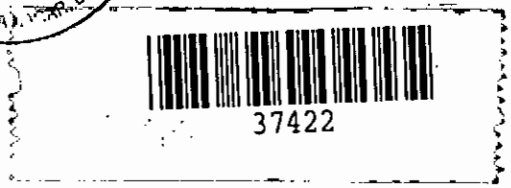
STUDY ON LEAF BLIGHT DISEASE (*PSEUDOMONAS* SP) OF LITCHI (*LITCHI CHINENSIS*) IN SELECTED AREAS OF BANGLADESH AND IT'S MANAGEMENT

TANIA AMIN



DEPARTMENT OF PLANT PATHOLOGY
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DHAKA-1207

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STUDY ON LEAF BLIGHT DISEASE (*PSEUDOMONAS* SP) OF LITCHI (*LITCHI CHINENSIS*) IN SELECTED AREAS OF BANGLADESH AND IT'S MANAGEMENT

BY
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REGISTRATION NO.05-1809

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CERTIFICATE

This is to certify that thesis entitled, **"STUDY ON LEAF BLIGHT DISEASE (*PSEUDOMONAS* SP) OF LITCHI (*LITCHI CHINENSIS*) IN SELECTED AREAS OF BANGLADESH AND IT'S MANAGEMENT"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by Tania Amin, Registration No.: **05-1809** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:20-09-2012
Dhaka, Bangladesh

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The Author

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

%	=	Percentage
et al.	=	and others
spp.	=	Species
J.	=	Journal
No.	=	Number
viz.	=	Namely
df.	=	Degrees of freedom
@	=	At the rate of
&	=	and
etc	=	Etcetera
PDA	=	Potato Dextrose Agar media
^{0}C	=	Degree Celsius
cm	=	Centimeter
BBS	=	Bangladesh Bureau of Statistics
Kg	=	Kilogram
CV%	=	Percentages of Co-efficient of Variance
LSD	=	Least Significant Difference
Sci.	=	Science
ANOVA	=	Analysis of variances
hr	=	Hour
cv.	=	Cultivar
T	=	Treatment
ft	=	feet
pv.	=	pathovar
syn.	=	synonym
var.	=	variety
mm	=	milimeter
µm	=	micrometer
USA	=	United State

STUDY ON LEAF BLIGHT DISEASE (*Pseudomonas* sp) OF LITCHI (*Litchi chinensis*) IN SELECTED AREAS OF BANGLADESH AND IT'S MANAGEMENT

ABSTRACT

The existing health situation of seedlings of litchi and management strategies of nursery diseases of seedlings of litchi species in the country is in a stage to be upgraded for successful fruit production to meet the national demand. Four experiments were carried out during 2010 to 2012 to study the status of leaf blight on seedlings of litchi, effect of weather parameters on the incidence of seedling diseases of litchi in different growing areas of Bangladesh with development of an environment friendly disease management practice. Important plant pathogen *Pseudomonas* sp was detected and identified by morphological study and by different biochemical test. Incidence and severity of leaf blight of litchi at seedling stage were studied and significant variations were observed that depends on weather factors. Occurrence of leaf blight was correlated with temperature, rain fall and relative humidity. Comparative effectiveness of BAU-biofungicide applied in the soil at the time of pot preparation as well as foliar spray and three fungicide viz. Indofil M-45, Bavistin 50 WP and Cupravit 50 WP were evaluated on litchi against leaf blight in the nursery. Among the treatments applied, Cupravit 50 WP showed better result in controlling leaf blight of litchi. BAU-Biofungicide, is also used as an alternate to chemicals can successfully be used.

Chapter I

INTRODUCTION

CHAPTER- I

INTRODUCTION

Litchi (*Litchi chinensis*) is considered as the popular fruits of Bangladesh. It belongs to the family Sapindaceae and sub-family Nepheleae. It is one of the most important sub-tropical evergreen fruit trees which grow well in Bangladesh. In spite of availability of different types of fruit in the market, demand for fresh litchi is always very high due to its unique taste, flavor, and color. The supply of litchi is insufficient and its availability is only for about 60 days per year. Dinajpur topped the list of highest litchi producers in the country although the fruit is now grown in almost all the northern districts including Thakurgaon, Panchagarh, Rajshahi and Chapainawabganj. There are about 3000 commercial litchi orchards in 13 upzilas in Dinajpur occupying 2,000 ha of land (Prothom-alo, 2011). About 3973 acre areas were cultivated during the period of 2008-2009 and total production was 55,288 m tons. (BBS, 2011).

Every year the amount of litchi cultivated land is increasing, as the fruit has the quality of providing returns in time. The expansion of the litchi area in these districts is relatively slow due to high mortality rate of young litchi plants. Litchi is playing a vital role in crop diversification and nutrition, economy and environment. Considerable successes have been achieved in the recent years in variety development and technology generation of litchi albeit their application is inadequate. Improved varieties of litchi are available in Bangladesh that can contribute in the poverty ridden economy and nutrition sector significantly. Litchi contributes a great share of the total production of fruits in the country. 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' iron and other nutrients cause debilitating illness among the population (HKI, 2005). We can improve this situation by increasing the production of litchi.

CHAPTER-1

INTRODUCTION

Litchi (*Litchi chinensis*) is considered as the popular fruits of Bangladesh. It belongs to the family Sapindaceae and sub-family Nephelieae. It is one of the most important sub-tropical evergreen fruit trees which grow well in Bangladesh. In spite of availability of different types of fruit in the market demand for fresh litchi is always very high due to its unique taste flavor, and color. The supply of litchi is insufficient and its availability is only for about 60 days per year. Dinajpur topped the list of highest litchi producers in the country although the fruit is now grown in almost all the northern districts including Thakurgaon, Panchagarh, Rajshahi and Chapainawabganj. There are about 3000 commercial litchi orchards in 15 upazilas in Dinajpur occupying 2,000 ha of land (Prothom-alo, 2011). About 70% trees were cultivated during the period of 2008-2009 and total production was 53,268 m tons (BBS, 2011).

Every year the amount of litchi cultivated land is increasing, as the fruit has the quality of providing returns in time. The expansion of the litchi area in these districts is relatively slow due to high mortality rate of young litchi plants. Litchi is playing a vital role in crop diversification and nutrition economy and environment. Considerable success have been achieved in the recent years in variety development and technology generation of litchi albeit more application is inadequate. Improved varieties of litchi are available in Bangladesh that can contribute in the poverty, rural economy and nutrition sector significantly. Litchi contributes a great share of the total production of fruits in the country. 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" iron and other nutrients cause debilitating illness among the population (UNI, 2005). We can improve this situation by increasing the production of litchi.

Success of an orchard or homestead gardening depends on the quality of the planting material. Diseases of litchi have been reviewed by few workers throughout the world (Chowdhury, 2009; Awasthi *et al.* 2005; Cooke and Coates, 2002 and Rawal, 1990). Seedling diseases of fruit trees play a major role in reducing yields of horticultural crops in the tropic (Ming Shen *et al.* 2001). It has been estimated that the production could be increased at least by 28% if the crop could be protected against 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 which causes hundreds of different diseases of crops (Fakir, 2001). The growers have desire to obtain plants root stock with the best sanitary and phonological qualities from nurseries. They should be guaranteed good quality healthy planting materials before planting. The first and most important step is to supply farmers with seedlings that are free from diseases. Seedling diseases are an important consideration for the growers and they have desire to obtain plants root stock with the best sanitary and phonological qualities from nurseries.

Seedling diseases are an important consideration for litchi. Although a huge number of nurseries are engaged in producing seedlings, they fail to produce quality seedlings due to lack of their knowledge about diseases. Diseases may cause mortality of many seedlings after plantation. For this reasons, seedlings are to be reared up with proper care in order to avoid the diseases and to ensure quality litchi production and increasing yield. Thus production of healthy seedlings ensures good plantation and save money, labor and energy of litchi gardener.

Anthracnose (Chowdhury, 2009), leaf spot, powdery mildew, (Rawal, 1990; Cooke and Coates, 2002) has been reported as litchi seedling. Red rust has also been found to attack on litchi and in this case alga attacks the foliage, bark and twig of litchi (Suit and DuCharme, 1946). Leaf blight of litchi is also an important disease of young litchi seedling (Chowdhury, 2009). The

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phytopathogenic *Pseudomonas* cause numerous plant diseases with diverse symptoms including canker, dieback, twig, leaf or kernel blights, leaf spots, soft or brown rots. Many *Pseudomonades* are associated with plants as foliar epiphytes or rhizosphere inhabitants.

Determining the effects of temperature, rainfall, leaf wetness and relative humidity on the inoculums formation, release and germination of inoculums in different pathosystems has been focused by many researchers worldwide (Sutton, 1980; Pinkerton *et al.* 1998; Mondal & Timmer, 2002). Understanding the disease epidemiology, effective control measures could be developed and implemented (Hopkins *et al.* 2000).

Determining the effect of temperature, rainfall and relative humidity on the formation of *Pseudomonas* have been studied by many researchers (Bultreys and Kaluzna, 2010; Agrios, 2005; Hirano and Upper, 2000; Chowdhury, 2009 and Cross, 1966). The level of infection occurring at different times of the year has been studied. Most of the growers as well as extension agents do not have the required knowledge and skill in litchi cultivation. As a result development of effective management strategies of litchi seeding diseases in the nurseries could minimize the use of expensive and hazardous chemicals that endanger people and the environment. Management of diseases in the fruit orchards as well as in nurseries has been investigated by many researchers.

The existing technology of cultivation of litchi in the country is in a stage that needs to be upgraded for successful litchi production in order to meet up the national demand. So, studies on the seed and seedling disease of litchi are an urgent need in the country. Therefore, attempt should be put forward to study the prevalence of various disease occuring on litchi seedlings in some selected nurseries of Dhaka, Gazipur, Barisal and Khagrachari. Considering the above facts, the present research program has been designed with the following objectives :

i. Survey on the prevalence of seedling disease of litchi in some selected nurseries.

ii. To identify the pathogen (s) associated with the disease (s).

iii. To study the epidemiology in relation to the effect of temperature relative humidity and rainfall on incidence and severity of nursery diseases of litchi.

iv. To study on environment friendly management of leaf blight of litchi.

Chapter II

REVIEW OF LITERATURE

CHAPTER-I I

REVIEW OF LITERATURE

Symptoms

Bultreys and Kaluzna (2010) observed that a long infection period of leaf scars and wounds by bacteria washed from leaves by rain begins early in autumn. Bacterial multiplication in cortical tissues occurs from late autumn to early spring, but no significant canker development occurs before spring. However, in late spring and early summer, the progress of overwintered cankers resulting from infections of the previous autumn is halted and the bacteria die out in infected tissues. Bacteria overwintering in and on buds, in the tissues around the leaf scars and at the margin of necroses and cankers multiply in spring in favorable fresh and humid conditions and spread on developing blossoms and leaves. Emerging leaves can be attacked in favorable wet conditions and leaf spots are then observed in spring and/or summer. These findings enabled the determination of optimal periods in autumn.

Zidari and Dreo (2008) pointed at a bacterial origin of the disease symptoms. Pathogenic bacteria belong to the genus *Pseudomonas* sp. Some peach, apricot and cherry samplings were carried out in the field. Among bacteria of the genus *Pseudomonas* sp, laboratory analyses confirmed only the presence of the bacterium *Pseudomonas syringae* on cherries and apricots. *Pseudomonas syringae pv. morsprunorum* and *Pseudomonas syringae pv. syringae* which was determined using IF and BIOLOG system.

Bultreys (2008) studied on *Pseudomonas syringae* and reported that symptoms on the leaves appear as small round lesions of various sizes, light brown initially, then changing to dark brown; they can be surrounded by a yellowish halo. The necrotized tissue often drops out of the leave (so-called shot-hole symptoms) with time. The pathogens can also attack blossoms, which become brown, shriveled and often fall down before full opening. The sunken

brown-black, irregular or regular necroses on immature fruits of susceptible cultivars of sweet and sour cherry are well known.

Kennelly *et al.* (2007) studied on *Pseudomonas syringae* and observed that *Pseudomonas* causes significant damage to nurseries and to plant's wood production reduces fruit yield and quality and limits tree and orchard life duration.

Canfield *et al.* (1986) isolated *Pseudomonas syringae* from all plants having the tip dieback symptom. Plants most commonly and most severely affected were maple, dogwood, filbert, blueberry, magnolia, lilac, oriental pear, aspen, and linden.

Pathogen

Firdous *et al.* (2010) characterized and identified virulence factors produced by virulent isolates of *Pseudomonas syringae pv. sesami* (Psse-08/NARC 1) and *Xanthomonas campestris pv. sesami* (Xcs-08/NARC) . HPLC analysis of cell free culture filtrates allowed characterizing toxins activity of a single active peak obtained from virulent isolate of Psse-08/NARC 1 that produced necrotic symptoms.

Kaluzna *et al.* (2010) studied on *P. syringae* disease of fruit trees and reported that *Pseudomonas* is responsible for diseases in cultivated cherry, plum, peach and apricots well as in wild cherry, and has been and still is of concern and often of economic importance in these crops worldwide.

Balestra *et al.* (2009) isolated isolates of *Pseudomonas syringae* that were Gram negative, aerobic, positive for catalase, production of levan, fluorescent pigment, tobacco hypersensitivity, gelatine liquefaction and acid fromsucrose. They were negative for oxidase, potato rot, arginine dehydrolase, nitrate reduction and utilization of 2-Keto- gluconate, and showed remarkable ice nucleation activity (INA) at -3°C.

Horky (2008) availed to detect, isolate and identify most of bacteria including genus *Pseudomonas*, from stone fruits laboratory is equipped for basic bacteriological methods, for IF microscopy, biochemical and GC identification. The biochemical laboratory uses PCR method in phytobacteriology range too. On the diagnostic working place is tested pathogenicity of isolates in quarantine greenhouse or in growing chambers. We are involved in the research to use the electro migration techniques such as capillary is electric focusing (CIEF) or capillary zone electrophoresis (CZE) with UV/Vis or fluorometric detection for the on-line rapid separation of microbes like *Pseudomonads*.

Bultreys (2008) described three different pathovars of *Pseudomonas syringae*, their occurance in Belgium in cherry and plum: *Pseudomonas syringae pv. syringae*, *Pseudomonas syringae pv. morsprunorum* race 1, *Pseudomonas syringae pv. morsprunorum* race 2, and *Pseudomonas syringae pv. avii.*

Zidari and Dreo (2008) conducted an experiment on bacterial diseases caused by *Pseudomonas sp* of stone fruits and nuts in Slovenia. They carried out some plants like peach, apricot and cherry sampling in the field and isolated pathogens. Laboratory analyses confirmed only the presence of the bacterium *Pseudomonas syringae* on cherries and apricots. *Pseudomonas syringae pv. morsprunorum* and *Pseudomonas syringae pv. syringae* which was determined using IF and BIOLOG system.

Sulikowska (2008) isolated almost 130 isolates of fluorescent *Pseudomonas* from symptomatic tissues of various organs of sweet and sour cherries, plums and peaches from different geographical regions in Poland. Using LOPAT tests 110 of them were identified as *P. syringae*, 5 did not induced a hypersensitive reaction (HR) on tobacco leaves and 11 isolates which were HR positive appeared to be not *P. syringae*. Their identification is now being conducted. *P. syringae* isolates were characterized by GATT'a tests. It was found that 31 isolates belonged to race 1 of *P. syringae pv. morsprunorum (Psm)* but 37 to race 2 of this pathovar. Remaining 42 isolates were classified as pathovar

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syringae (Pss). All isolates are maintained at -75°C in mixture of PBS (Phosphate Buffered Saline) and glycerol (200 µl/ml).

Palleroni (2005) reported that *P. syringae* is the most important plant pathogenic fluorescent *Pseudomonas*. It is an aerobic and motile, straight or slightly curved rod with one or several polar flagella.

Hirano and Upper (2000) commented that *Pseudomonas* was availed to grow as an epiphyte to high populations on sweet cherry leaves without causing symptoms, and that the high autumn epiphytic populations from leaves, and not necessarily bacteria originating from necrotic tissues, were the infecting agents of fresh leaf scars.

Carol *et al.* (1999) studied on mode of action of *Pseudomonas sp* and reported that coronatine, syringomycin, syringopeptine, tabtoxin are the most intensively studied phytotoxins *of Pseudomonas syringae* and each contributes significantly to bacterial virulence in plants. These make biological stress in plant.

Garden *et al.* (1999) determined DNA reletedness among pathovers of *Pseudomonas syringae.* They reported that *Pseudomonas syringae* is a phytopathogenic bacterial species currently divided into more than fifty pathovarsand nine genomospecies.

Rahme *et al.* (1997) used model plant hosts to identify *Pseudomonas aeruginosa*s a virulent factor Nine of nine TnphoA mutant derivatives of *P. aeruginosa* strain UCBPPPA14 that were identified in a plant leaf assay for less pathogenic mutants also exhibited significantly reduced pathogenicity in a burned mouse pathogenicity model, suggesting that *P. aeruginosa* utilizes common strategies to infect both hosts.

Younng *et al.* (1996) reported that *Pseudomonas* is currently subdivided into more than 50 pathovars that are pathogenic to more than 180 plant species,

including fruit plants, vegetables, ornamentals, and other annual and perennial species.

Khanna *et al.* (1990) conducted an experiment on isolation, characterization and screening of antagonist of *Pseudomonas.* They reported that fluorescent *Pseudomonas* sometimes represented more than 50 per cent of the total bacterial population belonging to *P. fluorescence* and *P. putida.*

Management

Gilbert *et al.* (2010) reported that the lack of chemicals for restraining bacterial diseases in the orchards creates a need for alternative methods, including approaches based on prevention, biological control and plant resistance. These approaches require accurate knowledge on the identity, ecology and pathogenicity of the different *P. syringae* strains encountered in the field. More information is needed to improve the knowledge on the ecology and epidemiology of the pathogens, to identify the most damaging organisms, to better differentiate, classify and detect the pathogens, and to allow for selection of correct reference strains when breeding stone fruit cultivars for resistance.

Ahmed *et al.* (2009) carried out a field survey in 2008 in all olive-growing areas of Egypt, bacterial knot symptoms were observed on twigs and branches of domestic olive cultivars in El-Fayoum governorate. From colonies resembling those of *Pseudomonas savastanoi pv.* savastanoi, isolated from oliveknots on nutrient agar, four representative isolates were selected, purified on 5% sucrose nutrient agar medium and compared with *P. savastanoi pv.* savastanoi reference strain LMG 2209T. All isolates were gram negative, fluorescent onKing's medium B and had only oxidative metabolism of glucose.They were negative for levan, oxidase, potato rot argenine dihydrolase and positive for tobacco hypersensitivity.

Kaiser and Shaha (2005) studied on Chemical control of the pathogen was achieved particularly against postharvest fruit rots of mango and litchi, citrus canker and dieback and seedling blight of cashew. Varietal resistance was

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Kaiser and Slaha (2005) studied on Chemical control of the pathogen was achieved particularly against psoriasis fruit rots of mango and litchi, citrus canker and dieback and wedding blight of cashew. Varietal resistance was

recorded against postharvest fruit rots of mango and litchi, seedling blight of guava and dieback of citrus.

Moore (1988) reported that fixed copper compounds (such as Bordeaux and copper hydroxide), streptomycin (an antibiotic) and coordination productions (such as Bravo CM) were used to control *Pseudomonas syringae* with various degrees of success. Adding spreader stickers to these bactericides had gotten longer lasting control under the cool, wet conditions of the Pacific Northwest.

Conover and Gerhold (1981) conducted an experiment to control bacterial spot of tomato by using mixture of copper and the fungicides maneb or mancozeb. This mixture produced a copper carbamate which is more effective than copper alone. Bravo CM, a mixture of chlorothalonil, copper oxychloride and maneb, had been registered to control *Pseudomonas syringae* on tomato, peach, and nectarine. Many fungal diseases were also controlled, adding to the benefit of this compound.

Epidemiology

Agrios (2005) observed that cold and wet weather in spring and frost events are favorable for infection of emerging buds and blossoms caused by *Pss* and subsequent twig and branch infections. The necroses on leaves and immature fruits caused by *Pss* and *Psm* evolve during the vegetation period: on the leaves, necrotic areas enlarge, dry with time, fall down and induce holes; mature fruits are no good for trade and consumption.

Chandler and Daniell (1976) studied on relation of pruning time and inoculation with *Pseudomonas syringae* and observed that pruning in fall and early winter also predisposed the trees to more severe damage from *Pseudomonas syringae* infections and the short-life syndrome.

Weaver and Wehunt (1975) reported effect of Soil Conditions Liming planting-site soil reportedly promoted peach tree growth and vigor to *Pseudomonas*

syringae. Altering soil pH affected the susceptibility of peach to *Pseudomonas syringae*.

Cross (1966) studied epidemiological relation of *Pseudomonads* of deciduous fruit and reported that the *Psm* race 1 cycle on sweet cherry is now well known facultative summer leaf spot stage and an obliged spring, summer and autumn leaf epiphytic stage, alternate with a winter canker stage on the stems and branches.

Related symptom (bacterial canker)

Vasinauskiene *et al.* (2008) collected leaf samples, injured branches and shoots with susceptible bacterial canker symptoms in four growth localities of stone fruits from seven observed. After maceration injured tissue fragments were streaked for isolation on plates with King's medium B, sucrose nutrient and nutrient dextrose agar media. *Pseudomonas syringae* strains were preliminary identified using conventional methods: Gram staining, metabolism of glucose, catalase reaction, LOPAT tests, and serologically. 66 isolates from injured stone fruit trees were selected for identification. Most tested isolates were Gram-negative, catalase-positive. Only 17 of them showed production of fluorescent pigment on King's medium B and utilized glucose oxidatively, produced levan, had negative oxidase and arginine dihydrolase activity were potato rot negative and induced positive reaction of tobacco hypersensitivity.

Pulaeska *et al.* (2008) proved that presence of bacterium *Pseudomonas morsprunorum* as a causal agent of sweet cherry bacterial canker. She also evaluated the susceptibility of breeding clones of Prunus avium to bacterial canker, proved some aspects of disease epidemiology and worked out its control program based on copper compounds.

Ozaktan *et al.* (2008) identified two related bacteria, *Pseudomonas syringae pv. syringae* and *P. s. pv. morsprunorum* that cause bacterial canker. Both pathogens affect sweet cherry, sour cherry, plums and peach in the Aegean

Region. These bacteria prefer cold, wet conditions to spread. Trees under stress (especially frost damage) are more susceptible to infection.

Hatttingh *et al.* (1989) isolated systemic Invasion *Pseudomonas syringae* from interior tissues of fruiting cherry trees; most of these trees typically had three to six cankers, but two showed no visible symptoms. Bacteria were detected as far as 20 ft from any obviously diseased tissue. The highest bacterial counts were from the trunk, roots, and lower scaffold limbs. However, of nearly 10,000 bacteria examined, fewer than 10% were identified as *Pseudomonas syringae*. In South Africa, bacteria introduced into leaves and leaf petioles during the growing season invaded leaves and shoot of plum and cherry trees and caused disease.

Chapter III

MATERIALS AND METHODS

CHAPTER- I I I

MATERIALS AND METHODS

Three experiments were carried out throughout the study period in order to study the seedling diseases of litchi .The experiment were as follows:

i. Laboratory experiment.
ii. Survey on the seedling diseases of litchi in some selected nurseries of Bangladesh.
iii. Epidemiology of disease incidence and severity.

3.1. Experiment I: Laboratory experiment

3.1.1. Collection of diseased specimen

Diseased leaves were collected from the infected plants representing the different areas of survey. The specimens were kept in the refrigerator at 4° C by following standard procedure of preservation of disease specimens until isolation was made.

3.1.2. Isolation of causal organisms was performed by following steps

3.1.2.1. Preparation of Nutrient Agar (NA)

For the preparation of 1 liter NA medium at first nutrient agar (15g) was taken in the Erlenmeyer flask containing 1000 ml distilled water. Peptone (5g) and beef extract (3g) were added to flask. For mixing properly the nutrient agar was shacked thoroughly for few minutes. Then P^H was adjusted at 7.0 by adding KOH. Flask was then plugged with cotton and wrapped with a piece of brown paper and tied with thread. It was then autoclaved at 121°C under 15 lbs pressure for 15 minutes. After autoclaving, the liquid medium was poured in the sterilize petridishes and solidified.

3.1.2.2. Isolation on Nutrient Agar plate

The diseased leaves were cut into small pieces from the young blighted portion. Then surface sterilized by dipping them in 0.01% $HgCl_2$ solution for 1.5

minutes and washed three times with sterile water. After surface sterilization the cut pieces were kept in a test tube containing 3-4 ml of sterile water and kept for 30 minute for bacterial streaming and get stock. From this stock 3-4 dilution were made. After preparing different dilution 0.1 ml of each dilution was spreaded over NA plate at three replications. The inoculated NA plates were kept in incubation chamber at 30 °C. The plates were observed after 24 hr and 48 hr. Then single colony grown over NA plate was taken with loop and again streaked on another plate to get pure colony. After purification bacterium incubated on NA plates and kept in refrigerator at 4°c for further use.

3.1.3.3. Morphological Study

Bacterial colony on NA plate was studied and different morphological characters were observed. The colony margin, color and shape were examined.

3.1.3.4. Biochemical Test

Different biochemical tests were done viz. gram staining, catalase test, oxidase test, motility test, citrate utilization test, starch hydrolysis, bacteriolytic test, salt tolerance test and tobacco hypersensitivity test.

3.1.3.4.a. Gram staining

A small drop of sterile water was placed on a clean microscope slide. Part of young colony was removed with a cold, sterile loop from the agar medium and smeared the bacteria on to the slide. The smear should be just discernible. Dried the thinly spreaded bacterial film in air without heat. Then the underside of the slide lightly flamed twice to fix the bacteria to the slide. Then the smear was flooded with crystal violet solution for 1 minute. It was washed with tap water for a few seconds and excess water was air dried. Then the smear was flooded with iodine solution (Lugol's Iodine) for 1 minute and then washed with tap water for few seconds and excess water removed by air. After that the smear was decolorized with 95% ethanol for 30 seconds and again washed with tap water and dried by air. Then the smear was counterstained with 0.5% safranine for 10 seconds and washed briefly in tap water and excess water was air dried. Finally it was examined under microscope at 40X\100X magnification using oil immersion.

3.1.2.4.b. Catalase test

One colony of the *Pseudomonas* was picked up from the agar plate with a sterile inoculating loop from the agar plate and was taken on a slide onto which one drop of H_2O_2 was added. Formation of bubbles within a few seconds indicated a positive test.

3.1.2.4.c. MIU test

One suspected isolated colony was touched with a sterile straight wire and stabbed into MIU agar carefully down the tube, without touching the bottom. The tube was incubated at 30° C for 18 to 24 hrs. Motility of organism was detected by the presence of growth along the time of inoculation on.

3.1.2.4.d. Citrate utilization test

A portion of the organism was picked up from the agar plate with a sterile inoculating loop and streaked into Simmon's citrate agar plates followed by incubation at 30^0c for 24 hrs. The changing of green bromothymol blue to Prussian blue indicating positive results.

3.1.2.4.e. Starch hydrolysis

For starch hydrolysis, it was inoculated on nutrient agar plate containing 0.2% soluble starch with the bacterium isolate to be tested. Then bacteria inoculated NA plates incubated at optimum temperature for at least 48 hours. After incubation the plates were flooded with indoles iodine. Blue color indicates no hydrolysis or (–) ve, while a clear zone indicates hydrolysis or (+) ve result.

3.1.2.4.f. LOPAT test

LOPAT test include levan, oxidase test, pectolytic test, arginine dihydrolase activity, tobacco hypersensitivity reaction.

3.1.2.4. f.1. Levan test

Streaked bacterial culture onto the nutrient agar containing 5% sucrose and incubated it for 3 to 5 days. After incubation observed the elevation of the colony.

3.1.2.4.f.2. Oxidase test

At first prepared 1 ml 1% aquous w/v solution of NNN'N-tetramethyl –p-phenylene-diamine-dihydrochloride solution. Then placed a few drops of

solution on a new piece of whatmanno.1 filter paper with a clean Pasteur pipette. Removed a part of a colony with a sterile toothpick and smeared onto the moistened filter paper. If the color changed to purple within 10 seconds. It will indicate positive.

3.1.2.4. f.3. Pectolytic test

Potato tubers were cut up into slices of about 7-8 mm thick, disinfected with 99% ethanol and made a nick in the centre of each slice. Then placed on moistened sterile filter paper in sterile petridishes. Added sterile distilled water to a depth of 3-4 mm. made a nick in the center of each slice. Bacteria isolate were grown in nutrient broth media. Approximately two loops of each bacterial isolate were uniformly spread onto the nick of the potato slices. One was disinfected for control. Development of rot on the slices was examined 24–48 h after incubation at 25°C. Observation was done for 5 days after inoculation. It was drown a inoculating loop across inoculated part to determine whether the slice had decayed beyond the point of inoculation. Decaying of potato was indicated positive reaction.

3.1.2.4.f.4. Arginine dihydrolase activity

A fresh culture was stabbed into a soft agar tube of Thornley's medium 2A, sealed with melted agar and incubated at 28^0c for 3 to 4 days. Finally the color change was observed.

3.1.2.4. f.5. Tobacco hypersensitivity reaction

At first prepared an opaque suspension (10^8-10^9 cells per ml) of the isolate in sterile distilled water. Then infiltrated the lower surface of a mature tobacco leaf by pressing a syringe containing the suspension against the leaf, forcing the suspension into the leaf and used distilled water as a negative control. This test was done in the evening.

3.1.2.5. Salt tolerance test

Nutrient broth was prepared by amount peptone (5g) and beef extract (3g) were added to the Erlenmeyer flask containing 1000 ml distilled water. There were 4 isolate and every isolate had 7 test tubes for 1%, 2%, 3%, 4%, 5%, 6% and 7% NaCl containing NA broth. At first, 10ml NA broth was poured in every test

tube. For preparing 1% NaCl conc., 0.1g NaCl was mixed in 10ml NA broth. Similarly for 2%, 3%, 4%, 5%, 6% and 7% NaCl conc., 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7g NaCl were mixed in each 10ml NA broth respectively and finaly autoclaved. After that, the 1, 2, 3, 4, 5,6 and 7% salt broths were inoculated very lightly from every fresh growth of isolates. These inoculated test tubes were transferred in incubating shaker machine maintaining 30 ° C temp and 80 rpm. Data were recorded after every 24hrs for 7 days.

3.1.2.6. Pathogenicity Test

Bacterial suspensions containing approximately 10^8 cfu/ml culture of *pseudomonas sp* were prepared from pure culture of *pseudomonas sp* (isolated from naturally infected plant). Pathogenicity tests were performed by injecting an aliquot of the inoculums suspension using a sterile syringe into the leaf of litchi seedlings (*litchi chinensis*) at the three to five leaf stages. Three litchi seedlings were inoculated for bacterial strain and kept in the net house. Regular observation was done 15 days. After appearance of the symptoms reisolation of the pathogen was done. Morphological and biochemical studies were performed to compare with the culture that was isolated from naturally infected litchi plant.

3.1.2.7 Bio assay of fungicides

Bio assay of fungicides were evaluated by agar well diffusion method (Vijayan *et al.* 2006). Nutrient agar plates were swabbed with *Pseudomonas* sp. *Pseudomonas* sp was grown at 30^0C for 2 days in Nutrient broth. In each plate, four wells of 4 mm in diameter were made by punching the medium with cork borer. Different volumes of fungicidal suspension at different concentration (1%, 2% and 3%) were added. In case of control only sterile water was used instead of fungicide per well. The plates were then incubated at 30 °C for 24 hrs. Zone of inhibition around the wells was measured and recorded. The tolerance of *Pseudomonas* to different fungicides was tested.

Table1. Detail of fungicides with dose used

Trade Name	Active ingredient	Chemical name	Amount used (µl/well)	Conc. used during treatment
Bavistin 50 WP	Carbendazim	Methyl benzimidazol-2-yl carbamate	0, 50, 80, 100	0.2%
Cupravit 50 WP	Copper oxychloride	Copper oxychloride	0, 50, 80, 100	0.2%
Indofil M-45	Mancozeb	N-(2,6 dimethyl phenyl)-N-(methoxyacetyl)-alanine methyl ester ($C_{14}H_{21}NO_4$)	0, 50, 80, 100	0.2%

3.2. Experiment II. Survey on the diseases of litchi in some selected nurseries of Bangladesh

3.2.1. Location of survey area

Prevalence of diseases occurring on Litchi seedlings raised in the selected nurseries was surveyed. The survey was carried out in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari.

3.2.2. Selection of Nursery

Name of District	Name of nursery
Dhaka	Green orchid nursery, Agargaon Barisal nursery, Savar
Gazipur	Gazipur nursery, Gazipur Laxmipur nursery, Gazipur
Barisal	Sarchina nursery, Barisal Riyad nursery, Barisal
Khagrachari	Hill Research Center, Khagrachari Ramghar nursery, Ramghar

The eight nurseries of four districts were surveyed.

3.2.3. Age (in Year) and number of seedlings

The age (year) and number (population) of the seedlings included for the survey are presented in Table 2.

Table 2. Age of the litchi seedlings and total number of seedlings in selected eight nurseries

Nursearies	Age of the seedling (Year)	Total number of seedlings (July,2010-July,2011)	Total number of seedlings (October,2010-October,2011)	Total number of seedlings (January,2011-January,2012)	Total number of seedlings (April,2011-April,2011)
Green orchid nursery, Agargaon, Dhaka	1	40	60	80	45
Barisal nursery Savar, Dhaka	1	70	100	100	100
Gazipur nursery, Gazipur	1	80	100	90	80
Laxmipur nursery, Gazipur	1	80	60	80	60
Sarchina nursery, Barisal	1	60	60	70	60
Riyad nursery, Barisal	1	50	50	50	50
Hill Research Center, Khagrachari	1	60	50	80	70
Ramghar nursery, Ramghar, Khagrachari	1	60	80	80	70

3.2.4. Observation of the seedlings

Seedlings present in different nurseries were studied carefully to identify the disease symptoms. Symptoms of the diseases were studied by visual observation. Sometimes hand lens was used for critical observation of the disease and the disease was identified (Vasinauskiene *et al.* 2008). After identifying the disease data were collected on incidence and severity of leaf blight.

3.3. Experiment III: Epidemiology of disease incidence and severity

3.3.1. 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.

3.3.2. Data collection during survey

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

3.4 Eco-friendly management trial

The study was conducted at Sher-e-Bangla Agricultural University, Dhaka during 2010 to 2011.

3.4.1 Preparation of nursery soil and seedlings

The substratum was prepared by mixing soil, sand and well decomposed cow dung and sterilized with 5 ml formalin (40%) diluted with 20 ml water for 4 kg soil. The prepared soil was heaped in square block. Soil heap was covered by a polythene sheet for 48 hr to make the soil free from soil borne inoculate. After 4 days of treatment, earthen pots were filled up with the sterilized soil. Seeds were sown in seed bed in July 2009. Seedling was transplanted in the earthen pots in July, 2010.

3.4.2 Treatments

For the management of nursery diseases seven different treatments were evaluated namely T_1 = BAU Bio-fungicide applied in soil at the time of pot preparation @ 2%, T_2 = BAU Bio-fungicide foliar spray @ 2%, T_3= BAU Bio-fungicide applied in soil and foliar spray @ 2%, T_4 = Indofil M-45 applied as foliar @ 0.2% , T_5 = Bavistin 50 WP spray as foliar @ 0.2%, T_6 = Cupravit 50 WP spray as foliar application @ 0.2%, T_7 = Untreated control (Normal tap water was used). Fungicide solutions were prepared separately by taking requisite amount of fungicides for each dose. The fungicides were sprayed at 30 days interval by a hand sprayer. Precautions were taken with ploythene barrier to avoid drifting of spray materials from plant to neighboring plants. One seedling per pot and 5 seedlings per treatment were used. The experiment was laid out in Randomized Completely Block Design (RCBD) in the net house.

Application of bio-agent

BAU Bio-fungicide is a formulated product of *Trichoderma harzianum*, developed by Prof. Dr. Ismail Hossain, Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. BAU-biofungicide was thoroughly mixed with the soil @ 6.4 g/m^2 soil. Spraying of seedlings with BAU-biofungicide was done at one month interval of the experimental period of 12 months. Seedlings of litchi were planted in the earthen pot after 7 days of soil treatment. Observations were made on seedling damping off, seedling blight, seedling spot etc. The data on % plant infection % leaf infection and plant height were collected at one month interval before each spray schedule.

3.5. Determination of disease incidence and disease severity

For calculation of incidence of disease every seedling was counted in the nursery and also counted the infected seedlings and then expressed in percentage. Percent disease incidence of foliar diseases was determined by the following formula (Rai and Mamatha, 2005):

$$\text{Percent Disease Incidence (Leaves)} = \frac{\text{Number of diseased leaves on each plant}}{\text{Number of total leaves on each plant}} \times 100$$

Percent Disease severity was determined by the following formula (Rai and Mamatha, 2005):

$$\text{Percent Disease Severity (Leaves)} = \frac{\text{Area of leaf tissue infected by disease}}{\text{Total area of leaf}} \times 100$$

Percent disease incidence (PDI) was calculated using the formula of Rai and Mamatha (2005):

$$\text{\% disease reduction (PD R)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

3.6. Meterological data collection

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

3.7. Satistical analysis

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

Chapter IV

RESULTS

CHAPTER- IV

RESULTS

4.1. Symptoms of the disease and identification of the pathogen

The infected leaf showed wilt, stripe, and spot symptoms. Moreover, necrotic leaf spots (entire clusters of younger, expanding leaves) were observed on litchi seedling. Discolored and blackened leaf veins and petioles resulting from systemic invasion and infection were also seen on the infected portion. A gummy substance often exudes from infected portion. The spots coalesce forming large blighted patches and the affected leaves drop. The spots were found mainly on the tip of the leaves (Fig 1).The symptoms causing pathogen was isolated.

4.1.1. Morphological study

The color of the colonies were creamy white, round in shape and margin was uneven (Fig 2) on nutrient agar plate.

4.1.2. Biochemical test of the isolates from NA medium

The bacterial strains collected and isolated from litchi leaf samples were grown on NA medium and different biochemical tests were performed.

Table3. Biochemical test of the bacterial isolates from NA media

Biochemical test	Result
Gram staining	- ve
Catalase	+ ve
MIU	+ ve
Citrate utilization test	+ ve
Starch hydrolysis	+ ve
Levan test	+ ve
Oxidase	+ ve
Pectolytic test	+ ve
Arginine dehydrolase	- ve
Tobacco hypersensitivity reaction	+ ve

[(-ve) means negative, (+ ve) means positive]

Fig 1. Leaf blight of litchi

Fig 2. Isolate of *Pseudomonas*

Fig 3. Microscopic view of gram staining

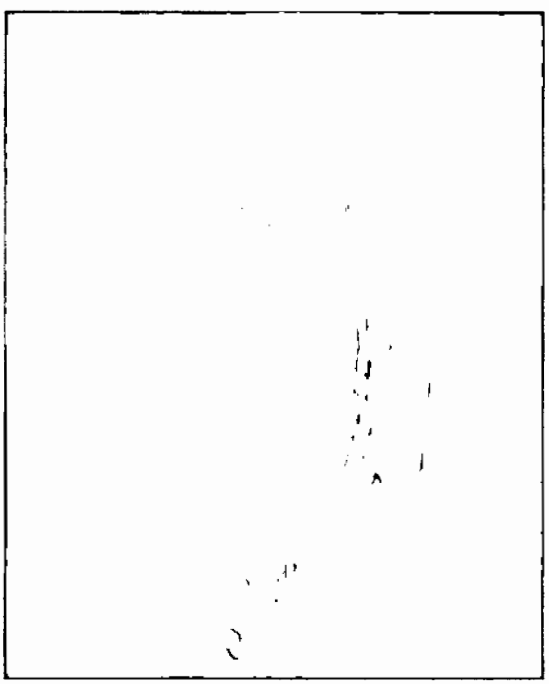

Fig 4. Catalase test

Based on these result the identification was done. The identified causal organism was *Pseudomonas* sp. Confirmation was done by growing the bacterium on specific medium for *Pseudomonas* sp i.e. Cetrimide Agar Medium.

In case of gram staining test the bacterial cell retained safranine color. So the bacterium was gram negative bacteria. Finally it was examined under compound microscope at 100x magnification at oil immersion objective. The bacterial isolate was gram negative rod shaped. (Fig 3)

In case of catalase test after adding 3% H_2O_2 onto the colony of the bacteria bubbles became visible within a few seconds. Therefore the test was positive (Fig 4).

In case of MIU test after incubating for 24-48 hours it was found the bacteria migrated away from the original line of inoculation. Thus the bacterium was motile (positive test).

In case of citrate utilization tests after 48 hrs at 30° c of incubation on simmon's citrate agar color changed from bromothymol blue to Prussian blue. Thus the result is positive (Fig 5). So the bacterium is capable of using citrate as a carbon source for their energy.

In case of starch hydrolysis the bacteria incubated plates resulted that starch stains became blue to black and a clear zone around growth indicates starch hydrolysis (amylase activity) as shown in Fig 6.

In case of levan test (Fig 7) it produced white, domed shaped, mucoid colonies in levan medium.

In oxidase test (Fig 8) a portion of bacteria was taken with a toothpick and rubbing was done for 10 seconds on the moistened filter paper. The moistened filter paper's color changed to purple within 10 seconds. It indicated positive.

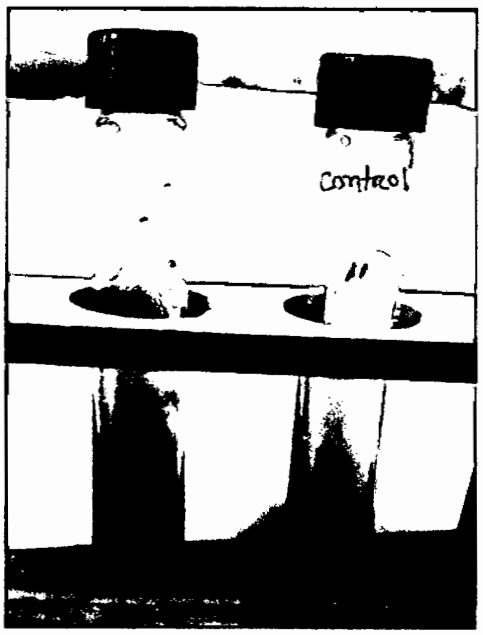

Fig 5. Citrate utilization test

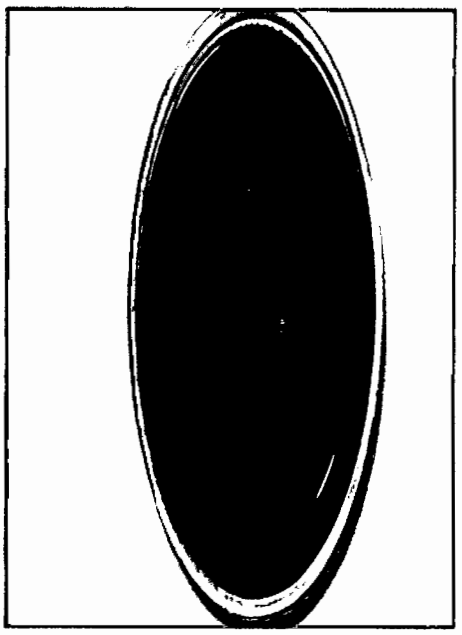

Fig 6. Starch hydrolysis test

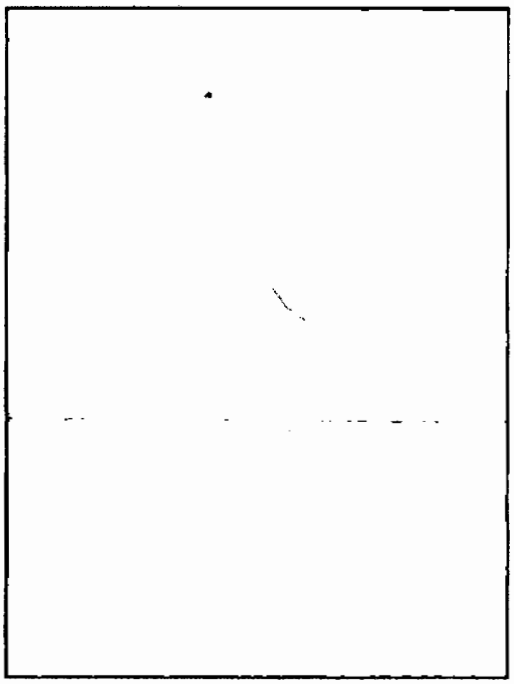

Fig 7. Levan test showing positive reaction with *Pseudomonas* sp

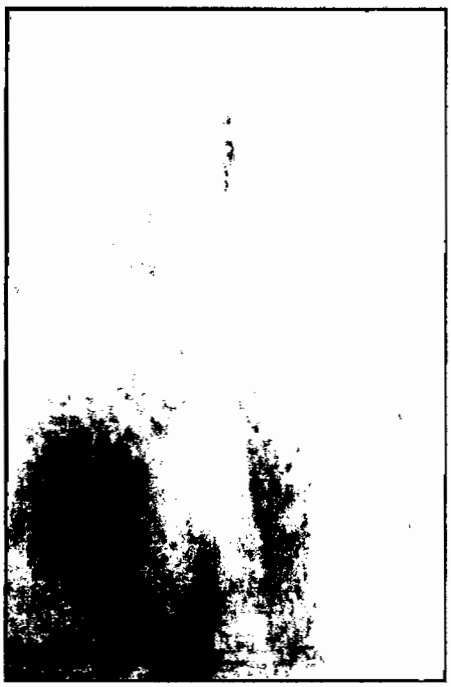

Fig 8. Oxidase test

Development of rot on the slices was examined 24–48 hrs after incubation at 25°C. After 3 days it was observed that the inoculated potato slices became rotten. Decaying of potato was indicated positive reaction (Fig 9).

After stabbed a fresh culture into a soft agar tube of Thornley's medium and incubation for 28^0C it observed no color change. Thus it indicated arginine dehydrolase negative.

After infiltration the lower surface of a mature tobacco leaf by pressing a syringe containing the suspension against the leaf. The infiltrated area becomes dry and necrotize within 24 hours. So it gave positive reaction (Fig 11). There was no change in control condition (Plate 10).

In case of salt tolerance test turbidity form after 24 hr, 48 hr, 72 hr up to 3% salt concentration. The result is as follows:

Table 4. Salt tolerance test of *Pseudomonas* sp in Nutrient broth

Time	Salt tolerance					
	1%	2%	3%	4%	6%	7%
24hr	+	+	-	-	-	-
48hr	+	+	-	-	-	-
72hr	+	+	-	-	-	-

[(-ve) means negative, (+ve) means positive]

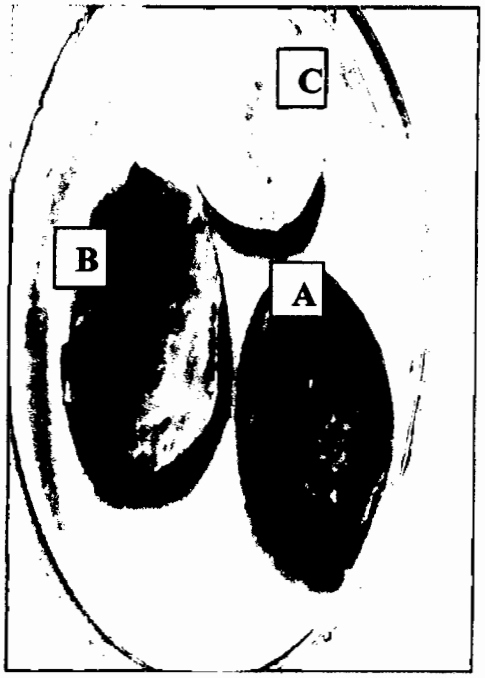

Fig 9. Pectolytic test
(A-bacteria inoculated,
B-bacteria inoculated,
C-Control condition)

Fig 10. Tobacco leaf (control)

Fig 11. Hypersensitivity positive
(*Pseudomonas* sp inoculated)

Fig 12. Pathogenicity test

4.1.3. Pathogenicity test

By injecting inoculums into the litchi seedling after 3 days it showed the same symptom (Fig 12). Bacteria were reisolated from artificially inoculated seedling. Morphological and biochemical test's results of reisolated *Pseudomonas* were similar to *Pseudomonas* which was isolated from naturally infected plant.

4.1.4. Efficacy of fungicides against *Pseudomonas* sp at different days after incubation

In case of cupravit 50 WP, after 24 hours incubation the significantly highest inhibition zone (2.7cm) was observed when concentration was 0.2% and amount of cupravit 50 WP used was 80µl/well. After 48 and 72 hours of incubation the inhibition zone was increased 3.9 cm, 4.0 cm, respectively. On the other hand no inhibition zone was observed in control condition (Table 5).

In case of indofil M-45, after 24 hours incubation the highest inhibition zone (2.4cm) was observed when concentration was 0.2% and amount of indofil M-45 used was 80µl/well. After 48 and 72 hours the inhibition zone was increased 3.9 cm, 4.0 cm, respectively. After 24 hours of incubation it was statistically similar with inhibition zone (2.3 cm) when concentration used 0.2% and amount was 100µl/well. On the other hand no inhibition zone occurred in control condition (Table 6).

In case of bavistin 50 WP, after 24 hours incubation the highest inhibition zone (1.5 cm) was observed when concentration was 0.2% and amount of bavistin 50 WP was 80 µl/well After 48 and 72 hours the inhibition zone was absent decreased (o cm, 0cm), respectively (Table 7).

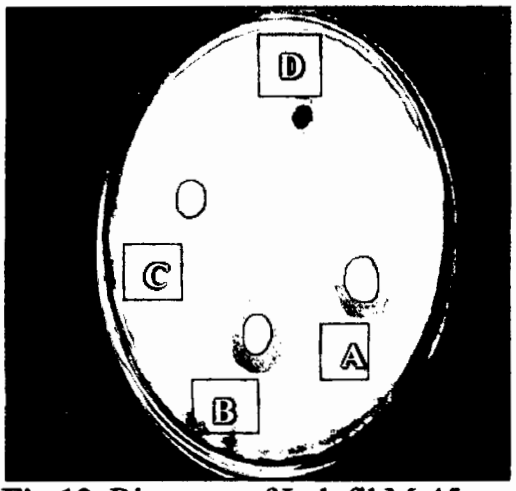

Fig 13. Bioassay of Indofil M-45 against *Pseudomonas* (A-100µl/well, B-80µl/well, C-50 µl/well, D-control)

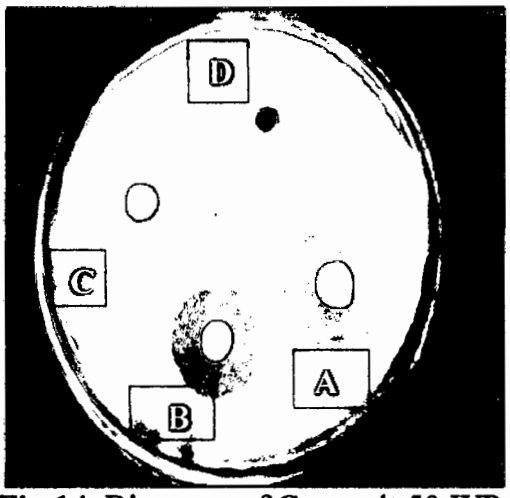

Fig 14. Bioassay of Cupravit 50 WP against *Pseudomonas* (A-100µl/well, B-80µl/well, C-50 µl/well, D-control)

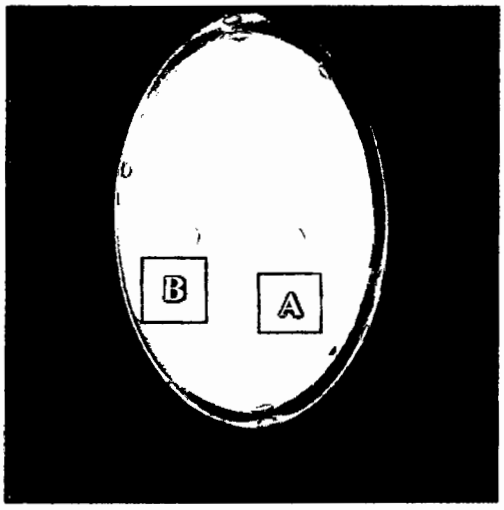

Fig 15. Bioassay of Bavistin 50WP against *Pseudomonas* (A-100µl/well, B-80µl/well, C-50µl/well, D-control)

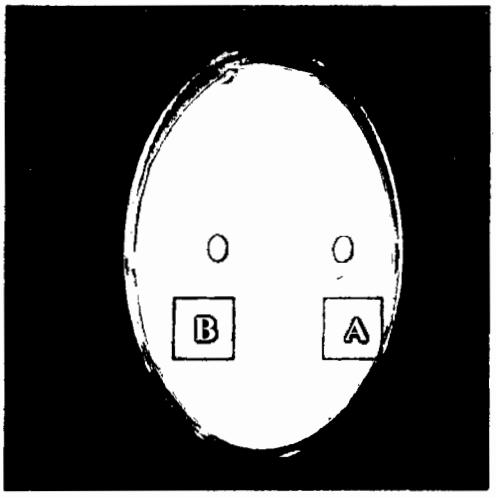

Fig 16. Efficacy of Cupravit 50 WP @ 80 µl/well and applied @0.2% (A-control B-80µl/well)

Table 5. Efficacy of Cupravit 50 WP

Cupravit 50 WP		Inhibition zone (cm)		
Used concen.	Amount used per well	24 hr	48hr	72 hr
0.1%	50 µl	1.5 h	3.0 e	3.2 e
0.2%		2.1 d	3.4 c	3.5 c
0.3%		1.4 i	2.8 g	3.0 f
0.1%	80 µl	2.3 b	3.6 b	3.8 b
0.2%		2.7 a	3.9 a	4.0 a
0.3%		2.2 c	3.0 e	3.2 e
0.1%	100 µl	1.6 g	2.9 f	3.4 d
0.2%		1.9 e	3.1 d	3.2 e
0.3%		1.8 f	2.9 f	3.0 f
0.1%	control	0.0 j	0.0 h	0.0 g
0.2%		0.0 j	0.0 h	0.0 g
0.3%		0.0 j	0.0 h	0.0 g
LSD(0.05%)		0.07573	0.07573	0.07573
CV%		3.10	1.90	1.79

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

Table 6. Efficacy of Indofil M-45

Indofil M-45		Inhibition zone (cm)		
Used concen.	Amount used per well	24 hr	48 hr	72 hr
0.1%	50 µl	1.7 cd	2.4 f	3.0 c
0.2%		2.0 b	3.1 b	3.2 b
0.3%		1.5 e	2.3 g	2.8 d
0.1%	80 µl	1.6 de	2.2 h	2.5 f
0.2%		2.4 a	3.6 a	3.7 a
0.3%		1.3 f	3.0 c	3.0 c
0.1%	100 µl	2.0 b	2.8 d	3.0 c
0.2%		2.3 a	3.0 c	3.2 b
0.3%		1.8 c	2.6 e	2.7 e
0.1%	control	0.0 g	0.0 i	0.0 g
0.2%		0.0 g	0.0 i	0.0 g
0.3%		0.0 g	0.0 i	0.0 g
LSD (0.05%)		0.1071	0.09275	0.07573
CV%		4.49	2.47	2.00

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

Table 7. Efficacy of Bavistin 50 WP

Bavistin 50 WP		Inhibition zone (cm)		
Used concen.	Amount used per well	24 hr	48 hr	72 hr
0.1%	50 µl	0.0 e	0	0
0.2%		1.0 c	0	0
0.3%		0.9 c	0	0
0.1%	80 µl	0.6 d	0	0
0.2%		1.5 a	0	0
0.3%		1.2 b	0	0
0.1%	100 µl	1.0 c	0	0
0.2%		1.2 b	0	0
0.3%		1.0 c	0	0
0.1%	control	0.0 e	0	0
0.2%		0.0 e	0	0
0.3%		0.0 e	0	0
LSD (0.05%)		0.09275		
CV%		8.25		

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

Table 8. Comparative efficacy of three fungicides against *Pseudomonas* sp at 80 µl @ 0.2% concentration

Fungicide	Inhibition zone (cm)		
	24hr	48hr	72hr
Cupravit 50 WP	2.7 a	3.9 a	4.0 a
Indofil M-45	2.4 b	3.6 b	3.7 b
Bavistin 50 WP	1.5 c	0.0 c	0.0 c
LSD(0.05%)	0.07169	0.1242	0.1242
CV%	0.00	2.31	2.25

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

4.1.5. Comparative efficacy of three fungicides @ 0.2% concentration and @ 80 µl per well against *Pseudomonas* sp

Among three fungicides Cupravit 50 WP showed statistically highest inhibition zone (2.7cm), where Bavistin 50 WP showed the lowest inhibition zone (1.5 cm) after 24 hours incubation @ 0.2% concentration and @ (80 µl/well) (Table 8).

Among three fungicides Cupravit 50 WP showed statistically highest inhibition zone (3.9 cm) where Bavistin 50 WP showed the no inhibition zone (0 cm) after 48 hours incubation @ 0.2% concentration @ (80 µl/well) (Table 8).

Among three fungicides Cupravit 50 WP showed the highest inhibition zone (4.0 cm) where Bavistin 50 WP showed the lowest inhibition zone (0 cm) after 72 hours incubation @ 0.2% concentration @ (80 µl/well) (Table 8).

In vitro bioassay of fungicides reveled that inhibition zone differed significantly when all fungicides applied at 0.2% concentration and 80 µl per well.

4.2. Survey on nursery diseases of litchi

Leaf blight of litchi was recorded in the survey conducted in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari district.

4.3. Epidemiology of disease incidence and severity

4.3.1.1. Incidence and severity of Leaf blight of litchi at different experimental locations of Bangladesh

Incidence of leaf blight of litchi varied from location to location and that ranged from 29.79-37.68% in 2010-2011 and 33.60-41.67% in 2011-2012 (Table 9). The statistically highest incidence was recorded at Gazipur and the lowest was recorded at Khagrachari for both the years. The severity of leaf blight of litchi also varied from location to location and that ranged from 19.53-25.60% in 2010-2011 and 25.28-27.06% in 2011-2012. The highest severity was recorded at Gazipur in 2010-2011 and Dhaka in 2011-2012 and the lowest was recorded at Barisal in 2010-2011 Khagrachari in 2011-2012.

Table 9. Incidence and severity of leaf blight of litchi at different locations of Bangladesh from July, 2010 to April, 2012

Location	Leaf blight			
	Incidence (%)		Severity (%)	
	2010-2011	2011-2012	2010-2011	2011-2012
Dhaka	33.06 b	35.99 b	24.08 a	27.06 a
Gazipur	37.68 a	41.67 a	25.60 a	26.36 b
Barisal	32.34 b	37.46 ab	19.53 b	25.57 c
Khagrachari	29.79 b	33.60 b	22.09 ab	25.28 c
LSD(p≥0.05)	3.805	5.191	4.377	0.5893
CV%	2.06	2.33	3.92	0.70

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

4.3.1.2. Incidence and severity of leaf blight of litchi in different growing seasons of Bangladesh

Incidence of leaf blight of litchi varied significantly from July 2010 to April 2012 and that ranged from 23.50-43.54% in 2010-2011and 26.06-48.26% in 2011-2012 (Table 10). The highest incidence (43.54%) and (48.26%) was recorded in October for both the years and the lowest (23.50%) and 26.06% were observed in the month of January for both the years. The severity of leaf blight of litchi varied significantly from July 2010 to April 2012 and that ranged from 17.47-27.02% in 2010-2011and 15.70-34.45% in 2011-2012. The highest (27.02%), (34.45%) severity was recorded in the month of October for both the years and the lowest (17.47%), (15.70%) was observed in the month of January for both the years.

Table 10. Incidence and severity of leaf blight of litchi during July, 2010 to April, 2012

Time of data collection	Leaf blight			
	Incidence (%)		Severity (%)	
	2010-2011	2011-2012	2010-2011	2011-2012
July	36.70 b	41.06 b	24.98 a	29.15 b
October	43.54 a	48.26 a	27.02 a	34.45 a
January	23.50 d	26.06 d	17.47 c	15.70 d
April	29.13 c	33.34 c	21.83 b	24.97 c
LSD(p≥0.05)	1.559	2.528	2.044	0.5302
CV%	2.06	2.33	3.92	0.70

Each data represents the mean value of three nurseries. In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

Table 11. Incidence and severity of leaf blight of litchi during July, 2010 to April, 2012 of different experimental locations in Bangladesh

Location	Data recording time(month)	leaf blight of litchi			
		Incidence(%)		Severity(%)	
		2010-2011	2011-2012	2010-2011	2011-2012
Dhaka.	July	35.65 fg	38.26 f	24.52 c	29.67 e
	October	45.72 b	47.67 c	30.07 a	35.34 b
	January	21.52 l	24.68 ij	19.29 ef	17.46 k
	April	29.34 h	33.34 g	22.42 cd	25.78 h
Gazipur	July	39.37 d	43.78 d	27.43 b	28.48 f
	October	49.31 a	54.25 a	31.37 a	36.25 a
	January	27.75 i	30.45 h	20.31 de	16.45 l
	April	34.29 g	38.22 f	23.30 c	24.28 i
Barisal	July	37.30 e	42.47 de	24.52 c	30.12 e
	October	42.80 c	50.28 b	22.23 cd	33.44 c
	January	23.73 k	26.55 i	12.87 g	15.35 m
	April	25.53 j	30.53 h	18.51 ef	23.38 j
Khagrachari	July	34.48 g	39.73 f	23.47 c	28.34 f
	October	36.33 ef	40.83 ef	24.40 c	32.78 d
	January	20.99 l	22.57 j	17.42 f	13.56 n
	April	27.35 i	31.28 gh	23.07 c	26.43 g
LSD($p \geq 0.05$)		1.559	2.528	2.044	0.5302
CV(%)		2.06	2.33	3.92	0.70

In a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT.

4.3.1.3. Incidence and severity of leaf blight of litchi during July, 2010 to April, 2012 of different experimental locations of Bangladesh

Incidence of leaf blight of litchi varied significantly and also varied from season to season as well as location to location and that ranged from 20.99-49.31% in2010-2011 and 22.57-54.25% in 2011-2012 (Table 11). The highest incidence was 49.31% in October, 2010 and 54.25% in October, 2011 of leaf blight of litchi recorded at Gazipur followed by in the month of October, 2010 at Dhaka (45.72%) and in the month of October, 2011 at Barisal (50.28%). The lowest incidence (20.99%) was observed in the month of January 2011 at Khagrachari, which was statistically similar 21.52% to the record in the month of January, 2011 at Dhaka. The lowest incidence (22.57%) was observed in the month of January, 2012 at Khagrachari. The severity of leaf blight of litchi also

varied significantly from season to season as well as location to location and that ranged from 12.87-31.37% in 2010-2011 and 13.56-36.25% in 2011-2012. The highest severity (31.37%) of leaf blight of litchi was observed in the month of October, 2010 at Gazipur which was statistically similar to (30.07%) in the month of October, 2010 at Dhaka. While the highest (36.25%) severity of leaf blight of litchi observed in the month of October, 2011 at Gazipur. The lowest severities (12.87%) at Barisal and at khagrachori (13.56%) were recorded in the month of January for both the years.

4.3.1.4. Effect of weather components on the incidence and severity of leaf blight of litchi seedling

The incidence of leaf blight of litchi was influenced by temperature, relative humidity and rainfall. The highest incidence (43.54%) and (48.26%) of leaf blight of litchi were recorded in October for both the years when average temperature, relative humidity and rainfall were 28.63°C, 80.20%, 7.13 cm and 29.5°C, 81.5%, 5.95 cm, respectively (Fig 17a and 17b). On the other hand, lowest incidence (23.50%, 26.06%) were recorded in January for both the years, having average temperature, relative humidity and rainfall 16.88°C, 73.80% and 0.52 cm and 18.46°C ,76%, 0.6 cm, respectively . In the month of July, 2010, 2012 the incidence of leaf blight of litchi were 36.70%, 41.06% when the temperature, relative humidity and rainfall were 29.65°C, 81.40% and 7.55cm and 30.5°C, 83.5%, 5.5 cm, respectively. In the month of April,2011 and 2012 the incidence of leaf blight of litchi were 29.13% and 33.34% while the temperature, relative humidity and rainfall were 20.5°C, 70.4% and 3.85 cm and 21.5°C, 71%, 3.5 cm, respectively.

The severity of leaf blight of litchi was influenced by temperature, relative humidity and rainfall. The highest severity (27.02% and 34.45%) of leaf blight of litchi were recorded in October for both the year when average temperature, relative humidity and rainfall were 28.63°C, 80.20% and 7.13 cm and 29.5°C, 81.5%, 5.95 cm, respectively in 2010 and 2011. On the other hand, lowest severity (17.47% and 15.7%) were recorded in January for both the years, having average temperature, relative humidity and rainfall 16.88°C, 73.80%

varied significantly from season to season as well as location to location and that ranged from 12.87-31.37% in 2010-2011 and 13.56-36.25% in 2011-2012. The highest severity (31.37%) of leaf blight of litchi was observed in the month of October, 2010 at Gazipur which was statistically similar to (30.07%) in the month of October, 2010 at Dhaka. While the highest (36.25%) severity of leaf blight of litchi observed in the month of October, 2011 at Gazipur. The lowest severities (12.87%) at Barisal and at Lhagrachori (13.56%) were recorded in the month of January for both the years.

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The severity of leaf blight of litchi was influenced by temperature, relative humidity and rainfall. The highest severity (27.02% and 34.45%) of leaf blight of litchi were recorded in October for both the year when average temperature, relative humidity and rainfall were 28.63°C, 80.20% and 7.13 cm and 29.5°C, 81.5%, 5.95 cm, respectively in 2010 and 2011. On the other hand, lowest severity (17.47% and 15.7%) were recorded in January for both the years, having average temperature, relative humidity and rainfall 16.88°C, 73.80%

and 0.52 cm and 18.46°C, 76%, o.6 cm, respectively in 2010 and 2011. In the month of July, 2010 and 2012 the severity of leaf blight of litchi were 24.98%, 29.15% when the temperature, relative humidity and rainfall were 29.65°C, 81.40% and 7.55cm, and 30.5°C, 83.5%, 5.5 cm, respectively and in the month of April, 2011, 2012 the severity of leaf blight of litchi were 21.83%, 24.9% while the temperature, relative humidity and rainfall were 20.5°C, 70.43% and 3.85 cm, respectively (Fig 17a and 17b).

4.3.1.5a. Relation between Leaf blight of litchi disease incidence as well as severity of litchi seedlings and temperature

A significant positive correlation between leaf blight disease incidence as well as severity and temperature was observed (Fig 18, 19). The relationship between disease incidence and temperature could be expressed by the equation $Y= 1.298x + 2.167$ (R^2 =0.852), $Y= 1.491x + 0.087$ (R^2 =0.849), where x=temperature and y=disease incidence. Here, the R^2 value indicates that the contribution of temperature was 85.2%, 84.9% on the incidence of leaf blight of litchi. On the other hand, the relationship between disease severity and temperature could be expressed by the equation $Y= 0.629x + 7.767$ (R^2 =0.8888), $Y= 1.197x + 3.848$ (R^2 =0.805), where x=temperature and y=disease severity. Here, the R^2 value indicates that the contribution of temperature was 88.88%, 80.5% on the severity of leaf blight of litchi.

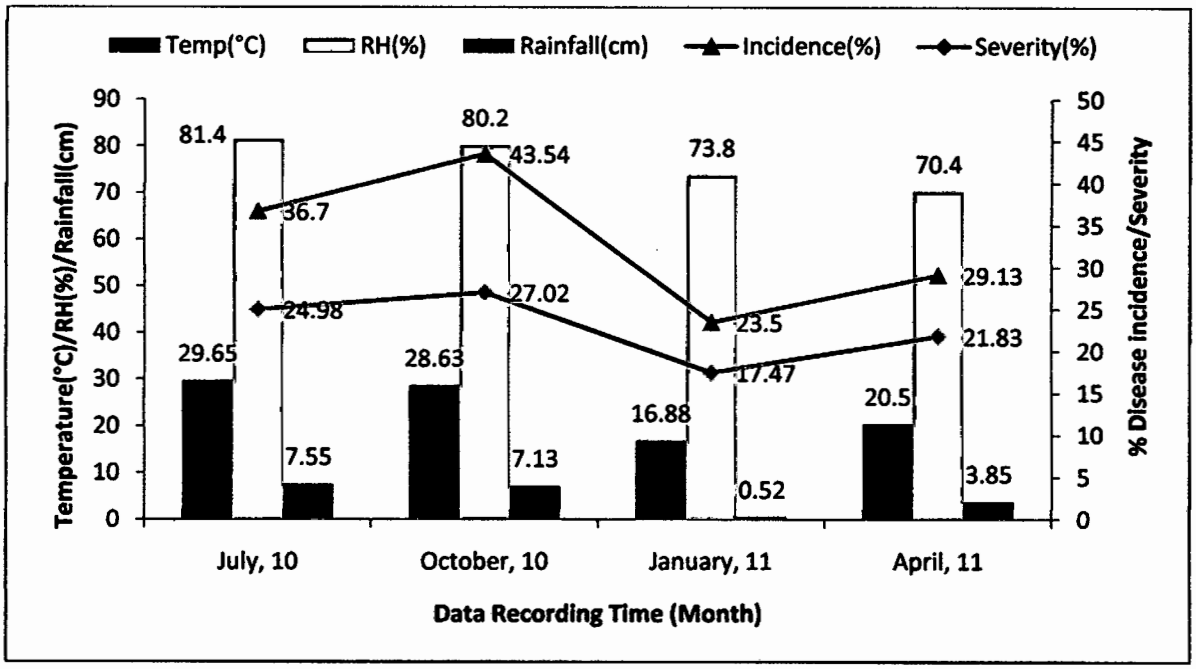

Fig 17a. Effect of different weather factors on the incidence and severity of leaf blight of litchi seedling during July, 2010 to April, 2011

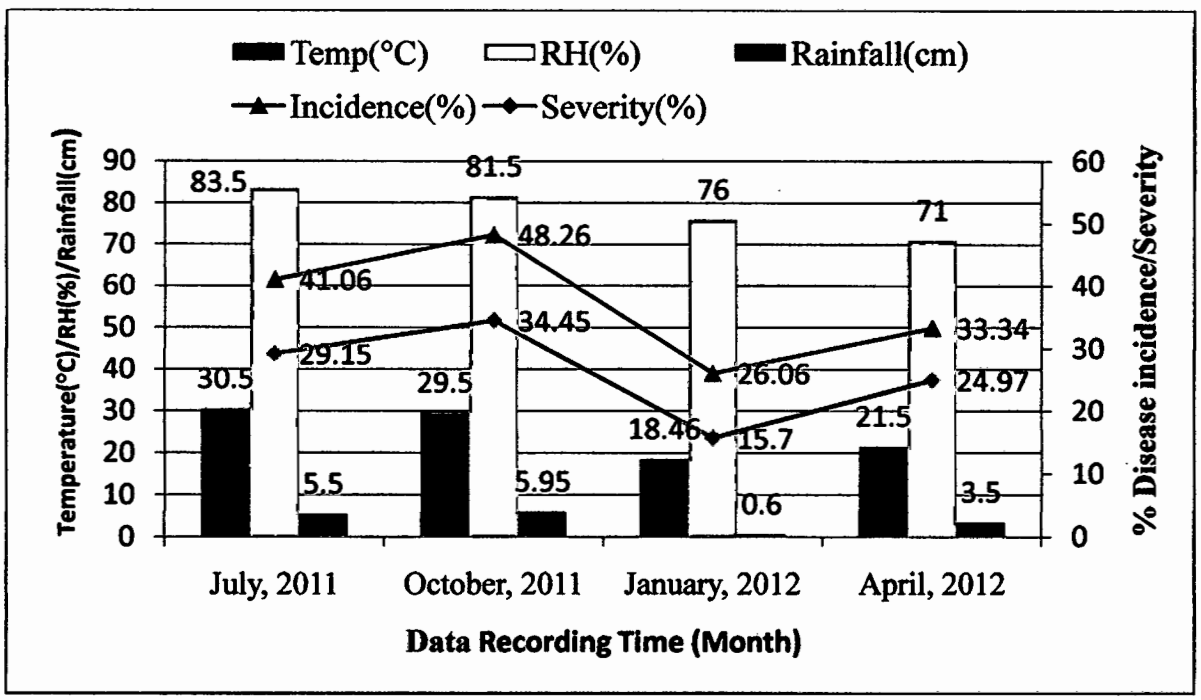

Fig 17b. Effect of different weather factors on the incidence and severity of leaf blight of litchi seedling during July, 2011 to April, 2012

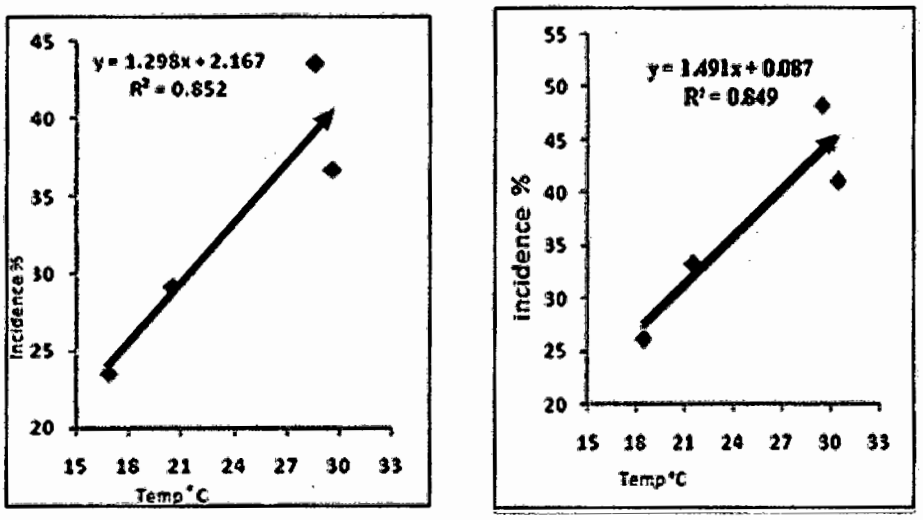

Fig 18. Linear regression analysis of the effect of monthly average temperature of associated four months on incidence of Leaf blight of litchi during July, 2010 to April, 2012.

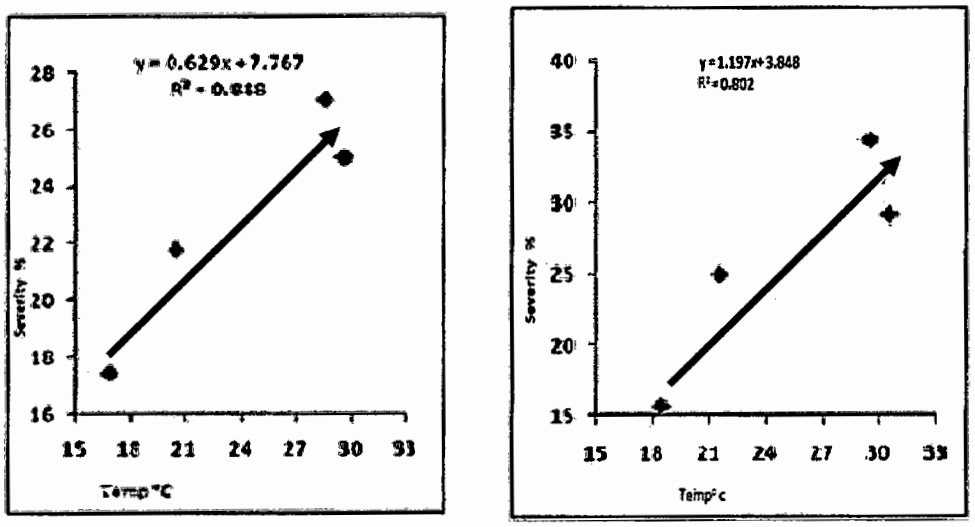

Fig 19. Linear regression analysis of the effect of monthly average temperature of associated four months on severity of Leaf blight of litchi during july, 2010 to April, 2012.

4.3.1.5b. Relation between leaf blight disease incidence as well as severity of litchi seedlings and relative humidity

A significant positive correlation between leaf blight disease incidence as well as severity and relative humidity was observed (Fig 20, 21). The relationship between disease incidence and relative humidity could be expressed by the equation Y= 1.294x -65.77 (R^2 =0.599), Y= 1.143x -52.04 (R^2 =0.452), where x= relative humidity and y=disease incidence. Here, the R^2 value indicates that the contribution of temperature was 59.9%, 45.2% on the incidence of leaf blight of Litchi. On the other hand, the relationship between disease severity and relative humidity could be expressed by the equation Y= 0.567x -20.52 (R^2=0.509), Y= 0.789x -38.36 (R^2 =0.152), where x= relative humidity and y=disease severity. Here, the R^2 value indicates that the contribution of temperature was on the severity of leaf blight of litchi.

4.3.1.5c. Relation between leaf blight disease incidence as well as severity of litchi seedlings and rainfall

A significant positive correlation between leaf blight disease incidence as well as severity and rainfall was observed (Fig 22, 23). The relationship between disease incidence and temperature could be expressed by the equation Y= 2.456x + 21.52 (R^2 = 0.845), Y= 3.770x + 22.52 (R^2 =0.916) where x= rainfall and y=disease incidence. Here, the R^2 value indicates that the contribution of rainfall was 84.50%, 91.6% on the incidence of leaf blight of litchi. On the other hand, the relationship between disease severity and rainfall could be expressed by the equation Y= 1.228x + 16.97 (R^2 =0.936), Y= 3.187x + 13.67 (R^2 =0.96) where x= rainfall and y=disease severity. Here, the R^2 value indicates that the contribution of rainfall was 93.6%, 96% the severity of leaf blight of litchi.

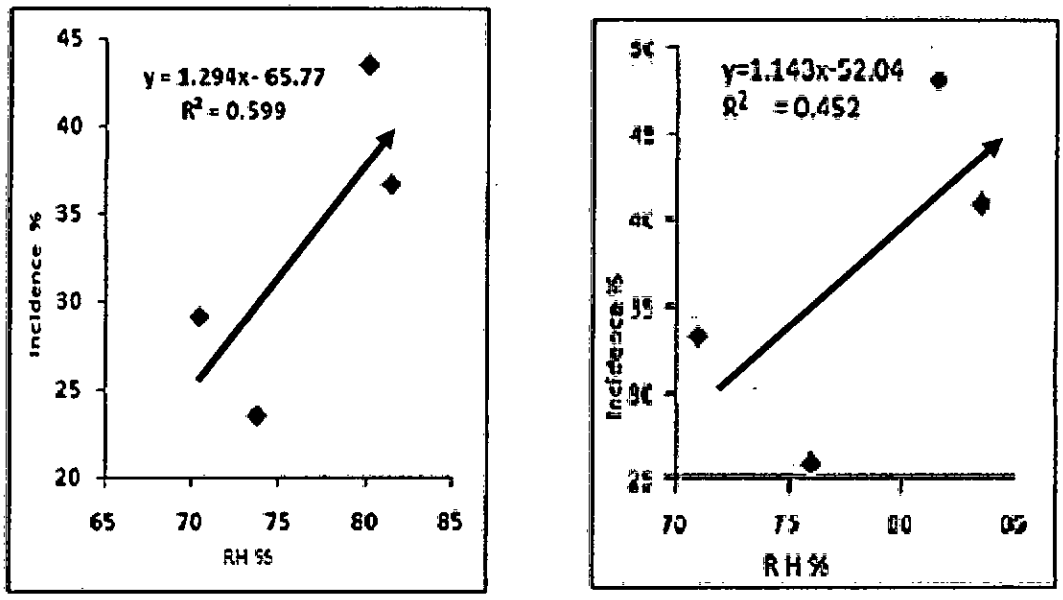

Fig 20. Linear regression analysis of the effect of monthly average relative humidity of associated four months on incidence of Leaf blight of litchi during July, 2010 to April, 2012

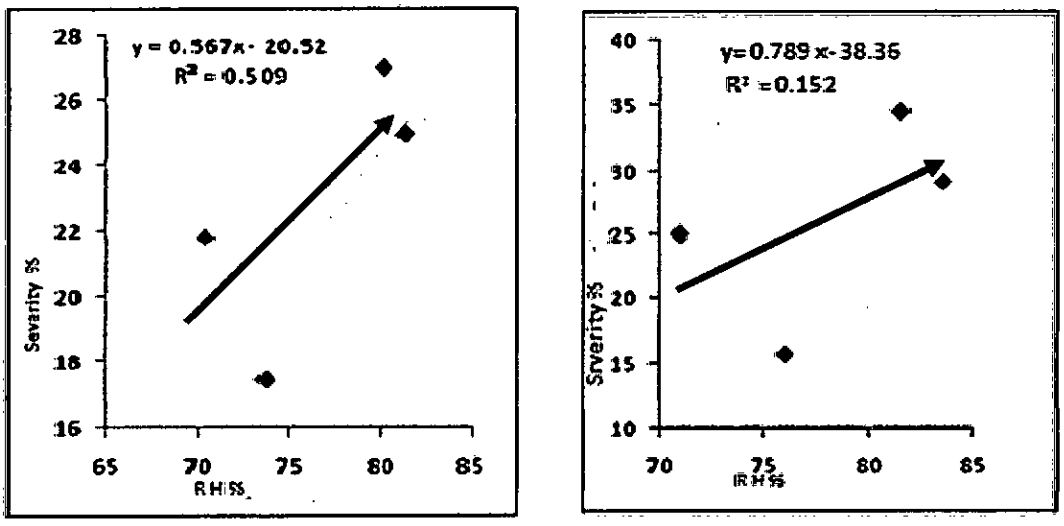

Fig 21. Linear regression analysis of the effect of monthly average relative humidity of associated four months on severity of Leaf blight of litchi during July, 2010 to April, 2012

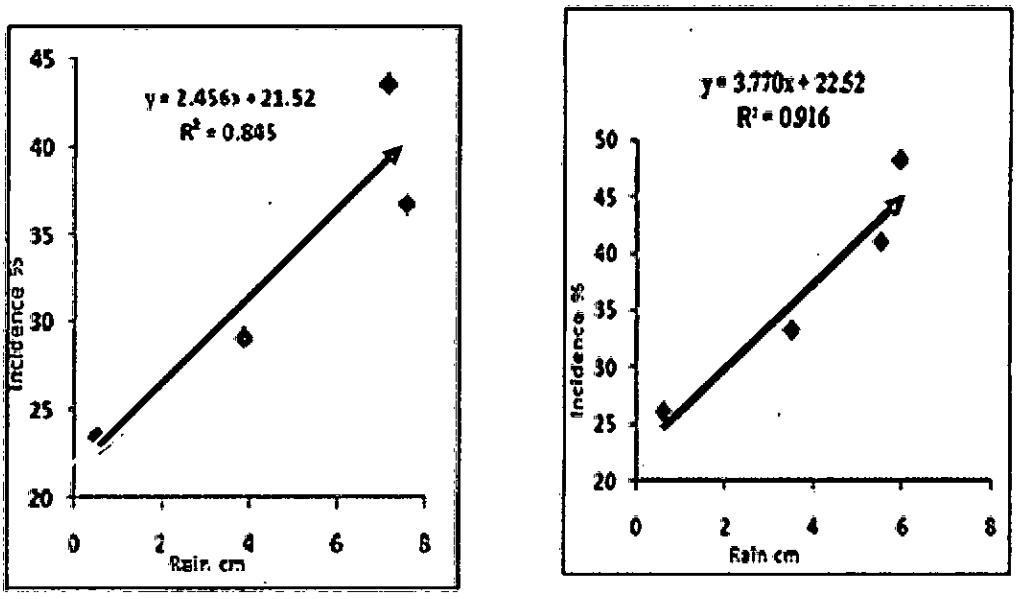

Fig 22. Linear regression analysis of the effect of monthly average rainfall of associated four months on incidence of Leaf blight of litchi during July, 2010 to April, 2012

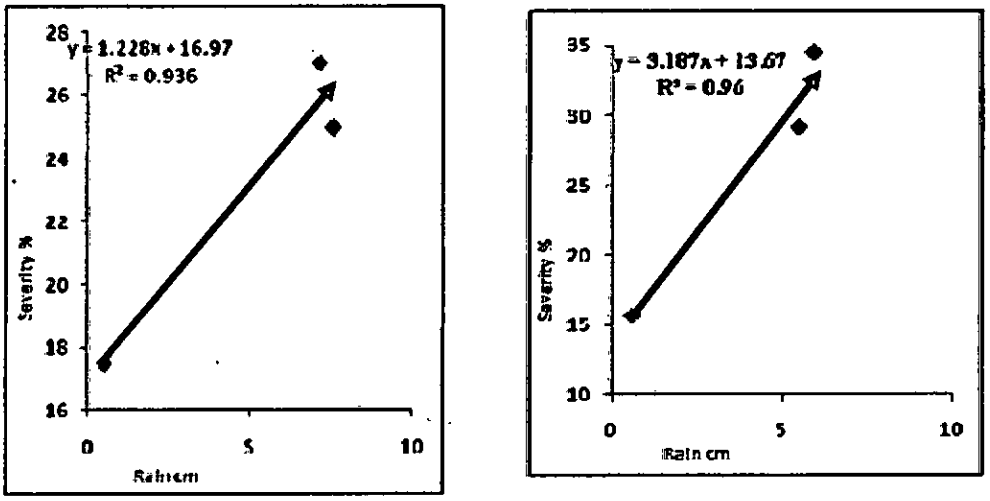

Fig 23. Linear regression analysis of the effect of monthly average rainfall of associated four months on severity of Leaf blight of litchi during July, 2010 to April, 2012

4.4 Developing of environment friendly disease management model of litchi seedling

Comparative effectiveness of BAU-biofungicide and three different chemical fungicides viz. Cupravit 50 WP, Indofil M-45 and Bavistin 50 WP were evaluated for controlling incidence and severity of leaf blight of litchi.

4.4.1 Effect of management practices on the incidence of leaf blight of litchi

All the treatments have significantly reduced the mean disease incidence of leaf blight of litchi seedling over untreated control (Table12). In all treatments applied, the incidence of disease decreased gradually in the month of November, 2010-January, 2011 and the lowest incidence was observed in the month of January, 2011. In the month of February, 2011 a little higher incidences was observed than the previous month. Considering the mean incidence, significantly highest incidence (33.95%) was observed in T_7 (untreated control) and the lowest incidence (6.31%) was observed in T_6 (Cupravit 50WP spray as foliar application @ 0.2%) followed by 9.07% in case of T_4 (Indofil M-45 spray as foliar application @ 0.2%). In case of percent reduction of disease incidence due to application of different management practices, the highest reduction by 81% of disease incidence over untreated control was observed in T_6 (Cupravit 50 WP spray as foliar application @0.2%) followed by 73.28% in T_4 (Indofil M-45 spray as foliar application @ 0.2%) and the lowest reduction by 30.99% over untreated control was observed in T_5 (Bavistin 50 WP spray as foliar application @ 0.2 %) followed by 40.56% in T_2.

4.4.2 Effect of management practices on the severity of leaf blight of litchi

Significant variations in severity were observed in application of different management practices throughout the growing period of litchi seedlings (Table 13). Out of all treatments applied, the lowest severity (0.73-4.38%) was found in the month of January, 2011 and it was gradually increased in the following months up to August, 2011 and it became higher and also remains almost static in the month of September, 2011 to October, 2011. Considering mean severity, In case of T_7 (untreated control) disease severity was highest (5.58%) which

was statistically different from all other treatments. On the other hand lowest severity (1.04%) was observed in T_6 (Cupravit 50WP spray as foliar application @ 0.2%) which was statistically similar with T_4 (Indofil M-45 spray as foliar application @ 0.2%). In case of reduction of disease severity over untreated control due to application of different management practices on litchi seedlings, the highest reduction (81.36%) of disease severity over untreated control was observed in T_6 (Cupravit 50WP spray as foliar application @ 0.2%) followed by in T_4 (Indofil M-45 spray as foliar application @ 0.2%) and the lowest reduction (30.47%) of disease severity over untreated control was observed in T_5 (Bavistin 50WP spray as foliar application @ 0.2 %) As a result, the order of efficiency different management practices regarding the percent of disease severity of litchi seedling was T_6, T_4> T_1> T_3> T_2> T_5> T_7.

Table 12. Effect of different management practices on the incidence of leaf blight of litchi seedling during the growing period of November 2010 to July 2011

Treatment	Disease Incidence(%)													
	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Mean	% reduction over control
T_1	9.49 cd	8.580 e	7.58 d	10.01 e	11.16 c	13.40 e	14.65 c	15.55 e	16.75 cd	15.34 e	12.55 c	10.39 d	12.12 e	64.3
T_2	15.85 b	14.50 c	12.33 bc	17.37 c	18.44 b	22.50 c	23.69 b	26.70 c	27.80 bc	26.04 c	20.59 b	16.46 b	20.18 c	40.56
T_3	10.34 c	9.250 d	8.17	10.60 d	11.96 c	14.80 d	15.34 c	16.60 d	17.59 bcd	16.25 d	13.16 c	11.65 c	12.97 d	61.8
T_4	7.44 cd	6.500 f	5.95 d	7.500 f	8.92 cd	9.550 f	10.70 cd	11.05 f	12.80 d	10.04 f	9.92 cd	8.560 e	9.070 f	73.28
T_5	18.84 b	17.84 b	15.68 b	20.70 b	21.58 b	27.35 b	28.39 b	28.50 b	29.62 b	27.78 b	24.12 b	20.90 a	23.43 b	30.99
T_6	5.04 d	5.0 g	4.18 d	4.487 g	5.58 d	6.180 g	7.16 d	7.500 g	8.20 d	8.010 g	6.54 d	4.550 g	6.310 g	81.41
T_7	26.17 a	24.15 a	22.82 a	29.50 a	30.14 a	37.45 a	38.42 a	44.58 a	45.65 a	41.97 a	34.22 a	8.000 f	33.95 a	
LSD%(0.01)	4.317	0.1258	4.217	0.0974 4	4.0474	0.0795 6	5.994	0.0795 6	11.76	0.0562 6	3.678	0.0562 6	0.0562 6	
CV%	18.34	0.56	11.76	0.40	14.96	0.26	17.14	0.23	19.37	0.00	12.02	0.32	0.04	

Data represent the mean value of 3 replications; each replication was derived from 7 plants per treatment, in a column means having similar letter (s) are statistically similar at 1% level of significant by DMRT

T_1= BAU-Bio-fungicide applied in soil at the time of pot preparation @2%

T_2=BAU-Bio-fungicide foliar spray @ 2%

T_3=BAU-Bio-fungicide applied in soil and foliar spray @2%

T_4=Indofil M-45 as foliar application @0.2%

T_5=Bavistin 50 WP as foliar application @0.25 %

T_6=Cupravit 50 WP as foliar application @0.2%

T_7=Untreated control

Chapter V

DISCUSSION

CHAPTER-IV

DISCUSSION

Litchi, one of the major fruit crops of Bangladesh, was surveyed during the research period of 2010-2011, 2011-2012. Litchi is now an emerging fruit all over Bangladesh. Leaf blight of litchi was recorded during the survey period of 2010 to 2012. This disease was recorded as a common disease in all the growing areas (Chowdhury, 2009).

Chowdhury, (2009) reported that seedlings of litchi were frequently affected by physical and physiological disorders as well as diseases caused by fungi, bacteria and viruses. Abdelmonem and Rasmi (2003) reported that seed borne pathogens affect nursery seedlings and reduce seedling vigor. Leaf blight is one of the most important diseases of litchi in Bangladesh. It was identified based on visual symptoms following the description of Balestra *et al.* (2009). The disease recorded in the present study had also been reported on different fruit seedlings from different countries of the world (Awasthi *et al.* 2005, Balestra *et al.* 2009). In the present study the pathogen isolated from leaf blight was *Pseudomonas* sp. The symptom and prevalence of this disease described here is in accordance with the findings of Chowdhury (2009). The pathogen has been reported by many researchers throughout the world in many fruit trees (Horkey, 2008, Vasinauskiene *et al.* 2008 and Kaluzna *et al.* 2010) reported that leaf blight caused by *Pseudomonas* sp is the major problem in the nursery of Czech Republic, Lithuania. But the causal organism of leaf blight of litchi in seedling was not reported by any workers from world earlier. It is the first time reported in Bangladesh. The present study find out the causal organism of leaf blight of litchi according to the findings of Gilbert *et al.* (2010), Palleroni (2005), Horkey (2008), Ozaktan *et al.* (2008) and Vasinauskiene *et al.* (2008). It causes numerous plant diseases with diverse symptoms including die back; twig, leaf, kernel blight; leaf spots; soft or brown rots (Bultreys and Kaluzna, 2010).

The disease causal agent of leaf blight of litchi was identified by different biochemical test following keys outline by Kaluzna *et al.* (2010), Firdous *et al.*

(2010), Vasinauskiene *et al.* (2008) and Moore (1988). According to the findings of Balestra *et al.* (2009) *Pseudomonas syringe* is gram negative, catalase positive, production of levan, show tobacco hypersensitivity which support the present study. Bio assay of three fungicides namely Cupravit 50 WP, Indofil M-45, Bavistin 50 WP were performed to find out the most effective control measure. From the present study it showed that Cupravit 50 WP showed the highest inhibition zone which is supported by Moore (1988), Conover and Gerhold (1981).

Leaf blight of litchi was recorded during the survey period of 2010 to 2012 in different location of Bangladesh. It was found in all the locations under survey viz. Green orchid nursery, Agargaon; Barisal nursery, Savar; Gazipur nursery, Gazipur; Laxmipur nursery, Gazipur; Sarchina nursery, Barisal; Riyad nursery, Barisal; Hill Research Center, Khagrachari; Ramghar nursery, Ramghar, Khagrachari. Now it is a common disease in Bangladesh.

The climate of Bangladesh harbors plant pathogens and provide favorable environment for the luxuriant growth and reproduction of pathogens which cause hundreds of different diseases of crops (Fakir, 2001). These diseases contribute to great losses of crops. Currently, there is very little information regarding the presence, prevalence, epidemiology and management of seedling diseases of litchi in Bangladesh. Since many fruit species are cultivated in close proximity in the nursery, there is a potential threat for spread of inoculates in the nursery. As diseases pose a potential threat to seedlings of litchi causing enormous loss in plant quality and disruption of production schedules, it is imperative to investigate nurseries to get information on the identity and epidemiology of the pathogens that cause diseases.

The growth and development of these diseases might be influenced by temperature, relative humidity and rainfall. That is why there was great variation of disease incidence and severity of litchi from one location to another as well as from one season to another season was recorded. This disease was recorded as a common disease in all the growing areas surveyed. The effects of temperature, rainfall and relative humidity on the incidence and

severity of noted diseases of litchi 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 (Anonymous, 1995). ANOVAs, correlation and linear regression analysis were performed to determine the relationship between different components of climatic factor (temperature, relative humidity and rainfall) and the incidence as well as severity of seedling disease of litchi. It observed that ANOVAs has been the fundamental method used by plant pathologist to determine the correlation between the prevalence and environmental parameters (Anonymous,1989). 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 like Bultreys and Kaluzna (2010), Agrios (2005), Hirano and Upper (2000), Chowdhury (2009) and Cross (1966).

In the present study diseases were recorded eight particular times during the period of two years survey from July, 2010 to April, 2012. Prevalence (Incidence and severity) of leaf blight was found to be increased in the month of October while the disease decreased in the month of January. Correlation regression analysis of prevalence of leaf blight disease along with generalized environmental parameters revealed that this increase and decrease were due the effect of temperature, relative humidity and rainfall. A positive correlation was observed between prevalence of leaf blight with temperature, relative humidity and rainfall. With the increase of temperature, relative humidity and rainfall both the incidence and severity increased significantly. The result of the present study corroborates with the study of Agrios (2005) that cold and wet weather in spring are favorable for infection of emerging buds and blossoms caused by *Pseudomonas* and subsequent twig and branch infections They also observed that *Pseudomonas* able to grow as epiphyte to high populations in autumn and the diseases showed a positive correlation with temperatures, relative humidity and rain and hence the period from July to October was identified as the most conducive for the development of leaf blight. Ozaktan *et al.* (2008) reported that *Pseudomonas* prefer cold and wet conditions to spread, trees under stress

condition are more susceptible. Chandler and Daniell (1976) observed that pruning in fall and early winter also predisposed the trees to more severe damage from *Pseudomonas syringae* infections and the short-life syndrome.

Comparative effectiveness of BAU-Biofungicide and three other fungicides viz. Cupravit 50 WP, Indofil M-45 and Bavistin 50 WP were evaluated on seedling diseases of litchi in the nursery. Significant effect of different management practices on incidence, severity and plant height were observed. Application of control measures resulted in gradual decrease of the incidence and severity of diseases over untreated control. The highest reduction of incidence and severity of leaf blight was observed in applying Cupravit 50 WP as foliar spray. Moore (1988) reported that fixed copper compounds were used to control *Pseudomonas syringae*. Performance of Indofil M-45 was found good in controlling incidence and severity of leaf blight of litchi. Conover and Gerhold (1981) reported that improved control of *Pseudomonas syringae pv. tomato* controlled from applying a mixture of copper and the fungicides maneb or mancozeb. This mixture produced a copper carbamate which is more effective than copper alone. Bravo CM, a mixture of chlorothalonil, copper oxychloride and maneb had been registered to control *Pseudomonas syringae* on tomato, peach, and nectarine. Many fungal diseases were also controlled, adding to the benefit of this compound.

Chapter VI

SUMMARY AND CONCLUSION

CHAPTER-VI

SUMMARY AND CONCLUSION

Litchi seedling are vulnerable to attack by various diseases in Bangladesh, but least concrete information regarding their distribution, incidence, severity, epidemiology and management is available. Therefore, the present study has been designed to study the occurrence and prevalence of leaf blight seedling disease of litchi and to study the correlation of disease development with environmental parameters in eight nurseries in four selected districts namely, Dhaka, Gazipur, Barisal and Khagrachari and to study the effective management strategies of the disease.

Four experiments were carried out throughout the study period from July, 2010 to April, 2012. The disease was identified based on matching the observed symptoms in the infected plants with the previously described symptoms. Besides, the symptoms of the diseases were recorded following the description of Balestra *et al.* (2009), Chowdhury (2009) and Zidari and Dreo (2008). The presence of bacteria in infected region was analyzed by isolation to nutrient agar culture and then confirmation was done by pathogenicity test and examining them by different biochemical test following keys outline by Bultreys and kaluzna (2010), Sulikowska (2008), Vasinauskiene *et al.* (2008), Kennelly *et al.* (2007), and Agrios (2005). After isolation the bacterial colonies were looked as creamy white, round in shape and uneven margin. In biochemical test it showed Gram staining negative, Catalase positive, MIU positive, Simmon's Citrate Agar test positive, starch hydrolysis positive, LOPAT positive, tobacco hypersensitive reaction positive. These result confirmed that the identified organism was *Pseudomonas* sp. In Bioassay Cupravit showed the highest efficacy and Bavistin showed the lowest efficacy against *Pseudomonas* sp.

Leaf blight of litchi was recorded during the survey period under four different geographical location viz. Dhaka, Gazipur, Barisal and Khagrachari and the effect of temperature, relative humidity and rainfall on incidence and severity

of noted diseases were observed. It has found that incidence and severity of leaf blight of litchi varied from location to location. Significant variations were observed in the incidence and severity under the variation of weather parameters. The intermittent addition and defoliation of leaves during different period of year responsible for significant reduction of disease incidence and severity. Different degrees of correlation were observed among the seedling diseases of litchi with temperature, relative humidity and rainfall. All the diseases were found to be influenced by the epidemiological parameters. In this study, it was observed that the incidence and severity of leaf blight of litchi varied from location to location. The incidence of leaf blight ranged from 29.79-37.68%, 33.40-41.67% in the year 2010-2011 and 2011-2012, respectively. The highest incidence was recorded at Gazipur and the lowest was recorded at Khagrachari. The severity of leaf blight ranged from 19.53-25.60%, 25.28-27.06% in the year 2010-2011 and 2011-2012 respectively.The highest severity was recorded at Gazipur and the lowest was recorded at Barisal in 2010-2011 and the highest severity was recorded at Dhaka and the lowest severity was recorded at khagrachori in2010-2012. In the present study, the diseases were recorded eight times during the period of ten month survey from July, 2010 to April, 2012. During the survey period leaf blight of litchi seedling was found to increase with the increase in time. Thus, the lowest prevalence (Incidence and Severity) of leaf blight of litchi seedling disease were recorded in January (23.50% and 17.47%) in 2010-2011 and (26.06% and 15.70%) in 2011-2012. It is highest prevalence (Incidence and Severity) of leaf blight of litchi seedling disease were recorded in October (43.54% and 27.02%) in 2010-2011 and in October (48.26%, 34.45%)in 2011-2012. The average temperature, relative humidity and rainfall was observed in January was 16.88°c, 73.80% and 0.52cm and 18.46°c, 76% and 0.6cm for both the year respectively. The temperature, relative humidity and rainfall were observed in October, 2010 it was 28.63°c, 80.20%, 7.13cm and in October, 2011 it was 29.5°c, 81.5%. 5.95 cm respectively. A positive correlation was observed between prevalence of leaf blight of litchi seedling with temperature, rainfall

and relative humidity. The incidence and severity of leaf blight was significantly increased with increasing of temperature and rainfall. Statistical analysis shows that the contribution of temperature, relative humidity and rainfall on the incidence of leaf blight of litchi seedling was 85.2%, 59.9% and 84.5% in 2010-2011 and 84.9%, 45.2%, 91.6% in 2011-2012. The contribution of temperature, relative humidity and rainfall on the severity of leaf blight of litchi seedling was 88.8%, 50.9%, 93.6% in 2010-2011 and 80.2%.15.2%, 96% in 2011-2012.

Effectiveness of BAU-biofungicide and three other fungicides viz. Cupravit 50 WP, Indofil M-45 and Bavistin 50 WP were evaluated on seedling diseases of litchi in the nursery. The management program especially aimed at leaf blight disease caused by *Pseudomonas*. Significant effect of different management practices in relation to incidence and severity of different disease were observed. Application of treatments resulted gradual decrease of incidence in the month of November 2010 to January 2011.This may be due to defoliation of leaves during winter season. The higher incidence in June may be due to conducive environment of the pathogen. The highest reduction (81.41%) of disease incidence over control was observed in T_6.i.e. while T_6= Cupravit 50 WP spray as foliar application @ 0.2% every month. In the study period the lowest severity of the disease was observed in the month of December and January and highest in the month of October this might be due to high humidity and rainfall. However, among the different management practices on litchi seedlings, the highest (81.36%) reduction disease over control was observed in case of use of Cupravit 50 WP spray as foliar application at o.2%. All the treatment has significant effect for controlling the incidence and severity of leaf blight over control. Applying BAU-biofungicide also has good result in controlling leaf blight of litchi but inferior in efficiency. Different management practices resulted significant variation on the controlling of disease. Among the treatments applied Cupravit 50 WP showed excellent result in controlling leaf blight of litchi seedling.

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Therefore, the present study on the occurrence of seedling disease in the nursery revealed that all the diseases studied are related to the temperature, relative humidity and rainfall. Other parameters of epidemiology viz. leaf wetness period, vapor presser deficit, sunshine hour, microclimatic parameters including canopy temperature, relative humidity etc, should be critically evaluated to have profound effects on over wintering formation, germination and development of inoculums in different pathosystem and these should be critically studied for each host-pathogen system to find out the most appropriate time to combat the disease at minimum effort. Use of BAU-biofungicide could be incorporated in the nursery disease management system that is a most important alternative to the hazardous chemical fungicides in Bangladesh.

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APPENDICES

APPENDIX-B

PREPARATION OF MEDIA AND REAGENTS

Preparation of Gram's staining reagents:

i) Gram's Crystal violet (Hucker's modification):

Solution A: Crystal violet (90% dye content)	2.0 g
Ethyl alcohol	20.0 ml
Solution B: Ammonium oxalate	0.8 g
Distilled water	80.0 ml

Solution A and B in equal volume to prepare crystal violate solution.

ii) Gram's Iodine (Gram's modification of Lugol's solution):

Iodine	1.0 g
Potassium iodide (KI)	2.0 g
Distilled water	300.0 g

Add iodine after KI is dissolved in water to prepare Gram's Iodine solution.

iii) Gram's alcohol (decolorizing agent)

Ethyl alcohol (95%)	98 ml
Acetone	2 ml

iv) Safranin (counter stain)

Safranin (2.5% solution in 95% ethanol)	10 ml
Distilled water	100 ml

Preparation of KOH solubility reagent:

3% aqueous solution of KOH was prepared from the KOH granules

Preparation of Starch hydrolysis media and reagent:

i) Culture medium

Nutrient broth (Difco)	8.0 g

	Soluble potato starch	10.0 g
	Bacto agar (Difco)	15.0 g
	Distilled water	1000 ml

ii) Reagent (Lugol's iodine)

Iodine	5.0 g
Potassium iodide	10.0 g
Distilled water	100 ml

NaCl	5.0 g
Bromothymol blue	80.0 mg
Agar	15.0 g
Distilled water	1000 ml

Preparation of Gelatin medium:

Gelatin	120g
Distilled water	1000 ml

Preparation of Thornley's medium 2A

Ingrediant	Per liter
Peptone	1.0 g
NaCL	5.0 g
K_2HPO_4	0.3 g
Agar	3.0 g
Phnol red	1.0 mg
Arginine HCl	1.0 mg

Adjust P^H to 7.2