STUDY ON SEEDLING DISEASES OF CITRUS (*Citrus* spp. L.) IN SELECTED AREA OF BANGLADESH AND THEIR MANAGEMENT

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JUNE, 2011

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REG. NO. 05-01693

A Thesis
Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JANUARY- JUNE, 2011

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CERTIFICATE

This is to certify that thesis entitled, **"STUDY ON SEEDLING DISEASES OF CITRUS (*Citrus* spp. L.) IN SELECTED AREA OF BANGLADESH AND THEIR MANAGEMENT"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **MD. MUSHFIQUR RAHMAN, Registration No. 05-01693** 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:
Dhaka, Bangladesh

(Dr. M. Salahuddin M. Chowdhury)
Professor
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DEDICATED

TO

MY BELOVED PARENTS

ACKNOWLEDGEMENTS

The author seems it a much privilege to express his enormous sense of gratitude to the almighty Allah for there ever ending blessings for the successful completion of the research work.

The author feels proud to express his deep sense of gratitude, sincere appreciation and immense indebtedness to his supervisor Professor Dr. M. Salahuddin M. Chowdhury, Department of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka, for his continuous guidance, cooperation, constructive criticism and helpful suggestions, valuable opinion in carrying out the research work and preparation of this thesis, without his intense co-operation this work would not have been possible.

The author feels proud to express his deepest respect, sincere appreciation and immense indebtedness to his co-supervisor Professor Dr. Md. Rafiqul Islam, Department of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka, for his scholastic and continuous guidance, constructive criticism and valuable suggestions during the entire period of course and research work and preparation of this thesis.

The author expresses his sincere respect to Associate Professor Chairman, Nazneen Sultana, Department of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka for valuable suggestions and cooperation during the study period. The author also expresses his heartfelt thanks to all the teachers of the Department of Plant pathology, SAU, for their valuable teaching, suggestions and encouragement during the period of the study.

The author expresses his sincere appreciation to his beloved father Md. Delwar Hossain, mother Bilkis Begum, wife Most. Najmunnahar Tasrifa Akter and other members of the family, well wishers and friends for their inspiration, help and encouragement throughout the study.

Finally, the author is grateful for financial help for conducting the research under the project “Surveillance of seedling diseases of some important fruit

species in Bangladesh with molecular characterization of pathogens and eco-friendly model development for their management" (Subproject ID-169) financed by PIU-BARC (NATP Phase-1), Bangladesh Agricultural Research Council, BARC, Farm gate, Dhaka-1215.

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ABSTRACT

Seedling diseases of citrus and the effect of weather parameters on the diseases were studied during 2010 to 2012 in different growing areas of Bangladesh with an effort to develop an environment friendly disease management approach. Important plant pathogens *Xanthomonas axonopodis* pv. *citri*, *Sphaceloma fawcetti* (*Elsinoe fawcetti*) and *Colletotrichum gloeosporioides* were detected and identified as causal agent of canker, scab and die-back of citrus seedlings, respectively. Greening of citrus at seedling stage was observed and this is the first time report of presence of the disease in citrus seedlings in Bangladesh. Effect of weather parameters on incidence and severity of diseases were studied and significant variations of disease development were observed in different weather conditions. Correlation studies revealed that occurrence of seedling diseases have significant relationship with temperature, rain fall and relative humidity. Comparative effectiveness of *Trichoderma harzianum* based BAU-biofungicide with three chemical fungicide viz. Mancozeb, Bavistin and Cupravit were evaluated on citrus seedlings in the nursery. Among the treatments applied *Trichoderma harzianum* based BAU-Biofungicide showed promising result in controlling canker, scab and die-back diseases. BAU-Biofungicide, may be used as an alternate to chemicals can successfully be used as eco-friendly option.

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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 (s)
cv.	=	Cultivar (s)
T	=	Treatment
ft	=	feet (s)
pv.	=	pathovar
syn.	=	synonym
var.	=	variety
mm	=	milimiter
µm	=	micrometer
USA	=	United State of America

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd.)

BAU	=	Bangladesh Agricultural University
SAU	=	Sher-e-Bangla Agricultural University
BARI	=	Bangladesh Agricultural Research Institute
NUV	=	Near Ultra violate radiation
WP	=	Wettable Powder
NA	=	Nutrient Agar (media)

CHAPTER I

INTRODUCTION

Citrus (*Citrus* spp. L.), belongs to the family Rutaceae, is one of the most important, popular and nutritious fruit crops in the world as well as in Bangladesh. It has a great demand due to its nutritive value, aroma and taste. It is thought to be originated in Indian sub continent because of maximum genetic diversity are grown in this region (Sohi and Kapoor, 1990). Considering the multipurpose use, the demand of fruit (citrus) is increasing day by day. Bangladesh produces only 21 thousand metric tons of citrus fruits every year according to a study of 2009-2010 (BBS, 2010). Eight species of citrus fruits are grown in Bangladesh. Among them, three species viz. Lemon (*Citrus limon* L.), Lime (*Citrus aurantifolia* Swing) and Pummelo (*Citrus grandis* L. Osbeck) are commonly cultivated in our country. In citrus growing area, Kagzi lime and Elachi lemon occupy 6,388.66 ha of land of the country (BBS, 2007). As the hilly and high land remains fallow round the year, there is a great opportunity to extend citrus cultivating area in the country.

Many people of our country are suffering from the deficiency of some limiting vitamins like Vitamin A and C and some other minerals like calcium and iron in their daily diet. About 91% people of Bangladesh are suffering from deficiency of vitamin C (Haque, 2005). Citrus serves as a potential source of vitamins and minerals (Alam *et al*., 2003). Vitamin C cannot be stored in human body like others and it is needed to be taken every day in the diet. Thus, citrus fruits play an important role in human health. Slices of lemon are served as a garnish on fish or meat or with iced or hot tea. Lemon juice is primarily used for flavoring cakes, cookies, cake icings, puddings, sherbet, preserves and pharmaceutical products. The rind of the fruit and leaves are used for the production of oil, which is used in cosmetics industries. It is also the source of pectin and citric acid. It is much used as a flavoring agent for hard candies. Bangladesh produces less than 30% of the fruits needed to meet the minimum

daily requirements for its population. Bangladesh produces less than 30% of the fruits needed to meet the minimum daily requirements for its population.

Various factors are responsible for lowering the yield of citrus. Among the factors, plant diseases play an important role in lowering yield as well as citrus decline. 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). Now a days, the demand of seedlings are very high in the nurseries. Citrus plants are very much prone to the attack of numerous diseases. Different species of citrus grown in the world suffers from more than 100 diseases (Klotz, 1973). In Bangladesh, twelve diseases are known to occur in different species of citrus seedlings. Among these diseases; scab, canker, die-back, sooty mould and citrus yellowing, citrus greening and gummosis are considered as major diseases in Bangladesh (Alam, 2003).

Seedling diseases are an important consideration for citrus production. Because healthy seedlings are prime need and basic raw material for establishment of orchard for production of citrus. But seedling diseases are one of the important problems in the tropics. 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 [Ramakrishnan, (1954); Aiyappa, (1958); Khucharek and Whiteside, (1983); Calvin *et al.*, (1986); Tripathi and Srivasttava, (1992); Huang and Huang, (2002); Varniere *et al.*, (2003); Agostini, (2003); Rawal, (2005); Chowdhury, (2009) and Hossain, (2011)]. Chowdhury (2009) studied the effect of weather factors on citrus seedlings and observed significant effect of temperature, relative humidity and rainfall on the prevalence of different diseases. Bal and Dhiman (2005) observed that citrus scab (*Elsinoe fawcetti*) and citrus canker (*Xanthomonas axonopodis* pv. *citri*) were serious diseases in Kinnow mandarin

nursery plants. The relationship between the disease development and environmental factors was studied and both citrus canker and scab were found to build up during the first week of June with the onset of rains. The highest incidence of citrus canker (73.3%) and scab (66.66%) was recorded during the second week of September. Both diseases showed a positive correlation with temperature, relative humidity and rain. The period from July to September was identified as the most conducive for the development of citrus canker and scab.

Effective management strategies of seedling diseases of citrus in the nurseries would minimize the use of expensive and hazaradous chemical that endanger people and the environment. Eradication of plant pathogen from the citrus seedlings in the nursery was needed to be ensured with appropriate measurement. Management of seedling diseases of citrus has been investigated by many researchers [Chowdhuri (1951); Rawal and Saxena (1997); Huang (1999); Mamatha *et al*., (2000); Zhong and Ling, (2002); Gade *et al*. (2005); Singh *et al*. (2005); Chowdhury, (2009) and Hossain, (2011)]. Singh *et al.* (2005) evaluated five antagonists and found antagonists *Trichoderma harzianum* suppressed the disease development significantly. Enhancement of seedling germination and vigor by using *Trichoderma harzianum* or *Trichderma* based formulations have been studied and reported by Chowdhury (2009). He also reported the performance of BAU biofungicide in controlling nursery diseases in Bangladesh.

Although a huge number of nurseries are engaged in producing seedlings, but they fail to produce quality seedlings due to lack of their knowledge about diseases. Thus production of healthy seedlings ensure good plantation and save money, labour, and energy of citrus gardeners. Citrus seedlings suffer from various diseases in Bangladesh but least concrete information regarding their distribution, incidence, severity and epidemiology is available. Moreover, the nursery people do not have adequate knowledge of management of nursery diseases. Therefore, attempt should be put forward to study the prevalence of

various disease occuring on citrus seedlings and their management in some selected nurseries of Dhaka, Gazipur, Barisal and Khagrachari.

Keeping in view of the above facts the present study was undertaken with the following objectives:

1. Survey on the prevalence of seedling diseases of citrus in selected nurseries of Dhaka, Gazipur, Barisal and Khagrachari.
2. To identify the pathogen(s) associated with the disease(s).
3. To study the effect of temperature, relative humidity and rainfall on incidence and severity of nursery diseases of citrus.
4. To find out suitable management strategies for controlling nursery diseases of citrus.

CHAPTER II

REVIEW OF LITERATURE

Citrus suffers from many diseases at all stages of growth of which scab, canker, die-back, citrus greening are considered as important diseases of seedlings. These are widely occurring throughout the citrus growing countries in the world. 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). Literature on disease symptoms and prevalence, management of the diseases with the application of different treatments are reviewed and presented in this chapter.

Adjei-Nsiah *et al.* (2009) conducted a survey in private nursery operators at Okumaning to examine their nursery management practices, production problems and the profitability of their operations using semi-structred questionnaires and focus group discussion. The result of the study revealed that problems of marketing, pests and diseases, high costs of inputs and lack of support services impede the enterprise. The study also indicates that indiscriminate use of pesticides in citrus nurseries at Okumaning.

Agostini (2003) conducted an experiment on citrus scab (*Elsinoe fawcetti*) and found external blemishes on citrus fruit, reducing acceptability of the fruit for the fresh market. In laboratory studies, rough lemon seedlings and grape fruit seedlings were inoculated with conidia of *Elsinoe fawcetti* and exposed to a range of temperatures and durations of leaf wetness. Scab as most severe at temperatures from 23.5 to 27°C and much less severe at 17, 20, 30 or 32°C. Leaf wetness duration of 4 hr was sufficient for some infection, but 12 hr of leaf wetness were needed for maximum infection with scab. In field studies, grapefruit seedlings with new shoots were placed beneath trees weekly and disease severity was evaluated in relation to environmental factors. Scab severity increased sharply with an increase in total weekly rainfall, leaf wetness durations of greater than 80 hr per week and average temperatures above 22°C.

Amador (2002) reported that die back affected young branches, start withering from the tip, sometimes producing gum exudation. Wood is discolored underneath the bark. Damage by twig die-back usually is severe.

Amador (2002) reported that citrus scab caused by fungus *Elsinoe fawcetti*, is an important disease in Texas. The disease is more severe on lemons, sometimes troublesome on grapefruit and seldom a problem on sweet orange. Sour orange is highly susceptible, thus nursery stocks may become infected before young sour orange trees are budded. Because citrus tissue is susceptible to scab only while young, the disease is mainly confined to new growth.

Aiyappa (1958) reported that all cultivated varieties of citrus and some wild species in Karnataka are highly susceptible to canker possibly due to heavy rainfall, high humidity and low temperature.

Awasthi *et al.* (2005) surveyed different nurseries during January 2002 to August 2003 in new alluvial plains of West Bengle, India. Survey revealed that prevalence of citrus twig blight (*Phytophthora* sp.) and canker (*Xanthomonas campestris* pv. *citrii*) were the major problem in the nursery.

Bal and Dhiman (2005) observed that citrus scab (*Elsinoe fawcetti*) and citrus canker (*Xanthomonus axonopodis* pv. *citri*) were serious diseases in Kinnow mandarin nursery plants. The relationship between the disease development and environmental factors was studied and both canker and scab were found to build up during the first week of June the onset of rains. The highest incidence of citrus canker (73.3%) and scab (66.6%) was recorded during the seconed week of September both diseases showed a positive correlation with temperatures, relative humadity and rain. The period from July to September was identified as the most conducive for the development of citrus canker and scab. The nursery should be provided with the required protection against these diseases during the period.

Citrus and Vegetable Seed Research Centre of BARI (1985) isolated *Colletotrichum*, *Diapporthe* and *Fusarium* as the causal agent of die-back in Bangladesh.

Bhatnagar and Prasad (1966) isolated *Fusarium solani* from twig die-back of lime (*Citrus aurantifolia*) and proved the fungus as the cause of the disease through artificial inoculations. The symptoms of the disease as observed by them were sudden dropping of leaves with withering tips of branches, yellowing and gradual die-back of infected branches mostly upto 6 to 8 inches.

Heavy damage of citrus due to die-back disease of an obscure etiology specially light and sandy soil in Florida was reported as the cause of citrus die-back (Burger, 1924). While, in Saint Lucia, West Indies, a die-back of lime trees characterized by yellowing and falling of the of leaves, casting fruits, browning of the young tips of the branches and subsequent extension to a main branch and drying up was associated with poor soil.

Calvin *et al.* (1986) stated that scab did not seem to establish well in Mediterranean climate or hot dry climates, but disease development was apparently favored in hot humid climates. The disease was more severe on sour orange (*Citrus aurantium*), lemon (*Citrus limon*), Temple orange (*Citrus reticulata* hybrid), Tangelo (*Citrus reticulata* x *Citrus paradisi*) and was less severe on grapefruit, Tahiti lime.

Chen *et al.* (2000) in Zhejiang, China evaluated some fungicide against citrus canker. They treated citrus canker affected plant with different concentrations of compounds including 56% cuprous oxide, Agro-streptomycine, 77% copper hydroxide (WP) and 50% shajunwang (WP). Eleven days after treatment the rate of infection of citrus canker (*Xanthomonas axonopodis* pv. *citri*) on leaves and fruit were investigated. They showed that the best treatment was 50% shajunwang which achieved up to 94.5% control.

Chowdhury (2009) studied on the effect of weather factors on the incidence and severity of scab disease of citrus seedling. Significant effect of temperature, relative humidity and rainfall on the incidence and severity of different diseases of citrus seedlings were observed. In case of scab disease of citrus seedlings, the highest incidence (57.53%) and severity (45.43%) were recorded during the month of July, 2007 at temperature, RH and rainfall of 28.87°C, 85.67% and 62.60 cm, respectively. On the other hand, lowest

incidence (21.12%) and severity (14.37%) were recorded during the month of April, 2008 at temperature, RH and rainfall of 28.53°C, 67.67% and 4.93 cm, respectively.

Chowdhury (2009) carried out trials to determine the effect of different management practices on the severity of scab disease of citrus seedlings. Considering the mean disease severity, the highest severity (56.33%) was observed in T_8 (untreated control), which was significantly different from all other treatments. On the other hand, the lowest severity (17.97%) was observed in T_5 (BAU Bio-fungicide applied in soil and top dressing @ 2%). In case of reduction of disease severity, the highest reduction (68.10%) of disease severity over control was observed in T_5 (BAU Bio-fungicide applied in soil and top dressing @ 2%) lowest reduction (29.93%) was observed in T_7 (BAU Bio-fungicide applied in soil @ 2% and Bavistin spray @ 0.2%).

Diware and Kolte (1990) observed that percent die-back rose with trees age from 8 to 20 years with the highest incidence (27.61%) in the 14 to 20 years age group.

Ebenzar *et al.* (1996) studied the effect of chemicals to prevent die-back of acid lime caused by *Colletotrichum gloeosporioides* in India (Tamil Nadu). They used 7 fungicides like Carbendazim, Mancozeb, Copper oxychloride, Zineb, Captafol, Bordeaux mixture and Aurafungin for testing their efficacy. All the fungicides reduce die-back of acid lime. The treatments which are used to control the disease with 1% Bordeaux mixture being the most effective followed by 0.1% carbendazim and 0.8% Bordeaux mixture.

Fantin and Kamati (1993) observed *Elsinoe austraiis* and *Elsinoe fawcetti* were the causal agents of citrus scab.

Ferguson (2002) described the symptoms, as scab was undoubtedly the ugliest disease of citrus, appearing as a rash of scabs or warts on leaves, twigs, and fruit. Since new generations of inoculum (infectious material) could be produced within five days, scab was a “compound-interest” disease that could spread rapidly, given the right conditions, from over wintering infections on leaves and stems. Water from rain, overhead irrigation and dew was, in fact,

the most important factor affecting disease development. Early stages of scab infection included well-defined, conical growths on one side of the leaf with a corresponding conical depression on the opposite side. These lesions might be occurred singly or grouped irregularly. The crests of these wart-like growths usually became covered with a scabby tissue ranging in color from pale to dark. Infected spots often run together and covered large areas with a corky, scab-like growth. Badly infected leaves and twigs became distorted and stunted. When fruit was infected at very young stage, it could become misshapen, with warty growths or projections.

Investigation in Zhejiang, China during 1993-1996, FU and XU (2001) showed that citrus canker (*Xanthomonas axonopodis* pv. *citri*) appeared in late April and early May; the rampart period being mid May to early June. Because higher the temperature, the earlier the occurrence. Spraying with different fungicides showed that a 500 fold solution of 77% copper hydroxide and 60% chlorothanil solution respectively, resulted in the efficient control of the disease.

Gade *et al*. (2005) reported that citrus was susceptible to *Phytophthora* spp. and the disease commonly spread through supply of plants from infected nurseries. Drenching with metalaxyl coupled with spraying of copper oxychloride effectively reduced seedling mortality (13.9%) and improved plant height (52.9 cm) and girth (3.2 cm).

Grace (2004) described that the symptoms of citrus scab which was appeared on young leaves. It was first as small, translucent spots, which rapidly became pustular. They were typically 2-3 mm in diameter and sometimes they coalesced. Color of the scab was pale rose and glossy to become chamois-beige later. Lesions developed on only one side of the leaf a feature that distinguishes citrus scab from citrus canker caused by *Xanthomonas campestris* pv. *citri.* Scab lesion development on fruit was similar to that on leaves, causing serious fruit deformities and premature fruit drop.

Hartmond *et al*. (2000) in Florida reported that citrus scab caused by the fungus *Elsinoe fawcetti* can occure on all varieties of citrus but it is of economic

importance for fruits production of Lemons, Temples, Page, Minneala, Taangelo and in some situations grape fruit. Citrus scab on foliage and shoots causes stunting of plants during seedling root stock production of rough lemon, sour orange, *Carizzo citroange*, trifoliate orange and Rangpur lime.

Hossain (2011) studied the nursery diseases of citrus in Bangladesh during the period of 2010-2011. He recorded canker, scab and die-back diseases in different citrus growing areas of Bangladesh.

Hossain (2007) conducted experiment in controlling die-back disease of citrus. He found die-back disease was less in seedling stage than older plants. Integrated application of MOC, cowdung and irrigation resulted die-back disease free healthy plants.

Hossain and Roy, (2006) isolated *Colletotrichum gloeosporioides*, *Fusarium solani*, *Botryodiplodia theobromae* as the causal agents of die-back of citrus.

Hossain and Roy, (2006) used different treatments viz. Mustard oil cake, (MOC), Urea, Tripple super phosphate (TSP), Murate of potash (MOP), cowdung and irrigation either alone or in different combination for the management of citrus diseases. They observed that irrigation has a strong effect in reducing the disease incidence in the field.

Huang and Huang (2002) reported that approximately 50% of citrus from Nannfengmiju trees were damaged by citrus scab (*Elsinoe fawcetti*). The main reasons were annual applications of multiple fungicides and unfavourable weather conditions (Much rainfall and high humidity and frost damage). Control was possible by spraying spring buds of 0.5 mm long with Bordeaux mixture followed by 2 sprayings of Bordeaux mixture between August and October.

Huang (1999) reported that citrus scab (*Elsinoe fawcetti*) attacked the expanded leaves of spring shoots of Satsuma mandarin and started attack the fruit-lets in mid-late May in Jiangxi, China. During the autumn, if the temperature and humidity are favoirable, it attacks the young shoots and fruit-lets causing up to 65.9-72.2% fruit-let drop.

Huang (1999) investigated the effects of fungicide against citrus scab caused by *Elsinoe fawcetti*. They used Bordeaux mixture or thiophanate methyl (as Topsin-M) for control of citrus scab on mandarin. In experiment on 8-year old pongan mandarins and poncirus trifoliate rootstock thiophanate methyl, mancozeb and jinmeng controlled citrus scab by 57%, 50.4% and 89.1% respectively. Again Huang (1999) reported that, spraying Bordeaux mixture at the bad break stage gave effective disease control.

Kale *et al.* (1994) conducted an experiment on the effects of foliar sprays to citrus canker of Kagozi lime caused by *Xanthomonas campestris* pv. *citri* in Maharashtra, India during July, 1988-Dec., 1990. They used 100 ppm of 7, 15 and 21 days and the most cost effective chemical control was achieved by spraying at 7 to 15 days.

Klotz (1948) observed that the disease was characterized by the killing back of twigs and small branches from a few inches to 2 ft or more. In severe cases, the infection often extended to the main twigs. The isolated species belonged to the genera of *Alternaria, Colletotrichum, Fusarium, Hormodendron* and *Stemphylium.*

The symptom of the diseases starts from the tips and extends some distance down may often become affected (Knorr *et al.*, 1957). Barks of the affected twigs develop a reddish-brown color and become dotted with small innumerable black structures which were the fruiting bodies of the fungus. They mentioned that *Dioplodia natalensis* pole Evans (syn. *Botryodiplodia theobromae*) was responsible for causing such die-back symptom. They also reported that wither-tip or die-back of citrus caused by *Gloeosporium limetticolum* and *Colletotrichum gloeosporioides* occurs on mature or senescent twigs and branches. Sometimes young twigs may be affected, particularly after damage from cold and drought. In general, the dying-back symptom progress slowly, leaves on the affected twigs turn yellow, wilt and drop, gumming may occure at the junction between the healthy and diseased tissues. Fruiting bodies of the fungi are found on the dead barks.

Koo and Reese (1977) have summarized the effects of irrigation on the exterior quality of fresh citrus fruit in four long-term field experiments in Florida's humid climate. Irrigation to maintain high soil moisture resulted in a higher proportion of green fruit in Valencia oranges and spring irrigation produced more green fruit in Temple oranges than fall irrigation and in lemon, more frequent irrigation resulted in fewer green fruit. Scab lesions were more prevalent in irrigated Temple oranges and Bearss lemon.

Kucharek and Whiteside (1983) conducted an experiment on citrus scab caused by *Elsinoe fawcetti*, in Florida. Scab might be more severe in groves located in flatwood areas than those planted on the ridge. The occurrence of scab was dependent upon available inoculum (spores) within the canopy of the tree or nearby trees. Apparently, scab pustules lost their capacity for spore production as they aged. Spore production was greatest in the morning following a night with heavy dew at 21-27°C. Two types of spores, colorless and colored, were produced within the acervulus. The colorless spore was dispersed by rain, overhead irrigation, nonfungicidal spray treatments, and perhaps to a limited extent by dew. This type spores were intolerant of even short periods of drying. The colored spore type could he dispersed by water or wind. Germination of spores needed a minimum wetting period of 3 hr and it occurred rapidly from 18-30°C with the optimum temperatures being 21-27°C. Temperatures below 21°C and above 27°C were inadequate for spore germination and subsequent infection. However, frequent or prolonged irrigation during this critical period greatly increased the chances for infection, thereby increasing the amount of inoculum produced to infect the fruit.

Miah and Fakir (1988) reported *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc., *Botryodiplodia theobromae* Pat, *Fusarium solani* (Mart.) Sacc., *Macrophoma mantegazziane* (Penz.), Berl & Voglino var. *limoni* Scalia, *Pestalotia* sp. and *Phomopsis cittri*. *Fawcetti* as the cause of citrus die-back in Bangladesh.

Nirvan (1961) reported that 43.2% of the cankered leaves exclusively owed the leaf miners infestation. Leaves affected by the miner and canker get distorted and usually drop off early.

Peralta (1949) reported a peculiar form of citrus die-back caused by *Colletotrichum gloeosporioides* from Malta. The disease was characterized by its rapid on-set, a complete absence of root symptoms and rapid death.

Qui and Ni (1988) in Fujian, China tested Monomolecular Film forming Substance and several fungicide treatments against citrus canker caused by *Xanthomonas campestris* pv. *citri*. The use of MMFS diluted 200, 300 and 500 times with water and each fungicide was applied at 5 kg/tree on 14 April (early flowering), 20 May (early fruiting) and 15 July (Mid-fruiting). They observed that all treatments gave good control of the disease on leaves, with 80% control by MMFS diluted 200 and 300 times. MMFS diluted 200 times was also effective in controlling the disease in fruits.

Ramakrishnan (1954) reported that young tissues of the plant are readily affected. In the nursery stages sweet oranges and other varieties also get infected by canker. High humidity, temperature between 20-35°C and the presence of moisture on the host surface for 20 minutes or more favor the incidence of the disease.

Ran *et al.* (2001) sprayed Mancozeb M-45, Carbendazim, Topsin-M (Thiophanate methyl) and Pyridaben in citrus orchard at different concentrations to control citrus disease and citrus rust mite. Spraying were applied at different stages. The results showed that the best control of citrus scab was achieved by spraying a 600 times solution of 80% M-45 when the shoots were 2 cm long, then twice more at 10 days intervals (total of 4 times) gave good control of citrus black spot. Also, spraying a 500-600 times solution of M-45 wettable powder gave good control of rust mite its effect could last for 30 days.

Randhawa (1970) has reported that nutrient imbalance in citrus plants can lead to adverse consequences and ultimately cause the die-back disease of citrus.

Rawal (2005) reported that the fungus *Sphaceloma fawcetti Jenkins* produced mostly the conidia stage (*Sphaceloma fawcetti Jenkins*) on the host. The pathogen perpetuated and survived in off-season as ascospores. Conidia were formed from 7-30°C with 66-100% humidity. The fungus could infect tissue only when the surface was wet, but prefers 16-23°C temperature. Its spread was through rains. In India, the disease was a problem in areas with low temperature and high humidity. The water was the most important factors influencing severity of the disease and could in feet fruits in summer as well as in spring.

Rawal and Saxana (1997) reported that anthracnose attacks the young leaves, shoots, blossoms and fruits of small acide limes. Young foliage and blossoms are blighted and distinct lesions formed on leaves and fruits. Affected fruits frequently drop prematurely. Wither tip is characterized by shedding of leaves and die-back of twigs. Leaves show light green spots which turn brown. On dead twigs, black dot like acervuli appear in concentric rings. The stem end of immature fruits results in fruit drop. In severe cases, branches show die-back and the tree dies in a few years.

Raychaudhuri *et al.* (1980) reported that numerous agents including greening virus were involved in the causal complex of the devastating citrus die-back diseases occurring in India. Broadbent *et al*. (1980) reported that citrus die-back was claimed to be caused by a mycoplasma like organism in Australia.

Reddy and Murti (1990) reported that canker infected leaves, twigs and branches constitute the source of inoculam to spread the disease from season to season. Since the infected leaves drop off early and the bacteria perish rapidly in the soil.

Senfu *et al.* (2004) conducted an experiment with 15-year-old Satsuma (Mandarin) trees. Fungicides used included 50% Xinling (Carbendazim + Mancozeb), 80% Bideli [of unstated composition], 20% Qingdaofu [of unstated composition], 50% Carbendazim, and 70% Mancozeb. Control of citrus scab (*Elsinoe fawcetti*) was highest (96%) with 600 times solution of 80% Bideli.

Sharma and Sharma (1969) identified certain toxic metabolites (S-metabolites) produced by *Colletotrichum gloeosporioides* causing citrus die-back, a widely prevailing diseases in the northern India.

Singh *et al*. (2005) evaluated five antagonists, i.e. *Trichoderma harzianum, T. viride, T. reesei* (*T. longibrachiatum*), *T. koningii* and *Epicoccum purpuurascens* (*E. nigrum*), against the most virulent isolate of *Elsinoe fawcetti* (Ef 20) *in vitro* using dual culture method and *in vivo* (with rough lemon, sweet orange and kinnow mandarin plants) for the management of citrus scab. The treatment of citru plants with the antagonists suppressed the disease development significantly.

Singh *et al.* (1998) conducted an experiment on the development of citrus scab. They collected a total of 50 scab-infected rough lemon leaves in the Indian Panjab to test the epidemiology and survival of *E. fawcetti.* Disease was shown to be initiated during August when the mean atmospheric temperature, relative humidity and rainfall were 29.8°C, 75% and 2.5 mm. respectively. A decrease in temperature and an increase in relative humidity in September favored the further development of the disease. The fungus was shown to survive on susceptible citrus species as dormant mycelium or conidia.

Singh *et al*. (1997) conducted a survey at Panjab in India and disease incidence recorded on 3 citrus species viz. rough lemon (*Citrus jambhiri*) 76.50-80.10%, sweet orange (*C. sinensis*) 10.8-20.30% and Kinnow (*C. nobiilus* × *C. deiicisa*) 46.25-81.07%. The highest disease incidence was recorded in the sitb-mounlarnous zone on rough lemon (81.10%) and Kinnow (80.07%) and the lowest incidence was recorded on sweet orange 10.80% in the arid irrigated zone.

Singh and Kapoor (1971) isolated *Colletotrichum gloeosporioides* from the diseased as well as healthy twigs of die-back affected citrus plants. In the pathogenicity test, they found the fungus most pathogenic to Kagzi lime among several citrus species including lemon.

Singh *et al.* (1971) observed that die-back of Kagzi lime and sweet orange were caused by fungi like *Colletotrichum gloeosporioides*, *Diplodia natalensis* (syn. *Botryodiplodia theobromae*).

Song *et al.* (1998) reported that citrus scab caused warty and scabby lesions on the surface of leaves, trees and twigs of mandarin cv. Satsuma. Warty lesions were mainly developed before July but scabby lesions developed during the summer season in Cheju Island, Korea republic. The causal organism of scab was morphologically identified as *Sphaceloma fawcetti* [*Elsinoe fawcetti*].

Talukder (1974) listed die-back or wither-tip caused by *Colletotrichum gloeosporioides* as one of the major disease of citrus occurring in Banladesh.

Thakore *et al.* (1999) tested some fungicides against die-back disease of citrus in Rajasthan. They treated die-back affected plant with Biltox-50 (Copper oxychloride), Dithane M-45 (Mancozeb) and Macuprex (Cupraneb+Bordeaux). They found all fungicides were effective against the disease. Then they use 500, 100 and 2000 ppm for each fungicide. They observed that 2000 ppm is most effective dose against the disease for all the fungicides.

Thind and Rawla (1961) reported that the disease was characterized by falling off of leaves from the top of the branches and the main axis. The twigs and stem parts were found to loose green color, die from top downwards, become, woody and white ashy membranous. Later on, black acervuli developed on these dead white membranous parts.

Timmer (2000) described citrus scab caused by *Elsinoe fawcetti* Bitencourt and Jenk. Syn. *Spheceloma fawcetti* Jenk. The telemorp of *Elsinoe fawcetti* in Brazil. The ascospores of *Elsinoe fawcetti* were 5-6 x 10-12 µm. Conidia of *Elsinoe* sp. were hyaline, one celled, elliptical, and 3-4 x 4-8 µm. The conidia were capable of reproducing by budding. In addition to the hyaline conidia, *Elsinoe fawcetti* produced colored, spindle shaped conidia on scab lesions which germinate to produce hyaline conidia. The pathogen had four pathotypes

viz. Florida Broad Host Range Pathotype, Florida Narrow Host Range Pathotype, Tryon's Pathotype and Lemon Pathotype.

Tripathi and Srivastava (1992) conducted an experiment on the effect of meteorological factors to the development of citrus scab. They found a correlation between the development of citrus scab disease (caused by *Sphaceloma fawcetti* [*Elsinoe fawcetti*]) in Srinagar, India, and the three most important environmental factors (humidity, rainfall and temperature). Although the disease on leaves and fruits appeared during summer (April-June), little progress was observed during these months. With the onset of the rainy season (July-September) the infection progressed well and was seldom exposed to severe conditions. A definite correlation was observed among the disease intensities on various cultivars of citrus and meteorological factors. The relative humidity of the area had a positive significant effect on the disease development; whereas rainfall showed a positive non-significant effect and temperature had a negative non-significant effect.

Verniere *et al.* (2003) studied the Asiatic citrus canker (ACC) and found expression of symptom depend on temperature where as relative humidity had no effect on disease suppression.

Whiteside (1990) conducted 3 years trial with 4 fungicides namely Dithianon and 2 sterol inhibiting fungicides, Diniconazole and Difenoconazole to evaluated them against citrus scab caused by *Elsinoe fawcetti* in Florida, USA. They showed that Dithianon performed better than copper fungicide treatments, recommended at the time of these field trials. However, difenoconazole usually gave better control of scab than diathianon. Where spray treatments were delayed until after some fruit had become infected, difenoconazole reduced scab severity even more than captafol (no longer available) because of its unique ability to inhibit the further development of existing pustules.

Zekri and Rouse (2002) reported citrus scab as corky outgrowths began on leaves, shoots and fruits as rounded pustules. Initially, scab lesions on fruit were consisted of slightly raised pink to light brown pustules. As these pustules developed, they become wart like, cracked, turn yellowish brown and

eventually dark gray. The outgrowths might be so numerous on fruit that it became distorted. Lesion on grape fruit and certain oranges could be confused with wind scar because they tend to be flatter than those on lemons, Satsuma mandarins, Temples, and sour oranges. This fungal disease could affect grapefruit, Temple orange, Murcott, tangelos, and some other tangerine hybrids.

Zhong and Ling, (2002) studied the occurrence of citrus canker disease *Xanthomonas axonopodis* pv. *citri* during 1995-1998. Results indicated that 15-20 days after bud burst, disease symptoms started to appear. Citrus canker occurrence had a close relationship with the daily mean temperature. When a daily temperature of 12°C occurred for 10-15 days, the spring shoots and fruit lets were found to be attacked. Experiments showed that spraying shoots with 77% kocide (Copper hydroxide) wettable powder at 20-30 days after bud burst and spraying summer-autumn shoots at 10-15 days after bud burst provided good citrus canker control.

Zhou *et al.* (2001) conducted an experiment to control citrus scab by chemical. They recorded that the most effective chemical control for preventing attack of young leaves by scab was spraying a copper and ammonium mixed solution in mid-late March (500 g Copper sulfate + 2.25 kg Ammonium Biocarbonate) and spraying three times a solution of 0.4-0.5% Jianganmycin at flowering stage.

CHAPTER III

MATERIALS AND METHODS

Four experiments were carried out throughout the study period in order to study the seedling diseases of citrus. The experiments were as follows:

I. Survey on the seedling diseases of citrus in selected nurseries of Bangladesh.
II. Identification of causal organisms of the seedling diseases of citrus.
III. Epidemiological survey on the disease prevalence of seedling diseases of citrus.
IV. Development of environment friendly disease management practices for the nursery diseases of citrus.

3.1. Experiment I. Survey on the diseases of citrus in selected nurseries of Bangladesh

3.1.1. Location of survey

Prevalence of diseases occurring on citrus seedlings raised in the selected nurseries were surveyed. The experiment was carried out in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari (Appendix I).

3.1.2. Selection of Nursery

The eight nurseries of four districts are surveyed:

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

3.1.3. Age and number of seedlings

The age and number (population) of the seedlings included for the survey are presented in Table 1.

Table1. Age of the citrus seedlings and total number of seedlings in selected eight nurseries from July, 2010 to April, 2012

Nurseries	Age of the seedlings (Years)	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, 2012)
Green orchid nursery Agargaon, Dhaka	1	40	60	60	45
Barisal nursery Savar, Dhaka	1	70	100	100	100
Gazipur nursery Gazipur	1	80	80	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	40	50	80	70
Ramghar nursery Ramghar, Khagrachari	1	60	80	80	70

3.1.4. Observation of the symptoms

Symptoms of the diseases were studied by visual observation. Sometimes hand lens were used for critical observation of the disease and sometimes a disease was identified based on matching the observed symptoms in the infected plants with the symptoms. Besides, the symptoms of the diseases were recorded

following the description of Amador, (2002); Ferguson, (2002) and Reddy and Murti, (1990). Identification of all the fungal diseases was finally confirmed by identification of the associated fungal organisms through isolation.

3.2. Experiment II: Identification of causal organisms

3.2.1. Collection of diseased specimen

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

3.2.2. Isolation of causal organisms was made by two methods as follows:

3.2.2.1. Moist blotter method

The pathogen associated with the diseased plant parts (leaves) were cut into several pieces by scissors and placed on the moist filter paper (Whatman no.1). Three pieces of filter paper were moistened by dipping in sterile water. The petridishes with the diseased specimens were incubated at 22 ± 2°C under 12/12 alternating cycles of NUV and darkness in the incubation room of the Seed Pathology Lab (SPL) for three to five days. After incubation the plates were examined under stereomicroscope for primary identification of the organisms (fungi). The fungi were transferred to PDA plates for proper sporulation and purification.

3.2.2.2. Preparation of Potato Dextrose Agar (PDA) media

200 g peeled potato chips and 500 ml of water were boiled together for 15 minutes so that the potato tissues were softened. Then the preapared pulp was sieved through a muslin cloth; 20 g of agar and 500 ml of water were heated together and a solution was made. Then 20 g of dextrose was added to it and 1000 ml of potato dextrose medium was prepared.

3.2.2.3. Preparation of Nutrient Agar (NA) media

Nutrient agar (28 g) was taken in the Erlenmeyer flask containing 1000 ml distilled water. For mixing properly the nutrient agar was shaken thoroughly for few minutes. 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 media was picked out from the autoclave and allowed to cool at room temperature in Laminar air flow cabinet until the temperature reach at approximately 50°C (feel warm in touch). Then the media was poured into sterilized glass petridishes for making NA plate and allowed to cool for solidification.

3.2.2.4. Agar plate method

At first the diseased plant parts (leaves) were thoroughly washed to remove soil and sand particles. Then infected plant parts were cut into 5 mm pieces from advancing end of the lesions. The cut portions were surface sterilized by dipping them in 0.001% $HgCl_2$ solution for 1.5 minutes and washed three times with sterile water and there after placed on PDA plates aseptically. The plates were incubated at 28°C ± 1°C for several days and examined daily for any fungal growth and continued for 10 days. After incubation period the inoculated PDA plates were observed to identify the causal organisms.

3.2.2.5. Bacteria isolation and identification

The bacterial strains isolated and collected from citrus leaf samples were identified using the following tests:

3.2.2.5.1. Gram staining test

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 smeared the bacteria on to the slide. The smear should be the just discernible. The thinly spreaded bacterial film was air dried without heat. For fixing the bacteria on glass slide, underside of glass slide was heated by passing it four times through a Bunsen flame, but does not over heated. Then the smear was

flooded with Crystal violet solution for 1 min. It was washed with tap water for a few seconds and excess water was removed by air. Then the smear was flooded with Iodine solution (Lugol's Iodine) for 1 min. 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 removed by air. Then glass slide was examined at 40x and 100x magnification using oil immersions. Strands of dark purplish color showed the bacterium Gram-positive and red color showed the bacterium Gram-negative (Gram H. C., 1884).

3.2.2.5.2. Starch hydrolysis test

For starch hydrolysis, bacteria from a well grown colony were inoculated on nutrient agar plate containing 0.2% soluble starch with the bacterium isolate to be tested. Then it was allowed to incubate at optimum temperature for at least 48 hr. After incubation the plate were flooded with indoles iodine. Blue color indicates no hydrolyis or negative (–ve), while a clear zone indicates hydrolysis or positive (+ve) result.

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. The times of data colletion were determined on the basis of variations in temperature, relative humidity and rainfall during the growing seasons.

3.3.2. Data collection during survey

During the survey in the nurseries, total numbers of citrus 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.

3.3.3. 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. Assessment of the disease incidence of citrus seedling was determined by the following formula:

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

Percent disease incidence (PDI) 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 (PDI) was determined by the following formula (Rai and Mamatha, 2005):

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

3.4. Experiment IV: Evaluation of Disease Management strategies for controlling the nursery diseases of citrus

3.4.1. Experimental site

The research work relating to determine the effective disease management strategies for controlling the citrus diseases was carried out at Sher-e-Bangla Agricultural University nursery, Dhaka-1207 (Appendix I).

3.4.2. Experimental period

The experiment was carried out from July 2010 to April 2012.

3.4.3. Preparation of nursery soil

The substratum was prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1 and sterilized with 5 ml formalin (40%) diluted with 200 ml water for 4 kg soil (Dashgupta,1988). 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 inocula. After 4 days of treatment, surface sterilized earthen pots were filled up with the sterilized soil.

3.4.4. Preparation of seedlings for Management trial

Air layering of lemon (Kagozi lemon) seedling was done in the month of June 2010. Shoots of 50-60 cm length from tip having 1.0 cm diameter were selected from healthy disease and insect free plants. The shoots were detached after 5 weeks i.e., in the first week of July, 2010 and planted in the earthen pots.

3.4.5. Treatments

For the management of nursery diseases of citrus seven different treatments were employed on the citrus (Kagozi lemaon) diseases. Three fungicides and one *Trichoderma* based preparation known as BAU-biofungicide were used. BAU-biofungicide reported to be effective against different disease of citrus was selected for management experiment. Fungicide solutions were prepared

separately by taking requisite amount of fungicides for each dose. The fungicides were applied as foliar spray at 30 days interval by a hand sprayer except BAU-biofungicide. BAU-biofungicide was applied as foliar spray and also used as soil treatment with specific dose. Normal tap water was used for control. The treatments were as follows:

Treatments		Dose used
T_1	BAU- Biofungicide was applied in the soil at the time of pot preparation.	2%
T_2	BAU- Biofungicide was applied as foliar spray.	2%
T_3	BAU- Biofungicide was applied in the soil as well as foliar spray.	2%
T_4	Mancozeb was applied as foliar spray.	0.2%
T_5	Bavistin was applied as foliar spray.	0.2%
T_6	Cupravit was applied as foliar spray.	0.2%
T_7	Untreated control.	No treatment was used

The detailed of BAU Bio-fungicide and fungicides used are as follows:

A. BAU Bio-fungicide

A formulated product of *Trichoderma harzianum*, developed by Prof. Dr. Ismail Hossain, Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh (Hossain, 2003).

B. Fungicide

Sl. No.	Fungicide	Chemical name	Active ingredient
1.	Mancozeb	Ethelene bisdithiocarbamate(EBDC)	Zineb & Maneb
2.	Bavistin 50 WP	Mythyl-2-Benzimidazole Carbamate	50% Carbendazim
3.	Cupravit 50 WP	Copper-oxychloride ($CuOCl_2$)	Copper

3.4.6. Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with five replications.

3.4.7. Soil treatment with bio-agent

BAU-biofungicide was thoroughly mixed with the soil @ 6.4 g/m^2 soil (Lo *et al.*, 1996). Seedlings of citrus were planted in the earthen pot after 7 days of soil treatment. Observations were made on citrus canker, citrus scab, die-back etc.

3.4.8. Spraying of fungicide

Fungicide solutions were prepared separately by taking requisite amount of fungicides for each dose. The fungicides were sprayed at 30 days interval by hand sprayer. Precautions were taken with polythene barrier to avoid drifting of spray materials from plant to neighboring plants.

3.4.9. Spraying of BAU-biofungicide as foliar spray

Spraying of seedlings with BAU-biofungicide was done at one month interval of the experimental period of 12 months. The data on incidence, severity and plant height were collected at one month interval before each spray schedule.

3.4.10. Assessment of disease incidence, severity, % disease reduction over control and % increase of height over first count

Assessment of disease incidence and severity was calculated using the following formula described in experiment 3.3.3.

Percent disease reduction (PDR) was calculated using the formula of Rai and Mamatha (2005) as:

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

Percent height increase/decrease over first count was calculated using the formula:

$$\% \text{ Height increase/decrease over first count} = \frac{\text{Height at final count} - \text{Height at first count}}{\text{Height at final count}} \times 100$$

3.4.11. Data collection

The data were recorded on the following parameters at an interval of 30 days as shown below:

a) Height of the seedlings (cm)
b) Total number of leaves/plant
c) Number of diseased leaves/plant
d) Percent diseased leaves/plant for different diseases viz. canker, scab and die-back of citrus
e) Percent leaf area diseased/plant for different diseases and
f) Number of branches/plant.

3.5. Meteorological data collection

Generalized environmental variables were selected for their effect on the incidence and severity of diseases of citrus. Day to day meteorological data on temperature, relative humidity and rainfall were collected from Meterological Department, Agargaon, Dhaka-1207. The data taken were analyzed and calculated for monthly mean of minimum and maximum temperature, mean of minimum and maximum relative humidity, and mean of minimum and maximum rainfall throughout the study period July, 2010 to April, 2012 of the respective locations.

Table 2. Average temperature, relative humidity and rainfall of Dhaka, Gazipur, Barisal and Khagrachari from July, 2010 to April, 2012

Month	Average temp. (°C)	Mean average temp. (°C) of 2 years	Average relative humidity (%)	Mean average relative humidity (%) of 2 years	Average rainfall (cm)	Mean average rainfall (cm) of 2 years
July, 2010	29.65	30.08	81.40	82.85	7.55	6.53
July, 2011	30.50		83.50		5.50	
Oct., 2010	28.63	29.07	80.20	80.85	7.13	6.54
Oct., 2011	29.50		81.50		5.95	
Jan., 2011	16.88	17.67	73.80	74.9	0.52	0.56
Jan., 2012	18.46		76.0		0.60	
April, 2011	20.50	21.0	70.43	70.72	3.85	3.68

April, 2012	21.50		71.0		3.50	

3.6. Statistical 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. Correlation and regression analysis were performed to find out the effect of different climatic factors, viz. temperature, relative humidity and rainfall on the incidence and severity of seedling diseases of citrus.

CHAPTER IV

RESULTS

4.1. Survey on Nursery Diseases of citrus

Four different diseases viz. Canker, Scab, Die-back and citrus greening were recorded in the survey conducted in eight nurseries of Dhaka, Gazipur, Barisal and Khagrachari districts.

4.2. Symptom of the diseases and identification of the pathogen

4.2.1. Canker of citrus

Observed plants infected with citrus canker had characteristic lesions on leaves, stems and twigs with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. It first appeared as small spots surrounded the tissue with a convex surface. With the advancement of diseases, the surface of the spots became white or grayish and finally ruptured as pustules. The size of the pustules varies in size and shape.

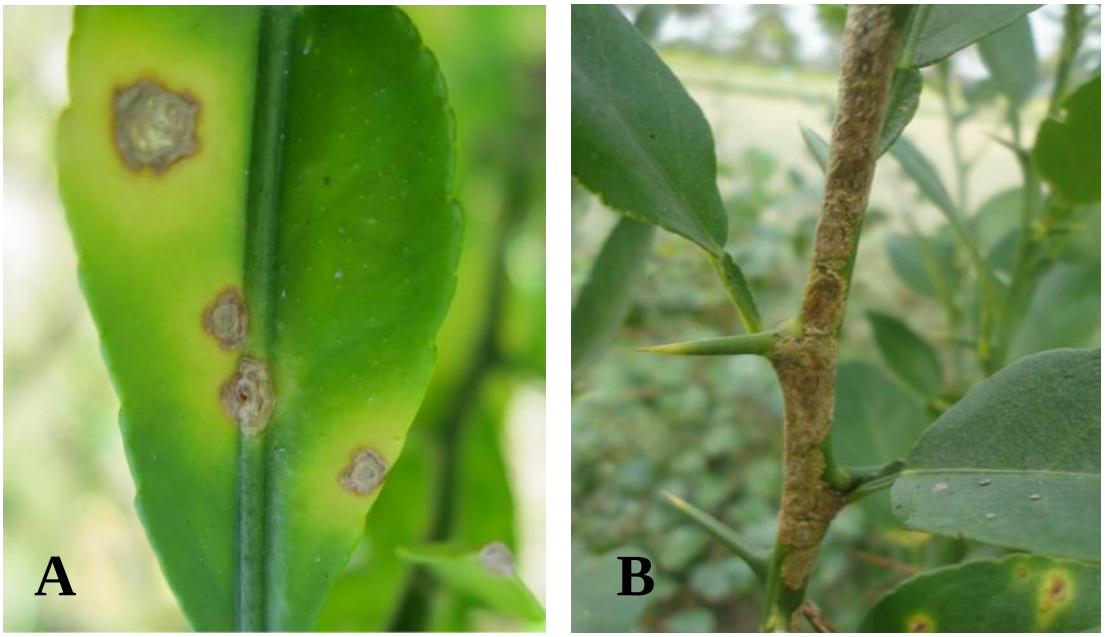
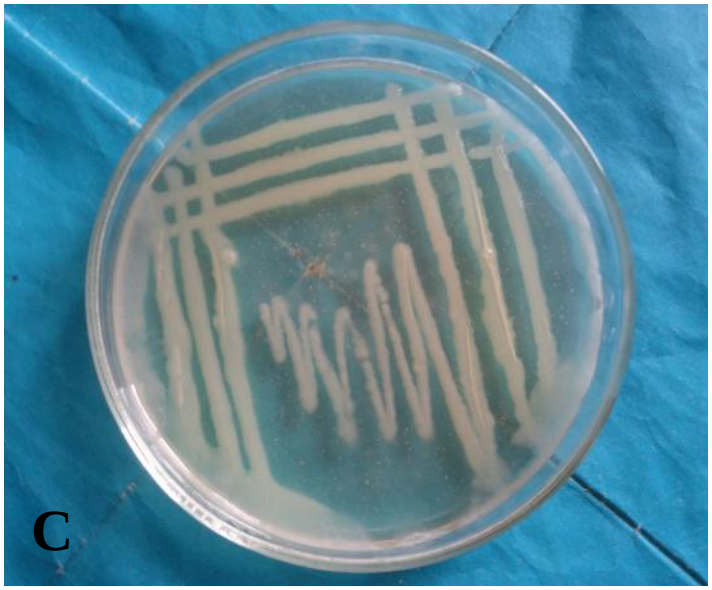
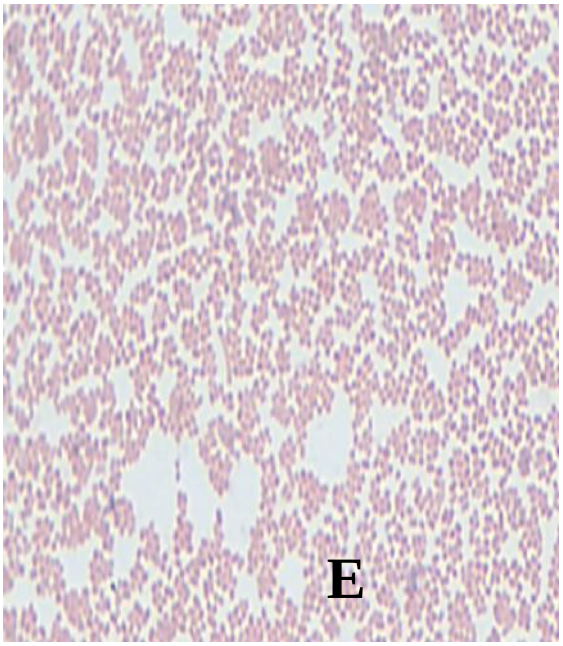


Plate 1. Canker symptoms of citrus seedling
A: Leaf, **B**: Twig

The pathogen isolated from the diseased symptom was identified as *Xanthomonas* sp. (plate 1 C). Bacterial strains isolated from leaf were used for gram staining, starch hydrolysis and inoculation test. Gram staining test reveals dark purplish color of the bacteria (plate 1 D). Development of blue-black with a clear zone around the growth indicates starch hydrolysis (Amylase activity) test (plate 1 E).



D



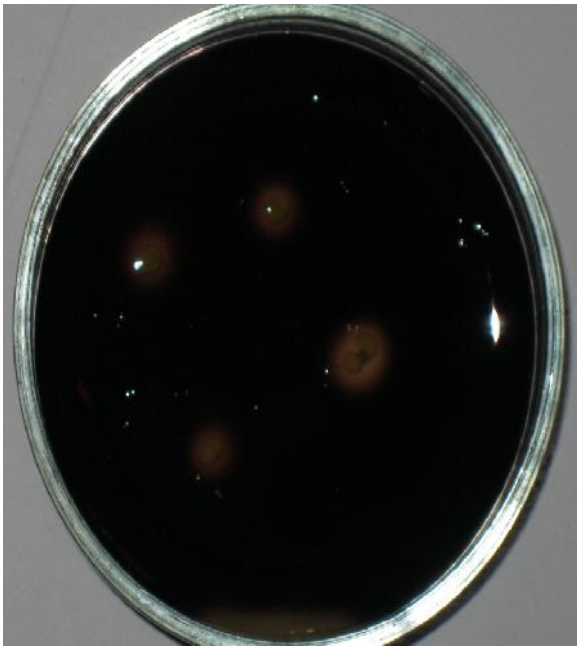


Plate 1. C. Pure culture of *Xanthomonas* sp. on Nutrient Agar medium, **D**. Gram staining (40x) and **E**. Starch hydrolysis tests of *Xanthomonas* sp.

4.2.2. Scab of citrus

Small, semi transluscent dots were found on the lower surface of leaves that becames well defined pustules. The lesions mostly appeared singly on one side of leaves. The opposite surface corresponding to warty growth showed a circular depression with a pink to red depression. The affected leaves became distorted, wrinkled, stunted and prematurely sheded. Mycelium of *Elsinoe fawcetti* was formed in pure culture (plate 2 B). The fungus identified as the conidial stage of *Sphaceloma fawcetti* and conidia was hyaline, single celled (plate 2 C).

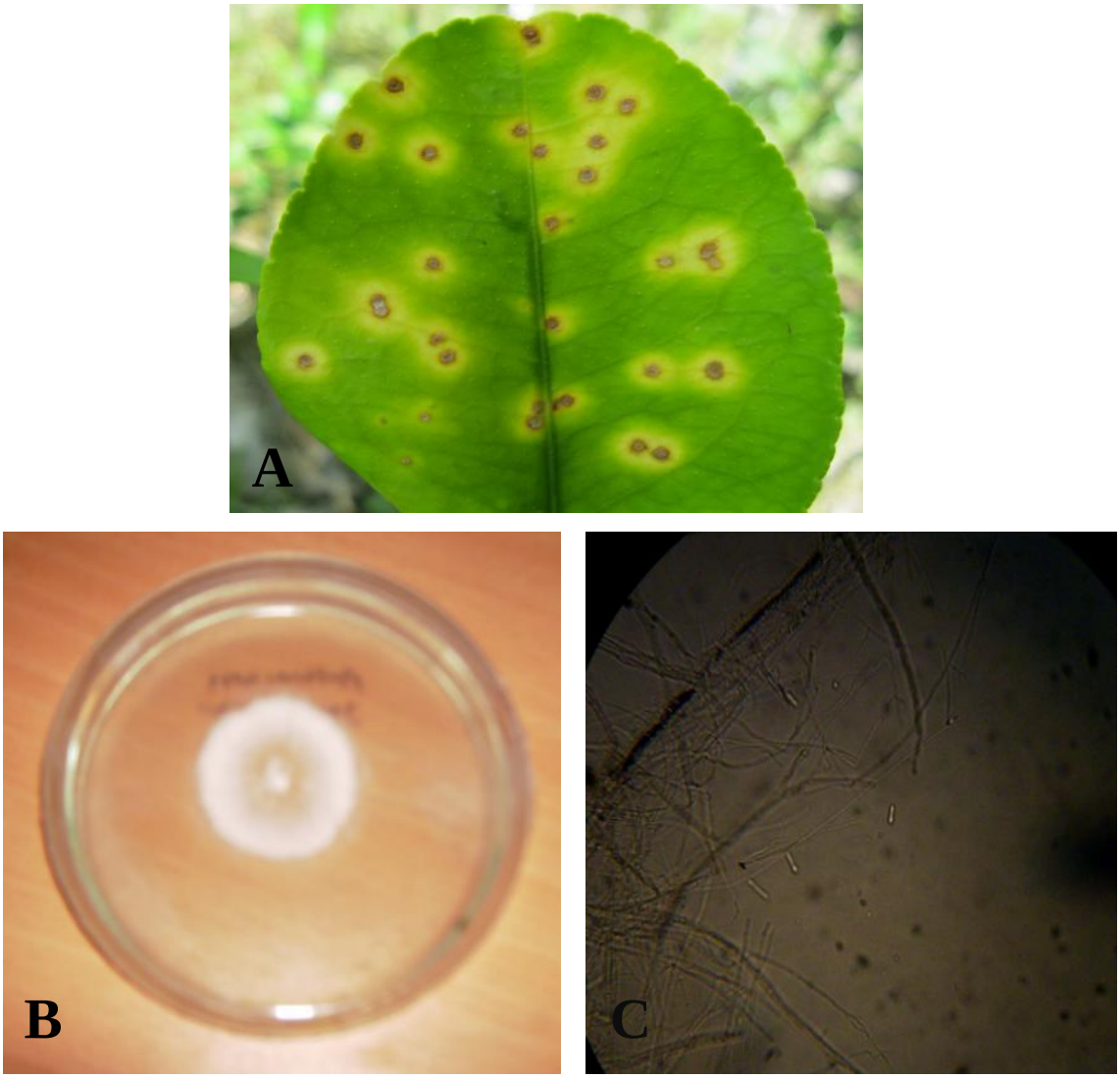


Plate 2. A. Scab symptom on citrus leaf, **B**. Pure culture on PDA medium and **C**. Conidia of *Elsinoe fawcetti* (*Sphaceloma fawcetti*)

4.2.3. Die-back of citrus

The disease symptoms was characterized as die-back of twigs in large numbers proceeded by the shedding of leaves. In the preliminary stage of the disease the young and small branches of the plants was died from the top to backward. Wilting of foliage was also seen. The first indication of blighted trees was dulling of the foliage followed by wilting. Symptoms appeared throughout the canopy.

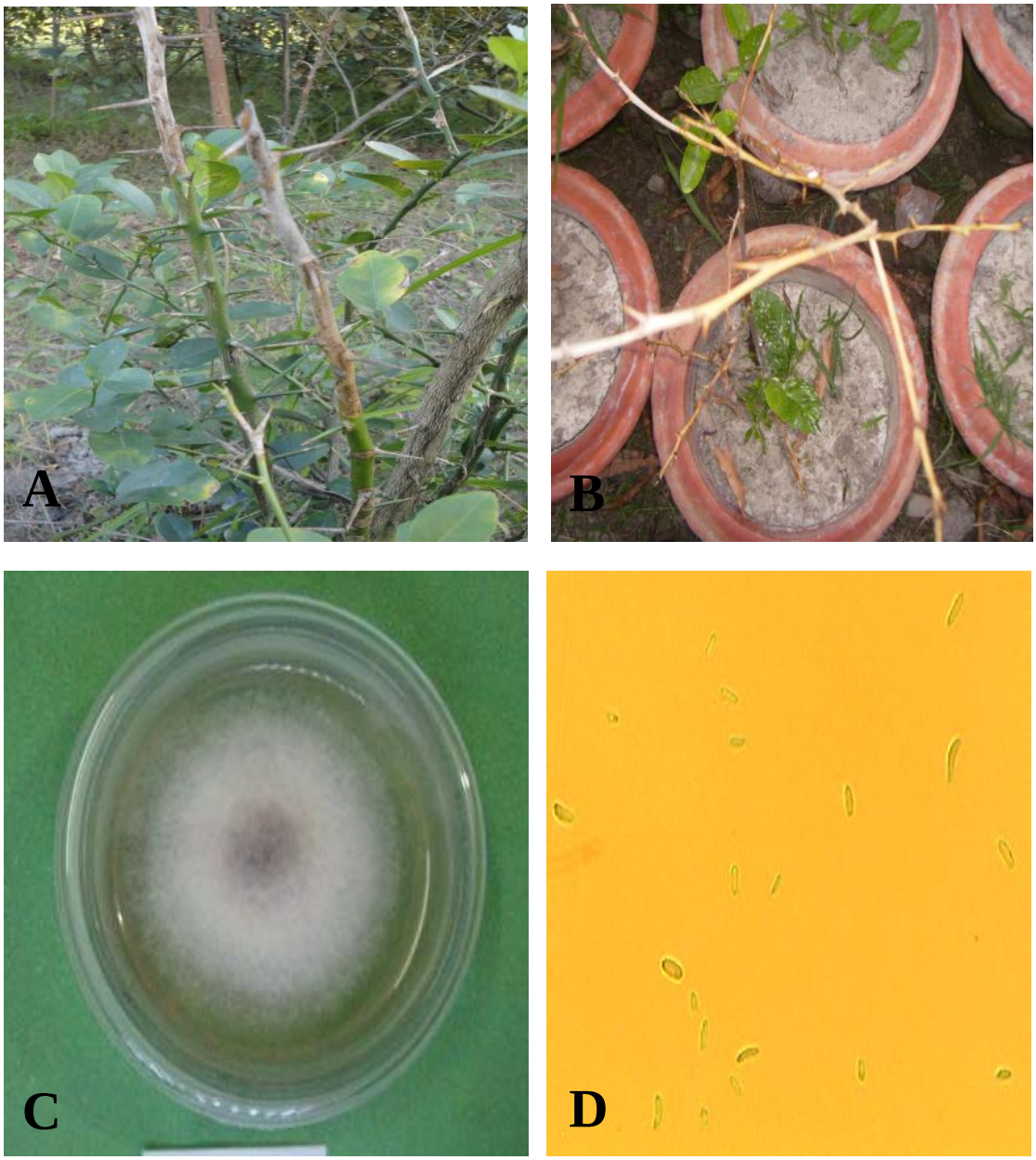


Plate 3. A & **B**. Die-back symptom on citrus seedlings; **C**. Pure culture on PDA medium and **D**. Conidia of *Colletotrichum gloeosporioides*

4.2.4. Citrus greening

The leaves of the plants were yellow and this was observed on the limb or sector of the tree canopy. Vein yellowing or a blotchy mottlling of all or part of the leaf blade was observed. Sometimes yellow area on the one side of the midvein and dark green area directly opposited was observed (Fig. 1).

Figure 1. Greening symptom on citrus leaves

4.3. Epidemiology of disease incidence and severity

4.3.1.1. Incidence and severity of canker of citrus at different experimental locations of Bangladesh during July, 2010 to April, 2012

Incidence of canker of citrus varied from location to location and that ranged from 40.69-48.18% (Table 3). The highest incidence was recorded at Khagrachari and the lowest was recorded at Dhaka. The severity of canker of citrus also varied from location to location and that ranged from 13.82-17.33% (Table 3). The highest severity was recorded at Khagrachari and the lowest was recorded at Dhaka.

Table 3. Incidence and severity of canker of citrus at different locations of Bangladesh from July, 2010 to April, 2012

Location	Canker of citrus	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka	40.69 a	13.82 c
Gazipur	44.72 a	14.64 bc
Barisal	46.39 a	15.72 b
Khagrachari	48.18 a	17.33 a
$LSD_{(p\geq 0.05)}$	9.880	1.413
CV%	8.87	6.05

Each data represents the mean value of three nurseries of two years

4.3.1.2. Incidence and severity of canker of citrus in different growing seasons of Bangladesh during July, 2010 to April, 2012

Incidence of canker of citrus varied significantly from July, 2010 to April, 2012 and that ranged from 30.97-60.83% (Table 4). The highest (60.83%) incidence was recorded in July (2010 & 2011) and the lowest (30.83%) was observed in the month of January (2011 & 2012). The severity of canker of citrus, varied significantly from July, 2010 to April, 2012 and that ranged from 6.86 - 24.09%. The highest (24.09%) severity was recorded in the month of July (2010 & 2011) and the lowest (6.86%) was observed in the month of January (2011 & 2012).

Table 4. Incidence and severity of canker of citrus during July, 2010 to April, 2012 of Bangladesh

Time of data collection	Canker of citrus	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
July	60.83 a	24.09 a
October	39.29 c	11.22 c
January	30.97 d	6.86 d
April	48.89 b	19.33 b
$LSD_{(p\geq 0.05)}$	6.723	1.567
CV%	8.87	6.05

Each data represents the mean value of three nurseries of two years

4.3.1.3. Incidence and severity of canker of citrus during different growing seasons at different experimental location

Incidence of canker of citrus varied significantly from season to season as well as location to location and that ranged from 28.33-70.0% (Table 5). The highest (70.0%) incidence of canker of citrus recorded in the month of July (2010 & 2011) at Khagrachari. The lowest (28.33%) incidence was observed in the month of January (2011 & 2012) at Dhaka. Statistically the similar incidence (28.89%) was recorded in the month of January (2011 & 2012) at Gazipur. The severity of canker of citrus also varied significantly from season to season as well as location to location and that ranged from 5.55-27.11%. The highest (27.11%) severity of canker of citrus observed in the month of July (2010 & 2011) at Khagrachari followed by in the month of April (2011 &

2012) at Barisal (20.0%) while the lowest severity (5.55%) was recorded in the month of January (2011 & 2012) at Dhaka.

Table 5. Incidence and severity of canker of citrus during July, 2010 to April, 2012 at different experimental locations of Bangladesh

Location	Time of data collection	Canker of citrus	
		% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka.	July	54.44 bc	21.50 cd
	October	32.78 fg	10.89 fg
	January	28.33 g	5.55 i
	April	47.22 cd	17.33 e
Gazipur	July	55.55 b	23.11 bc
	October	43.33 de	10.66 g
	January	28.89 g	6.55 hi
	April	51.11 bc	18.22 e
Barisal	July	63.33 a	24.66 b
	October	38.89 ef	10.89 fg
	January	33.89 fg	7.33 h
	April	49.44 bcd	20.0 d
Khagrachari	July	70.0 a	27.11 a
	October	42.16 de	12.44 f
	January	32.78 fg	7.99 h
	April	47.77 cd	21.77 c
$LSD_{(p\geq 0.05)}$		6.723	1.567
CV(%)		8.87	6.05

Each data represents the mean value of three nurseries of two years

4.3.1.4. Effect of weather components on the incidence and severity of canker of citrus seedling during July, 2010 to April, 2012

The incidence of canker of citrus was influenced by average temperature, relative humidity and rainfall. The highest incidence (60.83%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, lowest incidence (30.97%) was recorded in January (2011 & 2012) having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm, respectively. In the month of April (2010 & 2011) the incidence was 48.89% when the temperature, relative humidity and rainfall were 21.0°C, 70.72% and 3.68 cm, respectively and in the month of October (2010 & 2011) the incidence was 39.29% while the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively.

The severity of canker of citrus was influenced by average temperature, relative humidity and rainfall. The highest severity (24.09%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, lowest severity (6.858%) were recorded in January (2011 & 2012) having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm, respectively. In the month of April (2010 & 2012) the severity was 19.33% when the temperature, relative humidity and rainfall were 21.00°C, 70.72% and 3.68 cm, respectively and in the month of October (2010 & 2011) the severity was 11.22% while the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively (Fig. 2).

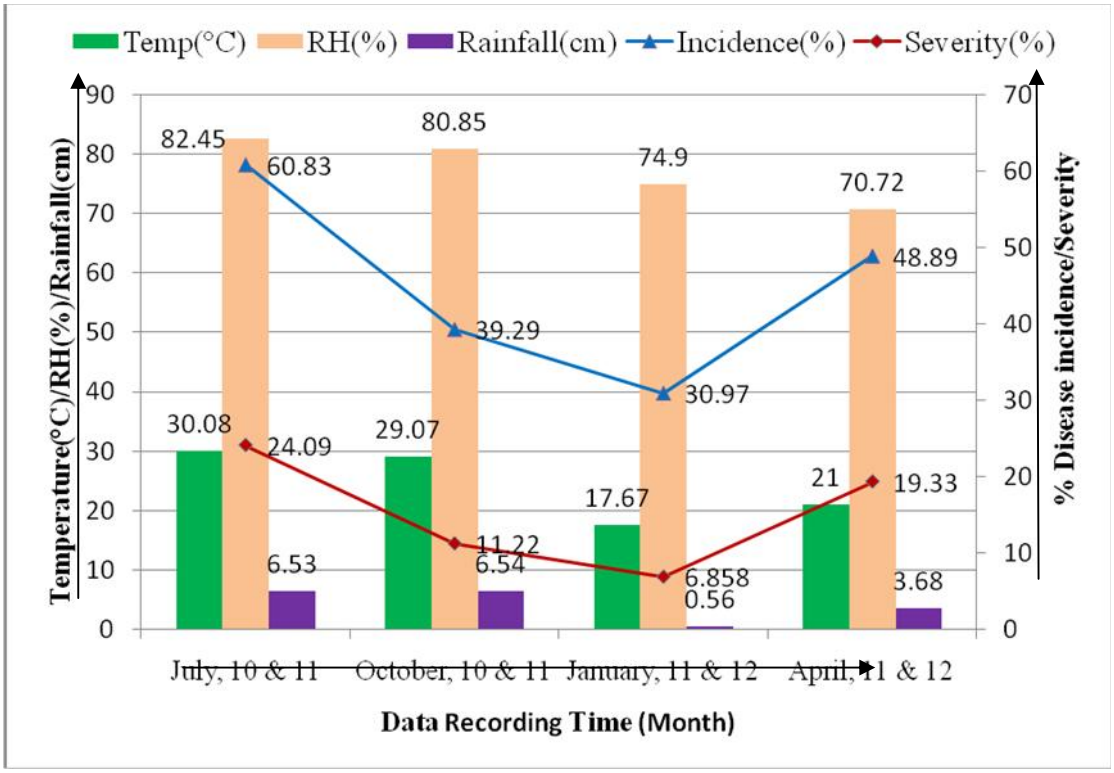


Figure 2. Effect of weather components on the incidence and severity of canker of citrus seedling during July, 2010 to April, 2012

4.3.1.5.a. Relation between canker disease incidence as well as severity of citrus seedlings and temperature

A positive correlation between canker disease incidence and severity with temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y= 1.302x + 13.14 (R^2 =0.379), where x = temperature and y = disease incidence. Here, the R^2 value

indicates that the contribution of temperature was 37.9% on the incidence of canker of citrus. On the other hand, the relationship between disease severity and temperature could be expressed by the equation Y= 0.666x – 0.935 (R^2 =0.272), where x = temperature and y = disease severity. Here, the R^2 value indicates that the contribution of temperature was 27.2% on the severity of canker of citrus.

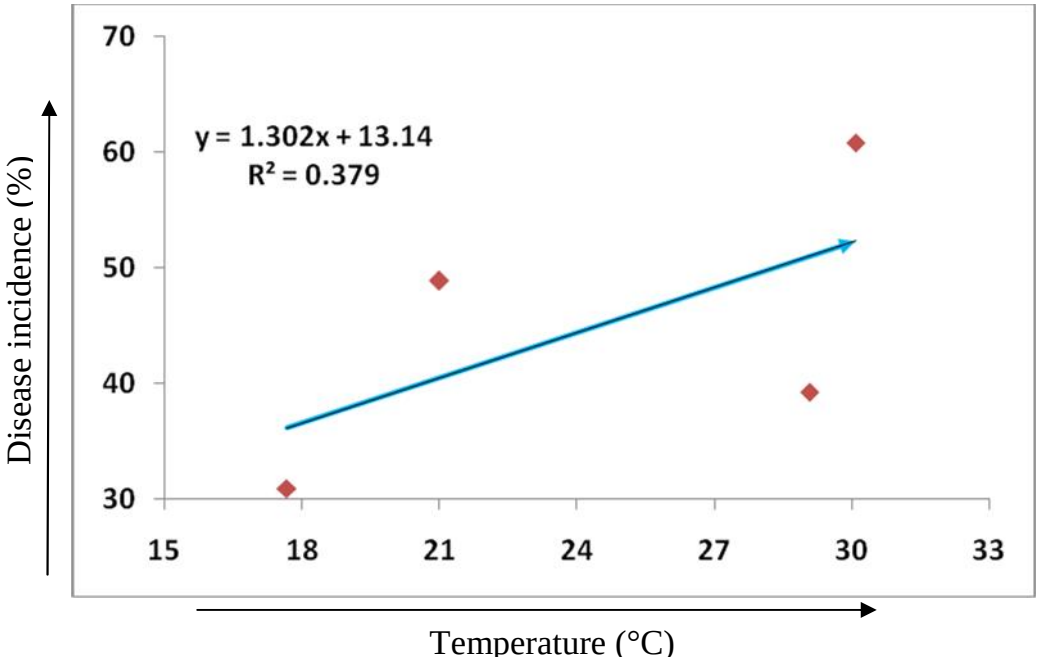


Figure 3. Linear regression analysis of the effect of temperature on the incidence of canker of citrus

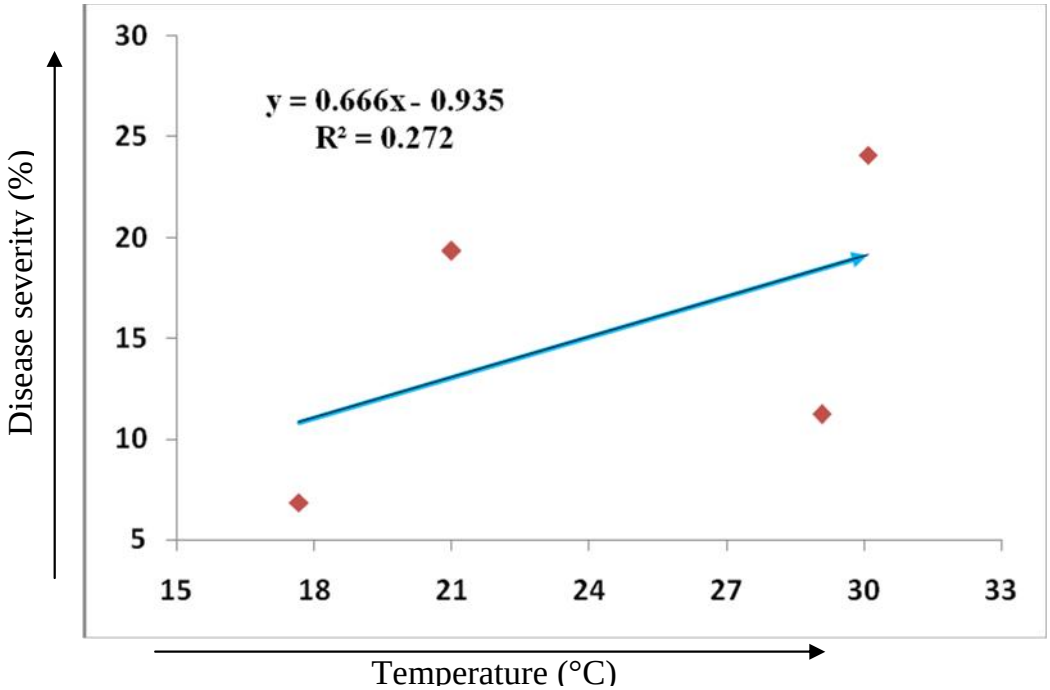


Figure 4. Linear regression analysis of the effect of temperature on the severity of canker of citrus

4.3.1.5.b. Relation between canker disease incidence as well as severity of citrus seedlings and relative humidity

A positive correlation between canker disease incidence and severity with relative humidity was observed. The relationship between disease incidence and relative humidity could be expressed by the equation Y= 0.786x – 15.73 (R^2 =0.110), where x = relative humidity and y = disease incidence. Here, the R^2 value indicates that the contribution of relative humidity was 11.0% on the incidence of canker of citrus. On the other hand, the relationship between disease severity and relative humidity could be expressed by the equation 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 relative humidity was 15.2% on the severity of canker of citrus.

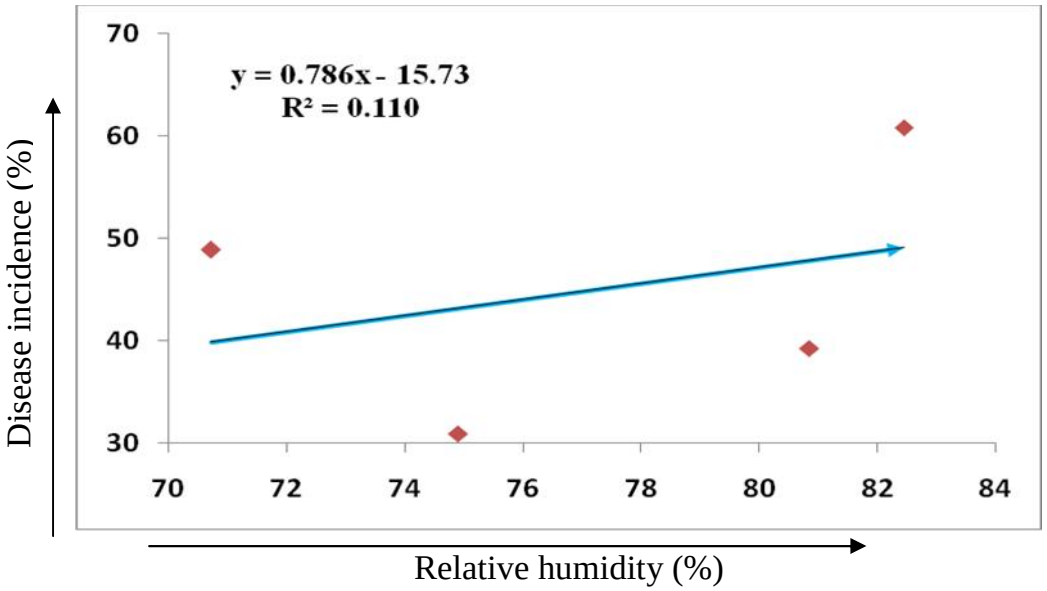


Figure 5. Linear regression analysis of the effect of relative humidity on the incidence of canker of citrus

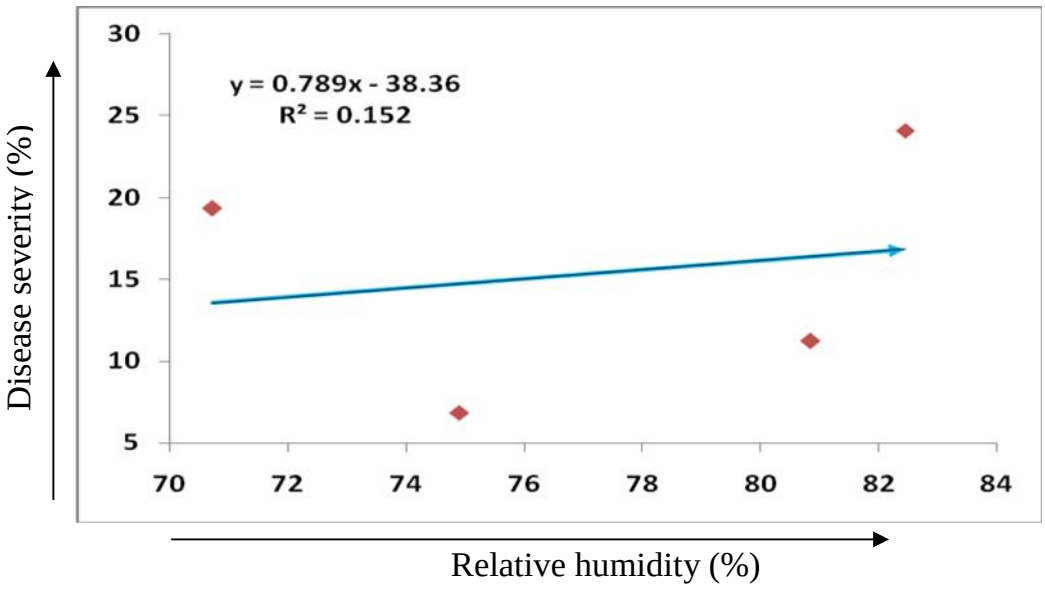


Figure 6. Linear regression analysis of the effect of relative humidity on the severity of canker of citrus

4.3.1.5.c. Relation between canker disease incidence as well as severity of citrus seedlings and rainfall

A positive correlation between canker disease incidence and severity with rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y= 2.979x + 32.10 (R^2 =0.436), where x = rainfall and y = disease incidence. Here, the R^2 value indicates that the contribution of rainfall was 43.6% on the incidence of canker of citrus. On the other hand, the relationship between disease severity and rainfall could be expressed by the equation Y= 1.622x + 8.352 (R^2 =0.353), where x = rainfall and y = disease severity. Here, the R^2 value indicates that the contribution of rainfall was 35.3% on the severity of canker of citrus.

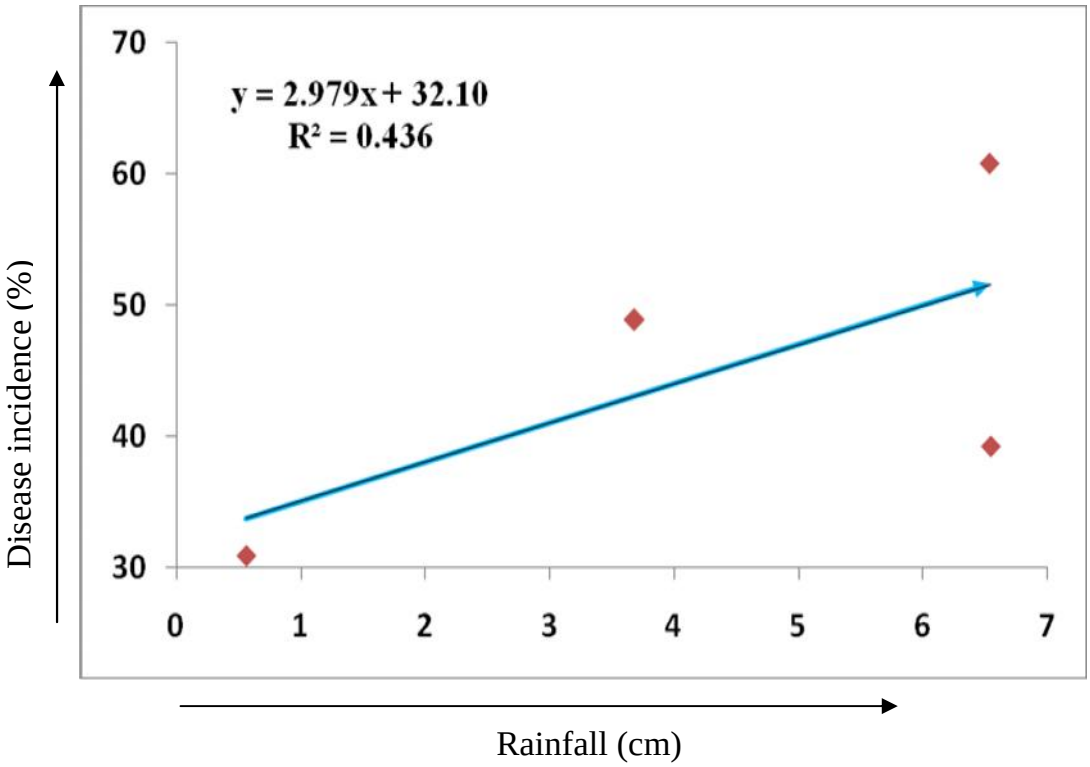


Figure 7. Linear regression analysis of the effect of rainfall on the incidence of canker of citrus

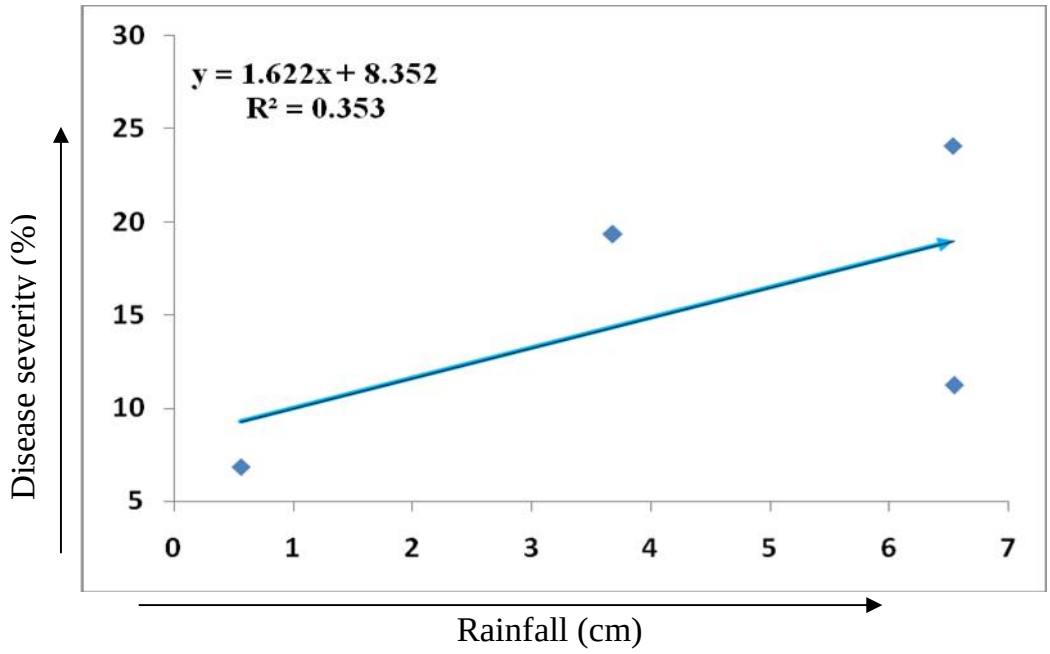


Figure 8. Linear regression analysis of the effect of rainfall on the severity of canker of citrus

4.3.2.1. Incidence and severity of Scab of citrus at different experimental locations of Bangladesh during July, 2010 to April, 2012

Incidence of scab of citrus varied from location to location and that ranged from 51.38-60.83% (Table 6). The highest incidence was recorded at Khagrachari and the lowest was recorded at Barisal. The severity of scab of citrus also varied from location to location and that ranged from 19.83-26.33%. The highest severity (26.33%) was recorded at Khagrachari and the lowest (19.83%) was recorded at Dhaka.

Table 6. Incidence and severity of scab of citrus at different locations of Bangladesh from July, 2010 to April, 2012

Location	Scab of citrus	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka	52.50 b	19.83 c
Gazipur	60.27 a	23.33 b
Barisal	51.38 b	21.00 bc
Khagrachari	60.83 a	26.33 a
$LSD_{(p\geq 0.05)}$	7.157	2.743
CV%	6.30	4.66

Each data represents the mean value of three nurseries of two years

4.3.2.2. Incidence and severity of scab of citrus in different growing seasons of Bangladesh during July, 2010 to April, 2012

Incidence of scab of citrus varied significantly from July, 2010 to April, 2012 and that ranged from 34.16-77.08% (Table 7). The highest incidence was recorded in July (2010 & 2011) and the lowest disease incidence was observed in the month of January (2011 &2012). The severity of scab of citrus, varied significantly from July, 2010 to April, 2012 and that ranged from 7.72-33.83%. The highest severity was recorded in the month of July (2010 & 2011) and the lowest disease severity was observed in the month of January (2011 & 2012).

Table 7. Incidence and severity of scab of citrus during July, 2010 to April, 2012 of Bangladesh

Time of data collection	Scab of citrus	
	% Disease Incidence	% Disease Severity

	(July, 2010-April, 2012)	(July, 2010-April, 2012)
July	77.08 a	33.83 a
October	50.00 c	22.66 c
January	34.16 d	7.72 d
April	63.75 b	26.27 b
$LSD_{(p\geq 0.05)}$	5.976	1.778
CV%	6.30	4.66

Each data represents the mean value of three nurseries of two years

4.3.2.3. Incidence and severity of scab of citrus during different growing seasons at different experimental location

Incidence of scab of citrus varied significantly from season to season as well as location to location and that ranged from 32.78-86.11% (Table 8). The highest incidence was recorded in the month of July (2010 & 2011) at Khagrachari followed by in the month of July (2010 & 2011) at Gazipur (82.78%). The lowest incidence was observed in the month of January (2011 & 2012) at Dhaka followed by in the month of January (2011 & 2012) at Barisal (33.33%). The severity of scab of citrus also varied significantly from season to season as well as location to location and that ranged from 7.33-40.22%. The highest severity (40.22%) was observed in the month of July (2010 & 2011) at Khagrachari followed by in the month of April (2011 & 2012) at Gazipur (28.66%). The lowest severity was observed in the month of January (2011 & 2012) at Dhaka followed by in the month of January (2011 & 2012) at Barisal (7.55%).

Table 8. Incidence and severity of scab of citrus during July, 2010 to April, 2012 at different experimental locations of Bangladesh

Location	Time of data collection	Scab of citrus	
		% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka.	July	71.11 b	29.77 cd
	October	43.89 f	19.55 g
	January	32.78 g	7.33 h
	April	62.22 cd	22.66 f
Gazipur	July	82.78 a	34.00 b
	October	53.89 e	23.55 f
	January	35.00 g	7.11 h
	April	69.44 b	28.66 d
	July	68.33 bc	31.33 c

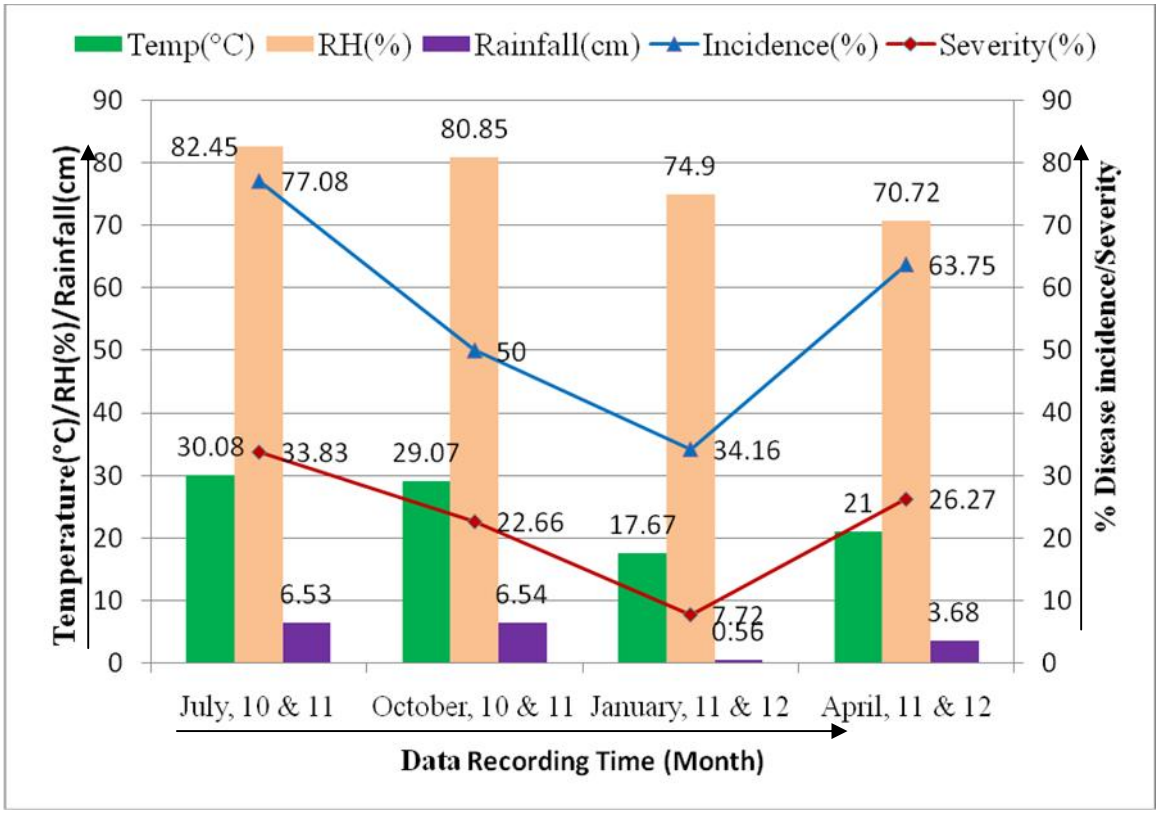
Barisal	October	46.66 f	22.22 f
	January	33.33 g	7.55 h
	April	57.22 de	22.89 f
Khagrachari	July	86.11 a	40.22 a
	October	55.55 e	25.33 e
	January	35.55 g	8.89 h
	April	66.11 bc	30.89 c
$LSD_{(p\geq0.05)}$		5.976	1.778
CV(%)		6.30	4.66

Each data represents the mean value of three nurseries of two years

4.3.2.4. Effect of weather components on the incidence and severity of scab of citrus seedling during July, 2010 to April, 2012

The incidence of scab of citrus was influenced by average temperature, relative humidity and rainfall. The highest incidence (77.08%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, lowest incidence (34.16%) was recorded in January (2011 & 2012) having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm, respectively. In the month of October (2010 & 2011) the incidence was 50.0% when the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively and in the month of April (2011 & 2012) the incidence was 63.75% while the temperature, relative humidity and rainfall were 21.0°C, 70.72% and 3.68 cm, respectively.

The severity of scab of citrus was influenced by average temperature, relative humidity and rainfall. The highest severity (33.83%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, lowest severity (7.72%) was recorded in January (2011 & 2012) having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm, respectively. In the month of October (2010 & 2011) the severity was 22.66% when the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively and in the month of April (2011 & 2012) the severity was 63.75% while the temperature, relative humidity and rainfall were 21.0°C, 70.72% and 3.68 cm, respectively.



Each data represents the mean value of three nurseries of two years

Figure 9. Effect of weather components on the incidence and severity of scab of citrus seedling during July, 2010 to April, 2012

4.3.2.5.a. Relation between scab disease incidence as well as severity of citrus seedlings and temperature

A positive correlation between scab disease incidence and severity with temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y= 1.914x + 9.439 (R^2 =0.399), where x = temperature and y = disease incidence. Here, the R^2 value indicates that the contribution of temperature was 39.9% on the incidence of scab of citrus. On the other hand, the relationship between disease severity and temperature could be expressed by the equation Y= 1.368x – 10.83 (R^2=0.575), where x = temperature and y = disease severity. Here, the R^2 value indicates that the contribution of temperature was 57.5% on the severity of scab of citrus.

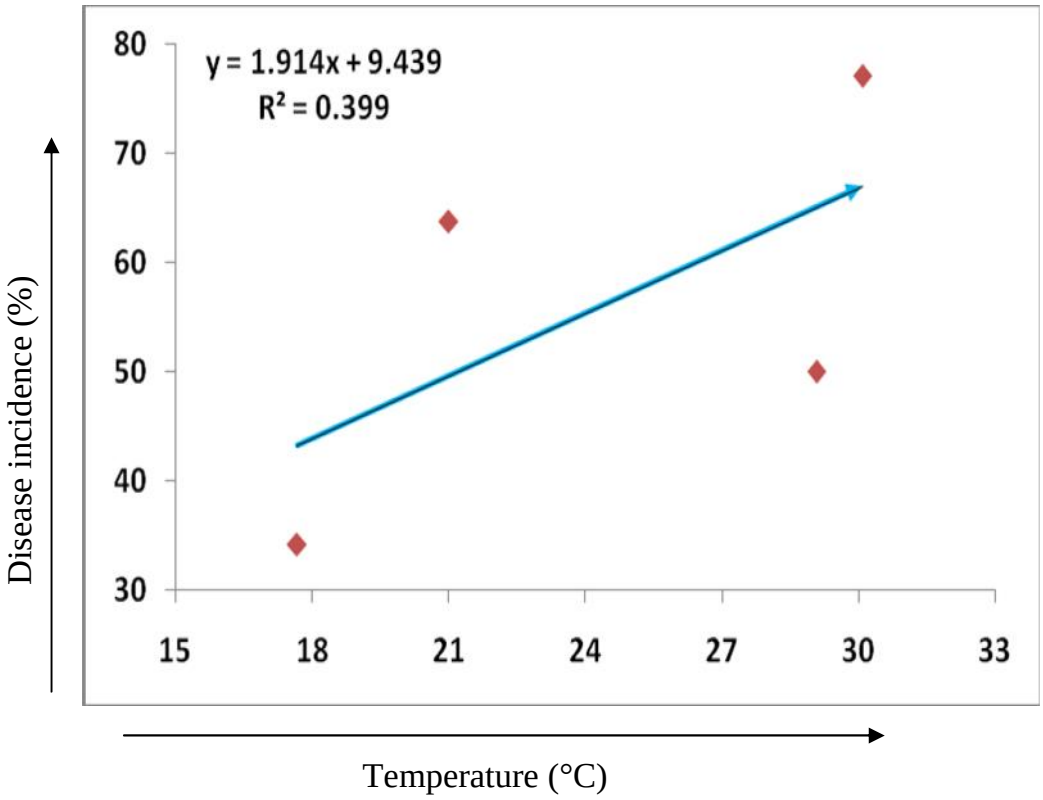


Figure 10. Linear regression analysis of the effect of temperature on the incidence of scab of citrus

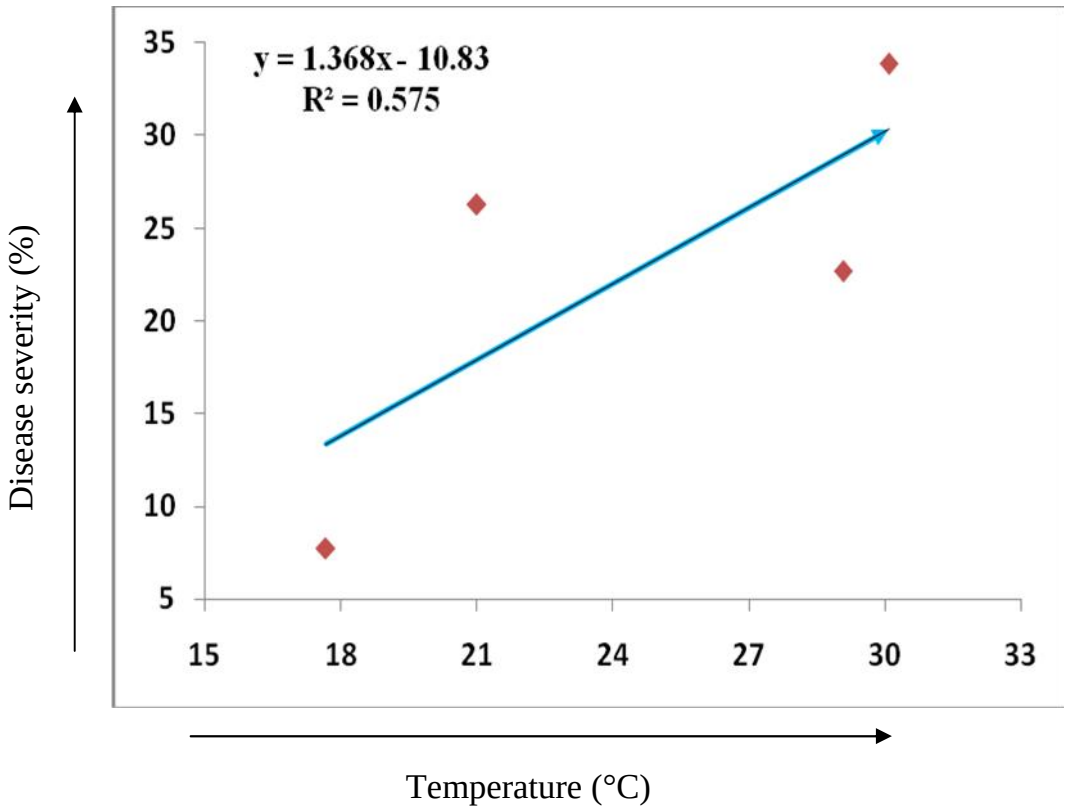


Figure 11. Linear regression analysis of the effect of temperature on the severity of scab of citrus

4.3.2.5.b. Relation between scab disease incidence as well as severity of citrus seedlings and relative humidity

A positive correlation between scab disease incidence and relative humidity was observed. The relationship between disease incidence and relative humidity could be expressed by the equation Y= 1.006x – 21.5 (R^2 =0.087), where x = relative humidity and y = disease incidence. Here, the R^2 value indicates that the contribution of relative humidity was 8.70% on the incidence of scab of citrus. On the other hand, also a positve correlation between scab disease severity and relative humidity was observed. The relationship between disease severity and relative humidity could be expressed by the equation 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 relative humidity was 15.2% on the severity of scab of citrus.

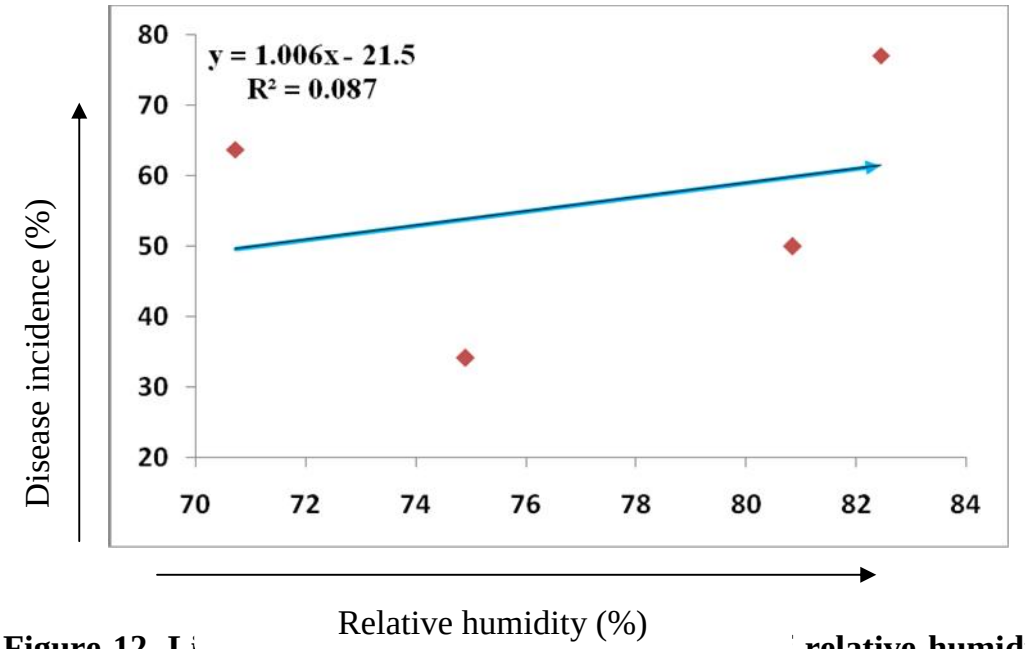


Figure 12. Linear regression analysis of the effect of relative humidity on the incidence of scab of citrus

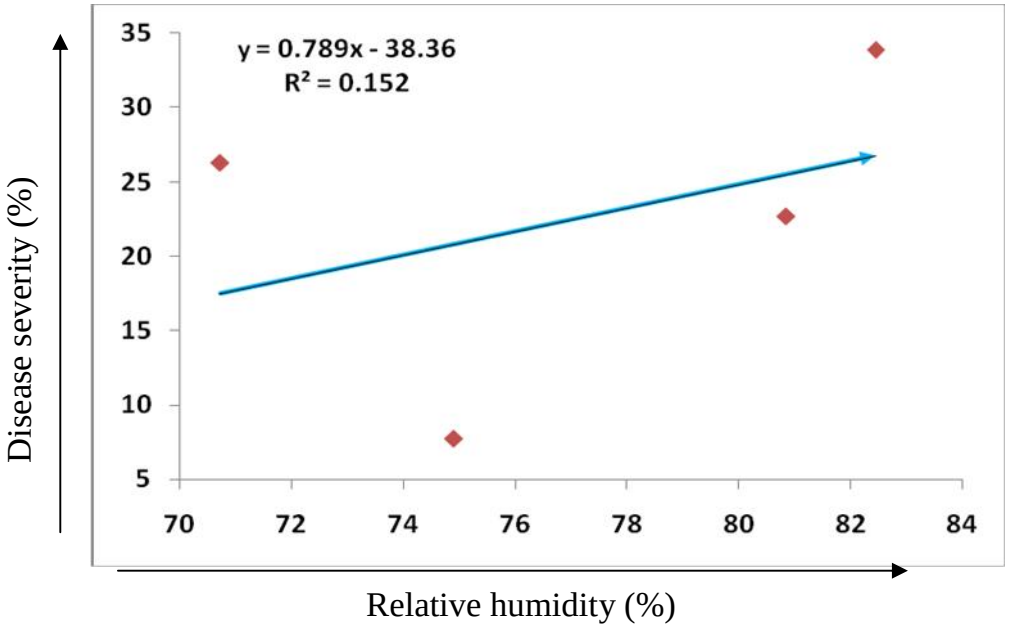


Figure 13. Linear regression analysis of the effect of relative humidity on the severity of scab of citrus

4.3.2.5.c. Relation between scab incidence as well as severity of citrus seedlings and rainfall

A positive correlation between scab disease incidence and rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y= 4.532x + 36.63 (R^2 =0.492), where x = rainfall and y = disease incidence. Here, the R^2 value indicates that the contribution of rainfall was 49.2% on the incidence of scab of citrus. On the other hand, a significant positive correlation between scab disease incidence and rainfall was observed. The relationship between disease severity and rainfall could be expressed by the equation Y= 3.224x + 8.664 (R^2 =0.701), where x = rainfall and y = disease severity. Here, the R^2 value indicates that the contribution of rainfall was 70.1% on the severity of scab of citrus.

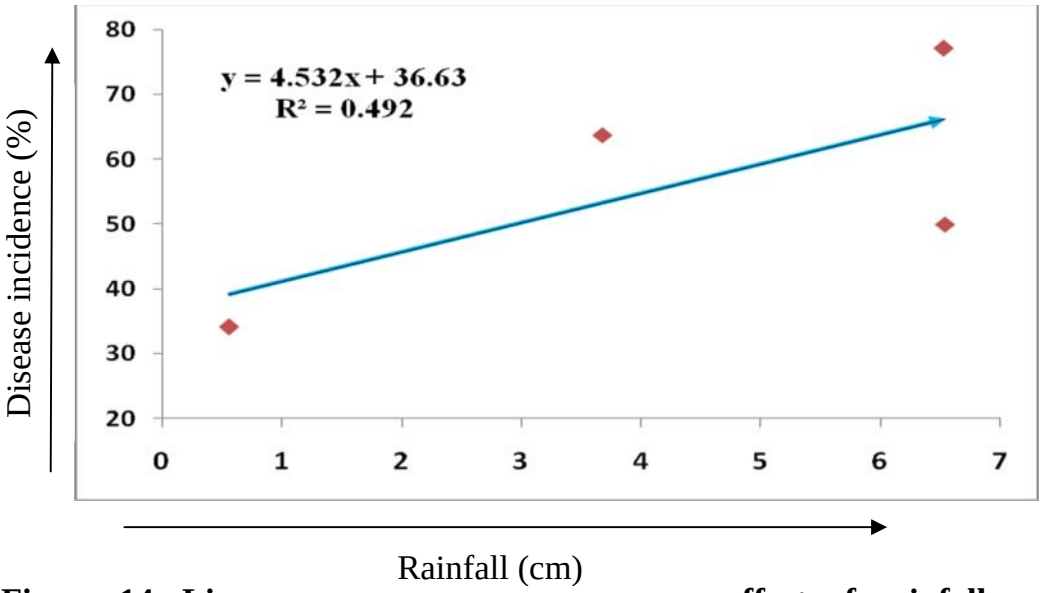


Figure 14. Linear regression analysis of the effect of rainfall on the incidence of scab of citrus

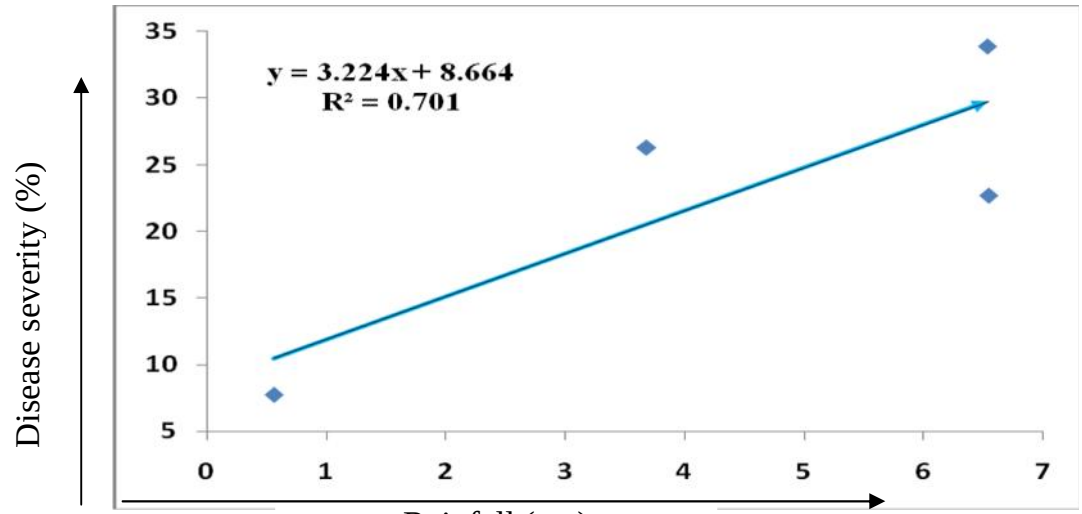


Figure 15. Linear regression analysis of the effect of rainfall on the severity of scab of citrus

4.3.3.1. Incidence and severity of die-back citrus at different experimental locations of Bangladesh during July, 2010 to April, 2012

Incidence of die-back of citrus varied from location to location and that ranged from 9.45-12.50% but no significant difference were found (Table 9). The highest incidence (12.50%) was recorded at Khagrachari and the lowest (9.45%) was recorded at Gazipur. The severity of die-back of citrus also varied from location to location and that ranged from 2.91-5.91% but no significant difference were found. The highest severity (5.91%) was recorded at Khagrachari and the lowest (2.91%) was recorded at Dhaka.

Table 9. Incidence and severity of die-back of citrus at different locations of Bangladesh from July, 2010 to April, 2012

Location	Die-back of citrus	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka	9.58 a	2.91 a
Gazipur	9.45 a	4.13 a
Barisal	11.39 a	4.76 a
Khagrachari	12.50 a	5.91 a
$LSD_{(p\geq 0.05)}$	10.22	8.643
CV%	9.86	9.70

Each data represents the mean value of three nurseries of two years

4.3.3.2. Incidence and severity of die-back in different growing seasons of Bangladesh during July, 2010 to April, 2012

Incidence of die-back of citrus varied significantly from July, 2010 to April, 2012 and that ranged from 0.00-26.11% (Table 10). The highest incidence was recorded in July (2010 & 2011) but no disease was observed in the month of January (2011 & 2012) and April (2011 & 2012). The severity of die-back of citrus varied from July, 2010 to April, 2012 and that ranged from 0.0-8.99%. The highest severity was recorded in the month of July (2010 & 2011) but no disease was observed in the month of January (2011 & 2012) and April (2011 & 2012).

Table 10. Incidence and severity of die-back of citrus during July, 2010 to April, 2012 of Bangladesh

Time of data collection	Die-back of citrus	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
July	26.11 a	8.99 a
October	16.81 b	8.71 a
January	0.00 c	0.00 b
April	0.00 c	0.00 b
$LSD_{(p\geq 0.05)}$	1.783	0.7229
CV%	9.86	9.70

Each data represents the mean value of three nurseries of two years

4.3.3.3. Incidence and severity of die-back of citrus during different growing seasons at different experimental location

Incidence of die-back of citrus varied significantly from season to season as well as location to location and that ranged from 0.00-28.33% (Table 11). The highest incidence was recorded in the month of July (2010 & 2011) at Khagrachari followed by in the month of July (2010 & 2011) at Dhaka (26.11%). The lowest incidence or no disease was observed in the month of January (2011 & 2012) and April (2011 & 2012) at Dhaka, Gazipur, Barisal and Khagrachari. The severity of die-back of citrus also varied significantly from season to season as well as location to location and that ranged from 0.00-12.44%. The highest severity (12.44%) was observed in the month of October (2010 & 2011) at Khagrachari followed by in the month of July (2010 & 2011) at Khagrachari (11.17%). The lowest incidence or no disease was observed in the month of January (2011 & 2012) and April (2011 & 2012) at Dhaka, Gazipur, Barisal and Khagrachari.

Table 11. Incidence and severity of die-back of citrus during July, 2010 to April, 2012 at different experimental locations of Bangladesh

Location	Time of data collection	Die-back of citrus	
		% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka.	July	26.11 b	6.89 f
	October	16.81 d	4.76 g
	January	0.00 f	0.00 h
	April	0.00 f	0.00 h
Gazipur	July	3.31 f	7.61 e
	October	14.44 e	8.89 d
	January	0.00 f	0.00 h
	April	0.00 f	0.00 h
Barisal	July	22.78 c	10.33 c
	October	15.00 e	8.72 d
	January	0.00 f	0.00 h
	April	0.00 f	0.00 h

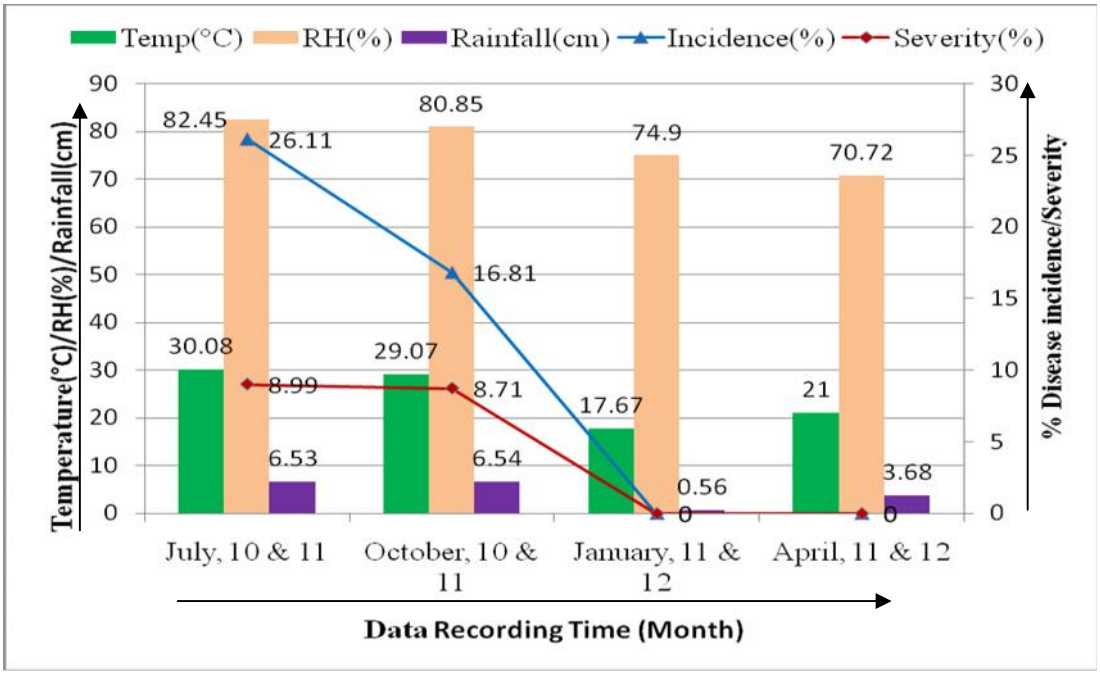
Khagrachari	July	28.33 a	11.17 b
	October	17.22 d	12.44 a
	January	0.00 f	0.00 h
	April	0.00 f	0.00 h
$LSD_{(p\geq0.05)}$		1.783	0.7229
CV(%)		9.86	9.70

Each data represents the mean value of three nurseries of two years

4.3.3.4. Effect of weather components on the incidence and severity of die-back of citrus seedling during July, 2010 to April, 2012

The incidence of die-back of citrus was influenced by average temperature, relative humidity and rainfall. The highest incidence (26.11%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, no incidence (0.00%) were recorded in January (2011 & 2012) and April (2011 & 2012), having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm and 21.0°C, 70.72% and 3.68 cm, respectively. In the month of October (2010 & 2011) the incidence was 16.81% when the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively.

The severity of die-back of citrus was influenced by average temperature, relative humidity and rainfall. The highest severity (8.99%) was recorded in July (2010 & 2011) when the average temperature, relative humidity and rainfall were 30.08°C, 82.45% and 6.53 cm, respectively. On the other hand, no severity (0.00%) were recorded in January (2011 & 2012) and April (2011 & 2012), having average temperature, relative humidity and rainfall 17.67°C, 74.90% and 0.56 cm and 21.0°C, 70.72% and 3.68 cm, respectively. In the month of October (2010 & 2011) the severity was 8.71% when the temperature, relative humidity and rainfall were 29.07°C, 80.85% and 6.54 cm, respectively.



Each data represents the mean value of three nurseries of two years

Figure 16. Effect of weather components on the incidence and severity of die-back of citrus seedling during July, 2010 to April, 2012

4.3.3.5.a. Relation between die-back disease incidence as well as severity of citrus seedlings and temperature

A positive correlation between die-back disease incidence and severity with temperature was observed. The relationship between disease incidence and temperature could be expressed by the equation Y= 2.023x – 38.75 (R^2 =0.901), where x = temperature and y = disease incidence. Here, the R^2 value indicates that the contribution of temperature was 90.1% on the incidence of die-back of citrus. On the other hand, the relationship between disease severity and temperature could be expressed by the equation Y= 0.818x – 15.58 (R^2=0.947), where x = temperature and y = disease severity. Here, the R^2 value indicates that the contribution of temperature was 94.7% on the severity of die-back of citrus.

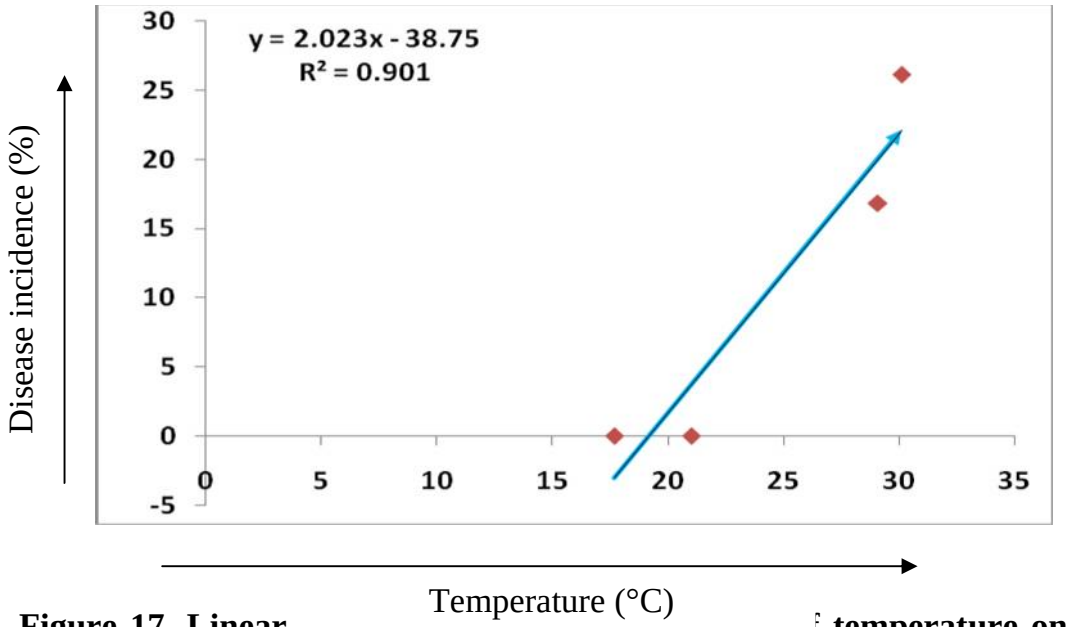


Figure 17. Linear [illegible] temperature on the incidence of die-back of citrus

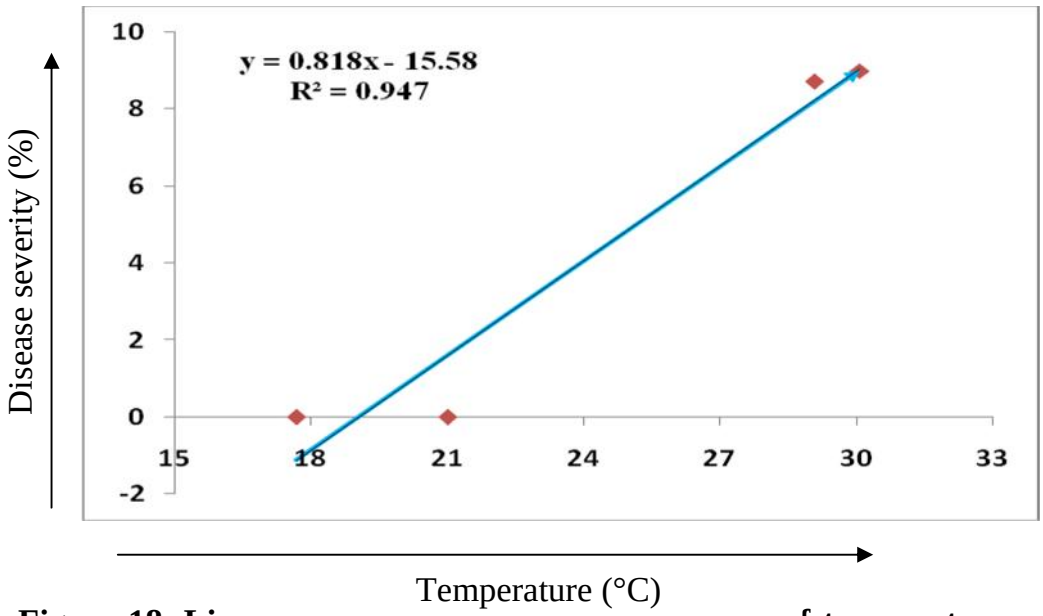


Figure 18. Linear [illegible] temperature on the severity of die-back of citrus

4.3.3.5.b. Relation between die-back disease incidence as well as severity of citrus seedlings and relative humidity

A significant positive correlation between die-back disease incidence and relative humidity was observed. The relationship between disease incidence and relative humidity could be expressed by the equation Y= 2.236x – 161.9 (R^2 =0.875), where x = relative humidity and y = disease incidence. Here, the R^2 value indicates that the contribution of relative humidity was 87.5% on the incidence of die-back of citrus. On the other hand, also a positve correlation

between die-back disease severity and relative humidity was observed. The relationship between disease severity and relative humidity could be expressed by the equation 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 relative humidity was 15.2% on the severity of die-back of citrus.

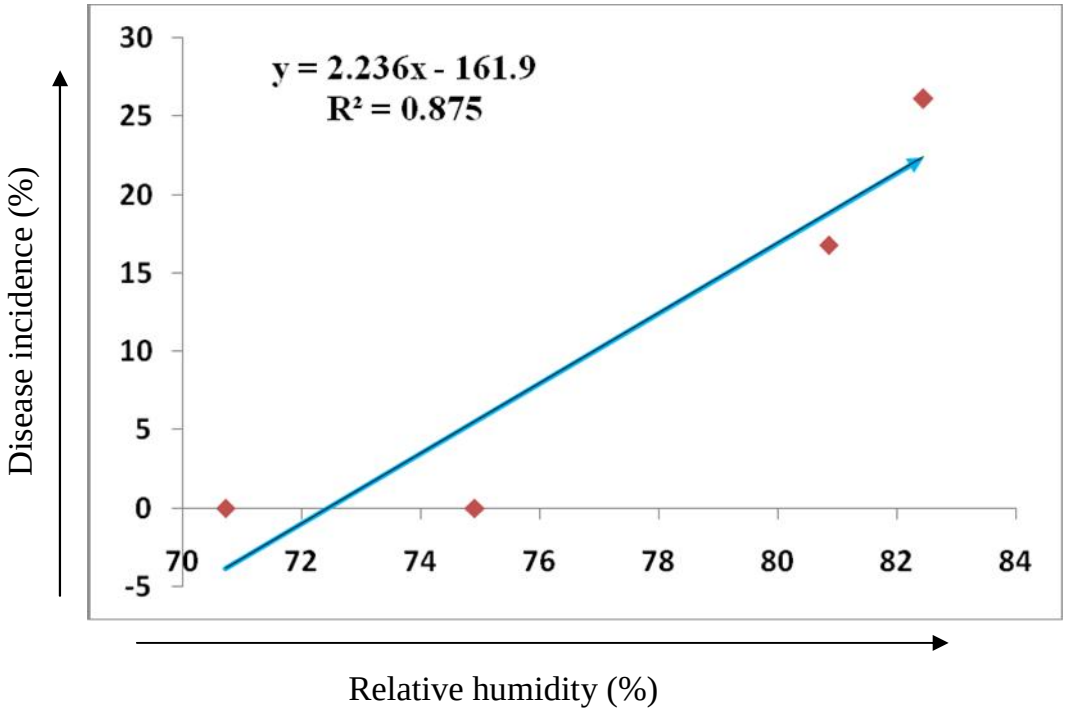


Figure 19. Linear regression analysis of the effect of relative humidity on the incidence of die-back of citrus

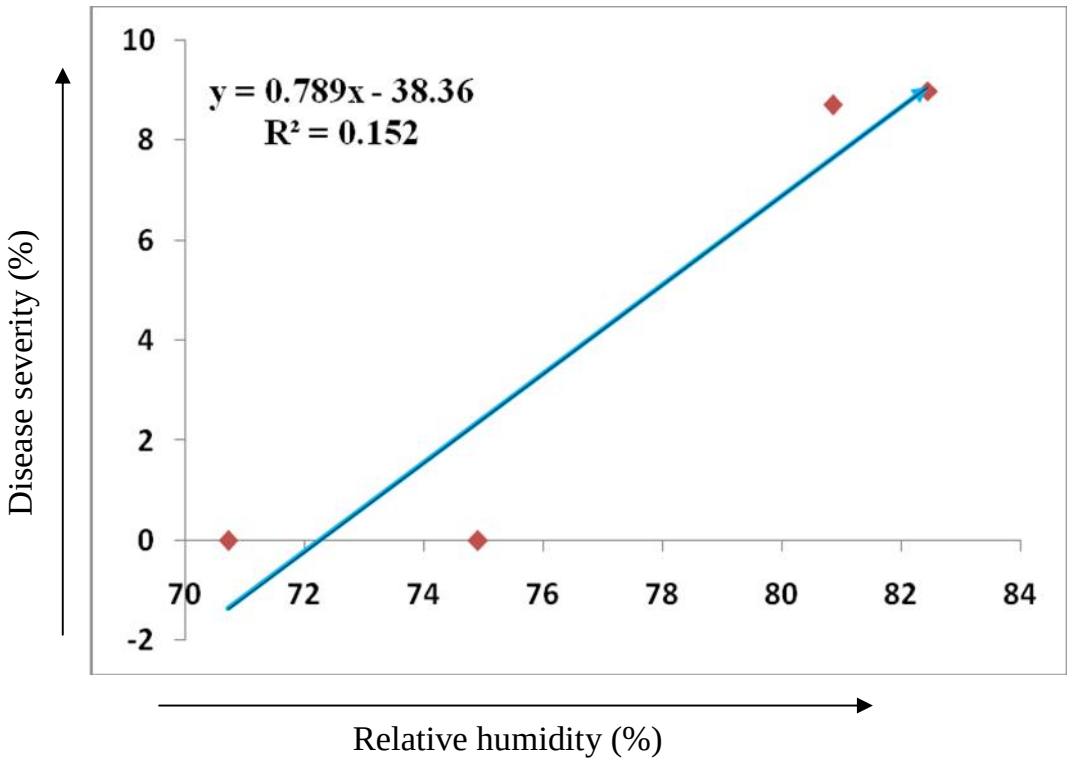


Figure 20. Linear regression analysis of the effect of relative humidity on the severity of die-back of citrus

4.3.3.5.c. Relation between die-back incidence as well as severity of citrus seedlings and rainfall

A significant positive correlation between die-back disease incidence and rainfall was observed. The relationship between disease incidence and rainfall could be expressed by the equation Y= 3.887x – 6.093 (R^2 =0.730), where x = rainfall and y = disease incidence. Here, the R^2 value indicates that the contribution of rainfall was 73.0% on the incidence of die-back of citrus. On the other hand, a significant positive correlation between die-back disease incidence and rainfall was observed. The relationship between disease severity and rainfall could be expressed by the equation Y= 1.603x – 2.515 (R^2 =0.799), where x = rainfall and y = disease severity. Here, the R^2 value indicates that the contribution of rainfall was 79.9% on the severity of die-back of citrus.

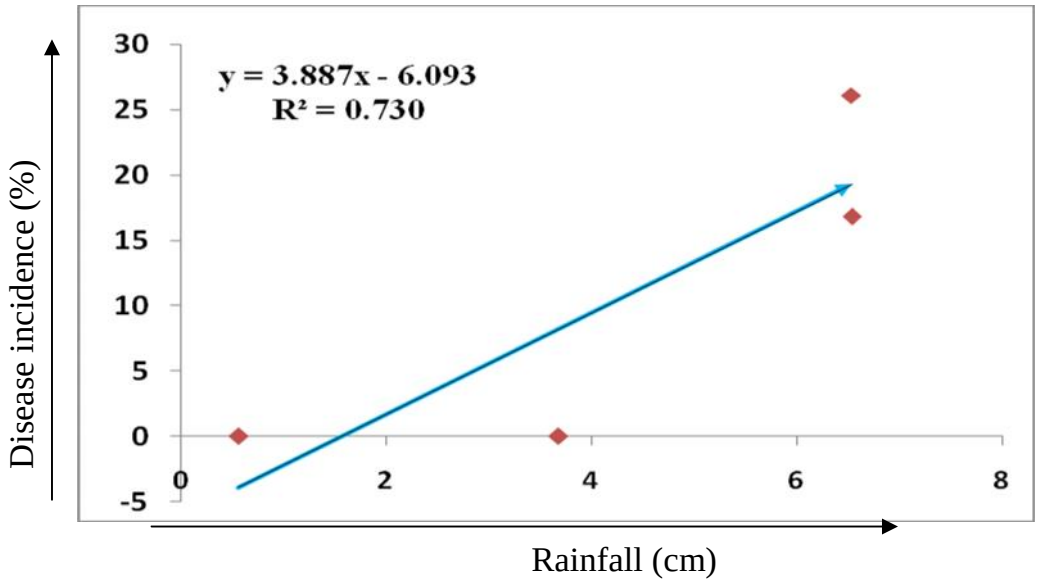


Figure 21. Linear regression analysis of the effect of rainfall on the incidence of die-back of citrus

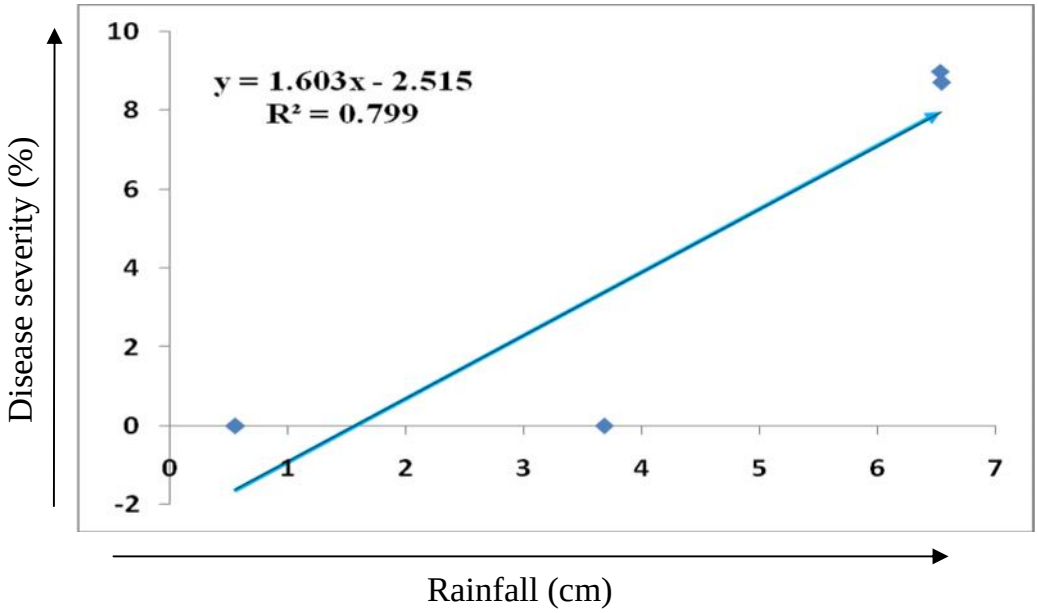


Figure 22. Linear regression analysis of the effect of rainfall on the severity of die-back of citrus

4.3.4.1. Incidence and severity of citrus greening at different experimental locations of Bangladesh during July, 2010 to April, 2012

The citrus greening only recorded at Barisal but no disease were recorded at Dhaka, Gazipur and Khagrachari during the survey period. Incidence of citrus greening at Barisal was 3.33% (Table 12). The severity of citrus greening at Barisal was 10.0%.

Table 12. Incidence and severity of citrus greening at different locations of Bangladesh from July, 2010 to April, 2012

Location	Citrus greening	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka	0	0
Gazipur	0	0
Barisal	3.33	10
Khagrachari	0	0

Each data represents the mean value of three nurseries of two years

4.3.4.2. Incidence and severity of citrus greening in different growing seasons of Bangladesh during July, 2010 to April, 2012

The prevalence (Disease incidence and Severity, respectively 3.33% and 10.0%) of citrus greening only recorded at Barisal in the month of July (2010 & 2011) but no disease were recorded in the month of October (2010 & 2011); January (2011 & 2012) and April (2011 & 2012). No disease was recorded at Dhaka, Gazipur and Khagrachari during the survey period (Table 13).

Table 13. Incidence and severity of citrus greening during July, 2010 to April, 2012 of Bangladesh

Time of data collection	Citrus greening	
	% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
July	3.33	10.0
October	0	0
January	0	0
April	0	0

Each data represents the mean value of three nurseries of two years

4.3.4.3. Incidence and severity of citrus greening during different growing seasons at different experimental location

The prevalence (Disease incidence and sverity, respectively 3.33% and 10.0%) of citrus greening only recorded at Barisal in the month of July (2010 & 2011) but no disease was recorded in the month of October (2010 & 2011); January (2011 & 2012) and April (2011 & 2012). No disease was recorded at Dhaka, Gazipur and Khagrachari during the survey period.

Table 14. Incidence and severity of citrus greening during July, 2010 to April, 2012 at different experimental locations of Bangladesh

Location	Time of data collection	Citrus greening	
		% Disease Incidence (July, 2010-April, 2012)	% Disease Severity (July, 2010-April, 2012)
Dhaka.	July	0	0
	October	0	0
	January	0	0
	April	0	0
Gazipur	July	0	0
	October	0	0
	January	0	0
	April	0	0
Barisal	July	3.33	10.00
	October	0	0
	January	0	0
	April	0	0
Khagrachari	July	0	0
	October	0	0
	January	0	0
	April	0	0

Each data represents the mean value of three nurseries of two years

4.5.1. Effect of management practices on the canker disease of citrus

4.5.1.1. Effect of management practices on the incidence of canker disease of citrus

All the treatments significantly reduced the incidence of disease of citrus seedlings over control (Table 15). Out of all the treatments applied except T_7 (untreated control), the highest incidence (100%) was observed in the month of July 2011 and the lowest incidence (40.67%) were observed in the month of April and May. Considering the mean incidence, the highest incidence (69.22%) was recorded in T_7 (untreated control), which was statistically different from all other treatments. On the other hand, the lowest disease incidence (45.46) was recorded in T_6 (Cupravit was applied as foliar spray @ 0.2%). In case of percent reduction of disease incidence over control highest reduction (34.32%) was observed in T_6 while the lowest reduction (5.77%) was observed in T_2 (BAU- Biofungicide was applied as foliar spray @ 2%).

4.5.1.2. Effect of management practices on the severity of canker disease of citrus

Significant variations in the disease severity of citrus seedlings were observed in the application of management practices during the period of December, 2010 to November, 2011 (Table 16). Out of all the treatments applied, the lowest severity (0.0 - 0.21%) were observed in the month of March and it was gradually increased in the following months up to July than gradually decrease up to December. Considering the mean severity, the highest severity (4.35%) was recorded in T_7 (untreated control), which was statistically different from al other treatments. On the other hand, the lowest disease incidence (1.29%) was recorded in T_3 (BAU Bio-fungicide was applied as foliar spray @ 2%). In case of percent reduction of disease severity over control due to application of different management practices, the highest reduction (70.45%) over control was observed in T_3 (BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%) and lowest reduction (33.21%) over control was observed in T_4 (Mancozeb was applied as foliar spray @ 0.2%). As a result the order of efficiency of different management practices regarding the percent reduction of disease severity of citrus seedlings were $T_3 > T_6 > T_5 > T_2 > T_1 > T_4$.

4.5.2.1. Effect of management practices on the incidence of scab disease of citrus

All the treatments significantly reduced the incidence of disease of citrus seedlings over control (Table 17). Out of all the treatments applied except T_7 (untreated control), the highest incidence (32.67-66.2%) were observed in the month of July 2011 and the lowest incidence (0.0 %) were observed in the month of January, February, March, September and October. Considering the mean incidence, the highest incidence (24.85%) was recorded in T_7 (untreated control), which was statistically different from all other treatments. On the other hand, the lowest disease incidence (9.89%) was recorded in T_5 (Bavistin was applied as foliar spray @ 0.2%). Incase of percent reduction of disease incidence over control due to application of different management practices, the highest reduction (60.21%) over control was observed in T_5 (Bavistin was

applied as foliar spray @ 0.2%) and lowest reduction (21.53%) over control was observed in T_4 (Mancozeb was applied as foliar spray @ 0.2%).

4.5.2.2. Effect of management practices on the severity of scab disease of citrus

Significant variations in the disease severity of citrus seedlings were observed in the application of management practices during the period of December, 2010 to November, 2011 (Table 18). Out of all the treatments applied, the lowest severity (0.00%) was observed in the month of January, February, March, September and October. Considering the mean severity, the highest severity (1.02%) was recorded in T_7 (untreated control), which was statistically different from all other treatments. On the other hand, the lowest disease severity (0.18%) was recorded in T_3 (BAU Bio-fungicide was applied as foliar spray @ 2%). In case of percent reduction of disease severity over control due to application of different management practices, the highest reduction (82.58%) over control was observed in T_3 (BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%) and lowest reduction (53.54%) over control was observed in T_4 (Mancozeb was applied as foliar spray @ 0.2%). As a result the order of efficiency of different management practices regarding the percent reduction of disease severity of citrus seedlings were $T_3 > T_6 > T_5 > T_1 > T_2 > T_4$.

4.5.3.1. Effect of management practices on the incidence of die-back disease of citrus

Out of all treatment applied, disease incidence with die-back disease did not follow a definite pattern of increase or decrease (Table 19). But die-back diseased plant was not found in March, April, May and June under all treatments. Mean disease incidence ranged from 0.40% to 8.56%, where the lowest count was made in T_6 (Cupravit was applied as foliar spray @ 0.2%) and highest in T_7 (Untreated Control). The order of efficiency of different management practices regarding the disease incidence were $T_6 > T_5 > T_3 > T_1 > T_2 > T_4 > T_7$.

4.5.3.2. Effect of management practices on the severity of die-back disease of citrus

There was a variation of disease severity with die-back disease that ranged from 0.00% to 1.67% (Table 20). Mean disease incidence ranged from 0.03% to 0.92%, where the lowest count was made in T_5 (Cupravit was applied as foliar spray @ 0.2%) and T_3 (BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%) and highest in T_7 (Untreated Control). The order of efficiency of different management practices regarding the disease severity were $T_3 > T_5 > T_1 > T_6 > T_2 > T_4 > T_7$.

4.5.4. Effect of different management practices on the height of citrus seedlings

Significant variations in the height increase over first count were found under different management practices (Table 21). The maximum 109.8 cm height was recorded in T_3 (BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%). The maximum increase by 45.90% over control was recorded in T_2 (BAU- Biofungicide was applied as foliar spray @ 2%) and the minimum 35.86% height increase over first count and increase 9.32% over control was observed in T_4 (Mancozeb was applied as foliar spray @ 0.2%).

CHAPTER V
DISCUSSION

A survey was conducted in eight nurseries under four districts of Bangladesh viz. Green orchid nursery, Barisal nursery, Gazipur nursery, Laxmipur nursery, Sarchina nursery, Riyad nursery, Hill Research Center and Ramghar nursery during the period of July, 2010 to April, 2012. In the present study four diseases of citrus were recorded namely scab, canker, die-back and greening of citrus. Among the recorded diseases two were fungal viz. Citrus scab and citrus die-back and two were bacterial viz. citrus canker and greening of citrus (Polek *et al.* 2007). The diseases recorded in the present study had also been reported on citrus seedlings from different countries of the world [Nirvan, (1961); Klotz, (1973); Reddy and Murti, (1985); Alam, (2003); Agostini, (2003); Hossain and Roy, (2006) and Bove, (2006)]. Again, in Bangladesh twelve diseases including four observed in the present study were recorded by Alam (2003) and Chowdhury (2009) in the citrus growing areas of Bangladesh. These diseases were recorded as a common disease in all the growing areas surveyed. The diseases in the present study were identified by observing the symptoms on the seedlings during survey and determination of presence of fungi was made either directly by preparation of slides and examining them under compound microscope or indirectly by isolation to agar culture following keys outline by Sing (1978), Pathak (1980), Peterson (1986), Sing (1998) and Ploetz *et al.* (1998). Greening of citrus was identified based on visual symptom following the description of Bove (2006) and Polek *et al.* (2007).

In the present study the pathogen isolated from Citrus canker was *Xanthomonas campestris* pv. *Citri.* The pathogen has been reported by many researchers throughout the world [Qui and Ni (1988) and Kale *et al.*1994)]. Awasthi *et al.* (2005) reported canker (*Xanthomonnas campestris* pv. *citri*) as the major problem in the nursery. Scab in the seedling was caused by *Sphaceloma fawcetti*, the conidial stage of (*E. fawcetti*) which is in accordance

with Pathak, (1980). Scab of citrus caused by *Sphaceloma fawcetti* have also been reported by many researchers throughout the world [McClean, (1960); Nirvan, (1961); Singh *et al*., (2005) and Chowdhury, (2009)]. The presence of *Sphaceloma fawcetti* has also been reported by others [Singh and Kapoor, (1971); Talukder, (1974); Qui and Ni, (1988); Kale *et al*., (1994); Ebenzer *et al*., (1996); Huang, (1999); Timmer, (2000); Zhong and Ling, (2002); Huang and Huang, (2002); Rawal, (2005); Hossin and Roy, (2006)]. Die-back of citrus was caused by *Colletotrichum gloeosporioides.* This has been reported by many researchers throughout the world [Klotz, (1948); Peralata, (1949); Knorr *et al.*, (1957); Sharma and Sharma, (1969); Singh and Kapoor, (1971); Singh *et al.*, (1971); Talukder, (1974); and Miah and Fakir, (1988)]. Citrus greening was observed on the citrus seedlings. The disease was identified by observing the symptom only since pathogen could not be isolated due to lack of laboratory facilities. However, Polek *et al*. (2007) stated that the HLB - causing bacteria could not be cultured in the laboratory and do not survive outside the host cells, making them difficult to study. The presence of greening of citrus in seedling was not reported by any workers from Bangladesh earlier and as such it is the first record of prevalence of greening in the seedling of citrus in the country. Greening of citrus caused by several species of the genus *Candidatus* Liberibacter and is reported by many researchers throughout the world [Da Graca and Korsten, (2004); Bove, (2006) and Polek *et al*. (2007)].

The prevalence of the recorded four diseases on citrus varied in respect of nursery and location. Similar variation in prevalence of seedling diseases in respect of nursery and location was recorded by Fraser, (1966); McClean, (1960); Nirvan, (1961); Syfullah, (2008) and Chowdhury, (2009) in different citrus growing regions. Regarding incidence of the four diseases recorded in the present study, citrus scab and canker were the most predominant while die-back and citrus greening had the least occurrence. In case of disease severity, citrus scab and citrus canker were the prevalent diseases while the prevalence of die-back and citrus greening had the least occurrence. Singh *et al*. (1997)

conducted a survey at Panjab in India and found the highest disease incidence of scab on rough lemon (81.10%) and Kinnow (80.07%) and the lowest incidence were recorded on sweet orange 10.80% in the arid irrigated zone. In the present study, it was also observed that the incidence and severity of scab, canker, die-back and greening of citrus varied from location to location. These variations may be due to effect of environment of different agro-ecological zone. The highest incidence and severity of scab, canker and die-back was recorded at Khagraachari. The Khagraachari is a south eastern hilly district of Bangladesh. This high prevalence may be due to environmental effect of that particular agro-ecological zone. Greening of citrus was only recorded at Barisal. This prevalence may be due to environmental effect of the particular agro-ecological zone and presence of insect vector Asian citrus psyllid, *Diaphorina citri* (Catling, 1970). Greening of citrus occur at lower elevations (360 m) under low humidity and at both cool and warm temperatures (heat-tolerant) up to 35°C (Garnier and Bove, 1993).

The effects of temperature, rainfall and relative humidity on the incidence and severity of noted diseases of citrus 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 citrus. Gilling (1986) observed that ANOVAs has been the fundamental method used by plant pathologist to determine the correlation between the prevalence and environmental parameters. 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 (Ramakrishnan, 1954; Aiyappa, 1958; Khucharek and Whiteside, 1983; Calvin *et al.*, 1986; Tripathi and Srivasttava, 1992; Huang

and Huang, 2002; Varniere *et al.*, 2003; Agostini, 2003; Rawal, 2005; Chowdhury, 2009; Hossain, 2011).

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 citrus scab and canker were found to be increased in the month of April and July while the disease decreased in the month of January and October. Correlation regression analysis of prevalence of scab and canker 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 canker 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 Bal and Dhiman (2005) who found that citrus canker was found to build up during the first week of June with the onset of rains. They also observed that highest incidence of citrus canker was recorded during the second week of September and the diseases showed a positive correlation with temperatures, relative humidity and rain and hence the period from July to September was identified as the most conducive for the development of citrus canker. Polek *et al.* (2007) reported that citrus canker occurs primarily in tropical and sub-tropical climates where considerable rainfall accompanies warm temperatures but it can also occur in drier climates. The highest prevalence (Incidence and disease severity) of citrus scab was observed in the month of July and April and the lowest prevalence (Incidence and disease severity) observed in the month of January and October. A positive correlation was observed between prevalence of scab 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 Nirvan (1961) who found that the scab was negligible in colder season than that of warmer seasons. The present study also

supported by the previous workers [Chowdhury, (1955); Reddy and Murti, (1990); Singh *et al*., (1997); Bal and Dhiman, 2005; and Chowdury, (2009)]. Reddy and Murti (1990) found that scab prevails in the regions where temperature and humidity remains low. Chowdhury (2009) reported that humidity and rainfall played more important role in the development of scab than by temperature. The prevalence (Incidence and severity) of die-back of citrus was recorded in the months July and October but no disease was found in the month of January and April. A positive correlation was observed between prevalence of die-back with temperature, relative humidity and rainfall. The present study also supported by the Peralta (1949); Knorr *et al.*, (1957); Singh and Kapoor (1971); Singh *et al.* (1971); Talukder (1974); Miah and Fakir (1988); Ebenzer *et al.* (1996); Hossain and Roy (2006). Greening was only observed in the month of July but no disease was found in the month of January, April and October. Greening of citrus occur at lower elevations (360 m) under low humidity and at both cool and warm temperatures (heat-tolerant) up to 35°C (Garnier and Bove, 1993).

Comparative effectiveness of BAU-Biofungicide and three other fungicides viz. Mancozeb, Bavistin and Cupravit were evaluated on seedling diseases of citrus in the nursery. Significant effect of different management practices on incidence, severity and plant height were observed. Employment of control measures resulted in gradual decrease of the incidence and severity of diseases over untreated control. The highest reduction of incidence of citrus canker was observed in applying Cupravit as foliar spray while highest reduction of severity over control was observed in application of BAU biofungicide. Zhong and Ling, (2002) reported that spraying shoots with 77% kocide (Copper hydroxide) wettable powder at 20-30 days after bud burst and spraying summer-autumn shoots at 10-15 days after bud burst provided good citrus canker control. Performance of Bavistin was found best in controlling incidence and severity of scab while treatment BAU biofungicide showed statistically similar effect in combating the disease. Chowdhury (2009) reported

that Cupravit and Bavistin alone and in combination with BAU biofungicide reduced the incidence and severity of scab of citrus seedlings in the nursery but inferior in efficiency. He also observed that the lowest severity of scab was resulted while applied BAU Bio-fungicide in soil and top dressing @ 2%. Mamatha *et al*., (2000) reported that soil amendment with *Trichoderma harzianum* have been shown to superior over other treatments like chemical, physical and plant extract treatments both in reducing seed mycoflora and in enhanching the germination and vigour in four different forest species tested. Gade *et al.* (2005) observed that drenching with metalaxyl coupled with spraying of copper oxychloride effectively reduced seedling mortality (13.9%) and improved plant height (52.9 cm) and girth (3.2 cm). Singh *et al*. (2005) evaluated five antagonists against the most virulent isolate of *Elsinoe fawcetti* (Ef 20) causing scab of citrus and found the antagonist *Trichoderma harzianum* suppressed the disease development significantly resulted the minimum disease index of 51.5%. Huang (1999) reported that, spraying Broadeaux mixture at the bad break stage gave effective control of scab. The disease is effectively checked through the spraying of Broadeaux mixture has also been reported by Chowdhuri (1951). Die-back disease did not follow a definite pattern of increase or decrease. Cupravit and Bavistin performed better in controlling die back disease. However, die-back diseased was not observed in March, April, May and June under all treatments. Rawal and Saxena (1997) found Bavistin (Carbendazim) effective against a wide range of fungal pathogens and controlling diseases caused by *C. gloeosporioides.* Different management practices resulted in significant effect in the height of citrus seedlings. Enhancement of seedling germination and vigor by using *Trichoderma harzianum* or *Trichoderma* based formulations have been studied and reported by many workers around the world [Mamatha *et al*., (2000); Chowdhry (2009) and Hossain, (2011)]. BAU biofungicide, a formulation based on naturally occurring fungus *Trichoderma harzianum* has been tested against disease of many crops like wheat, rice, maize, pulses and legumes and found to be effective [Hossain, (2007); Mostofa, (2009);

Shultana *et al.*, (2009) and Chowdhury, (2009)]. Chowdhury (2009) also reported the performance of BAU biofungicide in controlling nursery diseases of some fruits in Bangladesh. This new ecofriendly means of disease control may be incorporated in the management of nursery diseases in Bangladesh.

CHAPTER VI

SUMMARY AND CONCLUSION

Citrus seedling are vulnerable to attacked by various diseases in Bangladesh, but least concrete information regarding their distribution, incidence, severity, epidemiology and management is available. Therefore, the present study was designed to study the occurrence and prevalence of seedling diseases of citrus 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 dieseases.

Four experiments were carried out throughout the study period from July, 2010 to April, 2012. The diseases were identified based on matching the observed symptoms in the infected plants with the symptoms. Besides, the symptoms of the diseases were recorded following the description of Reddy and Murti, (1990); Rajput and Haribabu, (1985); and Bove (2006). The disease also identified by observing the symptoms on the seedlings during survey and determination of presence of fungi was made either directly by preparation of slides and examining them under compound microscope or indirectly by isolation to agar culture following keys outline by Sing (1978); Pathak (1980); Peterson (1986); Sing (1998); Ploetz *et al*. (1998) and Bove (2006).

Four different diseases viz. canker, scab, die-back and greening of citrus on citrus seedlings were 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. Incidence and severity of canker, scab, die-back and greening of citrus 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 were responsible for significant reduction of disease incidence and severity. Different degrees of correlation were observed among

the seedling diseases of citrus with temperature, relative humidity and rainfall. All the diseases were found to be influenced by the epidemiological parameters.

Effectiveness of BAU-Biofungicide and three other fungicides viz. Mancozeb, Bavistin and Cupravit were evaluated on seedling diseases of citrus in the nursery. Significant effect of different management practices in relation to incidence, severity and plant height were observed. Among the treatment applied, *Trichoderma harzianum* based BAU-Biofungicide showed the excellent result in controlling canker, scab and die-back diseases of citrus. Use of Mancozeb, Bavistin and Cupravit also reduced the incidence of incidence and severity of canker, scab and die-back diseases of citrus over control.

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 pressure 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 inoculam 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 should be incorporated in the nursery disease management system that is a most important alternative to the hazardous chemical fungicides.

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Table 15. Effect of different management practices on the disease incidence of canker disease of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Disease Incidence									
	Dec. 2010	Jan. 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept. 2011
T_1	30.87c	0.00d	0.00d	26.00d	30.87d	33.33c	100.0a	100	90.67b	90.67b
T_2	60.67a	40.67b	0.00d	36.67c	47.33a	36.67bc	100.0a	100	100.0a	100.0a
T_3	34.00bc	67.33a	40.67b	67.33a	34.00c	40.67b	82.00b	100	66.87c	66.87c
T_4	37.33b	0.00d	51.33a	0.00e	0.00e	0.00d	100.0a	100	100.0a	100.0a
T_5	34.00bc	0.00d	36.67c	0.00e	0.00e	34.00c	100.0a	100	100.0a	100.0a
T_6	30.87c	0.00d	0.00d	0.00e	0.00e	37.33bc	100.0a	100	100.0a	90.00b
T_7	34.00bc	33.33c	36.67c	40.67b	40.67b	45.33 a	100.0a	100	100.0a	100.0a
$LSD_{(p\geq 0.05)}$	3.409	2.421	2.438	2.769	2.769	4.089	2.206		2.560	2.224
CV (%)	6.98	9.18	7.91	8.70	9.7	9.65	1.73		2.09	1.84

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

Table 16. Effect of different management practices on the disease severity of canker disease of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Disease severity									
	Dec. 2010	Jan. 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept 2011
T_1	0.29 d	0.00 d	0.00 c	0.014 c	1.11a	1.11 a	5.47 c	6.65 d	4.88 c	2.69
T_2	0.20 e	0.35 c	0.00 c	0.10 b	1.11 a	1.11 a	3.74 d	4.76 e	3.39d	1.82
T_3	0.34 c	0.69 a	0.83 a	0.22 a	0.21 c	0.91 c	3.28e	3.27 f	2.69 e	1.84
T_4	0.52 a	0.00 d	0.54 b	0.00 c	0.00 d	0.00 d	6.61b	11.91b	8.81 b	3.71

T_5	0.25 e	0.00 d	0.02 c	0.00 c	0.00 d	0.00 d	3.74 d	9.56 c	2.69 e	1.42
T_6	0.31 cd	0.00 d	0.00 c	0.00 c	0.00 d	0.00 d	5.43 c	4.77 e	3.73 d	3.44
T_7	0.49 b	0.52 b	0.80 a	0.21 a	1.02 b	1.02 b	7.10 a	13.23a	12.20a	10.32
$LSD_{(p\geq0.05)}$	0.04128	0.04128	0.04128	0.04128	0.04128	0.05838	0.3646	0.591	0.5838	0.28
CV (%)	8.76	7.21	4.65	9.60	7.33	6.59	5.53	5.86	8.15	6.11

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

Table 17. Effect of different management practices on the disease incidence of scab disease of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Disease Incidence									
	Dec. 2010	Jan. 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept. 2011
T_1	34.0 c	0	0	0	39.33 b	12.0 cd	14.67c	32.67 c	25.33 b	0
T_2	40.67 ab	0	0	0	34.67 c	16.0 b	14.67 c	49.33 b	0.00 c	0
T_3	35.33 c	0	0	0	28.0 d	8.01 e	0.00 d	66.00 a	0.00 c	0
T_4	37.99 bc	0	0	0	34.0 c	14.67bc	22.0 b	64.67 a	24.67 b	0
T_5	34.0 c	0	0	0	37.33bc	12.0 d	0.00 d	35.33 c	0.00 c	0
T_6	35.33 c	0	0	0	28.0 d	12.0 d	0.00 d	52.0 b	0.00 c	0
T_7	42.67 a	0	0	0	50.67 a	28.00 a	28.67 a	66.20 a	32.0 a	0
$LSD_{(p\geq0.05)}$	4.053	0	0	0	3.192	2.539	1.632	3.544	1.503	0
CV (%)	8.36	0	0	0	6.79	13.26	10.94	5.19	9.83	0

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreatedcontrol

Table 18. Effect of different management practices on the disease severity of scab disease of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Disease severity										
	Dec. 2010	Jan. 2011	Feb. 2011	Mar. 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept. 2011	Oct 201
T_1	0.19 d	0	0	0	0.50 b	0.35 b	0.33 c	1.11 e	1.83 a	0	0
T_2	0.46 b	0	0	0	0.27 c	0.12 d	0.20 d	3.28 b	0.00 d	0	0
T_3	0.43 b	0	0	0	0.22 d	0.11 d	0.00 e	0.95 f	0.00 d	0	0
T_4	0.29 c	0	0	0	0.21 d	0.12 d	0.85 b	2.64 d	1.09 c	0	0
T_5	0.21 d	0	0	0	0.19 d	0.16 c	0.00 e	3.75 a	0.00 d	0	0
T_6	0.11 e	0	0	0	0.14 e	0.14cd	0.00 e	2.62 d	0.00 d	0	0
T_7	0.94 a	0	0	0	1.95 a	0.94 a	1.2 a	3.06 c	1.63 b	0	0
LSD($_{p\geq 0.05}$)	0.042	0	0	0	0.042	0.042	0.042	0.058	0.058	0	0
CV (%)	7.9	0	0	0	5.66	9.36	6.08	1.84	6.92	0	0

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

Table 19. Effect of different management practices on the disease incidence of die-back disease of citrus during the

Treatments	% Disease Incidence									
	Dec. 2010	Jan. 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept. 2011
T_1	6.00	0	0	0	0	0	0	6.0	5.33	0
T_2	9.33	0	0	0	0	0	0	8.67	8.67	0
T_3	0	0	0	0	0	0	0	7.34	8.02	0

T_4	10	0	0	0	0	0	0	0	5.33	3.99	
T_5	0	0	0	0	0	0	0	4.67	5.33	0	
T_6	0	0	0	0	0	0	0	0	4.67	0	
T_7	16.0	12.67	12.67	0	0	0	0	15.33	18.01	8.01	

growing period of December, 2010 to November, 2011

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

Table 20. Effect of different management practices on the disease severity of die-back disease of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Disease severity										
	Dec. 2010	Jan. 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sept. 2011	Oct. 2011
T_1	0.22	0	0	0	0	0	0	0.27	0.40	0	0
T_2	0.48	0	0	0	0	0	0	0.55	0.68	0	0
T_3	0	0	0	0	0	0	0	0	0.37	0	0
T_4	0.47	0	0	0	0	0	0	0.65	0.74	0.30	0.30
T_5	0	0	0	0	0	0	0	0	0.32	0	0
T_6	0	0	0	0	0	0	0	0.94	0.28	0	0
T_7	1.06	1.64	1.55	0	0	0	0	1.44	1.67	0.86	1.30

Data represent the mean value of 5 (five) replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

Table 21. Effect of different management practices on the disease on seedling height of citrus during the growing period of December, 2010 to November, 2011

Treatments	% Height (cm) of citrus seedling										
	Dec. 2010	January 2011	Feb. 2011	March 2011	April 2011	May 2011	June 2011	July 2011	Aug. 2011	Sep. 2011	
T_1	57.60d	62.60bc	66.20b	70.60b	74.40 b	77.20c	82.00bc	85.80cd	90.40c	94.40c	9
T_2	58.00cd	64.60abc	67.80b	72.80ab	79.20 a	83.80a	88.40a	91.80b	96.40b	100.4b	1
T_3	62.00b	65.00ab	70.20a	74.80a	79.40 a	83.60a	89.00a	94.40a	98.60a	103.2a	1
T_4	58.40cd	62.20c	66.20b	70.60b	73.40 b	76.40c	80.00c	83.40d	87.40d	91.20d	9
T_5	57.60d	62.60bc	66.20b	70.60b	74.40 b	77.20c	82.00bc	85.80cd	90.40c	94.40c	9
T_6	59.80c	63.80abc	67.40b	71.20 b	74.60 b	78.40bc	82.20bc	6.00c	89.20cd	92.80cd	96
T_7	64.00a	66.00a	68.00b	72.00b	74.80 b	80.00b	84.00b	86.20c	90.00c	94.00c	97
$LSD_{(p\geq 0.05)}$	1.894	2.361	2.156	2.368	2.198	2.569	2.157	2.336	1.831	2.235	1
CV (%)	2.43	2.83	2.45	2.53	2.22	2.48	1.97	2.04	1.53	1.79	

Data represent the mean value of 5 replications

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

T_2= BAU- Biofungicide was applied as foliar spray @ 2%

T_3= BAU- Biofungicide was applied in the soil as well as foliar spray @ 2%

T_4 = Mancozeb was applied as foliar spray @ 0.2%

T_5 = Bavistin was applied as foliar spray @ 0.2%

T_6 = Cupravit was applied as foliar spray @ 0.2%

T_7 = Untreated control

APPENDICES

Appendix I. Map showing the experimental site under study