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Bacterial leaf blight of guava saplings at Dhaka, Gazipur, Barisal and Khagrachori districts of Bangladesh

Md. Nazrul Islam[1], Md. Mushfiqur Rahman[2], Khondoker Mohammad Alam[1], Nazmun Naher[3], Sanzida Hoque[4] and Md. Mahfuz Alam[1,*]

[1]*Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[2]*Breeder Seed Production Centre, Bangladesh Agricultural Research Institute, Debigonj-5020, Bangladesh*
[3]*Pulse Research Sub-station, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
[4]*Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh*

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Correspondence:

Md. Mahfuz Alam
E-mail: mahfuzbari@gmail.com
Phone: +880 1704 605226

ABSTRACT

Bacterial leaf blight of guava caused by *Pseudomonas syringae* pv. *syringae* is an emerging disease and great threat for production of healthy guava saplings in different nurseries of Bangladesh. A survey was conducted in nurseries of some selected areas viz. Dhaka, Gazipur, Barisal and Khagrachari districts of Bangladesh to know the status of bacterial leaf blight of guava in terms of its incidence and severity and to observe the effect of temperature, relative humidity and rainfall on the occurrence of leaf blight disease incidence and severity on guava saplings. The highest incidence (55.55% and 67.22%) was recorded in July, in 2010-2011 at Gazipur and July, in 2011-2012 at Barisal, respectively and the lowest incidence (13.15% and 19.25%) was observed in January, at Khagrachari. The similar trend were found in case of disease severity, where highest severity (47.41% and 65.62%) was observed in July, in 2010-2011 at Gazipur and July, in 2011-2012 at Barisal, while the lowest (10.73% and 11.66%) was recorded in January, 2010-2011 at Khagrachari and in January, 2011-2012 at Dhaka, respectively. A significant variation in development of the leaf blight disease was observed in varied weather parameters. Occurrence of leaf blight disease was positively correlated with temperature, rainfall and relative humidity. That is why there were great variation of disease incidence and severity on guava from one location to another and season to season. These weather parameters should be critically addressed for host-pathogen interaction and to find out the most appropriate time for combating the leaf blight disease at minimum cost.

Introduction

Guava (*Psidium guajava* L.), is one of the most common fruits in tropical and sub-tropical regions of the world. It Guava has been found to be beneficial for people suffering from Asthma, high blood pressure, oral ulcers, scurvy, congestion of the lungs, bacterial infection etc. Bangladesh produces less than 30% of the fruits needed to meet the minimum daily requirements for its population. About 80% of families in the country consume less than the minimum recommended daily requirement of fruits. As a consequence widespread nutritional deficiencies in vitamin 'A' and 'C' and other nutrients cause debilitating illness among the people (HKI, 2005). The situation can be improved by growing guava fruit trees and increasing the production of guava which may fulfill the requirements of people in Bangladesh.

Bangladesh produces 190 thousand metric tons of guavas annually (BBS, 2012). Quantity and quality of guava in this country is far below the world standard. There are several factors responsible for low yield and poor quality of guava in Bangladesh, where diseases infection is the most important. It has been estimated that the production could be increased at least by 28% if the crop could be protected from various seedling diseases (Chowdhury, 2009). The climate of Bangladesh harbors plant pathogens and provide luxuriant environment for the growth and reproduction of large number of plant pathogens causing hundreds of different diseases (Fakir, 2001).

Like other fruits healthy saplings are prime need and basic raw material for establishment of orchard for the successful production guava. Seedling diseases are important consideration for guava production. Sapling is frequently affected by physical and physiological disorders as well as diseases caused by fungi, bacteria and viruses (Mittal & Mathur, 1990). Seed borne pathogens affect nursery saplings and reduce seedling vigor. So, sapling diseases of guava are one of the important problems in the tropics. Although a huge number of nurseries are engaged in producing saplings, they fail to produce quality saplings due to lack of their knowledge about diseases. Sapling of guava are affected by diseases such as wilt (*Fusarium oxysporium*), anthracnose (*Colletotrichum gloeosporioides*), seedling blight, fruit rot, *Phoma* rot, *Rhizopus* rot, collar rot, *Pestalotia* leaf spot, *Cercospora* leaf spot, stem canker , sooty mold, die back (Rahman et al., 2003).

The existing technology of guava cultivation in the country is in a stage that needs to be upgraded for successful guava production in order to meet up the national demand. So, studies on the sapling disease of guava are of urgent need in the country. Therefore, the attempts were taken to study the prevalence of leaf blight disease occuring on guava saplings in some selected nurseries of Dhaka, Gazipur, Barisal and Khagrachari and the effect of

temperature, relative humidity and rainfall on the incidence and severity of leaf blight disease of guava were undertaken.

Considering the above facts, the present research work was designed with the following objectives, i) to survey on the prevalence of leaf blight disease of guava sapling in some selected nurseries of Dhaka, Gazipur, Barisal and Khagrachari, ii) to identify the pathogen associated with the disease and iii) to study the effect of temperature, relative humidity and rainfall on the incidence and severity of leaf blight disease of attacking guava.

Materials and Methods

Location of survey area

A survey was carried out to know the status of bacterial leaf blight of guava in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari districts. The *eight* nurseries of four districts were surveyed: Dhaka (Green orchid nursery, Agargaon and Barisal nursery, Savar); Gazipur (Gazipur nursery and Laxmipur nursery); Barisal (Sarchina nursery and Riyad nursery) and Khagrachari (Ramghor Hill Research Center and Ramghor nursery). Prevalence of diseases occurring on guava saplings raised in the selected nurseries was surveyed.

Meterological data collection

Meterologial data of the experimental period were collected from Meterological Department, Agargaon, Dhaka.

Observation of the symptoms

Symptoms of the leaf blight disease were studied by visual observation. Sometimes hand lens was used for critical observation of the disease and sometimes a disease was identified based on matching the observed symptoms in the infected plants with the symptoms published in guava disease compendium.

Collection of diseased specimen

Diseased leaves were collected from the infected plants representing the different areas of survey. The specimens were preserved in the laboratory following standard procedure of preservation of disease specimens until isolation was made.

Bacteria isolation and identification

The bacteria strains isolated and collected from guava leaf, samples were identified using the following tests.

Potassium hydroxide solubility test

On glass slide a loop full of bacteria from a well-grown colony was mixed with a drop of 3% KOH aqueous solution. Mixing was continued for less than 10 seconds. A toothpick was used for picking bacteria from a colony as well as for sticking it. The toothpick was raised a few centimeters from the glass slides. Strands of viscid material showed the bacterium Gram-negative. Repeated strokes of the toothpick, which did not produced any strand, were Gram-positive (Mortensen, 1997).

Potato soft rotting test

The soft rotting test was done using well-washed, firm potatoes. The surface of the potatoes were sterilizes with rectified spirit, then cut into slices (3.5 cm long and 7-8 mm thick) aseptically with sharp sterile knife and places in sterile petri dishes, the bottom of which was lined with moist filter paper (Whatman filter paper no.1). Two slices were then inoculated with loop full of 24 hours bacterial colony previously grown on a NA media. Petri dishes were then incubated at room temperature (in darkness at 25 °C) for 24-48 hours for the detection of soft rot. A control for each isolate was maintained using loop full of sterile water instead of bacteria (Lelliott & Stead, 1987).

Epidemiology of disease incidence and severity Survey period

Altogether eight surveys were made during the period from July, 2010 to April, 2012. Where First, second, third, fourth, fifth, sixth, seventh, and eighth surveys were made in July, 2010; October, 2010; January, 2011; April, 2011; July, 2011; October, 2011; January, 2012; and April, 2012, respectively.

Data collection during survey

During the survey in the nurseries, total numbers of guava saplings as well as number of diseased saplings in the nurseries were recorded. Then 30 saplings were randomly selected for counting diseased leaves and disease free leaves. Moreover, five leaves per plant were randomly selected to determine the disease severity.

Determination of disease incidence and disease severity

For calculation of disease incidence every sapling was counted in the nursery and also counted the infected saplings and then expressed in percentage. The disease infection, incidence and severity of guava sapling were determined by the following formula (Rai & Mamatha, 2005).

$$\text{Percent plant infection} = \frac{\text{Number of diseased plants}}{\text{Number of total plants observed}} \times 100$$

$$\text{Percent disease incidence (leaves)} = \frac{\text{Number of diseased leaves on each plant}}{\text{Number of totatl leaves on each plant}} \times 100$$

$$\text{Percent disease severity (leaves)} = \frac{\text{Area of tissue infected by disease}}{\text{Total leaf area of the plant}} \times 100$$

Satistical analysis

Data on different parameters were analyzed in two factor randomized block design through computer software MSTAT-C (Anonymous, 1989). Duncan's Multiple Range Test (DMRT) and Least Significant difference (LSD) test were performed for mean separation of different parameters and to determine the level of significant differences.

Results

Survey on nursery diseases of guava

Bacterial leaf blight disease was recorded in the survey conducted in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari districts.

Symptom of the bacterial leaf blight disease of guava and identification of the pathogen

Minute water soaked lesions appeared in groups towards the tip of the blade that turned brown to black in color and surrounded by chlorotic halos. They were surrounded by the veins and hence angular in shape. Large necrotic patches were formed by coalescing of several lesions. The patches sometimes dried up, often rough and raised due to heavy bacterial exudates (**Fig. 1 A & B**). Petioles and tender stems were also infected and longitudinal cracks developed on the petiole.

The bacterial strains isolated and collected from guava leaf samples were used for potato soft rotting test or Pectolytic test (**Fig. 1 D**), potassium hydroxide solubility test (Plate 1 E), and Levan test (**Fig. 1 F**) that showing positive reaction with *Pseudomonas syringae* pv. *Syringae* isolate and producing distinctive domed shape colonies in NA medium containing 5% sucrose. In the potassium hydroxide solubility test, (**Fig. 1 E**) a strand of viscid material was found that indicated that the bacterium was Gram-negative. The isolated bacterium from the blighted leaf was *Pseudomonas syringae* pv. *Syringae* (**Fig. 1 G**).

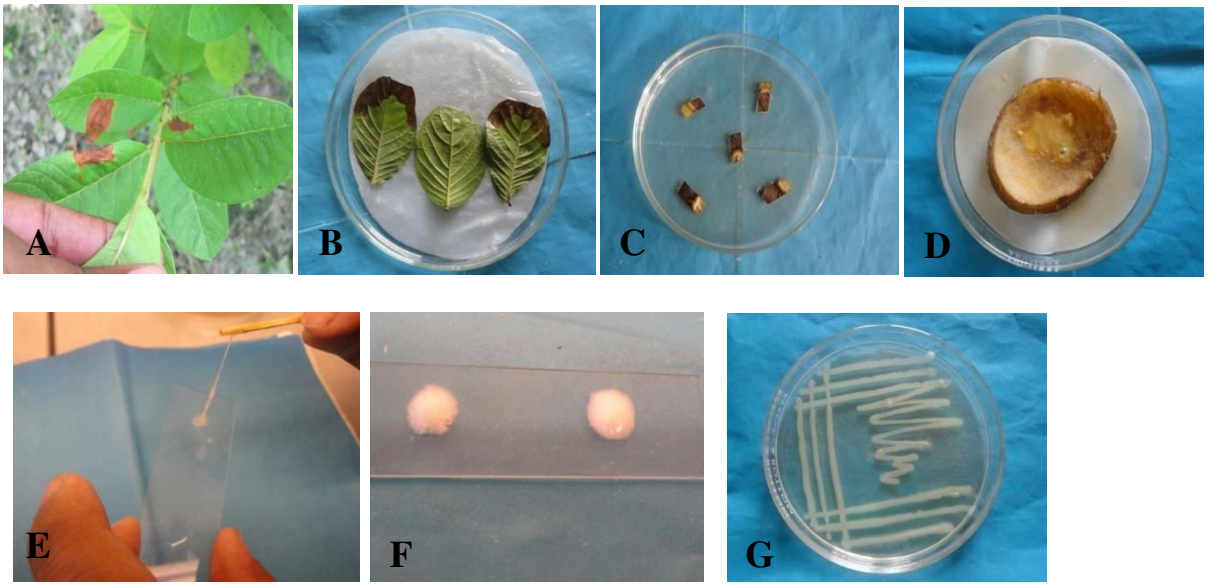

Fig. 1. A & B showing symptoms of leaf blight of guava. **C.** Isolation of bacteria by tissue culture method, **D.** Pectolytic test for *Pseudomonas syringae* pv. *syringae* isolate showing positive reaction producing rot on potato. **E.** Potassium hydroxide solubility test showing positive reaction as indicated by the elastic thread or viscous material with *P. syringae* pv. *syringae* isolate., **F.** Levan test showing positive reaction with *P. syringae* pv. *syringae* isolate and producing distinctive domed shaped colonies in NA medium containing 5% sucrose. **G.** Pure culture of *P. syringae* pv. *syringae* on NA medium.

Epidemiology of disease incidence and severity

Incidence and severity of leaf blight of guava at different locations of Bangladesh from July, 2010 to April, 2012. Incidence of leaf blight of guava varied from location to location and year to year that ranged from 39.93-23.48% in 2010-2011 and 42.54-26.75% in 2011-2012 (**Table 1**). The highest incidence (39.93%) was recorded at Gazipur in 2010-2011 and (42.54%) at Barisal in 2011-2012. The lowest incidence was at Khagrachari in both the years. The severity of leaf blight of guava also varied from location to location and year to year and was ranged from 33.65-19.05% in 2010-2011 and 38.54-25.62% in 2011-2012. The highest severity (33.65%) was recorded at Gazipur in 2010-2011 and (38.54%) at Barisal in 2011-2012. The lowest severity was recorded at Khagrachari in 2010-2011 and at Dhaka in 2011-2012.

Table 1. Incidence and severity of leaf blight of guava at different locations of Bangladesh from July, 2010 to April, 2012.

Locations	Incidence (%)		Severity (%)	
	2010-2011	2011-2012	2010-2011	2011-2012
Dhaka	32.40 b	38.70 ab	22.74 c	25.62 c
Gazipur	39.93 a	34.63 b	33.65 a	31.15 b
Barisal	31.48 b	42.54 a	24.81 b	38.54 a
Khagrachari	23.48 c	26.75 c	19.05 d	28.07 bc
$LSD_{(p\geq0.05)}$	1.626	4.350	0.6921	3.093
CV%	2.49	5.36	2.90	4.68

Each data represents the mean value of three nurseries.

Table 2. Incidence and severity of leaf blight of guava during July, 2010 to April, 2012 in Bangladesh.

Time of data collection	Incidence (%)		Severity (%)	
	2010-2011	2011-2012	2010-2011	2011-2012
July	42.67 a	54.75 a	35.77 a	50.88 a
October	36.14 b	36.42 b	30.81 b	39.06 b
January	20.32 d	21.86 d	15.31 d	14.27 d
April	28.18 c	29.60 c	18.36 c	19.17 c
$LSD_{(p\geq0.05)}$	1.335	3.223	1.227	2.431
CV%	2.49	5.36	2.90	4.68

Each data represents the mean value of three nurseries.

Incidence and severity of leaf blight of guava during July, 2010 to April, 2012 in Bangladesh

Incidence of leaf blight of guava varied significantly from July, 2011 to April, 2012 and that ranged from 42.47-20.32% in 2010-2011 and 54.75-21.86% in 2011-2012 (**Table 2**). Significantly the highest incidence (42.47% and 54.75%) was recorded in July, in both the years. And the lowest (20.32% and 21.86%) was observed in the month of January, in both the years. The severity of leaf blight of guava, varied significantly from July, 2011 to April, 2012 and that ranged from 35.77-15.31% in 2010-2011 and 50.88-14.27% in 2011-2012. Significantly the highest severity (35.77% and 50.88%) was recorded in the month of July, in both the years. And the lowest (15.31% and 14.27%) was observed in the month of January, in both the years.

Incidence and severity of leaf blight of guava during July, 2010 to April, 2012 of different study area of Bangladesh

Incidence of leaf blight of guava varied significantly from season to season as well as location to location and that ranged from 55.55-13.15% in 2010-2011 and 67.22-19.25% in 2011-2012 (**Table 3**). Significantly the highest incidence (55.55% and 67.22%) was recorded in the month of July, in 2010-2011 at Gazipur and July, in 2011-2012 at Barisal, respectively. Again significantly the lowest incidence (13.15% and 19.25%) was observed in the month of January, at Khagrachari, respectively. The severity of leaf blight of guava also varied significantly from season to season as well as location to location and that ranged from 47.41-10.73% in 2010-2011 and 65.62-11.66% in 2011-2012. Significantly the highest severity (47.41% and 65.62%) was observed in the month of July, in 2010-2011 at Gazipurand July, in 2011-2012 at Barisal, while the lowest (10.73% and 11.66%) was recorded in the month of January, 2010-2011 at Khagrachari and January, 2011-2012 at Dhaka, respectively.

Table 3. Incidence and severity of leaf blight of guava during July, 20 to April, 2012 at different study areas of Bangladesh.

Location	Data recording time (month)	Incidence (%)		Severity (%)	
		2010-2011	2011-2012	2010-2011	2011-2012
Dhaka	July	42.56 c	62.82 b	34.37 c	48.74 c
	October	37.67 e	43.30 d	27.92 e	26.70 f
	January	21.68 k	20.65 g	12.66 l	11.66 j
	April	27.71 h	28.03 f	16.02 jk	15.38 i
Gazipur	July	55.55 a	55.48 c	47.41 a	46.49 c
	October	45.14 b	35.21 e	41.86 b	52.63 b
	January	23.23 j	20.75 g	20.72 h	12.06 j
	April	35.81 f	27.08 f	24.61 g	13.41 ij
Barisal	July	41.18 d	67.22 a	35.38 c	65.62 a
	October	35.35 f	41.50 d	31.65 d	43.90 d
	January	23.20 j	26.79 f	17.11 ij	21.09 h
	April	26.17 i	34.64 e	15.08 k	23.56 g
Khagrachari	July	31.37 g	33.47 e	25.92 f	42.67 d
	October	26.39 hi	25.66 f	21.81 h	33.00 e
	January	13.15 l	19.25 g	10.73 m	12.26 j
	April	23.01 jk	28.63 f	17.73 i	24.33 fg
$LSD_{(p\geq0.05)}$		1.335	3.223	1.227	2.431
CV(%)		2.49	5.36	2.90	4.68

Each data represents the mean value of three nurseries.

Effect of weather components on the incidence and severity of leaf blightof guava sapling during July, 2010 to April, 2012

In different growing seasons of guava saplings,the highest incidence (42.67% and 54.75%) and the highest severity (35.77% and 50.88%) of leaf blight disease were recorded in July, in both the years when the average temperature, relative humidity and rainfall were 29.65 °C, 81.40% & 7.55 cm, and 30.50 °C, 83.50%, & 5.50 cm, respectively. On the other hand, lowest incidence (20.32% and 21.86%) and the lowest severity (15.31% and 14.27%) were recorded in January, in both the years when the average temperature, relative humidity and rainfall were16.88 °C, 73.80% & 0.52 cm, and 18.46 °C, 76%, & 0.60 cm, respectively (**Fig. 2 & 3**).

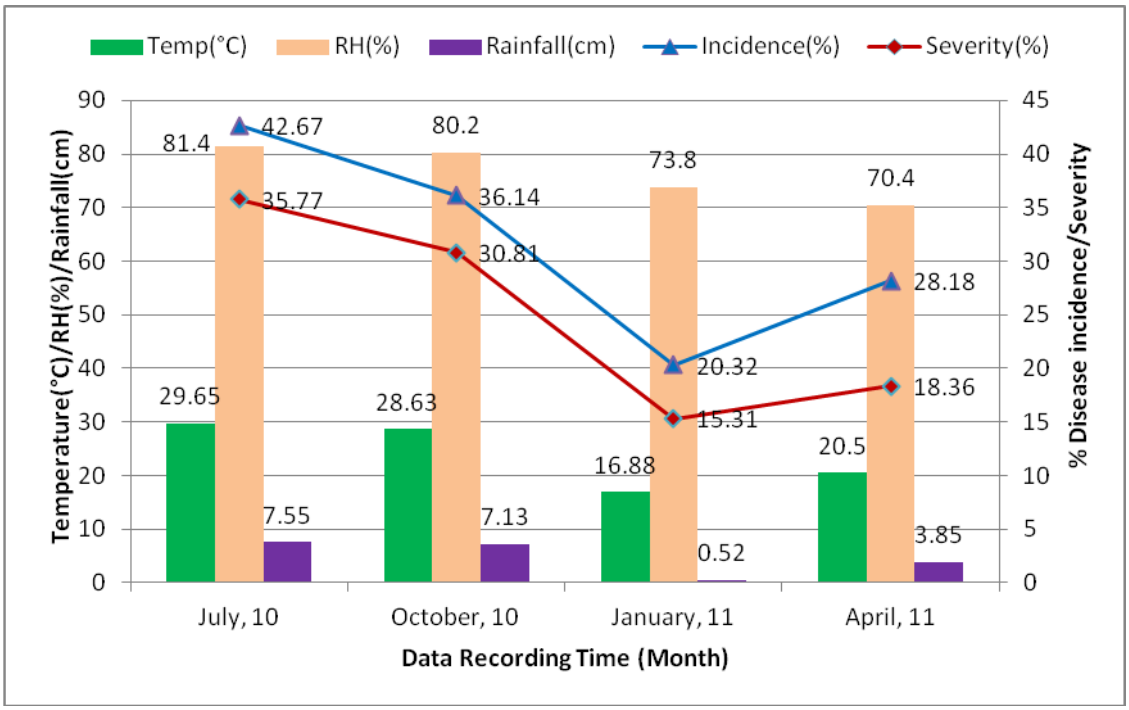

Fig. 2. Effect of different weather parameters on the incidence and severity of leaf blight of guava sapling during July, 2010 to April, 2011.

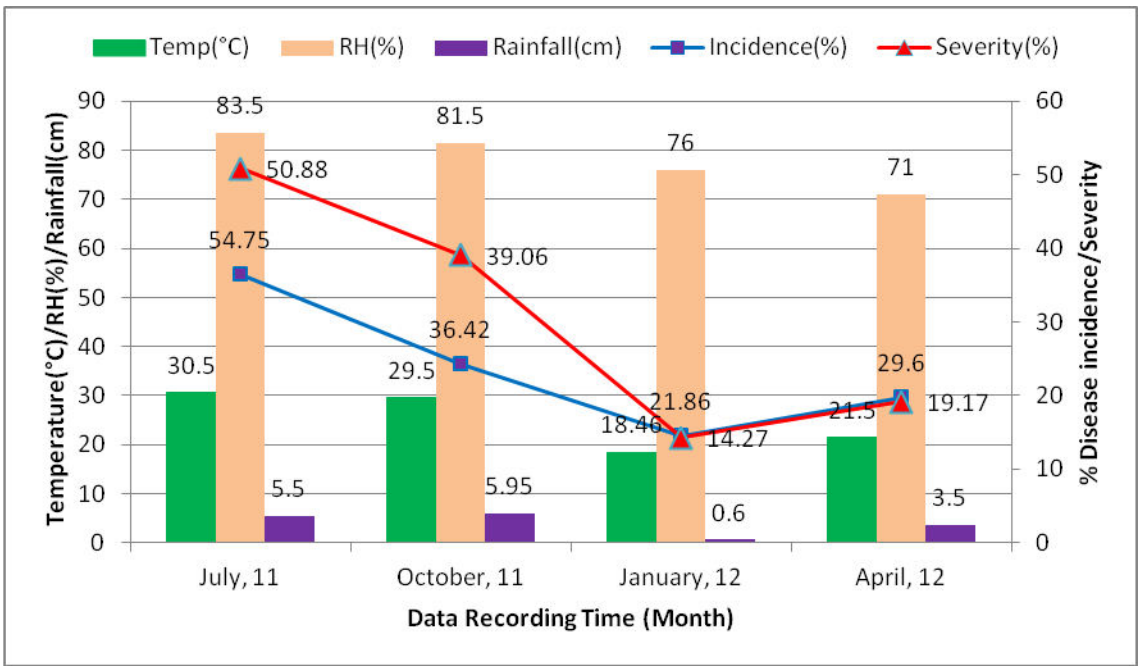

Fig. 3. Effect of different weather factors on the incidence and severity of leaf blight of guava sapling during July, 2011 to April, 2012.

Relationship between leaf blight disease incidence, severity of guava saplings and temperature

A positive correlation between incidence and severityof leaf blight disease with temperature was observed in both the years (**Fig. 4 & 5**). The relationship between disease incidence and temperature could be expressed by the equation Y=1.513x–4.365, (R^2=0.944) and Y=0.2.083x–16.41, (R^2=0.773), where x=temperature and y=disease incidence. Here, the R^2 values indicate that the contribution of temperature to the incidence of leaf blightof guava. On the other hand, the relationship between disease severity and temperature could be expressed by the equation Y=1.548x–11.96, (R^2=0.968), and Y=2.809x– 39.35, (R^2 = 0.946), where x=temperature and y=disease severity. Here, the R^2 values indicate that the contribution of temperature to the severity of leaf blight of guava.

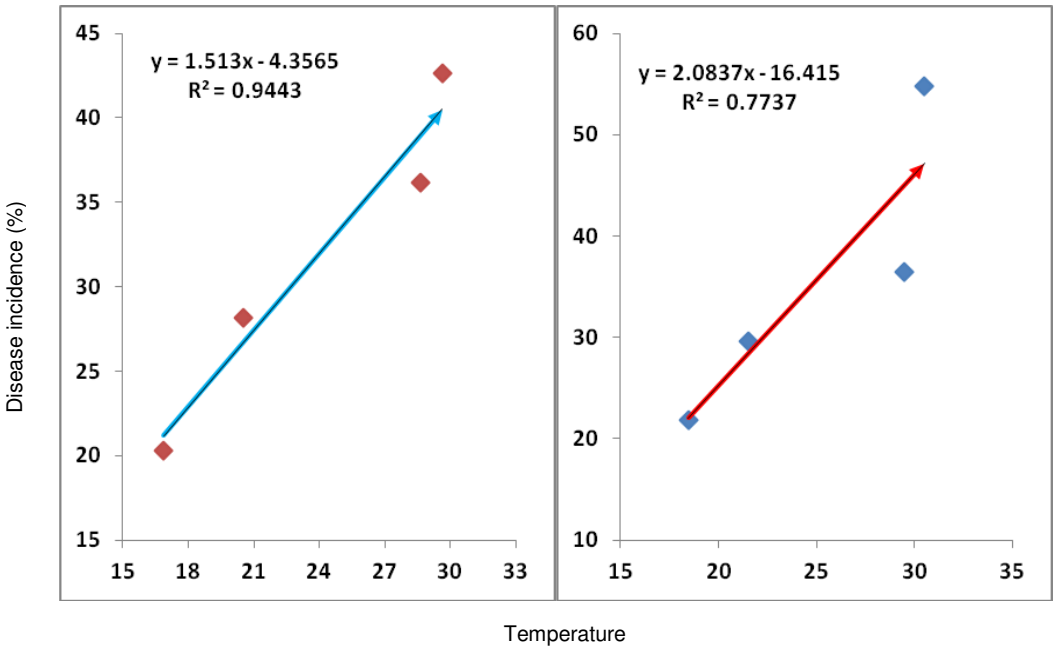

Fig. 4. Linear regression on the effect of temperature on the incidence of leaf blight of guava during July 2010 to April 2012.

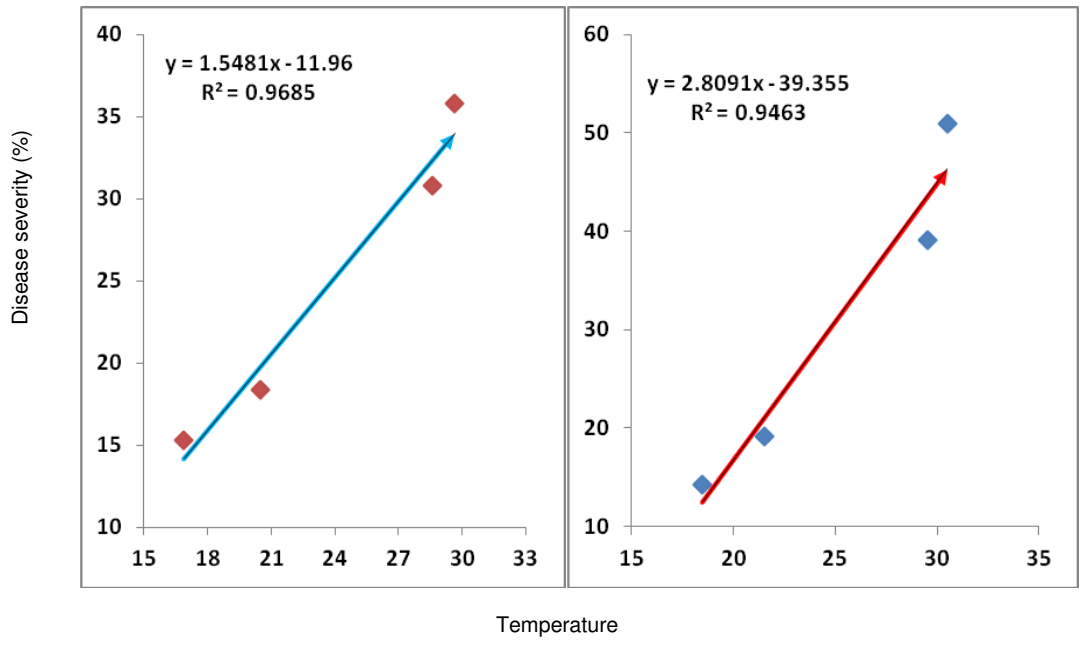

Fig. 5. Linear regression on the effect of temperature on the severity of leaf blight of guava during July 2010 to April 2012.

Relation between leaf blight disease incidence, severity of guava saplings and relative humidity

A positive correlation between incidence and severityof leaf blight disease with relative humidity was found in both the years (**Fig. 6 & 7**). The relationship between disease incidence and relative humidity could be shown by the equation Y=1.489x–82.02, (R^2 = 0.645) and Y=1.860x–109.4, (R^2=0.558), where x= relative humidity and y=disease incidence. Here, the R^2 values indicate that the contribution of relative humidity to the incidence of leaf blightof guava. On the contrary, the relationship between disease severity and relative humidity could be expressed by the equation Y=1.714x–106, (R^2=0.840), and Y=2.657x–176.4, (R^2 = 0.766), where x= relative humidity and y=disease severity. Here, the R^2 values indicate that the contribution of relative humidity to the severity of leaf blight of guava.

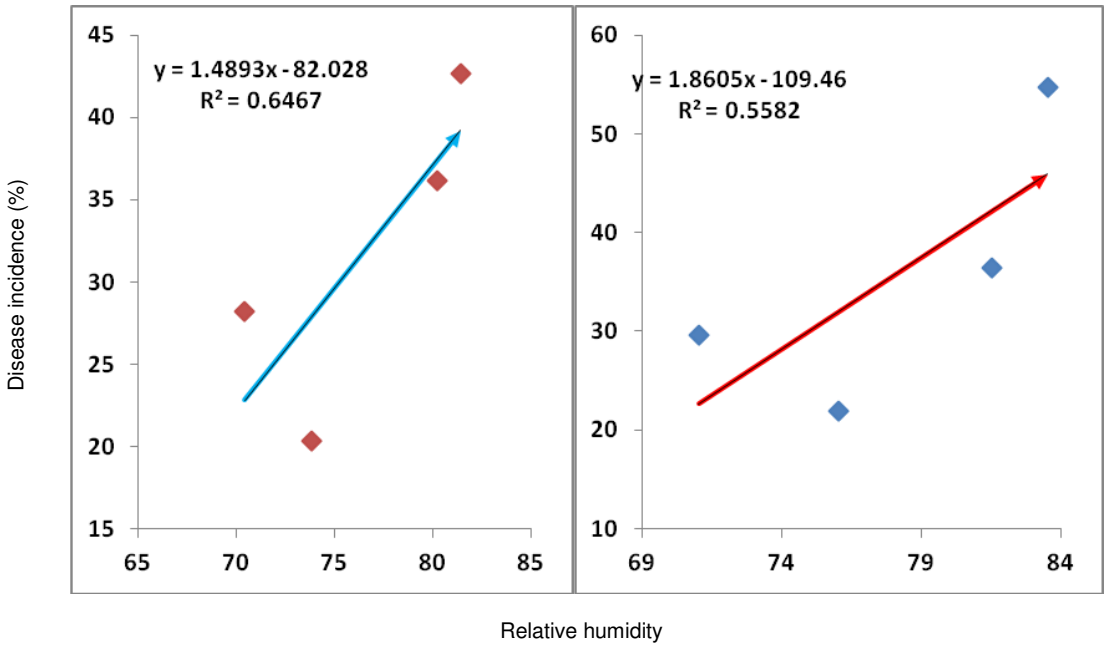

Fig. 6. Linear regression on the effect of relative humidity on the incidence of leaf blight of guava during July 2010 to April 2012.

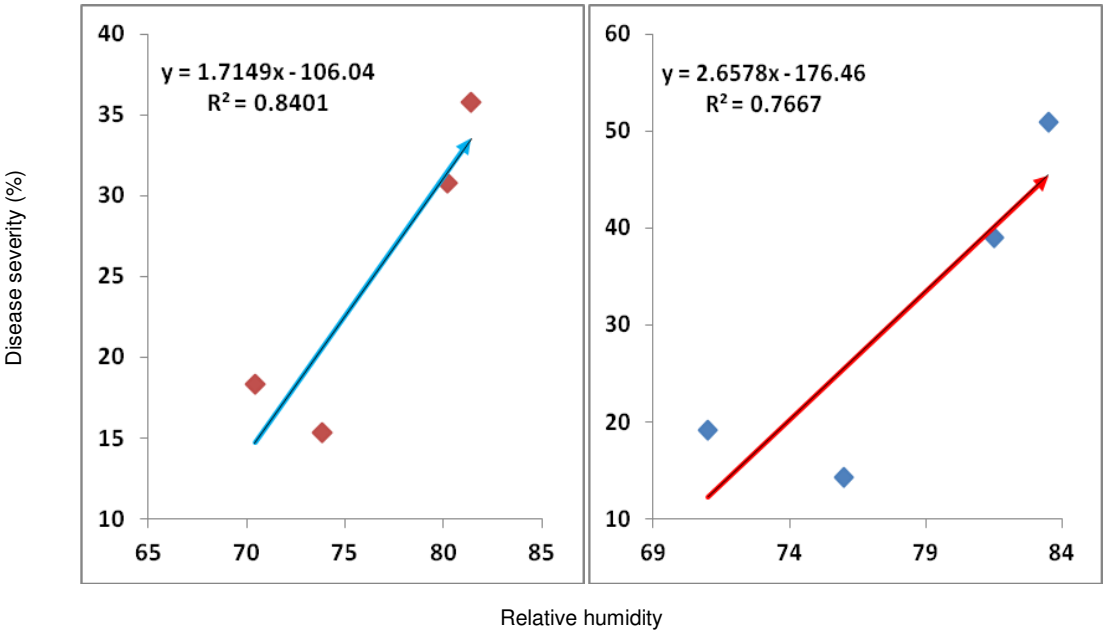

Fig. 7. Linear regression on the effect of relative humidity on the severity of leaf blight of guava during July 2010 to April 2012.

Relationship between leaf blightdisease incidence, severity of guava saplings and rainfall

A positive correlation between incidence and severityof leaf blight disease with rainfall was observed in both the years (**Fig. 8 & 9**). The relationship between disease incidence and rainfall could be expressed by the equation Y=2.874x+18.13, (R^2=0.944) and Y=4.495x+18.18, (R^2=0.607), where x=rainfall and y=disease incidence. Here, the R^2 values indicate that the contribution of rainfall to the incidence of leaf blight of guava. On the other hand, the relationship between disease severity and rainfall could be expressed by the equation Y=2.823x+11.61, (R^2=0.892), and Y=6.078x+7.216, (R^2=0.747), where x=rainfall and y=disease severity. Here, the R^2 values indicate that the contribution of rainfall to the severity of leaf blight of guava.

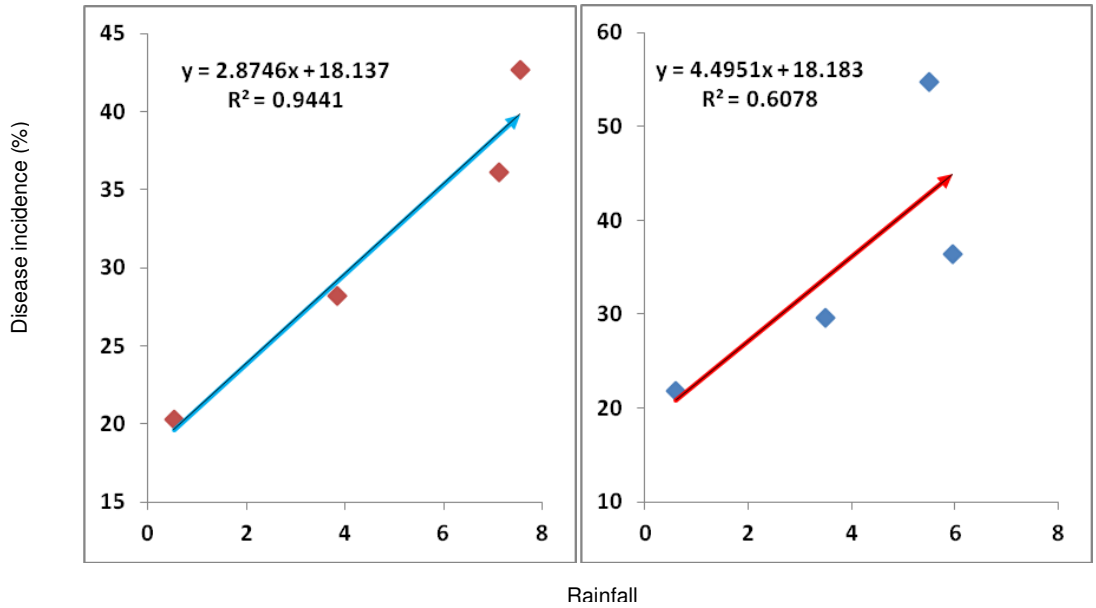

Fig. 8. Linear regression on the effect of rainfall on the severity of leaf blight of guava during July 2010 to April 2012.

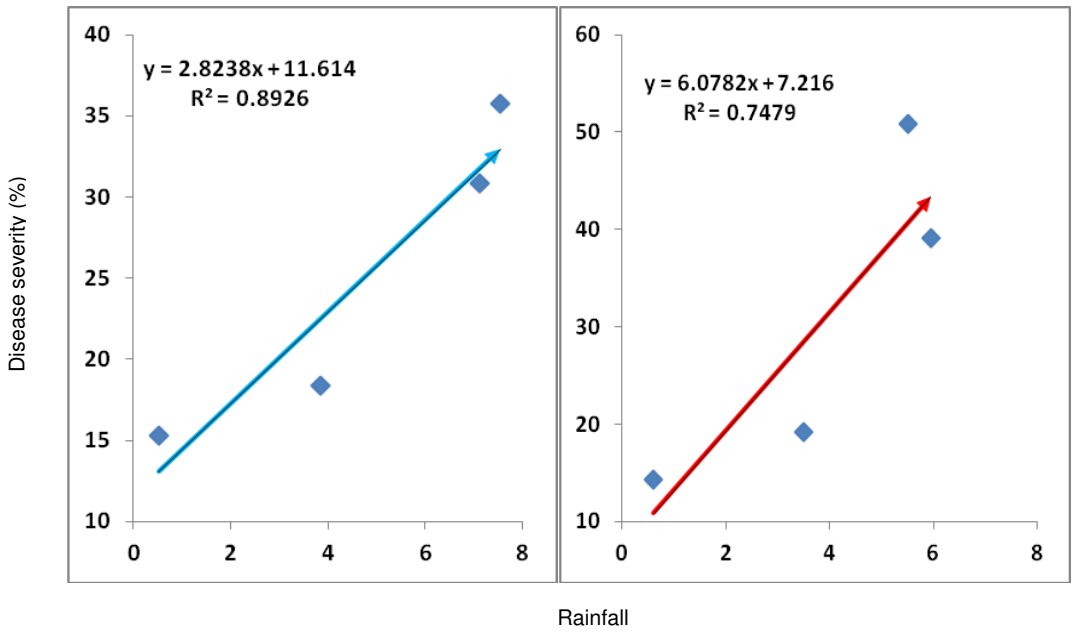

Fig. 9. Linear regression on the effect of rainfall on the severity of leaf blight of guava during July 2010 to April 2012.

Discussion

Bacterial leaf blight was identified as a new disease of guava in many nurseries of Bangladesh. The survey results indicated a regional variation in bacterial leaf blight incidence and severity. These variations of leaf blight incidence and severity may be attributed to the diversity of *Pseudomonas syringae* pv. *syringae* and also due to the host genotypes and variations of environmental factors prevailing in different locations surveyed. The disease recorded in the present study has also been reported on guava seedlings from different countries of the world by (Chowdhury, 2009; Hossain, 2011; Marques et al., 2007). The diseases were identified by observing the symptoms on the saplings during survey. The bacteria *Pseudomonas* was isolated from the blighted leaf of guava. Marques et al. (2007) reported the presence of *Pseudomonas* from leaf of guava. Leaf blight by *Psuedomonas* in litchi fruit plants is reported by (Bultreys & Kaluzna, 2010).

The prevalence of the recorded the leaf blight disease on guava varied in respect of nursery, location and time. Similar variation in prevalence of leaf blight seedling disease in respect of nursery location and time was recorded by Chowdhury, 2009 in different guava growing areas of the country. It was also observed that the incidence and severity of leaf blight of guava varied from location to location. These variations may be due to effect of environment of different agro-ecological zone. The highest incidence & severity of leaf blight of guava was recorded at Gazipur and the lowest prevalence was recorded at Khagrachari in both the years. This variation in the prevalence may be due to environmental effect of these particular agro-ecological zones.

The effects of temperature, rainfall and relative humidity on the incidence and severity of leaf blight disease of guava in selected location were observed. The climate of Bangladesh is characterized by high temperature, heavy rainfall, and often excessive humidity with fairly marked seasonal variations observed (Anonymous, 1995). Determining the effects of temperature, rainfall and relative humidity on the incidence and severity of disease in different pathosystems has been focused by many researchers worldwide (Chowdhury, 2009; Hossain, 2011; Marques et al., 2007).

In the epidemiological study, the disease was recorded eight times during the period of twenty month survey from July, 2010 to April, 2012. The incidence and severity of leaf blight of guava was found to be increased in the month of July and October while the disease decreased in the month of January and April in both the years. A positive correlation was observed between prevalence of leaf blight of guava with temperature, relative humidity and rainfall. With the increase of temperature, relative humidity and rainfall both the incidence and severity increased significantly. The finding of present study collaborates with the finding of Hossain, 2011. Chowdhury, 2009 identified this disease and did not mention any causal organism. Marques et al., 2007 also detected this disease in 56% of the orchards, and a correlation of 81.9% between the presence of symptoms and positive disease diagnosis in the laboratory was recorded.

Conclusion

Therefore, the findings on the incidence and severity of leaf blight disease of guava in different nurseries reveals that the disease studied is related to the temperature, relative humidity and rainfall and have a profound effect on the disease. These weather parameters should be critically observed for each host-pathogen interaction and to find out the most appropriate time to combat the disease at minimum effort. The findings of the study clearly indicated that leaf blight of guava is caused by *P. syringae* pv. *syringae* which was observed in all nurseries of different areas surveyed. The disease seems a potential threat for raising quality and healthy guava saplings in order to get higher fruit production. However, the management strategies still not developed due to lack of the identification of the causal organism. The results of the present study would definitely be useful to design a comprehensive molecular based analysis of the pathogen and to adopt a proper management strategy suitable for the integrated disease management programs.

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