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Research Article

Management of bacterial leaf blight disease of rice in farmer's field condition at Bhaktapur district of Nepal

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ABSTRACT
Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is considered as one of the major threats to rice production in Nepal. To identify the effective control measures, an experiment was done in a randomized complete block design with three replications in a farmer's rice field at Bhaktapur, during the summer seasons of 2018 and 2019. Three plants extracts; 1% Neem extract, 2% Asuro leaf extract, and 2 % Titepati leaf extract along with two fungicides viz. 0.1% Agricin and 0.2% Blitox 50, and one bio-control agent 1%Kisan Pseudomonas were used as treatments. The first spray was given as a prophylactic spray at the jointing stage, and they were applied 3 times at 50, 60 and 70 days interval. All the treatments significantly reduced disease and improved test weight higher than control. Among the tested treatments, 0.1 % Agricin performed as the best with 65.70 % disease control and increase in yield over control by 28.13 %. Among the tested botanicals, 2% stock solution of Titepati performed as the best with 50.59 % disease control and increase in yield by 13.51%. Therefore, three times application of 0.1 % Agricin at 10 day interval starting from jointing stage can be used to control BLB disease for higher rice production.

Keywords: Bacterial leaf blight, bio-control agent, botanicals, chemicals, disease control

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INTRODUCTION

In context of Nepal, rice ranks the first position in terms of area and production, covering 1.47 million ha with total production of 5.6 million ton in the country with the productivity of 3.80 t/ha (MoALD, 2022). Rice blast (*Pyricularia oryzae*), brown leaf spot (*Bipolaris oryzae*), sheath blight (*Rhizoctonia solani*) and bacterial leaf blight (*Xanthomonas oryzae*pv. *oryzae*) are the economically important and widespread diseases of rice in Nepal that causes

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higher yield loss. Rice bacterial leaf blight (BLB) is one of the most serious bacterial diseases. The disease was first observed in Japan in 1884 (Tagami & Mizukami, 1962). In Nepal BLB was first identified in Balaju, Kathmandu in 1965 (Khadka *et al.*, 1968) and has since been reported throughout the country. BLB is favored by warm temperature (25-30 °C), high humidity, rain and deep water and is more prevalent in wetland areas where these conditions often occur. Severe winds, which cause wounds and excess nitrogen, also favor the disease. The disease could be characterized mainly into two distinct phases; leaf blight phase, and the "Kresek phase" (acute wilting of young plants) which is the destructive one for the epidemic of disease (Reddy & Ou, 1976). In Nepal it reduced the yield from 5-60 % in terai and mid-hills during hot and humid periods (Burlakoti & Khatri-Chhetri, 2005).

The severity and significance of damages caused by the diseases have necessitated the development of strategies to manage the disease, to reduce the crop loss and to avert an epidemic. Although newly introduced Chinese hybrid has become more susceptible to BLB, it is favored by farmers due to its higher production and more suitable to make Chhyang (Local wine). Also, farmers do not want to leave some varieties despite their susceptibilities to diseases. At first, Bordeaux mixture was recommended to be sprayed prior to the occurrence of disease for the management of BLB (Hashioka, 1995). Also, copper, and mercuric compounds were then tested to control the BLB disease (Kiryu & Mizuta, 1955). In that case, other options for controlling diseases are required. Identification of less hazardous and effective measures, including bio control agent, botanicals and chemical control measure is needed for the effective management of BLB disease.

Therefore, identification of management option is the most for increasing production and productivity of rice and improves the food security status of people. An experiment was conducted to evaluate the different plant extracts, fungicides, and bio-control agent on Chinese hybrid variety DY 28 in farmer's rice field at Bhaktapur district of Nepal.

MATERIALS AND METHODS

An experiment was carried out during summer season of 2018 and 2019 in the farmer's field at Suryabinayak Municipality, ward no.9, Bhaktapur district of Nepal. The site lies in the sub-tropical zone with an altitude of 1579 meter above sea level and located between 27.67° N latitude and 85.47° E longitude. A set inclusive of commercially available bactericides, botanicals and bio-control agents were evaluated against bacterial leaf blight of rice. The field trial was laid out in Randomized Complete Block Design (RCBD) with three replications. There were seven treatments inclusive of control. The plots measuring 3 m × 2m were marked and five plants at random in each plot were selected and labeled. The Chinese hybrid variety DY 28 with a wide popularity among the Newari community has high yielding capacity, inspite of being susceptible to the disease. This variety was collected from ATC agrovet Khumaltar ,Lalitpur Nepal for this study. Twenty-four days old seedlings were transplanted in the field using two seedlings/hill with a spacing of 20 cm × 20 cm. The crop was transplanted 3rdweek of July of both experimental years. The required quantities of the chemicals were weighed and suitably dissolved in a requisite quantity of water to get desired concentrations. First spray was given as a prophylactic spray at jointing stage. Treatments were applied as a foliar spray for 3 times at 10 days interval. Disease scoring was done based on BLB scoring scale given by IRRI, 2002. 100 cm×100 cm square rod was thrown in the middle region of plots. All the data except grain yield were taken from those sample plants. Harvesting was done at full maturity stage of the crop and the grains were dried under sun for

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7 days after threshing and winnowing. As the moisture content was 12%, grain yield and thousand grain yield were taken. Treatment means were computed, and the grain yields were extended to t/ha.

Treatment details

The concentration details of the treatments and applied dose were as follows:

1. T1: Agricin (Streptomycin sulphate 9% + Tetracycline hydrochloride 1%) @ 1 g/L
2. T2: Blitox 50 (Copper oxychloride 50%WP) @ 2 g/L
3. T3: Nimbecidine (Azadirachtin 300 ppm) @ 10 mL/L
4. T4: Asuro leaf extract (1:1 weight/volume) @ 20 mL/L
5. T5: Titepatileaf extract (1:1 weight/volume) @ 20 mL/L
6. T6: Kisan Pseudomonas (Pseudomonas) @ 10 mL/L
7. T7: Control (Distilled water only)

Preparation of botanical extracts

Fresh leaves of mugwort (*Artimisia indica*) and Asuro (*Justicia adhatoda*) were collected from Lalitpur and Bhaktapur districts and thoroughly washed in tap water 3 to 4 times to remove any inert material on the surface. These materials were dried in the shed for six days. While preparing the extracts, the unwanted debris was removed, surface sterilized for 2 minutes in 70% ethanol. The paste was obtained by grinding 100g of leaf extract in 100 mL of sterile distilled water using a mortar and pestle. The paste was squeezed and filtered through 4 folds of sterile cotton wool into a 150 mL conical flask.

Fungicides and bio-control agents

The bio-control agent used for the experiment was commercial Kisan Pseudomonas collected from ATC agrovet located at Khumaltar, Lalitpur, Nepal.

Observation

Grain yields were taken from each plot and means were computed. Thousand grains were selected randomly from the grain yield of each plot immediately after harvest, weighed, with an electronic balance, and weight (g) was taken at 12% moisture content. AUDPC (Area Under Disease Progress Curve) values were computed from the following formula:

$$AUDPC=\sum_{i=1}^{n} [\{(Y_{i+1} + Y_i)/2\}\times(T_{i+1} - T_i)]$$

Where, Y_i = disease severity on the i^{th} date,

Y_{i+1} = disease severity on $(i+1)^{th}$ date and

n = number of dates on which disease scores

The increase in yield over the control by different treatments was calculated using the given formula:

Increase in yield over control (%) = (T-C)/C×100

Where, T= Yield from treatment plot

C= Yield from control plot

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Statistical analysis

The data were analyzed using R-studio ver. 1.1.6.1-5 (RStudio 2015) computer package programs. A one-way ANOVA was performed to see differences between the treatments. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% levels of significance.

RESULTS AND DISCUSSION

Agricin (210.83) had the least total Area Under Disease Progress Curve (AUDPC) value than all other treatments, showing it is the best treatment among the tested ones on BLB management. Kisan Pseudomonas had recorded the second lowest total AUDPC value (227.50). Among the plant extracts, Titepati leaf extract had lowest total AUDPC value (250.83) than others (Figure 1).

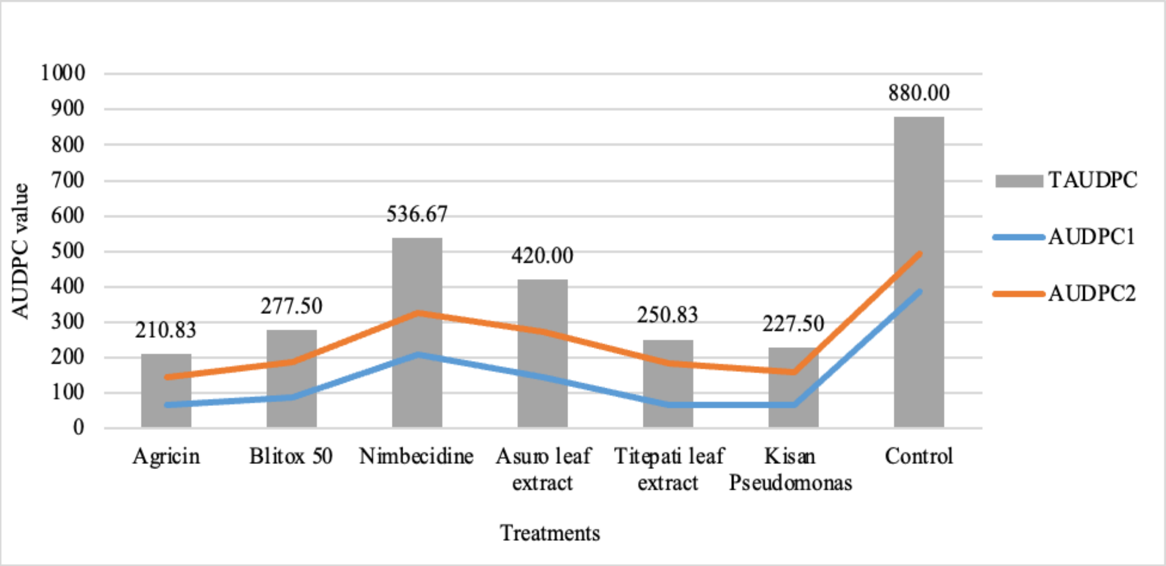

Figure 1: AUDPC of BLB disease of rice as influenced by different treatments

Agricin showed highest percentage disease control (65.70%) and highest increase in yield over control (28.13%), followed by Kisan Pseudomonas which showed 61.41% disease control and 24.26% yield increment over control. The least percent disease control (38.60) and percent increase in yield over control (10.72%) was found in treatment Asuro leaf extract (Figure 2).

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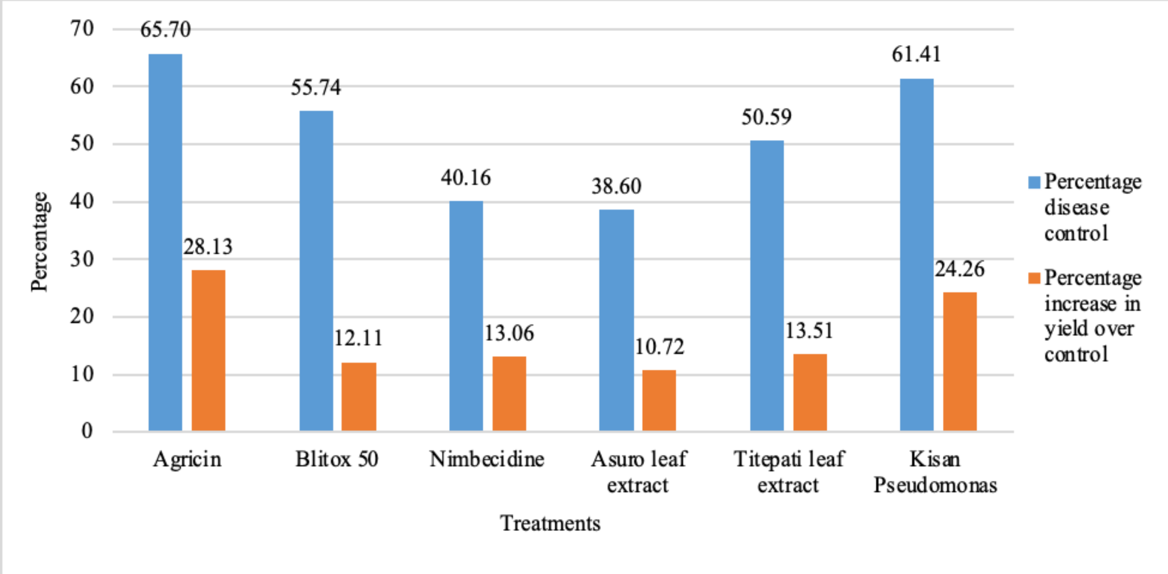

Figure 2. Effects of different treatments on BLB disease control and rice yield

Many researchers have reported the usefulness of some copper-based fungicides and antibiotics against BLB disease. Singh *et al.* (1980) stated that, encouraging effect of a antibiotics in suppression of causal pathogen of rice bacterial blight i.e., Agricin 500, Brestanol, Agric. Teramycin 17 and mixture of Cytoxan+Agricin100. Results of the present study also support by findings of Biswas *et al.* (2009) who studied the effect of antibiotics and fungicides on bacterial blight of paddy. Among the different chemicals tested, seed treatment with Streptocycline (100 ppm) along with foliar spray in combination with COC (100 ppm + 500 ppm) gave the best result against bacterial blight in all the consecutive years (2003, 2004 and 2005) showing 10.9%, 9.7 % and 10.8% disease intensity respectively. Likewise, Khan *et al.* (2012) achieved the suppression of *Xanthomonas oryzae* pv *oryzae* colonies during in-vitro studies by using the antibiotics (Benzylpenicillin, Ampicillin, Kanamycin, Streptomycin, Chloramphenicol and Sinobionic). This study revealed that Blitox 50 WP (copper oxychloride) gave 55.74 % control with 12.11% increase in rice yield over control, which had close conformity with Khan *et al.* (2005) who stated that copper oxychloride controlled the BLB disease in rice when used as foliar spray application and improved the rice yield. The finding of this experiment is also in agreement with the better control of bacterium growth by application of streptomycin solution as spray (Tagami & Mizukami, 1962).

Investigation also indicated *Pseudomonas* was effective in controlling BLB of rice. Various studies have been reported the use of *Pseudomonas* and *Bacillus* strains for the bio control of rice pathogens such as *Xanthomonas oryzae* pv. *oryzae,M. oryzae* and *R. solani* (Helene *et al*., 2011; Ji *et al.*, 2008; Spence *et al.*, 2014). Anuratha and Gnanamanickam (1987) observed that the roots of the rice plants possessed different isolates of *P. fluroresense* which possessed the antagonistic activity against the disease. They isolated the various strains of the *P. fluroresense* and studied their antagonistic activity against the *X. oryzae* under in vitro condition; *P. fluroresense* strains significantly controlled the growth of tested bacterium. Similarly, when they sow rice seeds after a presoaking treatment in the suspension of *P. fluroresense*, which provided a coating on the seed coat. After the seeding emergence they were sprayed with the *P. fluroresense* again and finally inoculated with the tested bacterium

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using the clipping method, this treatment considerably reduces the severity of the paddy bacterial blight in comparison with the non-treated control members of the *Pseudomonas* spp., both pathogenic as well as non-pathogenic strains, can produce various extracellular secondary metabolites. These metabolites exhibit diverse properties i.e., function as virulence factors, siderophores (having high-affinity of iron ions), biosurfactants, and antimicrobial agents as well as in cell-to-cell signaling etc. These metabolites enable *Pseudomonas* spp. to adapt in different environments, colonize different hosts and compete with other species. Among botanicals, Titepati leaf extract performed best. Several researchers like (Rukhsana, 2011) states that, the antibacterial activity of medicinal plants.

The treatments varied significantly in thousand grain weight and grain yield. Maximum grain yield (7.13 t/ha) and 1000 grain weight (30.31g) was obtained from Agricin which was then followed by Kisan Pseudomonas in which grain yield was 6.92 t/ha and 1000 grain weight 30.39g. Control has least grain yield and 1000 grain weight. There was no significant difference in grain yield among the treatments Blitox 50, Nimbecidine, Asuro leaf extract, Tittepati leaf extract and Kisan Pseudomonas (Table 1).

Table 1: Effect of different treatments on thousand grain weight and grain yield of rice

SN	Treatments	1000 grain weight* (g)	Grain yield* (t/ha)
1	Agricin @ 0.1%	30.31[a]	7.13[a]
2	Blitox 50 @ 0. 2 %	28.82[bcd]	6.24[b]
3	Nimbecidine @ 1%	28.46[cd]	6.29[b]
4	Asuro leaf extract @ 2 %	29.07[abc]	6.17[b]
5	Titepati leaf extract @ 2%	30.12[abc]	6.32[b]
6	Kisan Pseudomonas @ 1%	30.39[a]	6.92[b]
7	Control (Water spray only)	27.49[d]	5.57[c]
Grand mean		29.24	6.38
F-test		**	***
LSD (≤0.05)		1.4872	0.2446
CV (%)		2.86	2.16

¥Values are means of two years data (2018 & 2019). Means followed by the same letter in same column are not significantly different by DMRT (P=0.01).

The yield was negatively associated with disease severity. The relationship between disease severity and yield was linear with coefficient of determination (R^2) of 0.792. This indicated that disease severity alone reduced the yield by 79.2% and 20.8% remaining yield reduction was due to other factors (Figure 3).

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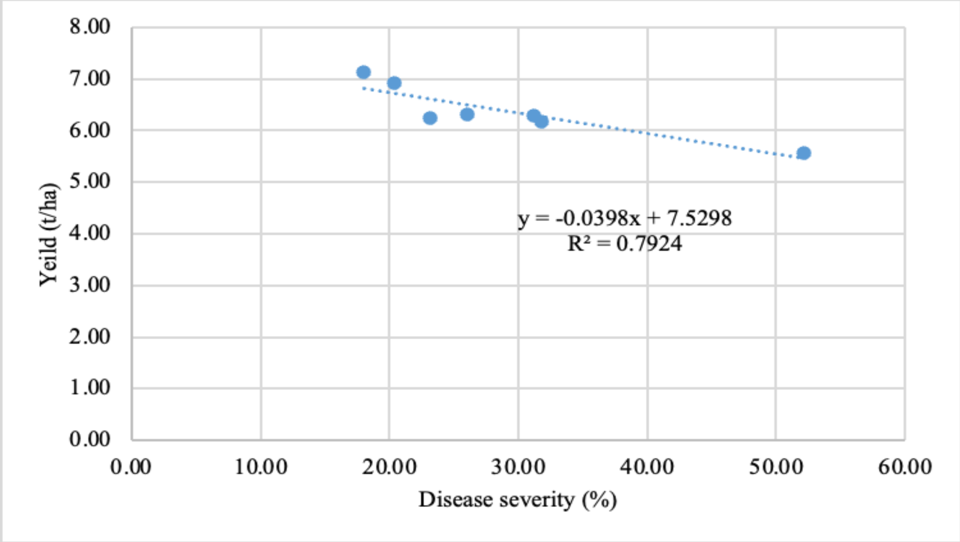

Figure 3: Relationship between BLB disease severity and rice yield

CONCLUSION

All treatments resulted in significantly better disease control with varying degree of success as compared to untreated control treatments. Among the tested treatments, 0.1% Agricin (Streptomycin sulphate 9% + Tetracycline hydrochloride 1%) performed as the best with 65.70% disease control and increase in yield over control up to 28.13%. One percent *Pseudomonas* gave disease control up to 61.41% with a yield increment by 24.26% over control. Among the tested botanicals, 2% stock solution of Titepati performed as the best with 50.59% disease control and increase in yield over control up to 13.51%. So, these treatments can be used to manage BLB of rice.

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Authors' contributions

P. Sharma designed and performed the experiment, and recorded and analyzed data together with S. Baidya, P. B. Magar, S. Kandel, and S. Chaudhary helped to edit the manuscript. S. Kandel helped to identify, isolate, and study the morphology of bacteria (*Xanthomonas oryzae* pv. *Oryzae)* at the lab.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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