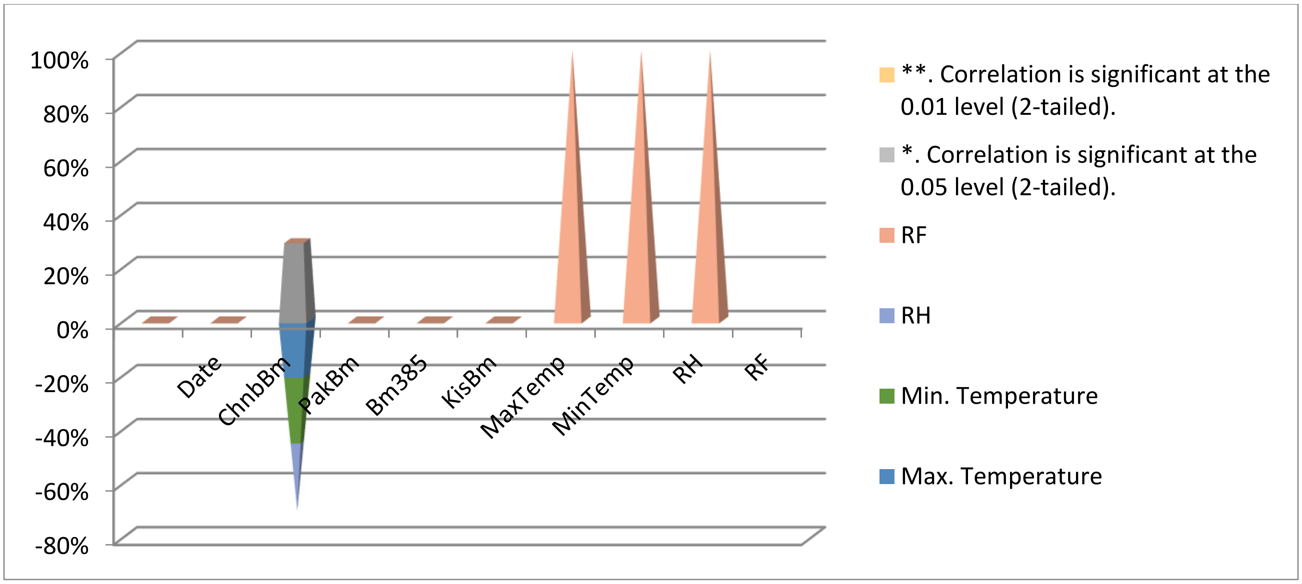

Fig. (1): Correlation of environmental conditions with bacterial blight disease recorded on rice varieties.

As minimum temperature gives negative effect on the disease incidence of variety Chenab basmati highly significant effect on disease incidence also Pak basmati showed highly significant effect on disease incidence with Basmati 385 and Kisan basmati. Relative humidity showed negative effect on disease incidence with the variety Chenab basmati and highly significant effect on disease incidence with Pak basmati, Basmati 385 and Kisan basmati. Rain fall showed highly significant correlated effect on disease incidence with Chenab basmati, Pak Basmati and Kisan Basmati and significant with Basmati-385.

Discussion

Rice is considered to be one of the most important grains consumed worldwide, as it supplies important nutrients to 2.7 billion people worldwide (Salim *et al*., 2003). Although many chemicals have been shown to inhibit bacteria in the laboratory, some studies have been found to be effective in preventing or reducing the disease outbreaks under field conditions. The mixtures of Bordeaux and other copper compounds have been used in Japan since 1909 against the fungi (Lindsey *et al*., 2020). In current study, all the cultivars depicted moderately resistant reaction against BLB disease. Rice germplasm was screened by many researchers to find out the resistant source against BLB disease. Wu et al., (2017) investigated the presence of major R genes and tolerance response correlation. The results are projected to provide necessary information for the rational use of the major R genes of the hybrid rice breeding program.

Lussewa *et al*., (2016) stated that the efficient way of administering BLB is only the introduction of host resistance and its development. The main objective of this study was to examine the genotype strength through the interaction of the environment (GxE) with bacterial blight resistance in rice. Rafi et al., (2013) stated that the incidence of bacterial blight disease was evaluated in all cultivated areas of Pakistan during 2005-2007. The incidence of Khyber Pakhtunkhwa disease (KPK) was 35 to 80.2% and the severity was 3.3 to 7.0. The incidence rates of Sindh and Baluchistan in Punjab were 36.8-7.46%, 11.67-46.67% and 12-21.67%, respectively. The severity of the disease was 3-6, 0.67-3.33 and 0.33-1.22. Khan *et al*., (2012) found that Xa21 and Xa13 genes are resistant to most Xoo isolates, followed by Xa14 and Xa7. This resistance gene can be integrated (individually and in combination) for the improvement of basmati rice cultivars grown in the Punjab region of Pakistan.

Nutrients are important for the growth and development of plants and microorganisms, and in disease control (Chakraborty *et al*., 2005). One of the first observations of the impact of nutritional amendments on the development of the disease is that fertilization at the time of plant deficiency has reduced the severity of the disease because fertilization optimizes plant growth (Ramli *et al*., 2018). Micronutrients take part in plant growth and development as well as these increases the aggressiveness of biocontrol agents against pathogens (Sharma *et al*., 2017).

The maximum and minimum percentage losses of grain weight (GW) due to BLB infection were 17.84% and 11.17% in Sialkot and Naoughal, respectively. These weight loss rates (15.59% -11.94%) due to this pathogenic infection in the Khyber Pakhtonkhwa region of Pakistan (Khan *et al*., 2015). Nutrients boost up the defence system of the plant and repairs the lost or damaged sites of and thus are helpful in managing the disease as the weak hosts are more prone to be attacked by the pathogens.

Several studies of epidemiology have shown that climatic variables such as rain, temperature and humidity affect the spread of diseases as an important factor. Temperatures, rain and wind accelerate disease outbreaks as temperatures rise, and wind speed and raindrops promote the spread of inoculums (DeVallavieille-Pope *et al*., 1995). Humidity has also been reported as the most promising factor, especially for wet periods for disease development (Huber and Gillespie, 1992). BLB is the most common occurrence in July with more than 200 mm of rainfall and an average annual temperature exceeding 24°C (Kumar *et al*., 2009). Therefore, the environment has been considered a source of strength in the development of the epidemic (Maanen & Xu, 2003). In this stage regression model, wind speed, relative morning humidity, precipitation and minimum temperature were the main environmental factors of the disease outbreak. The model showed that the maximum temperature did not have a significant impact on disease outbreaks. The most important factors of the disease epidemic are floods (Wang *et al*., 2003), which is considered a favorable environmental factor for precipitation, relative humidity, minimum temperature and flood season.

Rehman *et al*., (2016) developed a step-by-step regression model for destructive pathogenic systems. Three sensitive rice varieties (IRRI-24 and TN-1, Basmati Super) were tested for disease severity and environmental variables. Because climate conditions strongly influence disease development, data sets for environmental variables of increase and decrease in daily temperatures, relative humidity in morning time or in evening time, precipitation, wind speed and daylight hours were collected and correlations were analyzed for stepwise regression models. Samanta *et al*., (2016) performed regression analysis and showed that speed of wind and relative precipitation in the morning time were the most important environmental variables distressing disease development (P> 0.0001). Environmental factors influence the biological activities of the pathogen and also of the host plant thus played an important role in the development of disease. Under unfavorable conditions, the pathogen would not be able to incite infection in the host plant and thus plant escape the disease despite the pathogen is virulent.

Conclusion

It is concluded that NPK solution proved more effective against BLB disease of rice and all the agronomic attributes improved. It is the more eco-friendly approach for the management of disease.

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Conflicts of interest

There is no conflict of interest among authors.

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دراسات الوبائية ومقاومة مرض لفحة اوراق الرز البكتيرية

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المستخلص : يعد الرز من أهم المحاصيل في باكستان، ويزرع في جميع أنحاء العالم، هناك عدة اسباب وراء انخفاض إنتاج الرز منها التعرض لعدد من العوامل البيولوجية وغير الحيوية، ومنها مرض لفحة اوراق الرز البكتيرية من أقدم الأمراض المتسبب عن الاصابة بالمسبب المرضي هو.Xanthomonas oryzae pv . في هذه الدراسة ، تم زرع شتلات من أربعة أنواع من الرز المزروع تجاريًا باتباع تصميم القطاعات العشوائية الكاملة (RCBD) ، تم استخدام أربعة معاملات للسيطرة على المرض وتعزيز نمو النبات، استخدام تحليل الارتباط والانحدار لدراسة العلاقة بين مرض BLB والعوامل البيئية. لوحظ أن ارتفاع النبات زاد بإضافة NPK يليه السماد العضوي، البورون. اذ حققت معاملة البورون افضل طول ووزن في النباتات ، بينما كان الحد الأدنى في السماد الورق العضوي، تم تسجيل الحد الأدنى من شدة المرض في معاملة NPK متبوعًا بالبورون ، ثم السماد العضوي الورقي. أظهرت جميع المتغيرات البيئية علاقة سلبية مع تطور مرض، يستنتج من هذه النتائج امكانية تطبيق المعاملة الدراسة اعلاه ضد مرض BLB.

الكلمات المفتاحية: محصول الرز، الإدارة الغذائية، المتغيرات البيئية، علم الأوبئة.