The Agriculturists 15(1): 10-18 (2017) ISSN 2304-7321 (Online), ISSN 1729-5211 (Print)
A Scientific Journal of Krishi Foundation ***Indexed Journal***
Impact Factor: 0.568 (GIF, 2015)

Leaf Blight Disease caused by *Pseudomonas syringae* pv. *syringae* in the Nurseries of Litchi (*Litchi chinensis* Sonn.) and its Management

M.A.H. Khan[1]*, I. Hossain[2], M.U. Ahmad[2] and M.S.M. Chowdhury[3]

[1]*On-Farm Research Division, Bangladesh Agricultural Research Institute, Mymensingh*
[2]*Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh*
[3]*Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh*

**Corresponding author and Email: helim1367@gmail.com*

Received: 03 October 2016 Accepted: 12 June 2017

Abstract

Leaf blight of seven varieties of litchi caused by *Pseudomonas syringae* pv. *syringae* was surveyed in the nurseries of major litchi growing areas in Bangladesh viz. Rajshahi, Dinajpur, Rangpur, Mymensingh and Khagrachari. The mean level of incidence and severity were 8.58 and 7.88% in Rajshahi, 9.88 and 8.88% in Dinajpur, 8.44 and 7.76% in Rangpur, 6.50 and 6.18% in Mymensingh and 9.00 and 7.98% in Khagrachari. Incidence and severity of bacterial leaf blight disease of litchi varied significantly depending on weather conditions. Correlation studies revealed that bacterial leaf blight disease of litchi seedlings were positively correlated with temperature, rainfall and relative humidity, where temperature and rainfall was the major factor to the variations of both incidence and severity. Antibiotic sensitivity test revealed that among fifteen isolates, most of the isolates of *P. syringae* pv. *syringae* collected from litchi were sensitive to Gentamycin and Erythromycin. Under net house condition, six different treatments (i) Gentamycin @ 0.05%, (ii) Erythromycin @ 0.05%, (iii) Doxycycline @ 0.05%,(iv) Copper sulphate @ 0.05%, (v) BAU-Biofungicide @ 2% and (vi) Control were used for controlling bacterial leaf blight of litchi (Variety: China-3). BAU-Biofungicide was found to be superior in controlling bacterial leaf blight of litchi that reduced 33.64% disease incidence and 60.77 % disease severity in 2010-11 and 63.76% disease incidence and 61.40 % disease severity in 2011-12 over control when applied as foliar spray @ 2% followed by Copper sulphate (0.05%) and Erythromycin (0.05%).

Keywords: Antibiotic, BAU-Biofungicide, litchi, leaf blight, management.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is an important sub-tropical evergreen fruit crop belonging to family Sapindaceae, is believed to have originated in China. It is one of the most important tropical fruit widely grown in southern China, Taiwan, northern Vietnam, Indonesia and Philippines. It is widely grown all over Bangladesh with the quality litchi solely concentrated in the north-west areas especially greater Rajshahi, Dinajpur, Pabna and Rangpur (Sathe, 2011). World litchi production is estimated to be around 2.11 million tons with more than 95 % of the area and production share belonging to Asia. Bangladesh produced 17 thousand metric tons of litchi in 1609 ha of litchi orchard during the period 2012-13 (BBS, 2014).

Litchi is the most demanding fruit due to its excellent flavor, attractive colour and taste and

its demand is increasing day by day with growing population but declining in production which results in scarcity every year. Nursery disease is one of the predominating causes for lower production of litchi in Bangladesh (Meah and Khan, 1986). It is reported that litchi is to be attacked with 4 different diseases viz. leaf spot, leaf blight, die-back and red rust in Bangladesh. Among the 33 recorded diseases of the nurseries of different fruit species, a new disease bacterial leaf blight was recorded in litchi (Hossain, 2011). The symptoms of bacterial leaf blight disease are characterized by a rapid enlargement of necrotic lesions in buds leaves. Litchi buds, leaves and stems are all predisposed to infection but fruit lesions have not been detected. Bacterial leaf blight caused by *P. Syringae* pv. *syringae*. is an emerging disease and great threat for production of healthy litchi saplings in different nurseries of Bangladesh (Hossain, 2011). Bacterial diseases of plants are very difficult to manage. In addition to visible disease symptoms, bacteria can become systematic in a plant's vascular tissue making it impractical to eradicate the pathogen by pruning out symptomatic tissues or by applying a pesticide to the plant surface. Antibiotics are effective against most gram-positive and some gram-negative bacteria (Kanfer *et al*., 1998). In developed countries antibiotics are used in limited extent to control bacterial diseases of plants. Since no chemicals were screened in Bangladesh to control the dangerous disease. So, the present research works was undertaken to survey leaf blight of litchi in the nurseries of selected locations viz. Rajshahi, Dinajpur, Rangpur, Mymensingh and Khagracharito know its incidence and severity and to evaluate some antibiotics, Copper sulphate and BAU-Biofungicide for the management of bacterial leaf blight of litchi.

2. Materials and Methods

2.1. Survey on the incidence and severity of leaf blight disease of litchi

Survey was conducted at different nurseries of five major litchi growing areas of Bangladesh viz. Rajshahi, Dinajpur, Rangpur, Mymensingh and Khagrachari from 10 July 2010 to 10 April 2011 and 10 July 2011 to 10 April 2012. During the survey 30saplings of litchi for each variety were selected randomly from each location (nursery). In each nursery 30 tagged saplings of each variety were considered for disease incidence and severity. Altogether seven varieties of litchi were surveyed (Table 1). Number of saplings and number of healthy and diseased saplings were recorded from the selected nurseries. Each of the selected saplings was observed carefully and symptoms of leaf blight disease were recorded following the descriptions of Pathak (1980), Burhan (1987), Rawal (1997) and Singh (1998). The disease incidence and severity were evaluated following the formula of Rai and Mamatha (2005), and Johnston (2000), respectively. Meteorological data on temperature, relative humidity and rainfall were collected from weather stations of selected areas to determine the effect of these weather factors on incidence and severity of leaf blight of litchi.

2.2. Antibiotic sensitivity test of Pseudomonas syringae pv. *syringae*

Fifteen isolates of *P. Syringae* pv. *syringe* were isolated from blighted leaves of litchi then identified and used in this experiment. The isolates were preserved in 10% skimmed milk, kept at -20°C in refrigerator for antibiotic sensitivity test.

Sensitivity of *P. syringae* pv. *syringae* isolate to different antibiotics was determined *in-vitro* by employing Kirby-Bauer disc diffusion method (Larkin, 2006). Antimicrobial discs of 0.05% were used for the test. The used antibiotics were Gentamycin, Erythromycin and Doxycycline. With a sterile pipette a drop of test culture of bacteria was poured on NA plate. Sterile glass spreader was used to spread the culture homogenously on the medium. The plate was allowed to sit at room temperature at least 3 to 5 minutes. Then three antibiotic discs were placed aseptically onto the surface of the inoculated plates applying appropriate special arrangement

with the help of a sterile forceps. The plates were incubated at 35 ± 2 ˚C in the incubator. After 48 hrs of incubation the inhibition zones were measured and recorded. The plates were examined and the diameter of each zone of complete inhibition was measured in millimeter. At the time of measuring zone diameters, always rounded up to the next millimeter. For each antibiotic indicating on the recording sheet the zone size was reported as sensitive (S), intermediate (I), or resistant (R).

2.3. Management of bacterial leaf blight of litchi

The pot experiment was carried out from July 2012 to June 2013 in the net house, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. Three years old saplings of litchi variety China-3 were used and grown in pots. Six different treatments were employed viz. T_1= Gentamycin applied as foliar spray @ 0.05%, T_2= Erithromycin applied as foliar spray @ 0.05%, T_3= Doxycycline applied as foliar spray @ 0.05%, T_4= Copper sulphate applied as foliar spray @ 0.05%, T_5= BAU-Biofungicide applied as foliar spray @ 2% and T_6= Untreated control. The experiment was laid out in Completely Randomized Design (CRD) with three replications. The data were recorded on height of the saplings (cm), total number of branch/sapling, total number of leaves/sapling, number of diseased leaves/sapling and percent leaf area diseased/sapling. The incidence and severity of bacterial leaf blight of litchi was assayed following the formula of Rai and Mamatha (2005), and, Johnston (2000), respectively.

3. Results and Discussion

3.1. Incidence and severity of bacterial leaf blight of litchi

Seven varieties of litchi were evaluated to determine the incidence and severity of bacterial leaf blight disease in the nurseries of Rajshahi, Dinajpur, Rangpur, Mymensingh and Khagrachari as shown in Table 1. Bacterial leaf blight was found in all locations as well as in all nurseries. The survey results indicated a wide variation of blight disease incidence and severity. The results showed that the highest (9.88%) incidence was recorded in Dinajpur and the lowest (6.25%) incidence was in Mymensingh. At the same time the highest (8.88%) severity was recorded in Dinajpur and the lowest (6.18%) severity was recorded in Mymensingh (Figure 1).

Table 1. Litchi varieties evaluated in nurseries of five major litchi growing areas in Bangladesh during July 2010 to April 2012

Locations	No. of nursery surveyed	No. of variety screened	Name of variety
Rajshahi	10	05	China-3, Bombai, Bedana, BARILitchu-1 and BARILitchu- 3
Dinajpur	10	04	China-3, Bombai, Madragi and Bedana
Rangpur	10	04	China-3, Bombai, Madragi and Bedana
Mymensingh	10	03	China-3, Bombai and Mongalbari
Khagrachari	10	04	China-3, Bombai, Bedana andBARILitchu-1

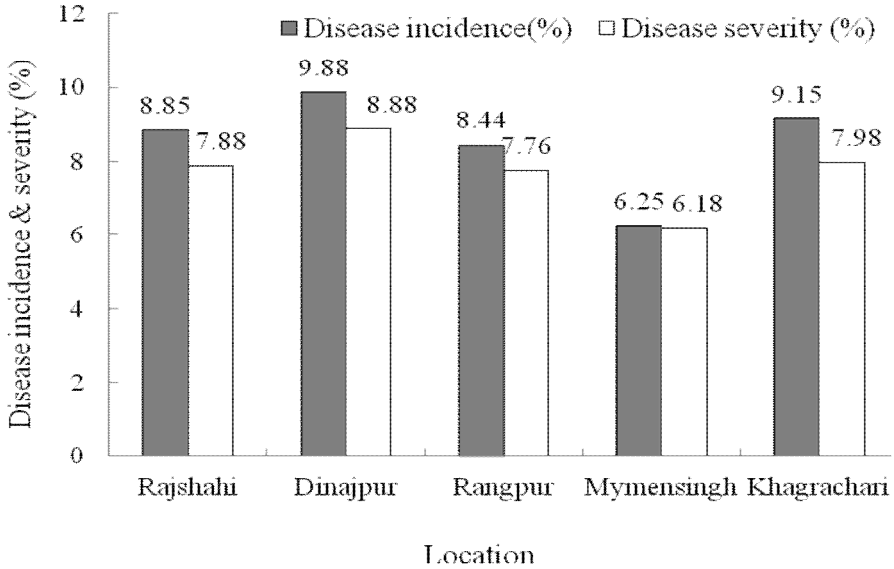

Figure 1. Incidence and severity of leaf blight of Lichi in major areas of Bangladesh

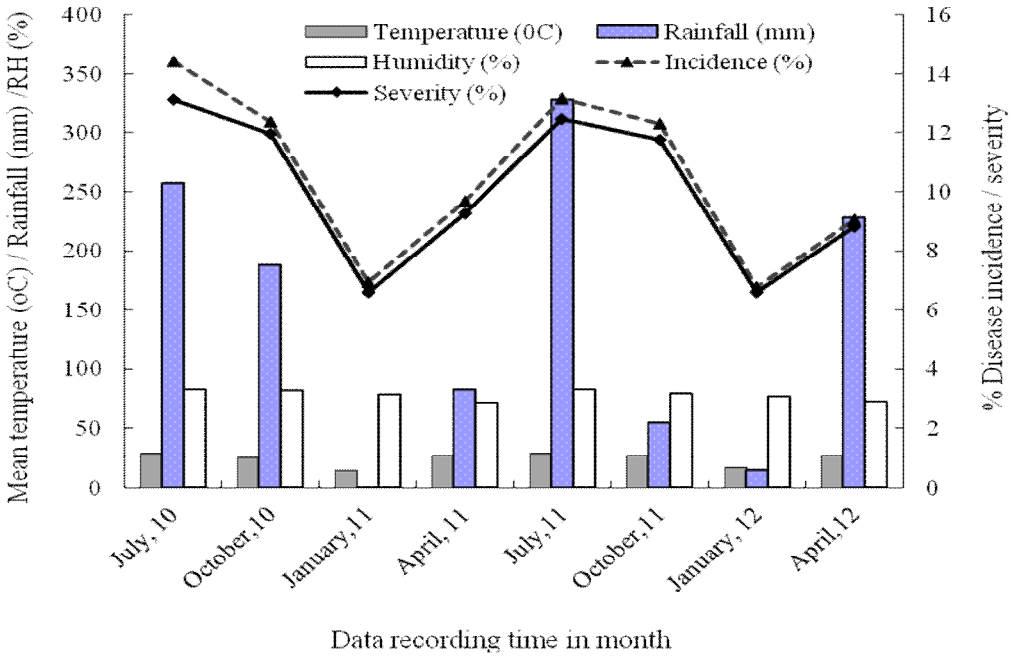

Figure 2. Effect of different weather factors on the incidence and severity of bacterial blight disease of Lichi seedlings

3.2. Effects of weather factors on the incidence and severity of bacterial leaf blight disease of litchi seedlings

The highest incidence and severity of bacterial leaf blight disease of litchi seedlings were recorded (14.43 and 13.11 %) in July 2010 at temperature, rainfall and relative humidity of 29.28^{o}C, 257.67 mm and 82.75 %, respectively and the lowest incidence and severity were recorded during the period of January 2012 at temperature, rainfall and relative humidity of 17.38^{o}C, 14.75 mm and 77.25%, respectively (Fig. 2).

It was revealed that the incidence (r = 0.66 and r = 0.09) and severity (r = 0.73 and r = 0.12) of bacterial leaf blight disease of litchi seedlings were positively correlated with temperature and rainfall in (Table 2), where the relationship with rainfall was very poor. On the other hand, the incidence (r =0.02) of bacterial leaf blight disease of litchi seedlings was correlated positively and severity (r = 0.05) was positively and poorly correlated with relative humidity. The correlation studies revealed that the incidence and severity of bacterial leaf blight disease of litchi seedlings were positively correlated with temperature, rainfall and relative humidity where temperature and rainfall was the major factor to the variations of both incidence and severity of bacterial leaf blight of litchi seedlings.

3.3. Antibiotics sensitivity test of P. syringae pv. *syringae collected from Rajshahi, Dinajpur, Mymensingh and Khagrachari*

Antibiotic sensitivity of fifteen isolates of *P. syringae* pv. s*yringae* were tested against three different antibiotics viz. Gentamycin, Erythromycin and Doxycycline and presented in Fig. 3 and Table 3.

Table 2. Correlation and linear regression analysis of temperature, rainfall and relative humidity on the incidence and severity of bacterial leaf blight disease of litchi seedlings

Bacterial leaf blight disease of litchi seedlings						
Weather factors	Coefficient of correlation (r)		Slope (b)		Coefficient of Determination (R^2)	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Temperature	0.66	0.73	-1.96	-1.68	0.77**	0.79**
Rainfall	0.09	0.12	-0.19	-0.20		
Relative humidity	0.02	0.05	-0.49	0.66		

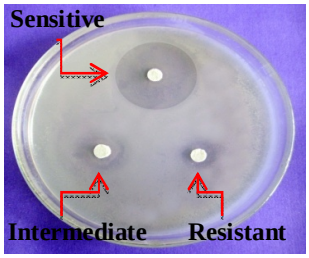

Figure 3. Antibiotic sensitive pattern of *P. syringae* pv. *syringae*

Table 3. Antibiotics sensitivity of *P. syringae* pv. *syringae* collected from blighted leaf of litchi of Rajshahi, Dinajpur, Rangpur, Mymensingh and Khagrachari during 2012

Location	Antibiotics	Reactions of isolates		
		Resistant (R)	Intermediate (I)	Sensitive (S)
Rajshahi	GEN-0.05	-	-	RL_1, RL_2, RL_3
	E-0.05	-	-	RL_1, RL_2, RL_3
	DO-0.05	-	RL_1, RL_2, RL_3	-
Dinajpur	GEN-0.05	-	-	DL_1, DL_2, DL_3
	E-0.05	DL_3	-	DL_1, DL_2
	DO-0.05	DL_2	DL_1, DL_3	-
Rangpur	GEN-0.05	-	-	RPL_1, RPL_2, RPL_3
	E-0.05	RPL_3	-	RPL_1, RL_2
	DO-0.05	RPL_2	RPL_1, RPL_3	-
Mymensingh	GEN-0.05	-	-	ML_1, ML_2, ML_3
	E-0.05	-	ML_3	ML_1, ML_2
	DO-0.05	ML_1	ML_2, ML_3	-
Khagrachari	GEN-0.05	-	KL_1	KL_2, KL_3
	E-0.05	KL_1, KL_2	-	KL_3
	DO-0.05	-	-	KL_1, KL_2, KL_3

Note: GEN=Gentamycin, E=Erythromycin and DO=Doxycycline

Among the three isolates of *P. Syringae* pv. *syringae* of Rajshahi none of the isolates were resistant against Gentamycin (GEN-0.05), Doxycycline (DO-0.05) and Erythromycin (E-0.05). Isolates RL_1, RL_2, RL_3 showed intermediate reaction to Doxycycline (DO-0.05) and were found sensitive to both Gentamycin (GEN-0.05) and Erythromycin. In case of Dinajpur isolates DL_2 and DL_3 showed resistant reaction against Doxycycline (DO-0.05) and Erythromycin (E-0.05), respectively. Isolates DL_1, DL_3 showed intermediate reaction to Doxycycline (DO-0.05). All the three isolates DL_1, DL_2 and DL_3 showed sensitive reaction to Gentamycin (GEN-0.05) and isolates DL_1, and DL_2were sensitive to Erythromycin. In Rangpur, all isolates RPL_1, RPL_2 and RPL_3 showed sensitive reaction to Gentamycin (GEN-0.05) and isolates RPL_1, and RPL_2 were found sensitive to Erythromycin, where RPL_3 was found resistant to Erythromycin. None of the isolates of Mymensingh resistant were found against Gentamycin (GEN-0.05) and Erythromycin (E-0.05). On the other hand $ML_1$was resistant to Doxycycline (DO-0.05). Intermediate reaction was found in isolates $ML_2$and ML_3 to Doxycycline (DO-0.05) and ML_3 to Erythromycin (E-0.05). All the isolates were sensitive to Gentamycin (GEN-0.05). In Khagrachari, none of the isolates showed resistant reaction against Gentamycin (GEN-0.05) and Doxycycline (DO-0.05). On the other hand isolates $KL_1$and KL_2 showed resistant reaction against Erythromycin (E-0.05), intermediate reaction was found in case of isolate KL_1 to Gentamycin (GEN-0.05). Isolates KL_2 and KL_3 were sensitive to Gentamycin (GEN-0.05). In case of Doxycycline (DO-0.05) all the isolates showed sensitive reaction. But only one isolate KL_3 was sensitive to Erythromycin (E-0.05). Orlans *et al.* (2011) reported that out of 189 isolates of *Pseudomonas* spp., 188 isolates were susceptible to Gentamycin. Akinbowale *et al*. (2007) also observed *Pseudomonas.* spp., were sensitive to Gentamycin. The findings also supported by Hossain *et al.* (2012). They reported that isolates of *P. syringae* pv. *syringae* collected from

different locations were sensitive to Gentamycin, Kanamycin, Erythromycin and Chloramphenicol.

3.4. Management of bacterial leaf blight of litchi

The effect of different treatments on incidence of leaf blight of litchi were determined and presented in Table 4. All the treatments significantly reduce incidence of bacterial leaf blight of litchi over control. In 2010-11, leaf blight incidence of litchi was the lowest (50.96 %) in T_5 (BAU-Biofungicide) resulting 33.64% reduction over control which was statistically at par with T_4 (Copper sulphate), T_2 (Erithromycin) and T_1 (Gentamycin) resulting reduction of 32.13%, 28.79% and23.91% over control, respectively. In 2011-12, leaf blight incidence of litchi was the lowest (26.01%) in T_5 (BAU-Biofungicide) resulting 63.76% reduction over control followed by T_4 (Copper sulphate) resulting reduction of 61.12. The lowest percent reduction (13.53 and 41.05%) of bacterial leaf blight incidence over control was observed in T_3 (Doxycyline) in both years. The findings of the present study supported the findings of Basak *et al*. (2014), Akter (2011) and Chowdhury (2009).

Significant variation in the percent disease severity was observed in different treatments (Table 4). All the treatments significantly reduced severity of bacterial leaf blight of litchi over control. In 2010-11, the lowest severity (22.81%) was recorded in T_5 (BAU-Biofungicide) which reduced 60.77% severity over control followed by T_4 (Copper sulphate) and T_2 (Erythromycin). Similar trend was observed in reduction of severity over control in 2011-12. The lowest reduction (40.47 and 43.72 %, respectively) of severity over control was observed in T_3 (Doxycycline. The findings of the present study have also been supported by Basak *et al*. (2014), Akter (2011) and Chowdhury (2009).

Table 4. Effect of antibiotics, Copper sulphate and BAU-Biofungicide on the incidence and severity of bacterial leaf blight disease of litchi seedlings during July 2010 to June 2012

Treatments	Incidence (%)		% reduction over control		Severity (%)		% reduction over control	
	2010-2011	2011-2012	2010-2011	2011-2012	2010-2011	2011-2012	2010-2011	2011-2012
T_1	58.43bc	33.59cd	23.91	53.21	32.57c	32.54bc	43.98	46.84
T_2	54.68c	30.46de	28.79	57.57	30.45cd	29.38cd	47.62	49.18
T_3	66.40ab	42.33b	13.53	41.04	34.61bc	30.74c	40.47	43.72
T_4	52.12c	27.91ef	32.13	61.12	27.74d	26.42d	52.29	54.31
T_5	50.96c	26.01f	33.64	63.76	22.81e	22.32e	60.77	61.40
T_6	76.79a	71.79a	-	-	58.14a	57.82a	-	-
CV (%)	8.17	7.33	-	-	9.23	11.12	-	-

Data represent the mean values of 3 replications; each replication was derived from 15 plants per treatments; in a column means having similar letter(s) are statistically similar at 5% level of significance by DMRT.

T_1= Gentamycin applied as foliar spray @ 0.05%
T_2= Erithromycin applied as foliar spray @ 0.05%
T_3= Doxycycline applied as foliar spray @ 0.05%
T_4= Copper sulphate applied as foliar spray @ 0.05%
T_5= BAU-Biofungicide applied as foliar spray @ 2%
T_6= Untreated control

4. Conclusions

Bacterial leaf blight (*Pseudomonas syringae* pv. *syringae*) of litchi is a great threat for raising quality and healthy saplings in order to get higher fruit production. Most of the isolates of *P.syringae*pv. *syringae* were sensitive to Gentamycin and Erythromycin. In net house test, BAU-Biofungicide (2%) was found superior in controlling bacterial leaf blight of litchi followed by Copper sulphate (0.05) and Erythromycin (0.05%) when applied as foliar spray. BAU-Biofungicide may be considered as an alternative means of controlling bacterial leaf blight of litchi in the country.

5. Acknowledgement

Authors are grateful to PIU-BARC (NATP phase-1), Bangladesh Agricultural Research Council, BARC, Farm gate, Dhaka-1215for a research grant to Dr. Ismail Hossain.

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