

## Phenotypic evaluation of rice germplasm against *Xanthomonas oryzae* pv. *oryzae* and its *in-vitro* management through antibiotics

Aysha Khan<sup>1</sup>, Safdar Ali<sup>1</sup>, Muhammad Sajid<sup>2</sup>, Muhammad Ahmad Zeshan<sup>4\*</sup>, Rana Binyamin<sup>5</sup>, Nadeem Ahmed<sup>3</sup>, Muhammad Usman Ghani<sup>6</sup>

<sup>1</sup>Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan

<sup>3</sup>Department of Plant Pathology, MNS-University of Agriculture, Multan, Pakistan

<sup>4</sup>Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan

<sup>5</sup>Department of Plant Pathology, University of Agriculture Faisalabad. Sub-Campus Burewala, Pakistan

<sup>6</sup>Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

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### Abstract

*Xanthomonas oryzae* pv. *oryzae* (Xoo) which causes bacterial leaf blight (BLB) disease is one of the most destructive pathogen of rice. BLB incites more than 70% yield losses in epidemic conditions thus deteriorating the rice industry and export revenue. The use of conventional synthetic bactericides causes remarkable environmental hazards such as water and air pollution. This study was focused at the management of bacterial leaf blight disease through the application of antibiotics because injudicious use of bactericides results in the development of resistance in bacteria. The objectives were to evaluation of rice germplasm against BLB, biochemical characterization of the pathogen and its *in-vitro* management. There were very limited studies on the phenotypic evaluation of BLB disease and biochemical analyses of its causal organism. Twelve rice cultivars were screened against the disease in randomized complete block design. The isolated pathogen was subjected to Gram staining and microscopic examination. Biochemical characterization of the pathogen was done by methyl red, indole production, citrate utilization and Voges-Proskauer tests. Three antibiotics (Kanamycin, Streptomycin and Ampicillin) were used at three concentrations (0, 50, 100 and 150 ppm) against the pathogen. Among all the screened cultivars, none was highly resistant and resistant. Maximum disease severity (89.01%) was depicted by the variety Super Basmati. All isolate were Gram negative and these showed positive response in case citrate utilization test indicating that citrate permeases enzyme is abundant. In-vitro results showed that Kanamycin is the most effective against pathogen at all concentrations than Ampicillin and Streptomycin. It is concluded that none of the screened cultivars showed resistance against Xoo, so the Basmati varieties may be sown with appropriate antibiotic like Kanamycin. It is recommended to devise effective breeding programs for the development of resistance rice cultivars against this economically important disease.

**Keywords:** Antibiotics, Xoo, *In-vitro*, Management, BLB

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\*Corresponding author email:  
muhammad.ahmad@uos.edu.pk

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## Introduction

Rice (*Oryza sativa* L.), is the second most important staple food after wheat. Its exports are the second-highest source of income in Pakistan (Cheng et al., 2020; Herawati et al., 2021). Rice yield is affected due to diseases like bacterial leaf blight (BLB) that poses huge qualitative and quantitative losses (Sere et al., 2005). Unfortunately, all varieties of Basmati are susceptible to BLB in Pakistan. BLB disease is accelerating in the latest years chiefly in the Kallar belt (Sheikhupura, Hafizabad, and Sialkot) which is known as high-quality rice-producing area in Pakistan (Khan et al., 2001). It was firstly reported in 1977 on rice Basmati-198, IR-6, Palman at Rice Research Institute Kala Shah Kaku, and some other farmer's field in Pakistan (Akhtar et al., 2008). Bacterial blight is viewed as an imperative illness in different parts of rice developing zones of Pakistan (Jabeen et al., 2012). The disease may destroy the seedling and in more established plants the loss of grain might be 4.5-29.1% (Shivalingaiah et al., 2013). There is a report of misfortune in rice trim because of blight up to 59-90% (Rangarajan et al., 2003).

BLB is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) that survives essentially in contaminated seeds, stubbles, straw, rations, and self-sown plants (Ahmed et al., 2020). The pathogen moves vertically through the leaf via primary veins. The vascular bundle of the plant is blocked by the physical presence and exudates of the bacteria which appear on the leaf surface as a characteristic sign (Nino-Liu et al., 2006). Xoo is a gram negative rod shaped bacteria with type III secretion system. It multiplies in xylem vessels and move systemically in plants (Furutani et al., 2009).

*In-vitro* screening of siderophore production by using Chrome Azurol S medium (CAS-medium) showed that 5 isolates were able to produce siderophore and it was confirmed by the development of orange halos surrounding those colonies (Manivannan et al., 2012). Seven isolates were found to produce more than 85% siderophore units (Tailor and Joshi, 2012). A subpopulation of 27 strains of plant associated with *Pseudomonas fluorescens* which could produce a compound and this compound inhibited the growth of the devastating rice bacterial blight pathogen Xoo in laboratory assays and suppressed rice bacterial blight up to 59-64% in net-house and field experiments (Velusamy et al., 2006). Jeyalakshmi et

al. (2010) found that the combination of seed treatment, soil application and foliar spray with *P. fluorescens* give the minimum disease incidence of bacterial leaf blight with maximum yield in comparison with the chemical treatment and control. The evaluation of immunity status of available rice germplasm against BLB is an important step in the sustainable management of disease (Malukani et al., 2020). Furthermore, the biochemical characterization is the most pivotal tool to map out the disturbances incited by the pathogen which would otherwise be helpful in devising suitable management strategies. There are very scarce studies on the Xoo isolates of Pakistan regarding biochemical characterization, phenotypic evaluation and antibiotic efficacy. Keeping in view the above mentioned facts, the present study was planned to elaborate different aspects of the Xoo including screening of rice germplasm against this pathogen, biochemical characterization of pathogenic isolate of BLB and *in-vitro* management of pathogen by using different antibiotics

## Material and Methods

### Screening of rice germplasm against bacterial leaf blight disease (BLB)

#### Experimental material and layout

Germplasm (Basmati-2000, Pak basmati, PS-2, Basmati-370, Super Basmati, Chenab basmati, Basmati-386, Punjab Basmati, Kisan Basmati, Basmati-385, Basmati-515 and Basmati-198) was collected from rice research institute Kala Shah Kaku. Randomized complete block design (RCBD) was used for the transplantation of nursery in the experimental field for screening of germplasm against the pathogen.

**Table-1: Disease rating scale of BLB of rice (Chaudhary, 1996)**

Highly Resistant	Less than 1% of leaf affected
Resistant	1-10% of leaf affected
Moderately Resistant	11-30% of leaf affected
Susceptible	31-70% of leaf affected
Highly Susceptible	71% or more of leaf affected

#### Disease assessment

Disease severity of all the varieties was evaluated after the appearance of salient disease symptoms in the screening nursery. Plants from each variety were



selected randomly and disease severity percentage was calculated in the field by using following formula;

$$\% \text{ disease severity} = \frac{\text{No. of infected leaves/plant}}{\text{Total no. of leaves/plant}}$$

All the varieties were classified according to the disease rating scale.

### Infected sample collection

BLB infected leaf samples were collected from Hassanpura, Riazabad, Thatha Kher-o-Matmal, Sukheki Chowki, Tootra, Madryanwala, Hafizabad bypass, Fatehpur, Rasoolpur Tarar, Thatha Jehadka Tibba and Shah Behloul areas of district Hafizabad, Punjab, Pakistan.

### Preparation of nutrient agar medium

Samples of infected leaves were preserved in refrigerator at 4°C for further processes. Nutrient agar media was prepared for the isolation of bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) from infected leaf samples (Basit et al., 2014).

### Isolation, identification and purification

Infected leaves were dipped into 70% ethanol for surface sterilization for 2 seconds and then into distilled water for 2 seconds to wash out extraneous disinfectant. After this infected leaves samples were cut into 2-3 mm portions. Five pieces of infected leaves were placed on each media containing plate, four in front of each other and one in the centre of plate. Plates were kept in incubator at 25°C for 24 hours. After 24 hours Petri plates were examined with yellowish growth of bacteria on sides of the leaf's cut portions. Isolated bacteria were characterized on the basis of colony formation pattern, color, size, distribution, and under UV light. Standard protocols of Koch's postulates were performed for the confirmation of pathogenicity. Then a specific test for gram negative bacteria (Gram's staining) was done.

### Gram's staining

Neat clean slide was taken and one drop of distilled water was put on it. By using a sterilized wire loop, small colony of bacteria was picked up and put on water; a thin film of a homogenous mixture was made. It was heat fixed by passing it 2-3 times on a fire flame (avoid killing of bacteria due to extra heat).

Crystal violet stain was added on it for 60 seconds. Then by applying Lugol's iodine, it was washed for 60 seconds. Decolorization was done then by using pure acetone. Safranin was poured on it for 60 seconds which acted as a counter stain. Washed with distilled water at last and dried with a tissue paper. It was observed at 100x under microscope to confirm shape, size and distribution of bacteria (Bradbury, 1970).

### Biochemical characterization of pathogen

Bacteria always produce some type of chemicals which are of great importance in characterization and identification of microorganism during some specific biochemical reactions (Klingler et al., 1992; Sultan et al., 2020).

These tests were performed in four different flasks. Initially, the ingredients of Methyl red test (Poly peptone, K<sub>2</sub>HPO<sub>4</sub>, Glucose and distilled water), Indole production test (Bactotrypton, NaCl and distilled water), Citrate Utilization test (Sodium ammonium phosphate, Sodium citrate, Potassium phosphate monobasic and Magnesium sulphate and distilled water), and Vogas Prosakure test (Poly peptone, K<sub>2</sub>HPO<sub>4</sub>, Glucose and distilled water) were mixed in different flasks as a media and poured in test tubes. Test tubes were autoclaved at 121°C at 15 psi for 15 minutes. Fresh and pure pathogen was inoculated to the medium with the help of inoculating loop. Test tubes were incubated at 35°C for 4 days. After this, for Methyl red test (Methyl red, Ethyl alcohol and distilled water), Indole production test (Para dimethyl amino benzaldehyde, Isoamyl alcohol and Hydrochloric acid), and Vogas Prosakure test (Alpha Naphthol, Ethyl alcohol, KOH and distilled water) were mixed to prepare a reagent. After removing test tubes from incubator 3-4 drops of the respective reagent were added in these test tubes and let the biochemical reaction begun. After 15 minutes the color of the reactants were observed (Padmaja et al., 2017; Ahmad et al., 2020).

### In-vitro management of pathogen by using antibiotics

#### Pure culture of virulent strain

An isolate was selected as most virulent on the basis of its most striking disease symptoms and high disease incidence and severity on pathogenicity test plants, rapid and most similar colony formation. Pure culture was prepared by using streaking method in Petri-dishes. It was subjected to evaluate different antibiotics at



different dilution levels to check that which antibiotic is best against *Xanthomonas oryzae* pv. *oryzae* and at which level of concentration.

### Antibiotics

Three antibiotics Kanamycin (Kanacillin Plain; manufactured by Fazul Ellahie (PVT) LTD.) Streptomycin (Streptomycin; manufactured by P.D.H. Pharmaceuticals (PVT) LTD.) and Ampicillin (Ampicillin; manufactured by Shifa Laboratories (PVT) LTD.) each at three levels of concentrations 50ppm, 100ppm and 150ppm. These concentrations were prepared by adding 50 mg, 100 mg and 150 mg of the antibiotics in 1 ml of distilled water.

### Poisoned food technique

Nutrient agar was mixed in 1000 ml distilled water and mixed thoroughly. It was autoclaved at 121°C for 15 minutes at 15psi. After that media was cooled down and shifted into small bottles. Different concentrations of the antibiotics were mixed into the media bottles. Media was poured into petri plates which were labeled by antibiotic name, replication number, concentration levels and pathogen name etc. Petri plates were allowed to solidify and then with the help of inoculating loop freshly purified pathogen (*Xanthomonas oryzae* pv. *oryzae*) was inoculated. Petri plates were incubated at 30°C (Gholve and Kurundkar, 2007).

### Data recording

Petri plates were observed and data was recorded at three intervals of time with the help on measuring scale. After 48 hours growth of bacteria on the inoculated petri plates was measured from the back side of petri plates with the help of scale then after 72 and 96 hours, respectively. The diameter of plate was measured and inhibition zone due to antibiotic application was recorded.

## Results

### Screening of rice germplasm against bacterial leaf blight disease

After three weeks of inoculation disease assessment data was collected and then till harvesting all the yield parameters were calculated. There was not even a single variety which found resistant against the pathogen. However, Pak Basmati, Kisan Basmati, Punjab Basmati, Chenab Basmati, Basmati 386 and Basmati-2000 were found susceptible while Super

basmati, PS-2, Basmati 370, Basmati 515, Basmati 198 and Basmati 385 were found highly susceptible against BLB disease (Table 2).

Out of twelve varieties of rice tested in the field against bacterial leaf blight of rice Super Basmati was showing (89.01%) disease severity followed by PS-2 showing (88.48%) disease severity, Basmati-370 was showing (80.55%) disease severity, Basmati-198 with (73.9%) disease severity, Basmati-515 was with (73.4%) disease severity, Basmati-385 was with (70.21%) disease severity, Basmati-2000 was found with (66.85%) disease severity, Basmati-386 with (64.28%) disease severity, Pak basmati with (54.69%) disease severity, Punjab basmati with (54.28%) disease severity, Kisan basmati was showing (47.11%) disease severity and Chenab basmati was showing (44.6%) disease severity (Table 3).

**Table-2: Response of rice germplasm against bacterial leaf blight of rice**

Sr. No.	Remarks	Varieties/Response
1	Highly resistant	0
2	Resistant	0
3	Moderately resistant	0
4	Susceptible	Chenab Basmati, Kisan Basmati, Punjab Basmati, Pak Basmati, Basmati-386, Basmati-2000
5	Highly susceptible	Super basmati, PS-2, Basmati-370, Basmati-515, Basmati-198, Basmati-385

**Table-3: Comparisons of means of disease severity among different rice cultivars**

Genotypes	Disease severity (%)	Response
Super Basmati	89.01 a	HS
PS-2	88.48 a	HS
Basmati-370	80.55 b	HS
Basmati-198	73.90 c	HS
Basmati-515	73.40 c	HS
Basmati-385	70.21 cd	HS
Basmati-2000	66.85 de	S
Basmati-386	64.45 e	S
Pak basmati	54.69 f	S
Punjab basmati	54.28 f	S
Kisan basmati	47.11 g	S
Chenab basmati	44.60 g	S

\*Means with similar letters are not significantly different from each other at 5% probability level (LSD = 1.26)



### Confirmation of pathogen by Koch's postulates and Gram staining

Symptom development of the plants subjected to Koch postulates was observed after 4 weeks of artificial inoculation of bacteria to the selected plants. Plants which were inoculated with the pathogen isolated from the samples collected from Hassan Pura, Riazabad, Thata Kher-o-Matmal, Sukheki Chowki, Tootra, Madryan Wala, Hafizabad Bypass and Thatha Jehadka were showing highly developed and most obvious symptoms of BLB. Plants which were inoculated with the isolates of Fateh Pur samples were showing medium symptom development while plants inoculated with the isolates of the leaf samples of Rasoolpur Tarar and Tibba Shah Behlool were showing low symptom development.

**Table-4: Gram staining and confirmation of pathogenicity by Koch's postulates**

Isolates	Gram staining	Disease severity	Symptom development
Hassan pura	Negative	99%	High
Sukheki chowki	Negative	95%	High
Thatha kher-o-matmal	Negative	89%	High
Hafizabad bypass	Negative	87%	High
Thatha jehadka	Negative	78%	High
Tootra	Negative	71%	High
Madryanwala	Negative	62%	High
Riazabad	Negative	56%	High
Fatehpur	Negative	43%	Normal
Rasoolpur tarar	Negative	42%	Low
Tibba shah behlool	Negative	34%	Low
Hassan pura	Negative	99%	High

Plants inoculated with Hassan Pura isolates were showing high disease severity which was 99% following Sukheki Chowki with 95% disease severity, Thatha Kher-o-Matmal with 89% disease severity, Hafizabad by pass with 87% disease severity, Thatha Jehadka with 78% disease severity, Tootran with 71% disease severity, Madryan Wala with 62% disease severity, Riazabad with 56% disease severity, Fatehpur with 43% disease severity, Rasoolpur Tarar with 42% disease severity and last was Tibba Shah Behlool with 34% disease severity (Table 4).

Gram's staining showed that all the tested isolates

were found gram negative as a result of this staining test but they were showing some differences in the sizes from small to medium with uniform rod shapes.

### Morphology of colonies

All the 11 isolates were observed and examined carefully. All the isolated pathogens were of yellow color and shape of colonies was circular with even margins. Sizes of colonies were medium to small and all the colonies were dark from center. Isolates of Hassan Pura, Thatha Kher-o-Matmal, Sukheki Chowki, Madryan Wala, Hafizabad Bypass and Thatha Jehadka were showing small colonies on nutrient agar plates. Isolates of Riazabad, Tootra, FatehPur, Rasoolpur Tarar and Tibba Shah Behlool were showing medium sized colonies grown on nutrient agar plates. All the isolates were having dark colored central portions of their colonies with the circular shape of colonies (Table 5).

**Table-5: Colonial morphology of *Xanthomonas oryzae* pv. *oryzae***

Isolates	Color	Margins	Centre	Shape
Hassan pura	Yellow	Even	Dark	Circular
Riazabad	Yellow	Even	Dark	Circular
Thatha kher-o-matmal	Yellow	Even	Dark	Circular
Sukheki chowki	Yellow	Even	Dark	Circular
Tootra	Yellow	Even	Dark	Circular
Madryanwala	Yellow	Even	Dark	Circular
Hafizabad bypass	Yellow	Even	Dark	Circular
Fatehpur	Yellow	Even	Dark	Circular
Rasoolpur tarar	Yellow	Even	Dark	Circular
Thathajehadka	Yellow	Even	Dark	Circular
Tibba shah behlool	Yellow	Even	Dark	Circular

### Biochemical characterization of pathogenic isolate of BLB

To assess the biochemical characteristics possessed by the pathogen methyl red test, indole production test, citrate utilization test and Voges Proskauer test were conducted.

**Methyl red test:** All isolates showed negative response towards the test because the color they were showing was yellow.

**Indole production test:** All isolates showed negative response towards the test because the color they were showing was yellow. All the isolates of *Xoo* were not able to produce indole.



**Citrate utilization test:** When test tubes were removed from incubator and color of the media was examined, it turned blue from green which showed that bacteria surviving in that medium were consuming carbon as food source from provided citrate compounds. It indicates that the bacteria were containing citrate permeases enzyme due to which they were consuming carbon from citrate compound.

**Voges Proskauere test:** When test tubes were removed from the incubator and color of each test tube was examined, they showed negative response toward test because there was yellow appearance of each test tube media (Table 6).

**Table-6: Biochemical characterization of Xoo**

Isolates	Biochemical tests			
	MR Test	IP Test	CU Test	VP Test
Hassan pura	-	-	+	-
Riazabad	-	-	+	-
Thatha kher-o-matmal	-	-	+	-
Sukheki chowki	-	-	+	-
Tootra	-	-	+	-
Madryanwala	-	-	+	-
Hafizabad bypass	-	-	+	-
Fatehpur	-	-	+	-
Rasoolpur tarar	-	-	+	-
Thatha jehadka	-	-	+	-
Tibba shah behloul	-	-	+	-

MR (Methyl Red), IP (Indole Production), CU (Citrate Utilization), VP (Voges-Proskauere)

**In-vitro management of pathogen by using different antibiotics**

Three different antibiotics were used for the *in-vitro* control of the pathogen. Four different levels of concentration (0 ppm, 50 ppm, 100 ppm and 150 ppm) of chemicals were used. Antibiotic Kanamycin showed much positive response in controlling the spread and multiplication of the pathogen (16.12 mm), 15.81 mm with Streptomycin and 15.81 mm radial growth was appeared in comparison to Ampicillin. It was obvious that with the increase in concentration of antibiotics the reduction in growth was decreasing that they linked in inversely proportional sequence. From the Tables it is clearly indicated that after 48 hours, Kanamycin at 50ppm concentration gave the maximum average control (0.69 mm) in all replications while the streptomycin

was giving maximum growth control (3.49mm) at 50 ppm and Ampicillin was reducing the maximum radial growth (5.13 mm) at 50ppm.

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**Table-7: Comparison of antibiotics against Xoo**

Level	Time (Hours)	Kanamycin	Streptomycin	Ampicillin
0 ppm	48	4.66 cd	5.37 de	5.68 h
50 ppm	48	0.69 e	3.64 f	5.86 g
100 ppm	48	5.64 c	5.68 de	7.96 e
150ppm	48	1.69 e	6.73 c	5.84 gh
0 ppm	72	3.28 cde	6.74 c	11.33 b
50 ppm	72	1.96 e	3.49 f	5.13 i
100 ppm	72	2.91 de	5.14 e	6.74 f
150ppm	72	11.41 b	11.33 b	8.96 d
0 ppm	96	16.12 a	6.21 cd	15.81 a
50 ppm	96	2.46 de	15.81 a	5.74 gh
100 ppm	96	2.67 de	5.41 de	5.74 gh
150ppm	96	3.36 de	6.21 cd	9.41 c

\*Means with different letters in each column are significantly different from each other at 5% level of probability (LSD =

**Discussion**

Rice is a primary nourishment for 2.7 billion individuals around the globe is one of the significant grains utilized everywhere throughout the world (Salim et al., 2003; Shivalingaiah and Umesha 2011; Shivalingaiah et al., 2012). Tropical and sub-tropical districts of the world are the significant rice-makers, with 90 % of generation happening in Asia (Jabeen et al., 2012). It is however heartbreaking that such a vital yield is assaulted by numerous sorts of maladies as well as BLB of rice caused by *Xoo*. As there is no single powerful control measure is accessible against this sickness (Chaudhary et al., 2009). Borines et al. (2003) announced that the utilization of safe cultivars has been the best and prudent method for controlling bacterial leaf curse of rice. In current study, none of the tested cultivars showed immune response against BLB which forces to find alternate ways for sustainable disease management tactics. These results are line with those of early researchers who



found 23 basmati genotypes susceptible to BLB (Shah, 2008; Zafar et al., 2020). It is the utmost requirement of for the management of this economically important disease by resistance breeding as maximum commercial basmati varieties showed moderately susceptible to highly susceptible response (Khan et al., 2008; Li et al., 2020).

Prompting plant barrier system by the utilization of organic operators is the new plant assurance procedure. Acceptance of systemic resistance by phytochemicals was recorded by numerous laborers (Vanitha et al., 2009; Kong et al., 2020). Phytochemicals are having extraordinary capacity in plant disease management; consequently they are utilized to treat numerous ailments. The green house tests demonstrated that maladies occurrence decreased when seeds treated and sown (Hari et al., 2013). The present study was gone for discovering contrast in the level of resistance appeared by changed genotypes in light of the visual side effects because of BLB contamination and overseeing disease through chemicals. The germplasm utilized for screening against BLB showed variable reaction. No variety was resistant against the disease. Distinction in resistance in rice germplasm was accounted for by less accessibility of safe sources use of pesticides in adjusted frame for the lessening of BLB is additionally a workable management choice (Arshad et al., 2013). The utilization of actuated resistance in plants is a promising, situation cordial procedure for controlling plant ailments; including those caused by microbes (Mohanbabu et al., 2003). During biochemical characterization of all the collected isolates were showing positive response towards citrate utilization which means that the isolates were gram negative and were consuming carbon as food from provided media. All the isolates were negative towards the indole production test, methyl red test as well as Voges-Proskauer test. Settlements of *X. oryzae* pv. *oryzae* are moderate developing, mucoid and straw-hued to yellow in shading, the disconnected microscopic organisms re-colored pink-red and demonstrated thin viscid mucoid strand showing positive for KOH dissolvability test and gram negative nature of the microorganisms. An unmistakable zone of hydrolysis was conformed to the bacterial states, when the plates were overwhelmed with Lugol's iodine. Thus the bacterium demonstrated positive for starch hydrolysis (Vera-Cruz et al., 1984).

The chemicals used against BLB displayed changing decrease in disease incidence rate. Among three chemicals it is indicated that after 48 hours, kanamycin at 50ppm concentration gave the maximum average control (0.690mm) in all replications while the streptomycin was giving maximum growth control (3.49mm) at 50ppm and ampicillin was reducing the maximum radial growth (5.137mm) at 50ppm. In the 1960s, various types of agrochemicals were created from rehashed field trials and made available on a vast scale, for the most part in Japan. They depended on chloramphenicol, nickel-dimethyldithiocarbamate, dithianon and fentiazon. Most were problematic, however inferable from changeability in affectability among the pathogen populace. In any case, synthetic control of bacterial leaf blight in tropical storm tropical Asia is unfeasible and no genuinely viable bactericide is monetarily accessible for malady control (Lee et al., 2005; Liu et al., 2006). Kanamycin inhibits protein synthesis by tightly binding to the conserved A site of 16S rRNA in the 30S ribosomal subunit of the bacteria (Su et al., 2015). A use of copper-oxchloride and streptomycin totally repressed of bacterial development. A streptomycin blend was tried in India for cleansing of rice seeds and it turned out to be compelling (Srivastava, 1972). The second most effective antibiotic in current study was streptomycin which irreversibly binds to the 16S rRNA and S12 protein within the bacterial 30S ribosomal subunit. As a result, this agent interferes with the assembly of initiation complex between mRNA and the bacterial ribosome, thereby inhibiting the initiation of protein synthesis (Aktan et al., 2013). Bacterial leaf blight injuries in rice were lessened by utilizing fading powder with 30 % chlorine (2 kg/ha) to clean rice seeds (Chand et al., 1979). The antibacterial exercises of various plant removes against plants ailments have been already researched (Okigbo and Nmeke, 2005). Antimicrobials, fungicides, and even organics, for example, dairy animals excrement were endeavored for the control of Bacterial leaf blight of rice, yet up until this point, just a halfway control of the illness has been conceivable (Mary et al., 2001).

The outcomes of the present research are supported by previous experiments as described above. These results would be valuable to manage BLB disease of rice that is an emerging and economically important disease in Pakistan. However, it is necessary to carry out field experiments to prove the effectiveness of these antibiotics because there are many factors in the



field which influence the chemical control of plant diseases. Further studies for chemical disease control in field conditions are needed.

## Conclusion

None of the cultivars showed resistance against BLB. However, Basmati varieties can be used with appropriate antibiotics. The results of screening provide practical useful information to find out the level of resistance in available rice germplasm. Biochemical characterization would be helpful in devising the probable losses caused by Xoo. The present study suggests that Kanamycin is the most effective antibiotic for the management of Xoo.

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### Contribution of Authors

Khan A: Conceived idea, designed research methodology and collected data  
Ali S: Supervised research and approved final draft  
Sajid M & Ghani MU: Collected, analysed and interpreted data  
Zeshan MA & Binyamin R: Collected data and wrote & edited manuscript  
Ahmed N: Helped in designing research methodology

