EFFICACY OF SOME CHEMICALS IN CONTROLLING CITRUS CANKER DISEASE

By

RAIHANA NUSRAT

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

DECEMBER, 2015

EFFICACY OF SOME CHEMICALS IN CONTROLLING CITRUS CANKER DISEASE

BY

RAIHANA NUSRAT

REGISTRATION NO. : 10-04143

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

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

SEMESTER: JULY-DECEMBER, 2015

Approved by

Prof. Dr. Nazneen Sultana	**Abu Noman Faruq Ahmmed**
Supervisor	**Associate Professor**
Department of Plant Pathology	**Co-supervisor**
Sher-e-Bangla Agricultural University	**Department of Plant Pathology**
	Sher-e-Bangla Agricultural University

Dr. Md. Belal Hossain
Associate Professor
Chairman
Department of Plant Pathology
Sher-e-Bangla Agricultural University

Department of Plant Pathology
Sher-e-Bangla Agricultural University,Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9Fax: +88029112649 Web site: www.sau.edu.bd

CERTIFICATE

*This is to certify that the thesis entitled, "**EFFICACY OF SOME CHEMICALS IN CONTROLLING CITRUS CANKER DISEASE**"submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY** embodies the results of a piece of bona fide research work carried out by **RAIHANA NUSRAT** bearing **REGISTRATION NO. 10-04143** 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 been duly acknowledged.

Dated: 01. 12. 2016
Dhaka, Bangladesh

(Prof. Dr. Nazneen Sultana)
Supervisor
Department of Plant Pathology
Sher-E-Bangla Agricultural University

DEDICATED

TO

MY BELOVED PARENTS

ACKNOWLEDGEMENT

First of all I would like to thank to Almighty Allah, the most merciful and compassionate. The most gracious and beneficent to whom every praise is due and his Prophet Muhammad (SM) who is forever a Torch of knowledge and guidance for humanity as a whole with whose delighting the present endeavor has been beautiful.

Now I would like to express my earnest respect, sincere appreciation and enormous indebtedness to my reverend supervisor, Dr.Nazneen Sultana, Professor, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka. I am grateful to her ever inspiring guidance, keen interest, helpful commentary, scholastic and constructive suggestions and polite behavior throughout the course of my study.

I wish to express my gratitude and best regards to my respected Co-Supervisor, Associate Prof. Abu NomanFaruqAhmmed, Department of Plant Pathology, Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.

I am also highly thankful to Dr. Md. Belal Hossain, AssociateProfessor and Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his necessary help and co-operation during the study period.

I am highly grateful to my honorable teachers Prof. Dr. M. Salahuddin M. Chowdhury, Prof. Mrs. Nasim Akhtar, Prof.Dr. Md. Rafiqul Islam, Prof Dr.F. M. Aminuzzaman,Associate Prof. Khadija Akhter, ,Associate Prof. Dr. NazmoonNaherTonu, Assistant Prof. Shukti Rani Chowdhury, Assistant Prof Md. Ziaur Rahman Bhuiya, Sayed Mohammad Mohsin and HosnaAra Chowdhury Nisha, Lecturer, Department of Plant Pathology, and Professor Dr. Md. Razzab Ali, Department of Entomology, Faculty of Agriculture, Sher-e –Bangla Agricultural University, for their valuable teaching, direct and indirect advice, and encouragement and co-operation during the whole study period.

I am pleased to thank all staffs and workers of Plant Pathology Department of Sher-e-Bangla Agricultural University, Dhaka, for their valuable and sincere help in carrying out the research work.

I also extending my heart fell thanks to my friends Golam Rasul Shimul, Kallol Das,Marzana Afrose, Sanjida Rahman,Nishat Tasnim, Nitu Ahmed, Moumita Momi,

Iftakhar Sajib, ShahrinMahbubaTanni and also thanks to my well-wishers Shahran Ahmed Nayem and Tanbin Hasan Shuvofor their help, support, inspiration and patience during my work.

I found no words to thanks my parents for their unquantifiable love and continuous support, their never ending affection, immense strength and untiring efforts for bringing my dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of my studies.

The Author

EFFICACY OF SOME CHEMICALS IN CONTROLLING CITRUS CANKER DISEASE

ABSTRACT

Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* is one of the most devastating disease hampering worldwide citrus production. A Laboratory and a pot experiments was conducted to evaluate the efficacy of some chemicals in controlling citrus canker disease under the Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka during the period from July, 2015 to September, 2016. *Xanthomonas axonopodis* pv. *citri* was purified by restreaking on nutrient agar medium and confirmation was done by pathogenicity test. The bacterium was gram negative, rod shaped with rounded ends. It showed negative result in oxidase test and positive result in KOH solubility test, starch hydrolysis test, catalase test, citrate utilization test, gelatine liquefaction test. On NA medium the bacterium appeared as circular, mucoid, convex, yellow to orange colour and on SX medium the bacterium produced yellow to slightly blue, mostly circular, small, flattened, mucoid colonies. *In-vitro* evaluation of chemicals indicated that 0.2% Dithane M 45 (Mancozeb) at 96 hours of incubation showed highest (31.91%) inhibition zone against *Xanthomonas axonopodis* pv. *citri* among the four tested chemicals. Cl_2 solution, Sulcox 50 WP and Companion showed moderate result against the bacterium. In pot experiment, among the seven different chemicals, Mancozeb (Dithane M 45) was found highly effective against Xanthomonas *axonopodis* pv. *citri*. The highest disease incidence and severity were found in control treatment and the lowest incidence and severity were found in Dithane M-45 treated plants.

LIST OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

No.	=	Number
%	=	Percentage
et al.	=	And others
^{o}C	=	Degree Celsius
@	=	At the rate
WP	=	Wettable Powder
EC	=	Emulsifiable Concentrate
etc.	=	Etcetra
J.	=	Journal
Viz.	=	Namely
Cm	=	Centimeter
Cfu	=	Colony forming unit
df.	=	Degrees of freedom
&	=	And
ppm	=	Parts per million
Kg	=	Kilogram
G	=	Gram
ml	=	Milliliter

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

hr	=	Hour (s)
i.e.	=	That is
T	=	Treatment
cv.	=	Cultivar (s)
var.	=	Variety
mm	=	Millimeter
µl	=	Microliter
µm	=	Micrometer
SAU	=	Sher-e-Bangla Agricultural University
BBS	=	Bangladesh Bureau of Statistics
USA	=	United States of America
NA	=	Nutrient Agar (media)
NB	=	Nutrient Broth (media)
TTC	=	TriphenylTetrazolium Chloride
ANOVA	=	Analysis of Variances
LSD	=	Least Significant Difference
CV%	=	Percentages of Co-efficient of Variance

CHAPTER I

INTRODUCTION

Citrus (*Citrus* spp. L.) stands as second most important fruit worldwide after grapes in area and production. It is one of the most important, popular and nutritious fruit crop in the world as well as in Bangladesh and a tropical and subtropical crop which belongs to the family Rutaceae.It has a potential to make up the gap of vitamin c storage. 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). It has gained popularity because of its nutritive value, taste, aroma and economic importance among the people and is still booming (Whitney and Rolfes, 1999). There is considerable evidence that citrus foods may help reduce the risk, or retard the progression, of several serious diseases and disorders such as cardiovascular disease, cancer, anemia etc (Harats *et al*., 1998 and Reuther *et al*., 1967). Annual total citrus production in the world is 117 million tons among which lime and lemon production is 6.8 million tons (FAO, 2014). Bangladesh is ranked 52 in the world and has a 0.1% share in citrus production worldwide (FAOSTAT, 2012). In Bangladesh, the total acreage under citrus cultivation is about 5,995 ha while the total production is around 144,000 mt (BBS, 2015). In 2014-2015 fiscal year Bangladesh has exported 60 thousand 180 kg citrus in Europe alone and the amount is USD 2,06,111 (EPB, 2015). Citrus grows well in Chittagong, Chittagong Hill Tracts, Sylhet, Narsingdi, Gazipur, Comilla etc. district in Bangladesh.Eight species of citrus are grown in Bangladesh. Among them, lemon (*Citrus limon*), lime (*Citrus aurantifolia*) and pummelo (*Citrus grandis*) are commonly cultivated in our country. There is a large scope of expanding citrus production in our country.

Various factors are responsible for lower citrus production in Bangladesh. Among them, plant disease is one of the major influential factors. 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). Now-a-days, the demands of seedlings are very high in nurseries because healthy seedlings are prime need and basic raw material for establishment of an orchard. 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). Among them twelve diseases are

known to occur in different species of citrus in Bangladesh (FAO, 2014). Most of the commercial citrus species grown in nursery around the world including Bangladesh are suffering from a bacterial disease, citrus canker caused by *Xanthomonas axonopodis* pv. *citri*(Graham *et al.*, 2004; Gottwald *et al.*, 2002 and Koizumi, 1985). The disease is endemic in many tropical and subtropical citrus growing areas (Goto, 1992) and has been spread to most citrus producing areas of the world. It is originated in Southeast Asia, is extremely persistent when it becomes established in an area (Wolf, 2016). The disease is believed to have originated in South East Asia which was first found around 1912, spread throughout the southeastern U.S. on imported seedlings from Japan (Schoulties *et al*., 1987).Citrus canker caused by the bacterium *Xanthomonas axonopodis* pv.*citri* is probably the worst enemy to the citrus plantation (Awam *et al*., 1992). It is mostly a leaf spotting and rind blemishing disease (Civerolo, 1984). In our country most citrus exporters could not export citrus fruit to Europe for the attack of canker disease on the fruits (DAE, 2012). Intensive research on citrus canker is being carried out throughout the world which has been reviewed by Sahi *et al.* (2007); Zekri *et al.* (2005); Brurning and Gabriel (2003); Schubert and Sun (2003); Gottwald *et al.* (2001); Bergamin-Filho *et al.* (2000); Gabriel *et al.* (2000); Stall and Civerolo (1991); Sohi and Kapoor (1990). Citrus canker is distributed over 30 countries of the world (Das, 2003). This disease hampers citrus production not only by impeding quality of produces but also reducing economic value of the fruits. According to USDA approximately 50 million dollars per year are spending for management of this disease (USDA, 2014).

The bacterium *Xanthomonas axonopodis* pv. *citri* is a rod-shaped, gram-negative, and has a single polar flagellum. Colonies on laboratory media are usually yellow due to ‘xanthomonadin’ pigment production. Still now four types of citrus canker are found. Canker A (Asiatic canker) is found in Asia, South America, Oceania and the USA (Carrera, 1933); canker B (Cancrosis B) in South America (Carrera, 1933); canker C (Mexican lime cancrosis) in Brazil (Schaad *et al*., 2005); and canker D (citrus bacteriosis) in Mexico (Rodriguez *et al*., 1985). It gives positive result in KOH solubility test, starch hydrolysis test, catalase test, asculine hydrolysis, urease production, milk proteolysis, tween 80 lypolysis, gelatine liquefaction test, salt tolerant test, tobacco hypersensitivity reaction and gives negative result in oxidase test (Kishun and Chand, 1991). The bacterium produces bright yellow colony on both

GYCA and YDC medium (Yenjerappa, 2009) and light yellow colony with clear zone surround them on SX medium (Vudhivanich, 2003). Asian citrus leaf miner is the vector of this pathogen wounding induced by the larvae increases infection by *X. axonopodis* pv. *citri*during the flush periods (Schubert *et al*., 2001; Gottwald *et al*, 1997). The most critical period for fruit infection is during the first 90 days after petal fall (Graham *et al*., 2004). The citrus leaf miner (CLM), *Phyllocnistis citrella* is a potentially serious pest of citrus and related Rutacease, and somerelated ornamental plants (Beattie, 1989). They remain active from March to November. Caterpillars of this insect feed on leaves by making shiny silvery serpentine mines. The mining injuries serve as foci of infection for the cause of citrus canker, a bacterial disease (Jayaraj and Baskaram, 2007). Environmental factors play foremost role in the susceptibility of citrus plants to canker. Temperatures between 15 to 20°C and 35 to 40°C are conducive for infection and development of citrus canker disease (Pria *et al*., 2006). The disease is mostly prevalent in area with more than 1000 mm rainfall per year (Verniere *et al*., 2003). The pathogen is dispersed by splashing rain and winds in excess of 8.0 ms-1 (Gottwald *et al.,* 1989).

Appropriate management of citrus canker has been investigated by many researchers (Singh *et al*., 2005; Canteros, 2004; Graham and Leite, 2004; Das and Shyam, 2003; Dixon *et al.,* 2000; Gottwald and Timmer, 1989; Civerolo, 1981. In the last 90 years, scientific, industry and regulatory personnel worldwide have made recommendations to eradicate citrus canker based on cost/benefit ratios for the planned actions (Broadbent *et al*., 1992; Schubert *et al*., 2001).Prophylactic sprays of copper oxychloride or other copper containing compounds provide protection against initial infection of citrus canker during growth flushes and fruit development (Das, 2003; Muraro *et al.,* 2001; Leite and Mohan, 1990 and Stall *et al.,* 1980). Citrus canker is managed with preventive sprays of copper-based bactericides. Such bactericides are used to reduce inoculum build up on new leaf flushes and to protect expanding fruit surfaces from infection. Agro chemicals are noxious for environment (Huang, 1997). Therefore, concentration is focused on propitious method of disease management that will be friendly for environment as well as for mankind (Sutton, 1996).

Considering the above facts this research program has been designed with the following objectives:

1. To isolate and identify citrus canker causing pathogen *Xanthomonas axonopodis* pv.*citri* from infected leaves.
2. To find out the efficacy of some selected chemicals against citrus canker disease caused by *Xanthomonas axonopodis* pv.*citri.*
3. To find out the effect of selected chemicals on disease incidence and severity of citrus canker.

CHAPTER II

REVIEW OF LITERATURE

Canker of citrus caused by *Xanthomonas axonopodis* pv. *citri*, once deemed as a disease of minor importance, become a serious threat for citrus production in recent years. The disease assumed it's severity in all the growing areas of the world resulting severe yield losses both in terms of quality and quantity. The information available on this disease, pathogen and management strategies are very meagre. Hence, the literature pertaining to the canker of citrus along with information on related crops disease and pathogen are reviewed here as under.

2.1. Symptomology

Balestra *et al*. (2008) proclaimed canker lesions as hyperplasia type, often surrounded by a water-soaked margin and yellow halo. Typical citrus canker lesions were found on 8 to 10 years old lime (*Citrus limetta*) and grapefruit (*Citrus paradisi*) trees in northern and southern Somalia, respectively.

Graham *et al*. (2004) reported that the earliest symptoms on leaves appear as tiny, slightly raised blister-like lesions beginning around 9 days post-infection. As the lesions age, they first turn light tan, then tan to brown, and a water-soaked margin appears, often surrounded by a chlorotic halo. The water soaked margin may disappear as the lesions age, and is not as prominent on resistant cultivars. The centre of the lesion becomes raised and spongy or corky. These raised lesions from stomatal infection are typically visible on both sides of a leaf. Eventually, the centres of the leaf lesions become crater-like. Defoliation becomes a problem as the disease intensifies. On twigs and fruit, citrus canker symptoms are similar: raised corky lesions surrounded by an oily or water-soaked margin. No chlorosis surrounds twig lesions but may be present on fruit lesions. Twig lesions on angular young shoots perpetuate the inoculum and prolong survival of *X. axonopodis*pv. *citri* in areas where citrus canker is endemic. If twigs are not killed back by girdling infections, the lesions can persist for many years, causing raised corky patches in the otherwise smooth bark.

Brunings and Gabriel (2003) noticed on citrus leaves that first appearance of *Xanthomonas axonopodis* was water soaked, 2-10 mm, similarly small sized, circular spots, usually on the abaxial surface. On leaves, stems, thorns and fruits, circular lesions became raised and blister-like, growing into white or yellow spongy pustules. These pustules then darkened and thickened into a light tan to brown corky canker, which was rough to the touch. On stems, pustules coalesced to split the epidermis along the stem length, and occasionally girdling of young stems may occur. Older lesions on leaves tend to have more elevated margins and were at times surrounded by a yellow chlorotic halo (that may disappear) and a sunken centre.

Vudhivanich (2003) observed that canker lesions at first were small, slightly raised, round, light green spots. Later, they became grayish white, rupture, and appear corky with brown, sunken centre. The margins of the lesions were often surrounded by a yellowish halo.

Braithwaite *et al*. (2002) observed that yellow/brown, raised and corky lesions were formed on leaves, twigs and fruits of cultivated citrus which darkened and developed central depressions with age. The raised edges of the lesions were surrounded by a chlorotic halo.

Pruvost *et al.* (2002) expressed that the bacterium multiplies in lesions in leaves, stems and fruit. When there is free moisture on the lesion surface, bacteria are released from an extracellular polysaccharide matrix and dispersed to new growth by rain splash.

Swarup *et al*. (1991) noted that citrus canker lesions were first appeared as pin-point spots that became small, slightly raised pustules or blister-like eruptions. Initially, those appear on the lower leaf surface about 7 days after infection. Subsequently, the blisters became visible on the upper leaf surface. The young lesions were usually translucent due to water-soaking of the tissue. Lesions were initially circular or irregular, light colored at first and became tan or brown later.The epidermis ruptured and the lesions became spongy or corky at matured stage. The lesions finally became crater-like with a raised margin and sunken centre. The centre of large, old lesions cracked and/or dropped out.

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.

2.2. Cultural characteristics of *Xanthomonas axonopodis*pv. *citri* on different culture media

Jabeen *et al*. (2012) noticed that *Xanthomonas* gave yellow, circular, smooth, convex and viscous bacterial colonies on yeast dextrose calcium carbonate agar medium (YDCA) after 48-72 h of incubation at 28^{o}C. On SX medium the bacteria gave light yellow, mucoid, round and smooth colonies (1mm in diameter) while whitish, mucoid and smooth colonies were observed on Wakimoto medium.

Yenjerappa (2009) conducted an experiment to study the growth of *Xanthomonas axonopodis* on different growth media and found that modified D-5 medium was significantly superior in promoting the luxurious growth of the pathogen followed by yeast extract nutrient agar medium. Colonies of the bacterium on MD-5 and YNA medium appeared as circular to irregular, flattened, colourless to light yellow, occurred singly or rarely in aggregate. Colonies of similar morphology with glistening character and bright yellow colour were observed on both GYCA and YDC medium. Circular to irregular, slightly raised, mucoid colonies were recorded on nutrient agar and starch agar medium. XTS agar supported the moderate growth of the bacterium with minute, slightly raised, circular, creamy white coloured colonies. Bacterium exhibited very poor growth with dull white and slightly raised colonies character on BSCAA medium. He also revealed that *Xanthomonas axonopodis* liquefied the gelatin, hydrolysed the starch, positive for H_2S production, catalase and oxidase, utilized various carbon sources viz. glucose, fructose, sucrose, dextrose and produced mild acid from these carbon sources but did not utilize lactose, maltose, mannose and mannitol.

Balestra *et al*. (2008) isolated yellow, xanthomonad like mucoid, convex colonies on YDC medium which were purified and stored on YDC slants. Upon conducting pathogenicity tests, they also observed symptoms typical of *X. citri* on inoculated plants.

Vudhivanich (2003) isolated *Xanthomonas axonopodis*pv. *citri* from diseased citrus by tissue transplanting method on SX agar. After incubated for 48 hours at room temperature (30^0C), the light yellow colony developed from plant tissue with clear zone surround them.

Chand and Kishun (1991) revealed that *Xanthomonas* produce mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium and on SX agar, pathogen produced a clear starch digestion zone.

Hingorani and Singh (1959) reported that nutrient agar, yeast glucose chalk agar and potato cylinders are the best media for the cultivation of *Xanthomonas axonopodis* because of luxuriant growth obtained on them. Colonies on nutrient agar were filiform, slightly raised, glistening, pale yellow and odourless. Similar characters were also found on yeast glucose chalk agar with an exception that colour of the colony was bright yellow in the beginning and gradually changed to quite dark brown with age. They also described that the bacterium utilizes xylose, glucose, mannose, galactose, sucrose, lactose and raffinose but not maltose, glycerine and salicin when grown in Durham's fermentation tubes containing one percent carbohydrates in a peptone free synthetic liquid medium. Ammonia was produced in peptone water after 15 days. Nitrites, hydrogen sulphide and indole were not produced. Starch was hydrolysed, methyl red and voges proskauer tests gave negative results. Growth on gelatin slabs was good. Stratiform type of liquefaction commenced after 48 hours and completed within 21 days. The yellow colour of the growth on gelatin gradually changed from usual bright yellow to dark brown on yeast glucose chalk agar and cooked potato.

2.3. Biochemical characteristics of *Xanthomonas axonopodis*pv. *citri*

Braithwaite *et al*. (2002) detected that gram negative *Xanthomonas axonopodis*pv. *citri*produced yellow pigmented, mucoid colonies on yeast dextrose agar, which were also isolated from the leaf lesions. They conducted pathogenicity test on potted citrus (*Citrofortunella mitis*) plants. Water-soaked lesions, 2-3 mm diameter, developed at the inoculation sites after 10 days and the bacteria were consistently re-isolated from the affected tissues.

Gottwald and Graham (1992) observed that the concentrations less than 10^4 cfu/ml of *Xanthomonas* were insufficient to cause infection on unwounded citrus leaves under an impact pressure of 8.05 kPa, however 10^6cfu/ml gave consistent and successful infection.

Kishun and Chand (1991) reported that *Xanthomonas* was negative in nitrate reduction, urease oxidative, fermentative metabolism of glucose and acid from adonitol and sorbitol. The bacterium was positive in KOH solubility, gelatin liquefaction, hydrolysis of Tween 80, H_2S production, starch hydrolysis, indole production, growth at 3.5 percent NaCl, sucrose utilization, milk proteolysis and acid from most of the sugars.

Chand and Pal (1982) studied biochemical characteristics of *Xanthomonas axonopodis* and they found that bacterial cells were positive for hydrolysis of starch, aesculin, casein, liquefaction of gelatin and production of tyrosinase, catalase, reducing substance from sucrose, and hydrogen sulfide. The bacterium was negative for nitrate reduction, indole production and for methyl red test.

Genus *Xanthomonas* consisted of gram negative, rod shaped, polarly flagellated bacteria whose members were commonly occurred as serious plant pathogens. Colonies were typically yellow in color due to the presence of a particular carotenoid pigment identified through relatively simple screening procedures (Starr and Stephens, 1964).

2.3. Management of citrus canker with chemicals

Behlau *et al.* (2010) studied the effects of copper sprays on annual and polyetic progress of citrus canker caused by *Xanthomonas citri* pv. *citri*in the presence of the Asian citrus leafminer (*Phyllocnistis citrella*) in a commercial orchard in northwest Parana state, Brazil. They observed although citrus canker incidence increased during each of the seasons studied, it decreased over the whole study period, more so in copper treated trees than in water sprayed controls. Copper treatment reduced disease incidence compared with controls in every year. Copper treatment also reduced estimated initial disease incidence and epidemic growth rates every year.

Dhakal *et al.*, (2009) studied different chemicals, copper oxychloride (2.5%), copper oxychloride +kasugamycin (1000X), Bordeaux mixture 1% and 2% were sprayed to the plants in citrus orchard at Dhulikhel and the decrease in disease severity after spraying of chemicals was calculated with reference to the plants that were not sprayed with the chemicals. It was observed that spraying of the chemicals help in decreasing the disease severity.

Gottwald *et al.* (2009) showed that prewashing fruit and treating with chlorine significantly reduced total bacterial recovery compared to chlorine treatment alone, and prewashing fruit followed by chlorine and detergent was the most effective method for killing bacteria on the fruit surface.

Samavi *et al.* (2009) evaluated on the efficiency of two novel compounds, thyme essential oil produced from zaatar (Zataria multiflora) and Nanosilver (NS), as well as some commonly used chemicals were evaluated against citrus bacterial canker using detached leaf assays in the laboratory and whole seedling in the greenhouse. Mexican lime seedlings were used for detached leaf assays. Treatments included 0.3% copper oxychloride (COC), 1.5% Bordeaux mixture (BO), 0.3% OC+0.04% Mancozeb (MZ), 1.5% BO+0.04% MZ, TEO at 10-2 dilution, three concentrations of NS (100, 150 and 200 ppm) and 100 ppm Streptomycin (S). Results showed that all treatments were significantly different at P<0.01%. The best results were obtained with BO+MZ and TEO at 10-2 dilution, with infection inhibition of 78.44% and 69.78% respectively. Moreover, COC+MZ, BO, S and COC alone reduced the disease by 60.15%, 55.15%, 45.78% and 41.09% respectively.

Behlau *et al.* (2008) investigated the benefit of wind breaks and copper sprays for control of citrus canker caused by *Xanthomonas axonopodis* pv. *citri*in southern Brazil. Control of canker was evaluated as incidence and severity of lesions on foliage and by the effect on premature leaf and fruit drop for three production seasons. Effect of the treatments on fruit production was evaluated as incidence of citrus canker on prematurely abscised fruits and harvested fruits. They found copper application significantly reduced damage to foliage and fruit, while windbreaks made little contribution to disease control. Copper sprays increased fruit yield for 3 years. This could be attributed to lower incidence of fruit with lesions and fewer fruits abscised due to canker infection. Incidence and severity on the leaves were inversely related to

the number of fruits harvested per tree and directly related to the number of fruits abscised per tree.

Ahmed and Sahi (2009) studied that application of botanical and microbes has not affected the leaf miner infestation in plants of citrus nursery and groves. They suggested that to control citrus leaf miner plants with new flushes of leaves should be sprayed with Dimethoate 40 EC or Carbosufan 20 EC or Malathion 57 EC @ 2.0 ml/litre of water.

Behlau *et al.* (2007) conducted an experiment to study citrus canker incidence and severity under natural conditions in an orchard of Pera Rio sweet orange (*Citrus sinensis*) planted in Brazil. Chemical control, by using copper oxychloride sprays and windbreak protection were evaluated to reduce citrus canker incidence and severity on leaves. Levels of disease incidence and severity were evaluated monthly using specific diagrammatic scales. The coefficient of determination (R2) between incidence and severity levels was also determined. While copper sprays significantly reduced citrus canker on leaves, windbreak did not contribute significantly to disease control. After 29 assessments, plants subjected to frequent copper sprays showed AUDPC values for citrus canker incidence which were 43.5% lower than those observed on plants not protected with chemical sprays. The same result was observed for citrus canker severity. After 18 assessments, plants sprayed with copper showed AUDPC values for severity which were 37.1% lower than those for the control plants. In both years, the values of R2 between incidence and severity levels were higher than 0.80 ($p<0.01$).

Hardy *et al.*, (2007) demonstrated a research in the drier climate of Gayndah, Queensland, using lower rates of copper (40-80g copper/100 L water) there was no increase in blemish on navels from either 2 or 4 applications of copper hydroxide or 4 applications of either copper ammonium acetate or cuprous oxide. There was a significant increase in blemish after 4 applications of copper oxychloride at 100g copper/100 Lwater.

Meneguim *et al.* (2007) conducted a study to determine the sensitivity of *Xanthomonas axonopodis* pv.*citri*strains to copper as well as to a mixture of copper with mancozeb. The highest copper concentration where *X. axonopodis* pv.*citri*grew was 50 µg/ml. However, 45.5% of the bacterial strains from orchards with regular

sprays of copper compounds grew in the presence of 50 µg copper/ml. In contrast, only 13.4% of the strains from citrus orchards that never received copper sprays grew in such a copper concentration. Mixing mancozeb with copper increased the tolerance of *Xanthomonas axnopodis* pv.*citri*to copper.

Stein *et al.*, (2007) worked on citrus leaf miner control and copper sprays for management of citrus canker in Tucuman, Argentina. Abamectin and copper bacteriocides applied alone or in combination with products for control of citrus leaf miner and citrus canker in lemon were evaluated for three seasons. Canker treatments were applied on six dates from petal fall to February each year at 28 days intervals with a high volume. CLM control treatment with abamectin were applied every 15 days during the period og high CLM pressure. Higher application rates of copper hydroxide (0.20%) were more effective in controlling the disease then the lower rates (0.15%).

Koller *et al.* (2006) reported that copper treatments were not effective in controlling citrus canker at high inoculum concentration, but at low inoculum concentration, both Bordeaux mixture and copper oxychloride controlled 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).

Maher *et al.* (2005) tested toxicants (streptomycin sulfate, Dithane M-45 [mancozeb], Agrimycin-100, Vitavax [carboxin], Benlate [benomyl] and Cobox [copper oxychloride]) at 1% concentration against multiplication of *Xanthomonas axonopodis* pv. *citri*(*in vitro*). They observed Agrimycin-100, Streptomycin sulfate, Vitavax and Dithane M-45 were more effective compared to other toxicants. Agrimycin-100, streptomycin sulfate, Dithane M-45 and Vitavax were further studied *in vitro* against the growth of X. *axonopodis* pv. *citri*at 0.01, 0.1 and 1% concentration. All the toxicants inhibited the multiplication of the bacterium at all concentrations, however streptomycin sulfate was the most effective among the toxicants used while Agrimycin-100, Vitavax and Dithane M-45, in the order, were effective against the multiplication of bacterium at 0.01, 0.1 and 1% concentration. They also sprayed Streptomycin sulfate, Agrimycin-100, Vitavax, Dithane M-45 and Benlate at 0.2%

concentration on the field grown citrus plants and then inoculated with *X. axonopodis* pv. *citri*for the control of citrus canker disease. Streptomycin sulfate, Vitavax, Dithane M-45 and Agrimycin-100 in the order proved effective also in reducing the disease intensity compared to the inoculated control.

Ren *et al.* (2005) conducted an experiment with 4 years old trees of Hongjianhcheng orange cultivar and 21 fungicides for control of the canker pathogen strain HCS-1 (*Xanthomonas axonopodis* pv. *citri*). They evaluated among the 21 fungicides for control of HCS-1 the best one was spraying 1000x diluted solution of 10% streptomycin and then the 1000x diluted solution of 53.8% Kocide [copper hydroxide] suspension. Both control effectiveness could reach 43.0 - 34.6% for the leaf canker.

Graham and Leite (2004) observed that prophylactic sprays of copper oxychloride (or other copper containing compounds) provide protection against initial infection in canker endemic areas during growth flushes and early fruit development (fruit approximately 2-6 cm diameter).

Pan (2004) tested the effect of different concentration of 77% Kocide [copper hydroxide] solutions on 11 to 13 years old trees of Sijiyou pomelo cultivar. The 500x dilution of 77% Kocide was the best one for control of pomelo canker (*Xanthomonas citri*). Spraying 500x dilution of solution of 77% Kocide wettable powder to the summer shoots could get 100% of the shoot leaves without infection. Spraying 500x dilution of 77% Kocide wettable powder removed infection. The efficacy could reach 98.2%.

Verona *et al.* (2004) studied the relationship between the frequency of copper oxychloride sprays and the incidence of citrus canker in sweet orange leaves caused by *Xanthomonas axonopodis* pv. *citri*. They found control of citrus canker with 4 sprays of copper oxychloride at 30 day intervals during the growth season was satisfactory.

Das (2003) reported that timely application of protective copper containing and/or antibiotic sprays were generally the most effective means of disease management.

Zhong and Ling (2002) reported that citrus canker occurrence had a close relationship with the daily mean temperature. When a daily temperature of 12^0C occured for 10-15 days, the spring shoots and fruitlets were attacked. The experiments also showed that spraying spring 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.

Fu and Xu (2001) reported that citrus canker (*Xanthomonas axonopodis* pv. *citri*) appeared in late April and early May, the rampant period being mid May to early June and found the occurrence was correlated with the temperature. They also observed spraying with 500 fold and 400 fold solution of 77% copper hydroxide and 60% chlorothalonil solution resulted the efficient control 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.

Ravikumar *et al.* (2001) reported that streptomycin sulfate (500 ppm) sprayed either alone or in combination with copper oxychloride (2000 ppm) was very effective in reducing the disease severity (2.23-2.51 number of lesions per fruit), followed by combination spray of streptocycline (500 ppm)+copper oxychloride (2000 ppm) (3.22) compared to the control (8.29).

Chen *et al.* (2000) 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.

Canteros *et al.* (2000) stated that postharvest treatments, such as chlorine or SOPP, guarantee the complete eradication of epiphytic *Xanthomonas campestris* pv.*citri*on fruits without symptoms.

Jadeja *et al.* (2000) conducted field experiments to test the efficacy of various chemical treatments for the control of canker (*Xanthomonas axonopodis* pv. *citri*) of citrus at the Horticultural farm of Gujarat Agricultural University, Junagadh, Gujarat, India. Six years old acid lime (Kagzi lime) trees were treated with Bordeaux paste [Bordeaux mixture], Ridomil MZ 72 [metalaxyl + mancozeb], streptomycin sulphate + copper oxychloride, fosetyl Al [fosetyl], and aureofungin sol, alone and in various combinations. The results revealed that all the treatments with a foliar spray reduced canker incidence significantly except for aureofungin sol. Among these higher canker control was achieved with foliar application of streptomycin sulphate + copper oxychloride.

Lai (2000) studied the infection dynamics of *Xanthomonas axonopodis* pv. *citri*(citrus canker) and it's prevention and control by various pesticides (based on laboratory tests). The tests showed Kocide (copper hydroxide) and Baijuntong had good control effects.

Zheng *et al*., (2000) showed that chlorine dioxide is the 4^{th} generation of generally acknowledged disinfectant, with wide application range, good effect and high safety. The laboratory test showed that ClO_2 was very effective in reducing pathogenic bacteria such as *Xanthomonas oryzicola, X. citri* and in reducing fungi spores of *Piricularia oryzae, Fusarium graminearum, Erysiphe graminis, Sphaceloma ampelinum, Penicillium italicum* and *P. digitatum.*

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.

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.

Kale *et al.* (1994), spraying of streptocycline + Copper oxychloride (0.1%) should preferably be done at 7 days or 15 days interval for better control of canker.

Leite and Mohan (1990) observed that sprays of copper oxychloride or other copper containing compounds reduce the prevalence of Xac infection in citrus field.

Stapleton (1986), showed that four strains of *Xanthomonas campestris* pv.*citri* did not survive a 2-min exposure to more than 10 ppm chlorine in aqueous suspension and suggest that bacteria would not survive in packing shed dip-tank NaOCl solutions.

Kuhara (1978) observed that six or seven sprays of copper are necessary to protect new growth from infection.

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.

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

The laboratoryexperiment was conducted in the Molecular Plant Pathology laboratoryof Department of Plant Pathology and pot experiment was conducted in the net house under Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, (SAU), Sher-e-Bangla Nagar, Dhaka, Bangladesh .

3.2. Experimental period

The experiments were conducted during the period on August, 2015 toSeptember, 2016.

3.3. Isolation and identification of causal organism of citrus canker

3.3.1. Collection of disease plant parts

Diseased leaves and fruits with typical symptoms were collected from the infected citrus (*Citrus aurantifolia*) plants (Figure 1). The specimens were kept in the refrigerator at 4^0C by following standard procedure of preservation of disease specimens until isolation was made.

3.3.2. Nutrient Agar (NA) Preparation

Nutrient agar media (Appendix-I) was prepared according to the method followed by Schaad (1988). At first 15 g bacto agar was taken in an Erlenmeyer flask containing 1000 ml distilled water. Then 5 g peptone and 3 g beef extract were added to it. The nutrient agar was shaken thoroughly for few minutes for mixing properly. The mixture was then autoclaved at 121 ^{0}C under 15 PSI pressure for 15 minutes.

3.3.3. Isolation and purification of canker pathogen of citrus

The diseased citrus leaves were washed with sterilized distilled water. Then the young lesions with green healthy portion of diseased leaves were cut into small pieces. It was then surface sterilized by dipping them in 0.1% sodiumhypochloride solution for 20-30 seconds. After that it was washed three times with sterile water. At the end of surface sterilization the cut pieces were kept in a Petri dish and chopped with a sharp sterile blade. Then it was taken in a test tube containing 3-4 ml of sterile water and kept for 30 minutes for bacterial streaming and getting stock. With the help of sterile pipette one ml of this stock solution was transferred into the second test tube containing 9 ml sterile water and shaken thoroughly resulting 10^{-1} dilution (Figure 2). Similarly, final dilution was made up to 10^{-4}. After finishing the preparation of different dilution, 0.1 ml of each dilution was spread over NA plate previously dried (to remove excess surface moisture) at three replications as described by Goszczynska and Serfontein (1998). The solution was spread with the help of alcohol flame sterilized glass-rod. The inoculated NA plates were kept in incubation chamber at 30^0C. It was observed after 24 hrs and 48 hrs. In order to get pure colony, single unmerged colony grown over NA plate was restreaked on another plate with the help of a sterile loop.

Figure1: Infected citrus leaves showing canker symptoms.

Figure 2: Laminar air flow showing the apparatus used in dilution plate method.

3.3.4. Growth on nutrient agar (NA) media

Freshly prepared Nutrient agar (NA) medium was poured into a sterile petri dish and cooled. Pure colony of bacterium was streak inoculated on the plate with the help of a sterile transfer loop. It was incubated at 30^0C for at least 24 hours in incubation chamber and observed the colony characters.

3.3.5. Preservation of *Xanthomonas axonopodis*pv. *citri*

A slant culture of purified bacteria was done on NA slant in small screw-cap test tubes in order to preserve the bacteria for future use and kept it in refrigerator at 4^0C.

3.4. Identification of the pathogen

Citrus canker pathogen was identified on the basis of studying morphological, biochemical and cultural features of the pathogen as per standard microbiological procedures.

3.4.i. Morphological characters

Morphological characteristics of the pathogen such as cell shape, gram's reaction and pigmentation were studied as per the standard procedures described by Schaad, (1992); Gerhardt, (1981) and Bradbury, (1970).

3.4.i.a. Gram's staining

A single drop of sterile water was placed on a clean microscope slide. Small amount of a young colony (18-24 hrs old) was taken with a cold, sterile loop from the nutrient agar medium and the bacteria were smeared on to the slide very thinly. The thinly spread bacterial film was air dried. Underside of the glass slide was heated by passing it four times through the flame of a sprit lamp for fixing the bacteria on it. After that the slide was flooded with crystal violet solution for 1 minute. The slide was rinsed under running tap water for a few seconds and excess water was removed by air. Then it was flooded with lugol's iodine solution for 1 minute. After that it was decolorized with 95% ethanol for 30 seconds and again rinsed with running tap water and air dried. Then it was counterstained with 0.5% safranine for 10 seconds. It was rinsed under running tap water for a few seconds and excess water was removed by air. Then the glass slide was examined at 40x and 100x magnification using oil immersion.

3.4.i.b. KOH solubility test

A single drop of 3% KOH (aqueous) was placed on a glass slide. One loop full of a single colony (18-24 hrs old) was taken from the NA plate using a cooled, sterile loop and it was mixed with KOH solution until an even suspension was obtained. The loop was raised a few centimeters from the glass slide and repeated strokes to have strands of viscid materials as described by Suslow *et al*. (1982).

3.4.ii. Biochemical characters

Biochemical tests such as oxidase test, gelatine liquefaction test, starch hydrolysis test, catalase testand citrate utilization test, were studied as per the methods described by Schaad (1992) and Salle (1961).

3.4.ii.a. Oxidase test

1ml 1% aqueous (w/v) solution of NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride solution was spread on the middle of filter paper and the paper placed on a petri dish. Then some colony part of the bacteria was picked with a sterile toothpick and smeared onto the moistened filter paper and observed up to 60 seconds whether it changed color to dark purple or not.

3.4.ii.b. Gelatin liquefaction test

A tube containing 12% (w/v) gelatin was stub inoculated with one loop-full bacterial culture with the help of a sterile transfer loop. It was incubated at 30^0C for 24 hours. By the formation of liquid culture after keeping it at 5^0C in refrigerator for 15 minutes, gelatin liquefied microorganism was determined.

3.4.ii.c. Starch hydrolysis test

Nutrient agar plate containing 0.2% soluble starch was spot inoculated with pure colony of bacterium. It was then incubated at 30^0C for at least 48 hours in incubation chamber. Then the plates were flooded with lugol's iodine solution and observed whether a clear zone appeared around the colony or not.

3.4.ii.d. Catalase test

Some drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) was added with 48 hours old pure culture of bacterium grown on NA plate and observed whether it produced bubbles within a few seconds or not.

3.4.ii.e. Citrate utilization test

Petri dish containing simmon's citrate agar media was slant inoculated by Pure colony of bacterium with the help of a sterile transfer loop. Then it was incubated at 30^0C for 24 hours in incubation chamber. After incubation it was observed to determine color changed from green to bright blue.

3.4.iii. Cultural characters

Pathovars of *Xanthomonas* can be comprehended by growth and colony morphology on different media (Schaad, 1992). Growth characteristics of the pathogen were studied by using various differential, selective media.

3.4.iii.a. SX Agar media preparation

SX agar (Appendix-I) was prepared according to the method followed by Goszczynska *et al*. (2000). 10 g soluble potato starch, 1 g beef extract, 5 g NH_4Cl, 2 g K_2HPO_4, 0.4 ml methyl violet 2B (1% in 20% ethanol), 2 ml methyl green (1% in water) and 15 g agar were taken in an Erlenmeyer flask containing 1000 ml distilled

water for the preparation of 1 liter SX agar medium. The mixture was autoclaved at 121^0C under 15 PSI pressure for 15 minutes. Then it was cooled to 50^0C and 2ml cycloheximide (100 mg/ml in ethanol) was added to it and mixed thoroughly.

3.4.iii.b. Growth on selective SX agar media

Freshly prepared SX agar medium was poured into a sterile Petri dish and cooled. Pure colony of bacterium was streak inoculated on the plate with the help of a sterile transfer loop. Later it was incubated at 30^0C for at least 24 hours in incubation chamber and observed the colony characters.

3.5. Pathogenicity Test

Five healthy seedlings were collected from the Krishibid nursery Agargaon, Dhaka. Then citrus plant (*Citrus aurantifolia*) grown on earthen pot under net house condition was used for examining the pathogenicity of *Xanthomonas axonopodis*pv. *citri* as per method described by Lin *et al*. (2008). Inoculum was prepared by growing bacterial cells overnight in NA broth and resuspending in sterile distilled water to a concentration of approximately 10^8 cfu/ml (OD:0.5_{650nm}). After that an aliquot of the inoculums suspension was injected forcedly into the lower surface of citrus leaf between two epidermal layers using a sterile syringe. Distilled water was used as a negative control. It was observed for 15 days. Visual symptoms were recorded and examined. Koch's postulates were confirmed by reisolating bacteria from the artificially infected leaves.

3.5.1. Nutrient Broth (NB) Preparation

Nutrient broth (Appendix-I) was prepared according to the method followed by Schaad (1988). Five gram peptone and Three gram beef extract were taken in the Erlenmeyer flask containing 1000 ml distilled water and mixed well. The mixture was then autoclaved at 121^0C under 15 PSI pressure for 15 minutes.

3.6. *In vitro* evaluation of some agrochemicals against *Xanthomonas axonopodis* pv. *citri*

3.6.1. Preparation of sterile swabs

A supply of cotton swabs on wooden applicator sticks was prepared. The swabs were held in a dry tube and were sterilized in the autoclave (Vandepitte *et al*., 1991).

3.6.2. Bioassay ofagrochemicals against the bacteria

Bioassay of agrochemicals against the bacteria was done by well diffusion method measuring the inhibition zone (Grainger *et al*, 2010).*Xanthomonas axonopodis*pv. *citri* spread over NA plate with the help of sterile cotton swab. Then one hole of 4mm was punched into NA plate. Different chemical suspension were prepared and added into the hole @ 80 µl/hole. Three replication were used for each chemical. In case of control, instead of chemicals only sterile water was added into the hole. Then the plates were incubated at 30^0 C in incubation chamber.After incubation, an inhibitory effect on the test organism is indicated by a clear zone (no growth) around the test substance; microbial growth is visible to the naked eye in areas of the plate that are unaffected. Zone of inhibition around the holes were measured and recorded after every 24 hours for 4 days.

Inhibition diameter at every plate under each treatment was measured by scale. Percent inhibition zone was estimated by the following formula as suggested by Johnson and sekhar(2012).

$$\text{Percentage inhibition of growth} = \frac{C-T}{C} \times 100$$

C= Growth of bacteria in control
T= Growth of bacteria in treatment

Table 1. Name of theagrochemicals used in bioassay

Trade Name	Active Ingredients	Chemical Name	Concentration (%)
Companion	Mancozeb+carbendazim	Copper chloride oxide hydrate	0.2
Dithane M-45	Mancozeb	N-(2,6dimethyl phenyl)-N(methoxyacetyl)-Alanine methyl ester ($C_{14}H_{21}NO_4$)	0.2
Sulcox 50 WP	Copper oxychloride	Copper chloride oxide Hydrate	0.1
Clorox	Sodium hypochlorite	Cl_2 solution	0.5

3.7. Effect of agrochemicals on citrus canker disease development

3.7.1. Collection of citrus plants

Thirty six disease free and healthy citrus seedlings were collected from different nurseries of Dhaka and arranged in the net house of Department of Plant Pathology with four replications.

3.7.2. Preparation of Pots

Soil that used as potting media was collected from Agronomy field of Sher-e-Bangla Agricultural University (SAU), Dhaka. The entire quantity of cowdung was applied to the soil.Urea, triple super phosphate (TSP), murate of potash (MoP), zinc sulphate andgypsum were given at the rate of 375 g, 200 g, 200 g, 175 g and 100 g, respectively (100:60:40 kg/ha). TSP, Zinc sulphate, gypsum were given asbasal during final pot preparation.

The earthen pots were bought from the market which height was 14 inches anddiameter 0.16m2 per pot. Then pots were filled with sterilized soil @ 10kg/ pot.

3.7.3. Planting materials used for experiment

Eight months old lemon (*Citrus aurantifolia*) seedlings were used as planting material for experiment. The seedlings were vigor, healthy, disease and insect free. Collected lemon seedlings were potted in earthen pot (Figure 3).

Figure 3: Planting materials used to study the effect of some chemicals on canker disease.

3.7.4. Intercultural operation

Seedlings were irrigated everyday early in the morning with running tape water. Weeding was done occasionally to remove weeds from the potwhen necessary.

3.7.5.Treatments

Following treatments were used as foliar spraying of citrus seedlings:

Treatments	Active Ingredient(s)	Dose/Rate
T_1= control (sterile water)		
T_2= Companion	Mancozeb + Carbendazim	2mg/L (0.2%)
T_3= Dithane M-45	Mancozeb	2mg/L (0.2%)
T_4= Sulcox 50 WP	Copper oxychloride	1mg/L (0.1%)
T_5= Cl_2 solution	Cl_2	5 mg/L (0.5%)
T_6= Companion+ Malation 57 EC		2mg/L (0.2%)+2.0ml/L(0.2%)
T_7= Dithane M-45 + Malathion 57 EC		2mg/L(0.2%)+2.0ml/L(0.2%)
T_8= Sulcox 50 WP+ Malathion 57 EC		1mg/L(0.1%)+2.0ml/L(0.2%)
T_9= Cl_2 solution + Malathion 57 EC		5mg/L(0.5%)+2.0ml/L(0.2%)

3.7.6. Preparation of spray solution

The spray solutions were prepared separately mixing with water and requisite amount of chemicals to get required concentration.

3.7.7. Application of spray solution

All chemicals were sprayed with compressed hand sprayer. Four plants were sprayed with each chemical. First spray was done in 8 August, 2015 when few spots were appeared. The plants were sprayed at 30 days intervals. Control plants were sprayed with sterile water.

3.7.8. Data collection

Data from the pot experiment were collected based on the following parameters:

1. Number of lesions on leaf per plant
2. Size of lesion (mm)
3. Disease incidence (%)
4. Disease severity (%)

5. Number of lesions on fruit per plant
6. Fruit weight (g)

3.7.9. Computation of disease incidence (%)

Each inoculated leaf infected with canker disease was counted to calculate disease incidence and was expressed in percentage. Percent disease incidence (PDI) of foliar diseases was determined by the following formula (Rai and Mamatha, 2005)

$$\text{Percent Disease Incidence (Leaves)} = \frac{\text{Number of diseased leaves among the infected leaves on each plant}}{\text{Total Number of inspected leaves on each plant}} \times 100$$

3.7.10. Computation of disease severity (%)

The disease severity was recorded by using the following scale developed by Rai and Mamatha(2005).

Grade	Percent leaf infection
0	0.00
1	Up to 1
2	>1-10
3	>10-20
4	>20-40
5	>40-100

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual disease rating}}{\text{Total number of leaf examined} \times \text{maximum grade}} \times 100$$

3.8.Experimental design

Both the laboratory and pot experiments were conducted following complete randomize design (CRD).

3.9. Statistical analysis of data

Data collected during experiment period were tabulated and analyzed following Statistical package MSTAT-C. Treatment means were compared with Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS

4.1. Symptoms of canker disease of citrus

Symptom expression of citrus canker varied depending on the age of the lesionsand the plant part affected. On leaves,first appeared as small, blister-like lesions, usually on the leaf surface. At later stage, this lesion turned grey to tan brown which were surrounded by yellow halo. The center of the lesions were corky and erupted.

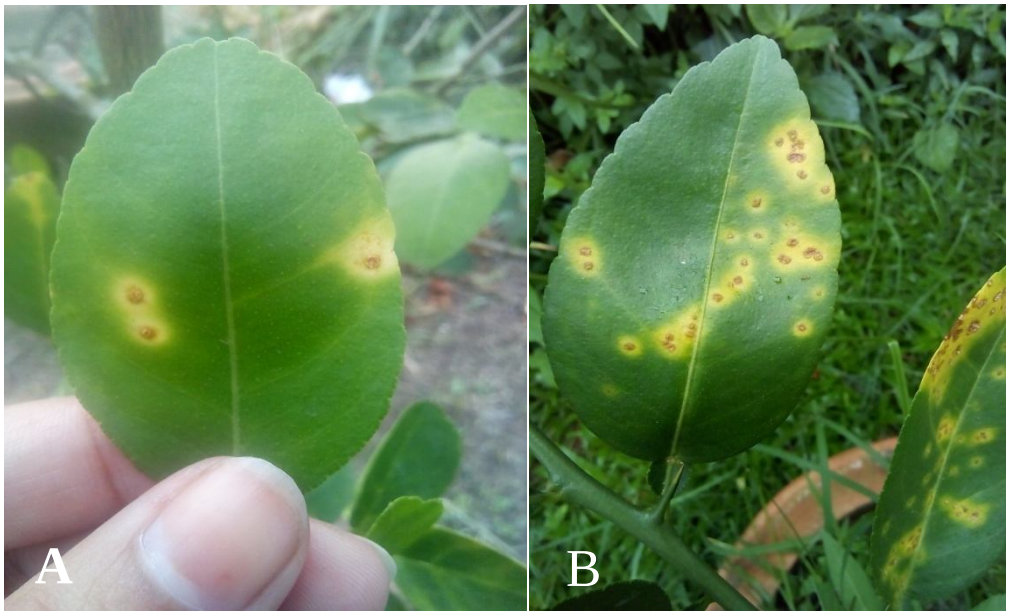

Figure 4: Symptoms of canker on citrus leaves

A.Early stage

B.Later stage

4.2. Isolation and identification of canker pathogen of citrus

4.2.1. Isolation and purification of canker pathogen

The causal organism was isolated from the infected leaves of citrus showing typical symptoms of citrus canker. Dilution plate method was used to isolate causal organism. Typical, yellow, convex, mucoid colonies of bacterium on nutrient agar medium were found after 48 hours of incubation at 30^0C (Figure5). Colonies were purified by restreaking the isolated colony on nutrient agar plate.

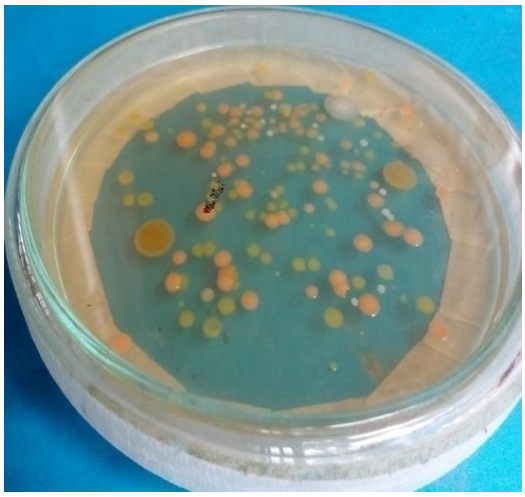

Figure 5: Yellow, convex, mucoid colonies of pathogenic bacteria isolated from infected citrus leaves

4.2.2. Preservation pathogen

Purified bacterium on NA slant was kept in refrigerator at 4^0c in test tubes. It was served as a stock culture for further studies (Figure 6).

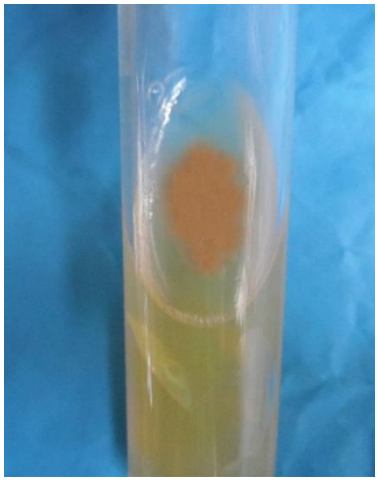

Figure 6: Slant culture of pathogenic bacteria.

4.2.3. Identification of the pathogen

Thecitrus canker pathogen was identified by studying morphological, biochemical and cultural characteristics of the pathogen as per standard microbiological procedures.

4.2.3.1. Morphological characters

The bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative (red color) and capsulated under the compound microscope at 100x magnification with oil immersion. The bacterium cells were stained with crystal violet (Figure 7).

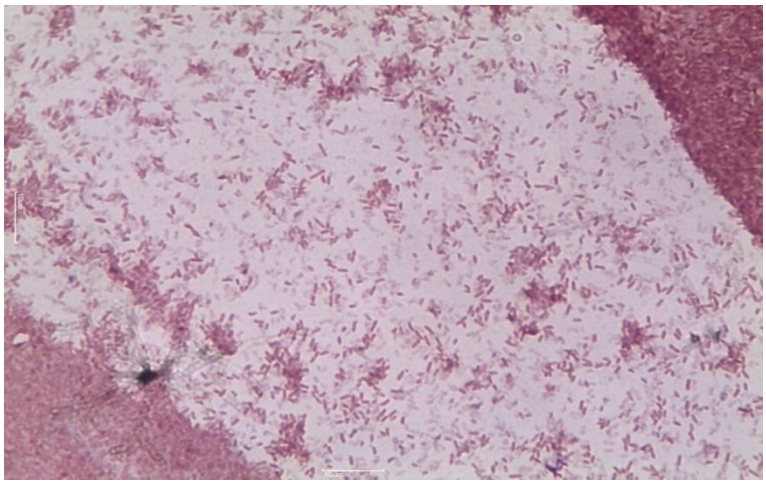

Figure 7: Microscopic view of pathogenic bacteria of citrus canker after gram's staining at 100x magnification.

4.2.3.2. Biochemical characters

Biochemical tests results for the pathogen are presented in Table 2.

Table 2. Biochemical characteristics of pathogenic bacteria of citrus canker

Biochemical tests	Results
KOH solubility test	Positive
Gelatine liquefaction test	Positive
Catalase test	Positive
Starch hydrolysis test	Positive
Oxidase test	Negative
Citrate utilization test	Positive

In catalase test, bubbles were formed within a few seconds onto the colony of the bacterium after adding 3% H_2O_2(Plate 3A), it indicated that the test was positive.

In KOH solubility test, mucoid thread was produced by the bacterium (Plate 3B), that indicates the bacterium was gram negative.

Gelatin was liquefied after 15 minutes of refrigeration at 4^oC in gelatin liquefaction test by the causal organism (Plate 3C). Hence the bacterium showed the positive result.

Green color of simmon's citrate agar slant changed into a bright blue color after 24 hours of incubation. That indicated the citrate utilization test was positive. The bacterium used citrate as a carbon source for their energy (Plate 3D).

In starch hydrolysis test, when lugol's iodine was added a clear zone was formed around the bacterial colony revealed starch hydrolysis (amylase activity) .The test was positive (Plate 3E).

In oxidase test, the bacterium when rubbed did not form any color in moistened oxidase disk (Plate 3F), which indicated that the test was negative.

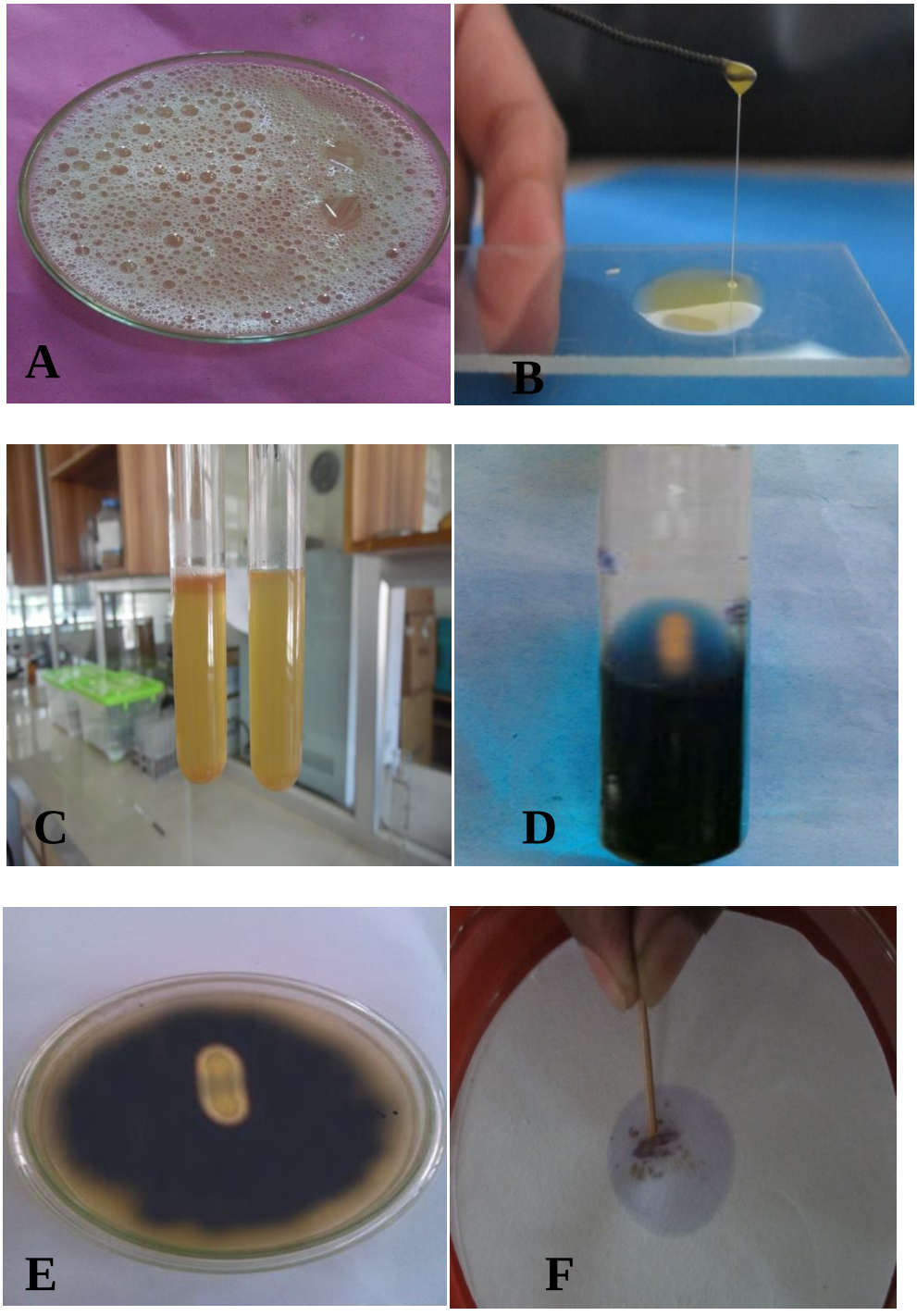

Plate 1: Biochemical characters of *Xanthomonas axonopodis*pv. *citri*

A. Catalase test.
B. KOH solubility test.
C. Gelatine liquefaction test.
D. Citrate utilization test.
E. Starch hydrolysis test
F. Oxidase test

4.2.3.3. Cultural characters

4.2.3.3.1. Colony morphology on different growth media

Colonies of pathogenic bacteria on NA medium shows as circular, mucoid, convex and orange color (Figure 8A). On SX medium bacterium showed very poor growth with light yellow to slightly blue, small, flattened, growth (Figure 8B).

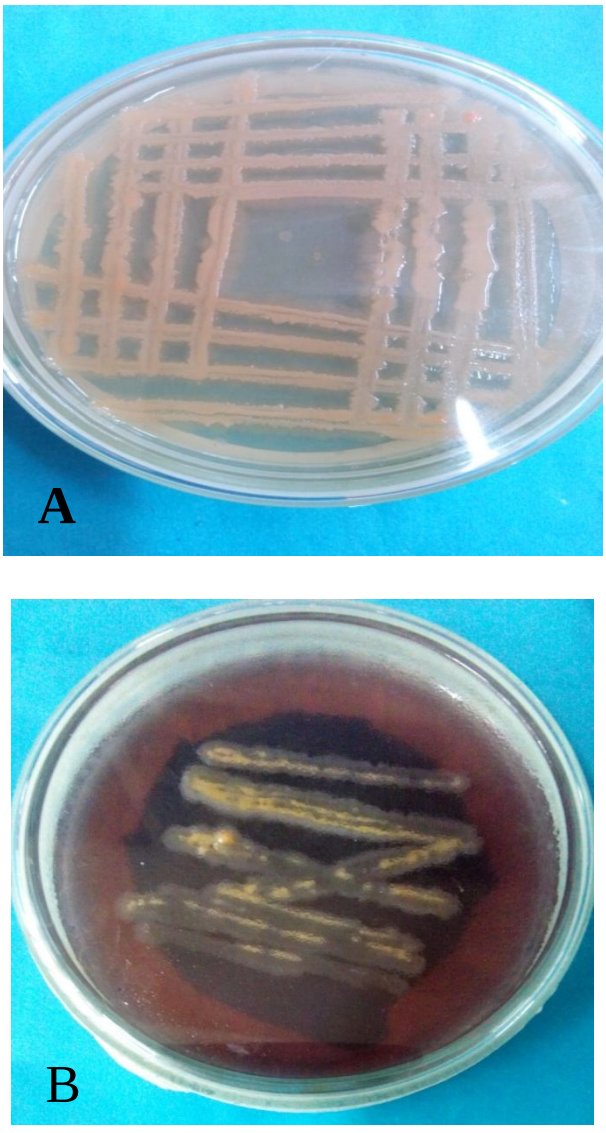

Figure 8: Cultural characteristics of *Xanthomonas axonopodis*pv. *citri* on different growth media
A. On Nutrient agar (NA) medium.
B. On SX agar medium.

4.3. Pathogenicity test

Bacterial cell suspension (10^8 cfu/ml, OD: 0.5_{650nm}) was injected into the lower surface of citrus leaf (Kagozi lemon) as described in "Materials and Methods" section. The inoculated leaves showed characteristic symptoms after fourteen days of inoculation as small, blister-like lesions, which later on turned gray to tan brown surrounded by a yellow halo (Plate 2). Bacteria were re-isolated from these lesions and comparisons were made with the original culture to confirm the identity of the pathogen. Both the colonies were similar.

On the basis of morphological, biochemical and cultural results it can be concluded that the pathogenic bacteria isolated from infected citrus leaf was *Xanthomonas axonopodis*pv. *citri.*

Plate 2: Pathogenesity test

A. Inoculation of bacteria on lower surface of leaf.

B. Typical symptom develops at 20 days after inoculation.

4.4.*In vitro* evaluation of chemicals

4.4.1. Efficacy of chemicals against *Xanthomonas axonopodis*pv. *citri* at different days after incubation

Statistically significant difference in case of inhibition zone was found among different treatments (Table 3; Figure 9). After 24 hours of incubation the highest inhibition zone (28.92%) was formed by 0.2% of Dithane M45 followed by 0.5% Cl_2 solution (24.50%), 0.1% Sulcox 50WP (14.75%) and 0.2% Companion (12.71%) respectively.

At 48 hours of incubation the highest inhibition zone (29.92%) was formed by 0.2% Dithane M45 followed by 0.5% Cl_2 solution (24.50%), 0.1% Sulcox 50WP (15.75%) and 0.2% Companion (13.71%) at respectively.

At 72 hours of incubation the highest inhibition zone (31.58%) was formed by 0.2% Dithane M45 followed by 0.5% Cl_2 solution (24.50%), 0.1% Sulcox 50 WP (15.75%) and 0.2% Companion (14.04%) respectively.

At 96 hours of incubation the highest inhibition zone (31.91%) was formed by 0.2% Dithane M45 followed by 0.5% Cl_2 solution (24.50%),0.1% Sulcox 75WP (15.75%) and 0.2% Companion (14.38%) respectively.

Inhibition zone significantly increased from 24 hours to 96 hours by each treatment.

Table 3. *In vitro* evaluation of chemicals against *Xanthomonas axonopodis* pv. *citri*

Treatments	% Inhibition zone			
	24 hour	48 hour	72 hour	96 hour
Sulcox 50 WP @ 0.1%	14.75 c	15.75 c	15.75 c	15.75 c
Companion @0.2%	12.71 d	13.71 d	14.04 d	14.38 d
Dithane M 45 @ 0.2%	28.92 a	29.21 a	31.58 a	31.91 a
Cl_2solution @ 0.5%	24.50 b	24.50 b	24.50 b	24.50 b
CV (%)	3.57	2.18	3.36	3.35
LSD	1.0504	0.6643	1.0504	1.2428
Level of Significance	**	**	**	**

Each data represent the mean value. Values followed by the same letter within a column are not significantly different ($p \leq 0.01$) according to Duncan's multiple range test.

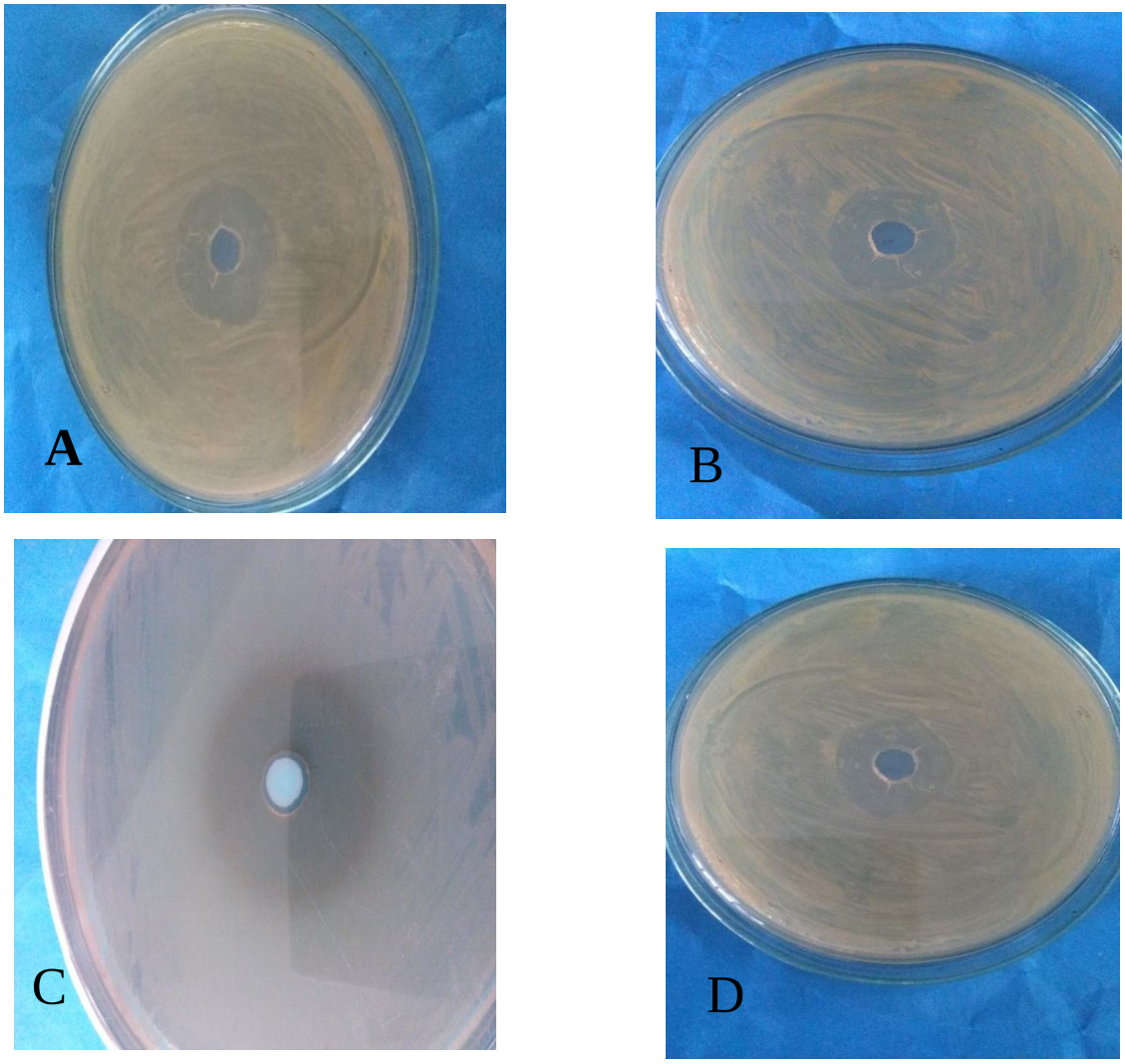

Figure 9: In vitro evaluation of chemicals against *Xanthomonas axonopodis*pv. *citri*

A. Companion @ 0.2%
B. Sulcox 50 WP @ 0.1%
C. Dithane M 45 @ 0.2%
D. Cl_2solution @ 0.5%

4.5.Effect of some chemicals on citrus canker disease development

4.5.1. Number of lesions and lesion size of citrus canker disease on leaf

Statistically significant interaction was found between treatments and development of citrus canker caused by *Xanthomonas axonopodis*pv. *citri*. Number of lesions varied from treatment to treatment and ranged from 15.50-33.75 (Table 4). At 30 days after spray (DAS), among the treatments the highest number of lesion (33.75) was found in T_6(Champion 75 WP+Malathion 57 EC) and the lowest number of lesion (15.50) was found in T_3 (Dithane M 45). In case of lesion number significant difference was found between T_4 (sulcox 50 WP), T5 (Cl_2 solution), T_7 (sulcox 50 WP+Malathion 57 EC) and T_8 (Dithane M45+Malathion 57 EC) treatments. Statistically similar lesion number was found in T_1 (control), T_2 (Champion 75 WP), T_4 (Sulcox 50 WP), T_5 (Cl_2 solution), T_8(Dithane M45+Malathion 57 EC) and T_9(Cl_2 solution+ Malathion 57 EC) treatments.

Statistically significant difference was found on lesion size under different treatments. At 30 days after spray (DAI), among the treatments the highest lesion size (5.32mm) was found in T2 treatment which was statistically similar with T_1 (4.12),T_4 (4.67mm), T_6 (4.00mm) and T_9 (4.87mm).The lowest lesion size (2.12mm) was found in T_3treatment which was statistically similar with T_1 (4.12mm),T_5 (3.00mm), T_6 (4.00mm) and T_8 (2.37mm)treatments.

Table 4. Number of lesions and lesion size of citrus canker disease at different treatments

Treatments	Lesion number of Leaf/plant	Lesion size (mm)
T_1	25.75 bcd	4.12 abc
T_2	24.50 bcde	5.32 a
T_3	15.50 f	2.12 c
T_4	29.00 abc	4.67 ab
T_5	22.00 def	3.00 bc
T_6	33.75 a	4.00 abc
T_7	29.75 ab	4.37 abc
T_8	18.75 ef	2.37 c
T_9	22.75 cde	4.87 ab
CV%	18.84	40.34
LSD	6.7346	2.2679
Level of significance	**	**

**- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P= 0.01

T_1= Control T_6= Companion+Malathion 57 EC
T_2= Companion T_7= Sulcox 50 WP + Malathion 57 EC
T_3= Dithane M45 T_8= Dithane M45 + Malathion 57EC
T_4= Sulcox 50 WP T_9= Cl_2 solution + Malathion 57 EC
T_5= Cl_2 solution

4.5.2. Disease incidence and Disease severity of citrus canker disease in leaf

Statistically significant difference on incidence was found at different treatments (Table 5). At 30 DAS, the highest incidence (20.00%) was found in T1 and T4 treatment followed by T2 (19.37%), T6 (19.31%), T7 (18.75%) and T9 (18.43%) treatments. All of these treatments showed significantly similar result. At the same time lowest incidence (11.56%) was found in T3 treatment which was statistically similar with T8 (13.43%) treatment.

Statistically significant difference on severity was found at different treatments. At 30 days after spray, among the treatments highest severity (30.25%) was found in T1 treatment which was statistically similar with T2 (25.33%)and T7 (25.00%) treatments followed by T4 (24.75%), T9 (24.25%) and T6 (23.75%) treatments. The lowest severity (17.50%) was found in T3 treatment which was statistically similar with T5 (20.00%) and T8 (19.50%) treatments.

Table 5. Disease incidence and disease severity of citrus canker disease at different treatments

Treatments	Disease incidence (%)	Disease severity (%)	% Disease reduction over control
T_1	20.00 a	30.25 a	-
T_2	19.37 a	25.33 ab	16.26
T_3	11.56 d	17.50 d	42.15
T_4	20.00 a	24.75 bc	18.18
T_5	15.62 bc	20.00 bcd	33.88
T_6	19.31 a	23.75 bc	21.49
T_7	18.75 ab	25.00 ab	17.36
T_8	13.43 cd	19.50 cd	35.54
T_9	18.43 ab	24.25 bc	19.83
CV%	12.97	15.95	
LSD	3.2725	5.4067	
Level of significance	**	**	

**- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P= 0.01

T_1= Control
T_2= Companion
T_3= Dithane M 45
T_4= Sulcox 50 WP
T_5= Cl_2 solution
T_6= Companion+Malathion 57 EC
T_7= Sulcox 50 WP + Malathion 57 EC
T_8= Dithane M45 + Malathion 57EC
T_9= Cl_2 solution + Malathion 57 EC

4.5.3. Number of lesions and lesion size of citrus canker disease on fruit

Statistically significant interaction was found between treatments and development of citrus canker caused by *Xanthomonas axonopodis*pv. *citri*. Number of lesions varied from treatment to treatment and ranged from 1-4 (Table 6). At 30 days after spray (DAS), among the treatments the highest number of lesion (4.00) was found in T_6(Champion 75 WP+Malathion 57 EC) which was statistically similar with T_4(Sulcox 50 WP) treatment and showed significant difference between T_1 (Control), T_2 (Champion 75 WP), T_3 (Dithane M45), T_5 (Cl_2 solution), T_7 (Sulcox 50 WP + Malathion 57 EC), T_8 (Dithane M45) and T_9(Cl_2 solution) treatments. The lowest number of lesion (1.00) was found in T_3 (Dithane M 45) treatment which was statistically similar with T_1 (Control), T_8(Dithane M 45+Malathion 57 EC) and T_9 (Cl_2 solution+Malathion 57 EC) treatments and showed significant difference between T_2 (Champion 75 WP), T_4 (Sulcox 50WP), T_5 (Cl_2 solution), T_6 (Champion 75 WP+Malathion 57 EC) and T_7 (Sulcox 50 WP+ Malathion 57 EC) treatments.

Statistically significant difference was found on lesion size under different treatments. At 30 days after spray (DAI), among the treatments the highest lesion size (4.00mm) was found in T_1 and T_2 treatments which were statistically similar with T_4 (3.68mm), T_7 (3.38mm) and T_9 (3.37) treatments. The lowest lesion (2.25mm) was found in T_3 and T_8 treatments which were statistically similar with T_5 (3.00mm), T_6 (2.88mm) T_7 (3.38mm)and T_9 (3.37mm) treatments.

Table 6. Number of lesions and lesion size of citrus canker disease at different treatments

Treatments	Lesion number of Fruit/plant	Lesion size (mm)
T_1	2.00cd	4.00a
T_2	2.50bc	4.00a
T_3	1.00d	2.25c
T_4	3.25ab	3.68ab
T_5	2.75bc	3.00bc
T_6	4.00a	2.88bc
T_7	2.50bc	3.38abc
T_8	1.75cd	2.25 c
T_9	1.00d	3.37abc
CV%	35.66	24.58
LSD	1.1928	1.1707
Level of significance	**	**

**- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P= 0.01

T_1= Control
T_2= Companion
T_3= Dithane M 45
T_4= Sulcox 50 WP
T_5= Cl_2 solution
T_6= Companion+Malathion 57 EC
T_7= Sulcox 50 WP + Malathion 57 EC
T_8= Dithane M45 + Malathion 57EC
T_9= Cl_2 solution + Malathion 57 EC

4.5.4. Disease incidence and Disease severity of citrus canker disease on leaf

Statistically significant difference on incidence was found at different treatments (Table 7). At 30 DAS, the highest incidence (66.67%) was found in T_1 treatment which was statistically similar with T_2 (54.79%) and T_4 (56.07%) treatment and showed significant difference with T_3 (7.5%), T_5 (29.18%), T_6 (46.86%), T_7 (43.90%), T_8(15.18%) and T_9(28.66%) treatments. At the same time lowest incidence (7.50%) was found in T_3 treatment which was statistically similar with T_8 (15.18%) treatment and showed significant difference with T_1, T_2, T_4, T_5, T6, T_7 and T_9 treatments

Statistically significant difference on severity was found at different treatments. At 30 days after spray, among the treatments highest severity (37.50%) was found in T1 treatment followed by T6 (25.00%), T7 (22.50, T2 (20.00%), T4 (20.00%) and T9 (17.50%) treatments which are statistically similar. The lowest severity (1.67%) was found in T3 treatment which was statistically similar with T5 (10.00%) and T8 (5.00%) treatments.

Table 7. Disease incidence and disease severity of citrus canker disease at different treatments

Treatments	**Disease incidence (%)**	**Disease severity (%)**	**% Disease reduction over control**
T_1	66.67 a	37.50 a	-
T_2	54.79 ab	20.00 b	46.67
T_3	7.50 d	1.67 d	95.55
T_4	56.07 ab	20.00 b	46.67
T_5	29.18 c	10.00 cd	73.33
T_6	46.86 b	25.00 b	33.33
T_7	43.90 b	22.50 b	40
T_8	15.18 d	5.00 d	86.67
T_9	28.66 c	17.50 b c	53.33
CV%	23.40	38.09	
LSD	13.157	9.7727	
Level of significance	**	**	

**- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P= 0.01

T_1= Control
T_2= Companion
T_3= Dithane M 45
T_4= Sulcox 50 WP
T_5= Cl_2 solution
T_6= Companion+Malathion 57 EC
T_7= Sulcox 50 WP + Malathion 57 EC
T_8= Dithane M45 + Malathion 57EC
T_9= Cl_2 solution + Malathion 57 EC

4.5.5. Yield performance of different treatments against citrus canker disease per plant

The yield performance of different treatments was recorded as number of fruits per plant which showed significant variation among the treatments. The highest yield (14 NF/plant) was found in T3 treatment followed by T8 (11 NF/plant), T6 (8 NF/plant), T7 (7 NF/plant), T2 (6 NF/plant) and T4 (6 NF/plant) which were statistically similar. The lowest yield (3 NF/plant) were found in T1 and T9 treatments which were statistically similar with T5 (5 NF/plant) treatment.

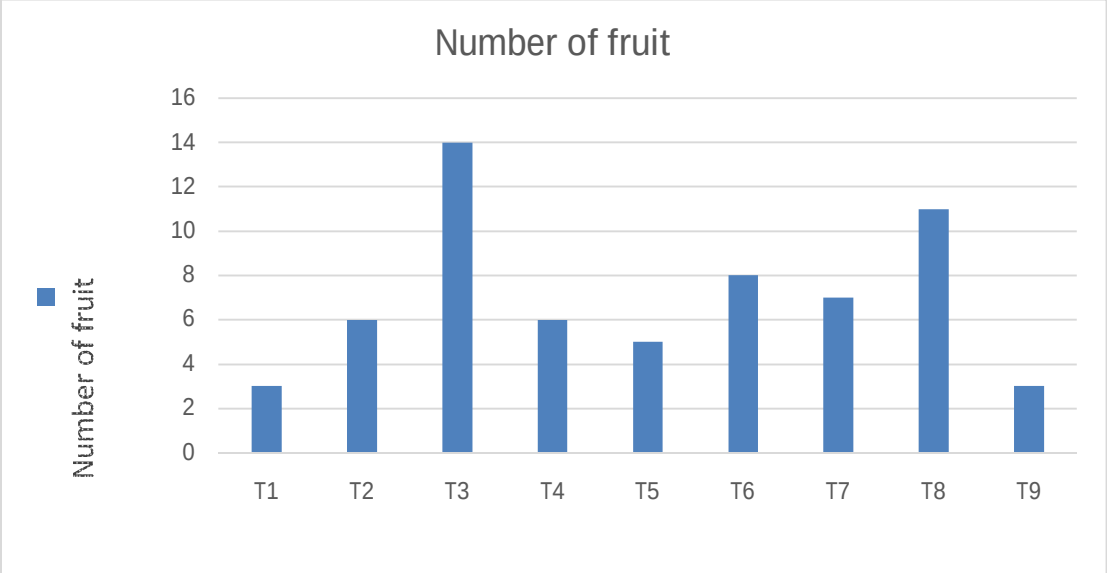

Figure10: Yield performance of citrus plants against citrus canker under different treatments.

CHAPTER V

DISCUSSION

Experiment was carried out to find out the effect of some chemicals to control citrus canker disease of citrus caused by *Xanthomonas axonopodis* pv. *citri.*

In the present study the disease recorded on visual symptoms following the description of Brunings and Gabriel (2003), Agrios (2006) and Civerolo (1981). The disease had been reported by Hossain (2011) and Chowdhury (2009) in the citrus growing areas of Bangladesh. The disease recorded in the present study had also been reported on citrus seedlings from different countries of the world (Burhan *et al.,* 2007; Graham *et al.,* 2004; Gottwald *et al.,* 2002; Schubert *et al.,* 2001;Awasthi *et al.,* 2005 and Koizumi, 1985).The causal organism of canker canker (*Xanthomonas axonopodis* pv. *citri*) was isolated from infected leaves following standard dilution plating technique using nutrient agar medium. After 48 hours of incubation at 30^0C yellow, convex, mucoid, colonies of bacterium were found on nutrient agar medium. The pathogen has also been reported by many researchers throughout the world (Vudhivanich, 2003; Kale *et al.,* 1994 and Qui and Ni, 1988). Chand and Kishun (1991) reported that *Xanthomonas* produce mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium. Lin *et al.* (2008) isolated the bacterial pathogen from the canker infected leaves and proved pathogenicity.

Xanthomonas axonopodis pv. *citri*, the causal agent of citrus canker was identified by studying morphological, biochemical and cultural features following procedures stated by Braithwaite *et al*.(2002) and Schaad (1992). After gram staining the bacterium was proofed as gram negative that was supported by KOH solubility test which are found by Kishun and Chand (1991), Gerhardt(1981), Bradbury(1970) and Starr and Stephens (1964). In the present study the bacterium *Xanthomonas axonopodis* pv. *citri* showed positive results in starch hydrolysis test, gelatine liquefaction test catalase test, citrate utilization test, and negative result in oxidase test which are reported by Yenjerappa (2009), Chand and Pal (1982) and Kishun and Chand (1991). It was observed that the bacterium, *Xanthomonas axonopodis* pv. *Citri* produce light yellow to slightly blue, mostly circular, small, flattened, mucoid colonies on SX medium. The similar result has also been reported by Balestra *et al*. (2008), Vudhivanich (2003) and Braithwaite *et al*. (2002).

Among the different chemicals evaluated in the present *in vitro* evaluation, Dithane M-45 [Mancozeb] (31.91%) and Cl_2 solution (24.50 %) exhibited significantly superior efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *citri* while Sulcox 50 WP (15.75%) and Companion [Mancozeb+Carbendazim] (14.38 %) were found moderately effective. In field experiment, significant effect of different chemicals on the incidence and severity of citrus canker of citrus were observed. The highest reduction of incidence and severity of canker of citrus was observed in applying Dithane M-45 [Mancozeb] as foliar spray. Cl_2 solution, Companion [Mancozeb+Carbendazim] and Sulcox50 WP [Copper oxychloride] were found moderately effective in reduction of incidence and severity of canker of citrus. Maher *et al.* (2005) reported that Dithane M-45 [Mancozeb] was effective against the multiplication of bacterium as well as effective in reducing the disease intensity of citrus canker. Meneguim *et al.* (2007) reported that copper as well as mixture of copper with mancozeb were effective in controlling citrus canker. Behlau *et al.* (2010), Graham and Leite (2004), Pan (2004), Verona *et al.* (2004), Das (2003), Zhong and Ling (2002), Chen *et al.* (2000) and Leite and Mohan (1990) reported that copper containing chemicals were effective in controlling citrus canker.

For the above study it can be said that Dithane M 45 had better potentiality in reducing disease incidence and severity of citrus canker both *in vitro* and *in vivo* test. Besides Cl_2solution, Sulcox 50 WP and Companion had moderate influence in controlling citrus canker. However, further investigation in the farmer's field at different agro-ecological zones of Bangladesh is needed to prove the efficacy of the recommended chemicals against citrus canker.

CHAPTER VI

SUMMARY AND CONCLUSION

A laboratory and a pot experiments were carried out to find the efficacy of some agrochemicals against citrus canker caused by *Xanthomonas axonopodis* pv. *citri*. Infected leaves having typical symptoms were collected fromnaturally infected plant and isolated the pathogen in the laboratory by following dilution plating method using nutrient agar medium. The causal organism was purified by restreaking on nutrient agar medium with single colony and confirmation was done by pathogenicity test. The bacterium was gram negative, rod shaped with rounded ends. It showed negative result in oxidase test and positive result in KOH solubility test, starch hydrolysis test, catalase test, citrate utilization test, gelatine liquefaction test. On NA medium the bacterium appeared as circular, mucoid, convex, yellow to orange colour and on SX medium the bacterium produced yellow to slightly blue, mostly circular, small, flattened, mucoid colonies. On the basis of morphological, biological and cultural characteristics it can be terminated that the pathogen was *Xanthomonas asonopodis* pv. *citri*.

Significance of different management practices *in vitro* was evaluated by measuring the inhibition zone and field evaluation was done by observing the incidence and severity of the disease. A pot experiment was conducted in the net house of Department of plantpathology, Sher-e-Bangla Agricultural University, to find out the effect of chemicals in controlling citrus canker disease. The chemicals were first sprayed at 8 August, 2015. Finally one time data were collected and disease symptoms of the infected plants were determined with the compare of control plants. *In vitro* of different agrochemicals were evaluated by measuring the inhibition zoneat different days of incubation. Among the seven different chemicals, *In vitro* evaluation of chemicals indicated that 0.2% Dithane M 45 (Mancozeb) at 96 hours of incubation showed highest (31.91%) inhibition zone against *Xanthomonas axonopodis* pv. *citri*. Cl2 solution, Sulcox 50 WP and Companion showed moderate result against the bacteria. In pot experiment, among the chemicals tested Mancozeb (Dithane M 45) was highly effective against *Xanthomonas axonopodis* pv. *citri.* The highest disease incidence was found in control and the lowest incidence was found in plant spraed

with Dithane M 45. Disease severity was recorded also highest in control and lowest in plant sprayed with Dithane M 45.

From the above study it may concluded that Dithane M 45 is most effective in suppressing the *Xanthomonas axonopodis* pv. *citri*. Further biological study is suggested to be carried out for citrus canker management as eco-friendly alternatives to chemical compounds.

REFERENCES

Ahmed, S. and Sahi, S. T. (2009). Management of citrus leaf miner to suppress citrus canker. Directorate of Pest Warning and Quality Control of Pesticides, Sargodha, Pakistan. p. 25.

Alam, M. N., Biswas, M., Bhuya, M. A. J. and Afroz, D. (2003). Phol Brikha Ropon O Poricharja. Prochalita Phol: Prothom Khando. Bangladesh Agricultural Research Council, Farmgate, Dhaka. p. 45.

Anon. (1996). The Indian Pharmacopoeia. 3rd ed. Government of India, New Delhi. Ministry of Health and family welfare.

Agrios, G. N. (2006). *Xanthomonas*. **In**: Plant Pathology. 5th ed. Academic Press. San Diego, CA. pp. 671-672.

Awam, M.Z., M. Ishfaq, I.A. Hafiz, M. Ijaz and C.M. Ayyub. 1992. Incidence of citrus canker in Barani area at Chakwal. 1st Inter. sem. citriculture in Pakistan pp: 315-8.

Awasthi, D. P., Sarkar, S., Mishra, N. K. and Kaisar, S. A. K. M. (2005). Disease situation of some major fruit crops in new alluvial plains of west Bengal. *Environ. Ecol.* **235**(Special-3): 497-499.

Balestra, G. M., Sechler, A., Schuenzel, E. and Schaad, N. W. (2008). First report of citrus canker caused by *Xanthomonas citri* in Somalia. *Plant Dis*. **92**(6): 981**.**

BBS (2015). Bangladesh Bureau of Statistics. Statistical Year Book of Bangladesh. Statistics Division. Ministry of Planning. Government of the Peoples Republic of Bangladesh. p. 375.

Behlau, F., Amorim, L., Belasque, J. J., Bergamin-Filho, A., Leite, R. P. J., Graham, J. H. and Gottwald, T. R. (2010). Annual and polyetic progression of citrus canker on trees protected with copper sprays. *Plant Pathol*. **59**(6): 1031-1036.

Behlau, F., Belasque, J. J., Bergamin-Filho, A., Graham, J. H., Leite, R. P. J. and Gottwald, T. R. (2008). Copper sprays and windbreaks for control of citrus canker on young orange trees in southern Brazil. *Crop Protect*. **27**(3/5): 807-813.

Behlau, F., Belasque, J. J., Bergamin-Filho, A. and Leite, R. P. J. (2007). Citrus canker incidence and severity on 'Pera Rio' sweet orange under chemical control and windbreak protection. *Fitopatol. Bras.* **32**(4): 311-317.

Bergamin-Filho, A., Amorim, L., Laranjeria, L. and Gottwald, T. R. (2000). Epidemiology of citrus canker in Brazil with and without the Asian citrus leafminer. **In:** Proceedings of the International citrus canker Research Workshop, Ft. Pierce, Florida.

Bradbury, J. F. (1970). Isolation and preliminary study of bacteria from plants. *Rev. Pantl. Path*. **49**: 213-218.

Braithwaite, M., Leite, R. P. J., Smith, J. J., Boa, E. and Saddler, G. S. (2002). First report of citrus canker caused by *Xanthomonas campestris*pv. *citri* on Citrus sinensis in Bolivia. *Plant Pathol*. **51**(3): 383-385**..**

Broadbent, P., Fahey, P. C., Gillings, M. R., Bradley, J. K. *and* Barnes, D. *(*1992*).* Asiatic citrus canker detected in a pummelo orchard in northern Australia. *Plant Dis.* Vol. 76:824–829.

Brunings, A. M. and Gabriel, D. W. (2003). *Xanthomonas citri*: Breaking the surface. *Mol. Plant Pathol*. **4** (3): 141–157.

Carrera, C., (1933). Informe preliminar sobre una enfermedad nueva comprobada en los citrus de Bella Vista (Corrientes). *Bol. Mens. Min. Agric. Nac. Buenos. Aires.***34**:275-280.

Canteros, B. I. (2004). Management of citrus canker in Argentina. *A review. Proc. Intn. Soc. Citric*. Paper No. 90, 10th International Citrus Congress, Agadir, Morocco.

Canteros B.I., Naranjo M., Rybak M., (2000). Production offruit free of *Xanthomonas axonopodis* pv. *citri*in selectedplots in areas of endemic canker in Argentina. *Proceedingsof the IX Congress of the International Society of Citriculture,Orlando 2000*: 1136-1137.

Chand, J. N. and Pal, V. (1982). Citrus canker in India and its management. **In**: Problems of citrus diseases in India (S.P. Raychaudhuri and Y.S. Ahlawat, Eds.). Surabhi Printers and Publishers, New Delhi. pp. 21-26.

Chand, R. and Kishun, R. (1991). Studies on bacterial blight (*Xanthomonas campestris* pv. *punicae)* of pomegranate. *Indian Phytopath*. **44**: 370-371.

Chen, Y. F., Song, C. Q., Xu, H. Z. and Wu, L. T. (2000). Experiment of screening the fungicide for control of citrus canker. *South China Fruits*. **29**(4): 18.

Chowdhury, M. S. M. (2009). Seed and seedling diseases of some selected fruits of Bangladesh. Ph.D. Thesis. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. 97-124.

Civerolo, E. L. (1981). Citrus bacterial canker disease: An overview. *Proc. Intn. Soc. Citric*. **1**: 390-394.

Civerolo, E.L. 1984. Bacterial canker disease of citrus. J. Rio. Grande Val. Hortic. Soc., 37: 127-46.

Civerolo, E. L. (1985). Citrus bacterial canker disease: The bacterium *Xanthomonas campestris* pv. *citri*. Citrus canker: An international perspective. Citrus Research and Education Center, University of Florida, Lake Alfred. Nov. 11-17.

Cook, A. A. (1988). Association of citrus canker pustules with leaf miner tunnels in North Yemen. *Plant Dis*. **72**: 546.

DAE. (2012). Export of Citrus from Bangladesh during 2010-2011. Annual Report. P.44.

Das, A. K. and Shyam, S. (2003). Integration of chemicals and cultural practices for management of bacterial canker (*Xanthomonas axonopodis* pv. *citri*) in acid lime (*Citrus aurantifolia*). *Indian J. Agric. Sci*. **73**(10): 570-571.

Das, A. K., (2003). Citrus canker – A review. *J. Appl. Hort*. **5**(1): 52-60.

Dhakal, D., Regmi, C. and S. R. Basnyat. (2009). Etiology and Control of Citrus Canker Disease in Kavre. *Nepal Journal of Science and Technology*. Vol. 10: 57-61.

Dixon, W. N., Levy, L. and Gottwald, T. R. (2000). Proceedings of the International Citrus Canker Research Workshop, Ft. Pierce, FL, USDAARS.

FAOSTAT, (2014). Food and Agricultural Organization. Citrus fruit (Fresh and processed). Annual statistics, 2012. Statistical Division. Economic and Social Department. Rome, Italy.

Fu, Z. H. and Xu, Z. H. (2001). Study on the occurrence of citrus canker and its control. *South China Fruits*. **30**(4): 15.

EPB (2015). Export Promotion Bureau. Ministry of Commerce,Government of the Peoples Republic of Bangladesh.

Gabriel, D. W., Duane, Y. P. and Ramadugu, C. (2000). The molecular mechanism of citrus canker pathogenicity and a gene engineering approach to control. *Intn. Soc. Citric. Cong.* Dec. 3-7, Orlando, Florida (Abst.). p. 51.

Gade, R. M.; Giri, G. K. and Sangale, U. R. (2005). Management of Phytophthora root rot in citrus nursery. *Pesticide-Research-Journal*. **17**(1): 28-29.

Gerhardt, P. (1981). Manual of methods of General Bacteriology. American Society of Microbiology Washington, D.C. p. 127.

Goszczynska, T., Serfontein, J. J. and Serfontein, S. (2000). Media and diagnostic tests. In: Introduction to Practical Phytobacteriology. 1st ed. Ultra Litho (Pty) Ltd. Johannesburg, South Africa. pp. 61-73.

Goszczynska, T. and Serfontein, J. J. (1998). Milk tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae*pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*. *J. Microbiol. Method*. **32**(1): 6572.

Goto, M. (1992). Citrus canker. In: Kumar, J.. Chaube, H. S Singh, U. S. and Mukhopadhyay, A. N. Plant diseases of international importance. **3**. Prentice-Hall, Englewood Cliff, NJ. pp. 170-208.

Goto, M. (1962). Studies on Citrus Canker, I. Bull. Fac. Agric. Shizuoka Univ. **12**: 3-72.

Gottwald T., Graham J., Bock C., Bonn G., Civerolo E., IreyM., Leite R., McCollum G., Parker P., Ramallo J., Riley T.,Schubert T., Stein B., Taylor E., 2009. The epidemiologicalsignificance of post-packinghouse survival of *Xanthomonascitri* subsp. *citri* for dissemination of Asiatic citrus cankervia infected fruit. *Crop Protection* **28**: 508-524.

Gottwald, T. R., Sun, X., Riley, T., Graham, J. H., Ferrandino, F. and Taylor, E. L. (2002). Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Phytopathology*. **92**: 361-377.

Gottwald, T. R., Hughes, G., Graham, J. H., Sun, X. and Riley, T. (2001). The citrus canker epidemic in Florida: the scientific basis of regulatory eradication policy for an invasive species. *Phytopathol*. **91**: 30-34.

Gottwald, T. R., Graham, J. H. and Schubert, T. S. (1997). An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leaf miner. *Fruits.***52**: 371378.

Gottwald, T. R. and Graham, J. H. (1992). A device for precise and nondisruptive stomatal inoculation of leaf tissues with bacterial pathogens. *Phytopathology*. **82**: 930-935.

Gottwald, T. R., Timmer, L. W. and Mcguire, R. G. (1989). Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology*. **79**: 1276-1283.

Graham, J. H. and Leite, R. P. J. (2004). Lack of control of citrus canker by induced systemic resistance compounds. *Plant Dis*. **88**(7): 745-750.

Graham, J. H., Gottwald, T. R., Cubero, J. and Achor, D. S. (2004). *Xanthomonas axonopodis* pv. *citri.*factors affecting successful eradication of citrus canker. *Mol. Plant Pathol.***5**(1): 1-15.

Grainger, J., Hurst, J. and Burdass, D. (2010). Basic Practical Microbiology: A Manual. Society for general Microbiology.

Hardy, S., Fallow, K. and P. Barkley. (2007). Using copper sprays to control diseases in citrus. *Australian J. of Pathol.* Vol. 22: 45-48.

Haque, M. A. (2005). Lebu pholer Adhunik Udpadan project, Horticultural Research Centre, BARI, Gazipur.

Harats, D., Chevion, S., Nahir, M., Norman, Y., Sagee, O. and Berry, B. (1998). Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E in vivo. *American J. Clin.l Nutrition*.**67**: 240-245.

Hingorani, M. K. and Singh, N. J. (1959). *Xanthomonas* sp. Nov. on *Punica granatum* L. *Indian J. Agric. Sci.* **29**: 45-48.

Hossain, I. (2011). Nursery diseases of some selected fruit species in Bangladesh. Eco friendly Plant Disease Management Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 13-16.

Huang, H. H. C. (1997). Biological Control of Soilborne Diseases in Canada, In: International Symposium on Clean Agriculture, Sapporo: OECD. pp. 52-59.

Jabeen, R., Iftikhar, T. and Batool, H. (2012). Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae*pv. *oryzae* causing BLB disease in rice. *Pak. J. Bot.* **44**(1): 261-265.

Jadeja, K. B., Mayani, N. G., Patel, V. A. and Ghodasara, M. T. (2000). Chemical control of canker and gummosis of citrus in Gujarat. *J. Mycol. Pl. Pathol.* **30**(1): 87-88.

Jayaraj, J. and R. K. M, Baskaran. (2007). Management of citrus leaf miner Department of Entomology, Agricultural College and Research Institute, TNAU, Madurai – 625, p.104.

Johnson, M. and Sekhar, C.V. (2012). Principles of Plant Pathology: Practical manual. p. 67.

Kale, K. B., Kolte, S. O. and Peshney, N. L. (1994). Economics of chemical control of citrus canker caused by *Xanthomonas campestris* pv. *citri*Under field conditions. *Indian Phytopath*. **47**(3): 253-255.

Kishun, R. and Chand, R. (1991). Outbreak of black rot of radish in India. *Indian J. Pl. Pathol*. **9**(1and 2): 7-13.

Klotz, L. J. (1973). Color Hand Book of Citrus Disease. University of California. Div. Agric. Sci. p. 122.

Koizumi, M. (1977). Relation of temperature to the development of citrus canker lesions in the spring. Proc. Intn. Soc. Citric. **3**: 924-928.

Koizumi, M. (1985). Citrus canker: The world situation. In: Timmer, L. W. Citrus Canker: An International Perspective. ed. University of Florida, Lake Alfred. pp. 2-7.

Koller, O. C., Oliveira, R. B. L., Nunes, D. S., Soglio, F. D., Panzenhagen, N. V., Sartori, I. A. and Manteze, F. (2006). Chemical control of citrus canker in young plants managed in conventional and organic systems. *Ciencia Rural*. **36**(4): 1043-1048.

Lai, H. Q. (2000). Inquiry into the dynamics of *Xanthomonas campestris* pv. *citri*and its prevention and cure. *J. Zhejiang Forest. Sci. Tech*. **20**(6): 43-45.

Leite, R. P. J. and Mohan, S. K. (1990). Integrated management of the citrus bacterial canker disease caused by *Xanthomonas campestris* pv. *citri*in the State of Parana, Brazil. *Crop Protect*. **9**(1): 3-7.

Lin, H. C., Chang, H. and Tzeng, K. C. (2008). Characterization of novel strains of citrus canker bacteria from citrus in Taiwan. *J. Taiwan Agric. Res.* **57**: 265-278.

Maher, S, M., Sahi, S. T., Ghazanfar, M. U., Haq, M. I.; Iftikhar, Y., Sarwar, M. S. and Ahmad, T. (2005). Evaluation of different toxicants against *Xanthomonas campestris* pv. *citri*(Hasse) Dows. *Int. J. Agr. Biol*. **7**(1): 121-124.

Meneguim, L., Rinaldi, D. A. M. F., Santos, A. C. A., Rodrigues, L. S., Silva, M. R. L., Canteri, M. G. and Leite, R. P. J. (2007). Sensitivity of *Xanthomonas axonopodis* pv. *citri*to copper and mancozeb. *Fitopatol. Bras*. **32**(3): 247-252.

Muraro, R. P., Roka, F. M., Spreen, T. H. (2001). An overview of Argentina's Citrus Canker Control Program. Publication of the Department of Food and Resource Economics, Florida Cooperative Extension Service, University of Florida University of Florida Extension, Institute of Food and Agricultural Sciences.

Nirvan, R. S. (1961). Citrus canker and its control. *Hort. Adv*. **5**: 171-175.

Pan, X. Q. (2004). Experiment of control of citrus canker disease by Kocide fungicide. *China Fruits*. **5**: 57.

Pria, M. D., Christiano, R. C. S., Furtado, E. L., Amorim, L. and BergaminFilho, A. (2006). Effect of temperature and leaf wetness duration on infection of sweet oranges by Asiatic citrus canker. *Plant Pathol*. **55**(5): 657-663.

Pruvost, O., Boher, B., Brocherieux, C., Nicole, M. and Chiroleu, F. (2002). Survival of *Xanthomonas axonopodis*pv. *citri* in leaf lesions under tropical environmental conditions and simulated splash dispersal of inoculum. *Phytopathology***. 92**: 336- 346.

Rai, V. R. and Mamatha, T. (2005). Seedling diseases of some important forest tree species and their management. In working papers of the Finish Forest Research Institute. p. 11.

Ran, C., Lei, H. D., Li, H. Y., Hu, J. H., Lin, B. M. and Zhang, O. B. (2001). Experiment on the use of mancozeb M-45 to control citrus diseases and citrus rust mite. South China Fruits, **30**(6): 14-15.

Ravikumar, M. R., Somasekhara, Y. M., Shamarao J. and Ryagi, Y. H. (2001). Field evaluation of antibiotics against citrus canker caused by *Xanthomonas axonopodis* pv. *citri*. *Agri. Sci. Dig*. **21**(4): 253-255.

Reddy, G. S. and Murti, V. D. (1990). Citrus Disease and their control. The Indian Council of Agricultural Research. pp. 5-50.

Ren, J. G., Huang, S. L., Chen, Z. L. and Li, Y. R. (2005). Experiment of control of citrus canker with 21 fungicides. *South China Fruits*. **3**: 13-15.

Reuther, W., Bachelor, L. D. and Webber, H. J. (1967). The citrus industry. University of California, USA.

Rodriguez, G. S., Garza, L. J. G., Stapleton, J. J., Civerolo, E. L., (1985). Citrus bacteriosis in Mexico. *Plant Dis.* **69**(9): 808-810.

Rossetti, V. (1977). Citrus canker in Latin America. A review. *Proc. Intn. Soc. Citric.* **3**: 918-924.

Sahi, S. T., Ghazanfar, M. U., Afzal, M., Rasheed, A. and Habib, A. (2007). Incidence of citrus canker disease caused by *Xanthomonas campestris* pv. *citri*(Hasse) dows on kinnow (*Citrus reticulata*) and its chemotherapy. *Pak. J. Bot.* **39**(4): 1319-1327.

Salle, A. J. (1961). Fermentation of carbohydrates and related compounds. In: Laboratory manual on fundamental principles of bacteriology. 5th ed. McGraw Hill Book Company, Inc. New York. pp. 94-98.

Samavi, S., Hassanzadeh, N., Faghihi, M. M. and Y. R. Danesh. (2009). Effects of thyme (Zaatar) essential oil and some chemical compounds in the control of citrus bacterial canker in Iran. *J. of Plant Pathol.*Vol. 91 (3):691-696.

Schaad, N. C., Postnikova, E., Lacy, G. H., Sechler, A., Agarkova, I., Stromberg, P. E., Stromberg, V. K., Vidavar, A. K., (2005). Emended classification of Xanthomonad pathogens on citrus. *Syst. Appl. Microbiol.* **29**: 690-695.

Schaad, N. W. (1992). *Xanthomonas.* In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. International Book Distributing Co. Lucknow. p. 165.

Schaad, N. W. (1988). Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. American Phytopathological Society, St. Paul, Minnesota. pp. 185-197.

Schoulties, C. L., Civerolo, E. L., Miller, J. W. and Stall, R. E. (1987). Citrus canker in Florida. *Plant Dis.* **71**: 388-395.

Schubert, T. S. and Sun, X. (2003). Bacterial citrus canker. Plant Pathol. Circular No. 377. Fl. Dep. Agric. and Cons. Services. Division. Plant industry. pp. 1-6.

Schubert, T. S., Rizvi, S. A., Sun, X., Gottwald, T. R., Graham, J. H., and Dixon, W. N. (2001). Meeting the challenge of eradicating citrus canker in Florida- again. *Plant Dis*. **85**: 340-356.

Serizawa, S., Inoue, K., and Goto, M. (1969). Studies on citrus canker disease. I. Dispersal of the citrus canker organism. *Bull. Fac. Agric. Shizuoka Univ.* **8**: 81-85.

Serizawa, S., and Inoue, K. (1976). Studies on citrus canker. III. The influence of wind on infection. *Bull. Shizuoka Pref. Citrus Exp. Sta.* **11**: 54-67.

Sohi, H. S. and Kapoor, S. P. (1990). Disease problem in citrus nursery with special references to Kinnow. Citri culture in north-west India. P. A. U., Ludhiana. pp. 139-142.

Sohi, G. S. and Sandhu, M. S. (1968). Relationship between citrus leaf miner (*Phyllocnistis citrella*) injury and citrus canker (*Xanthomonas citri*) incidence on citrus leaves. *J. Res. Punjab Agric*. **5**: 66-69.

Sinha, M. K., Batra, R. C. and Uppal, D. K. (1972). Role of citrus leafminer (*Phyllocnistis citrella*) on the prevalence and severity of citrus canker (*Xanthomonas citri*). *Madras Agric. J*.**59**: 240245.

Singh, D., Singh, R. S. and Kaur, K. (2005). Biological management of citrus scab in Panjab. *Plant Dis. Res.* **20**(1): 76-82.

Stall, R. E. and Civerolo, E. L. (1991). Research relating to the recent outbreak of citrus canker in Florida. *Ann. Rev. Phytopathol*. **29**: 339-420.

Stall, R. E., Miller, J. W., Marco, G. M. and Echenique, B. I. C. (1981). Timing of sprays to control cancrosis of grapefruit in Argentina. *Proc. Intn. Soc. Citric.* **1**: 414-417.

Stall, R. E. and Seymour, C. P. (1983). Canker, a threat to citrus in the gulf-coast states. *Plant Dis*. **67**: 581-585.

Stall, R. E., Miller, J. W., Marco, G. M. and Echenique, B. I. C. (1980). Population dynamics of *Xanthomonas citri* causing cancrosis of citrus in Argentina. *Proc. Fl. State Hort. Soc.* **93**: 10-14.

Stapleton J.J., 1986. Effects of postharvest chlorine and waxtreatments on surface microflora of lime fruit in relation tocitrus bacteriosis disease. *Plant Disease* **70**:1046-1048.

Starr M. P. and Stephens. W. L. (1964). Pigmentation and taxonomy of the genus *Xanthomonas. J. Bacteriol*. **87**: 293-302.

Stein, B., Ramallo, J., Foguet, J. and J. H. Graham. (2007). Citrus leaf miner control and copper sprays for management of citrus canker in Tucuman, Argentina. Proc. Fla. State Hort. Soc. Vol.120: 127-131.

Suslow, T. V., Schroth, M. N. and Isaka, M. (1982). Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology*. **72**: 917-918.

Sutton, T. B. (1996). Changing options for the control of deciduous fruit tree diseases. *Ann. Rev. Phytopathol*. **34**: 527-547.

Swarup, S., Feyter, R., Brlansky, R. H. and Gabriel, D. W. (1991). A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit cankerlike lesions on citrus. *Phytopathology*. **81**: 802-809.

Thakore, B. B. L.; Singh, R. B.; Singh, R. D. and Mathur, S. (1999). Chemicals control of citrus die-back in Rajasthan. Agric. Sci. Digest, India. **4**(l): 36-38.

USDA. (2014). United states Department of Agriculture. Citrus fruit (Fresh and processed). Annual statistics, 2014. Statistical Division.

Vandepitte, J., Engbreak, K., Piot, P. and Heuck, C. C. (1991). Basic Laboratory Procedure in Clinical Bacteriology. World Health Organization, Geneva.

Verniere, C. J., Gottwald, T. R. and Pruvost, O. (2003). Disease development and symptom expression of *Xanthomonas axonopodis*pv. *citri*in various citrus plant tissues. *Phytopathology*. **93**(7): 832-843.

Verona, L. A. F., Theodoro, G. F. and Nesi, C. N. (2004). Relationship between the frequency of copper oxychloride sprays and the incidence of citrus canker in sweet orange leaves. *Agropecu. Catar*. **17**(2): 64-66.

Vudhivanich, J. (2003). Efficacy of Thai herbal extract for growth inhibition of *Xanthomonas axonopodis*pv. *citri*, the bacterial canker of citrus. *Kasetsart J. Nat. Sci*. **37**: 445 - 452.

Whitney, E. and Rolfes, S. (1999). Understandingnutrition. Belmont, Ca., USA, West/Wadsworth. Eighth ed.

Wolf, F. (2016). Citrus canker, *J. Agril. Res*. **6**: 68.

Yenjerappa, S. T. (2009). Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis*pv. *punicae*. Ph.D. Thesis. Department of Plant Pathology, University of Agricultural Sciences, Dharwad. pp. 21-123.

Zekri, M., Chamberlain, H., Timmer, P., Roberts, P. and Muchove, R. (2005). Field identification of citrus canker symptoms and decontamination procedures. Uni. Florida. IFAS extension.

Zheng H. H., Liu Z. L.,Ou Z. L.,Lai Z. H., Ying Z. D. and Xiang H. C. (2000) Preliminary study on chlorine dioxide for controlling crop pathogens. Acta Agriculturae Zhejiangensis Vol. 12 No. 5 pp. 281-284.

Zhong, D. Z. and Ling, N. C. (2002). Observation on the occurrence regulation of citrus canker disease and its control. *South China Fruits*. **31**(2): 20-21.

APPENDICES

Appendix I.Preparation of culture media and reagents

The compositions of the media used in this thesis work are given below. All media were autoclaved at 1210C for 15 minutes at 15 lb pressure.

Nutrient Agar (NA)

Beef extract (Difco)	3.0 g
Peptone (Difco)	5.0 g
Bacto agar	15.0 g
Distilled water	1000 ml

Nutrient Broth (NB)

Beef extract (Difco)	3.0 g
Peptone (Difco)	5.0 g
Distilled water	1000 ml

SX Agar

Potato starch (Soluble)	10.0 g
Beef extract (Dico)	1.0 g
NH4Cl	5.0 g
K_2HPO_4	2.0 g
Methyl violet 2B (1% in 20% ethanol)	0.4 ml
Methyl green (1% in water)	2.0 ml
Bacto agar	15.0 g
Cycloheximide	2.0 g
Distilled water	1000 ml

Gelatine Liquefaction Media

Beef extract	3.0 g
Peptone	5.0 g
Gelatine	120 g
Distilled water	1000 ml

Simmon's Citrate Agar

Magnesium sulphate	0.2 g

Sodium citrate	2.0 g
NaCl	5.0 g
Dipotassium Phosphate	1.0 g
Monopotassium Phosphate	1.0 g
Bromothymol blue	0.08 g
Bacto agar	20.0 g
Distilled water	1000 ml

Starch hydrolysis media and reagent

Culture medium	
Nutrient broth (Difco)	8.0 g
Soluble potato starch	10.0 g
Bacto agar (Difco)	15.0 g
Distilled water	1000 ml
Reagent	
(Lugol's iodine) Iodine	5.0 g
Potassium iodide	10.0 g
Distilled water	100 ml

Gram's staining reagents

Gram's Crystal violet (Hucker's modification)

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

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

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

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

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

Gram's alcohol (decolorizing agent)

Ethyl alcohol (95%)	98 ml

Acetone	2 ml
Safranin (counter stain)	
Safranin (2.5% solution in 95% ethanol)	10 ml
Distilled water	100 ml

KOH solubility reagent

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

Catalase reagent

3% aqueous solution of H_2O_2 was prepared from the H_2O_2 absolute solution.

Oxidase reagent

1% aqueous solution of NNN'N-tetramethyl-p-phenylene-diamine dihydrochloride was prepared from the absolute solution

Appendix II. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour during the experimental period (May 2015 to October 2015)

Month	Average RH (%)	Average Temperature(^{0}C)		Total Rainfall	Average Sunshine Min. Max. Hours
		Min	Max		
May	81	32.1	34.5	339.4	4.7
June	84	31.4	33.4	415.6	4.8
July	89	30.5	32.8	512.5	4.7
August	91	29.1	32.2	412.8	4.6
September	90	27.2	30.4	350.3	4.6
october	93	26.8	29.5	201.9	4.5

Source: Bangladesh Meteorological Department (Climate division), Agargaon Dhaka-1207.