EFFICACY OF SOME SELECTED TREATMENTS FOR THE MANAGEMENT OF SCAB AND DIE-BACK OF CITRUS

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By

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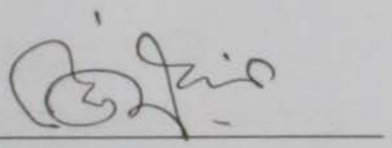

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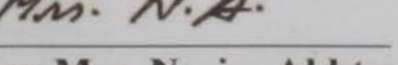

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This is to certify that thesis entitled, ***"EFFICACY OF SOME SELECTED TREATMENTS FOR THE MANAGEMENT OF SCAB AND DIE-BACK OF CITRUS"*** *Submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE IN PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by Tanvir Ali Siddiquee, Reg. No- 27572/00733 under my supervision and guidance. No part of the thesis has been submitted for any other degree in any other institutes.*

I further certify that any help or sources of information, received during the course of this investigation have been duly acknowledged.

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DEDICATED TO
MY
BELOVED PARENTS

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EFFICACY OF SOME SELECTED TREATMENTS FOR THE MANAGEMENT OF SCAB AND DIE-BACK OF CITRUS (*Citrus limon* L.)

ABSTRACT

A research work was conducted on the standing citrus plant raised at citrus orchard in Sher-e-Bangla Agricultural University, Dhaka, during the period from March to September 2007 to investigate the effect of some selected treatments in controlling the scab and die back of citrus. Allamanda leaf (*Allamanda cathertica*) leaf extract and seven fungicides *viz*. Dinthane M-45 (0.3%), Rovral 50WP (0.1%), Bavistin 50 WP (0.1%), Champion 77 WP (0.2%), Cupravit 50WP (0.2%), Tilt 250EC (0.25%) and Proud 250EC (0.25%) were tried against *Elsinoe fawcettii* in controlling scab and dieback of citrus. A marked reduction of disease incidence and severity of scab and die back of citrus was observed after commencement of spraying and reduction of disease, reached to the highest after three month of spraying. The highest reduction of PDI (leaf) and PDI (fruit) of scab of citrus was recorded in champion 77WP (85.43% and 80.12% respectively) at three months after sixth sprays and the highest reduction of PDI (twig) of die back of citrus was recorded in case of Dithane M-45 (58.01%). Allamanda leaf extract also showed better performance in reducing the severity of scab and die back of citrus.

Chapter 1
Introduction

CHAPTER-1
INTRODUCTION

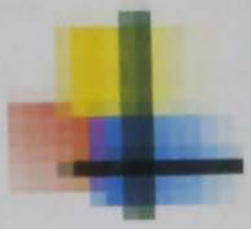
Citrus (*Citrus limon* L.) is one of the important items used in our daily food. It is one of the important fruit crops in the world as well as in Bangladesh. It has a great demand due to its nutritive value, flavor, appealing color and taste. It is observed that 93% people of Bangladesh are suffering from the deficiency of vitamin C (Anonymous, 1980). Citrus serves as a potential source of vitamins and minerals (Reuther *et al.*, 1967)). In Bangladesh, citrus is cultivated in about 15,008 ha. of land with a total production of 31,750 metric tons per annum (BBS, 2005). In Bangladesh, eight species of citrus fruits are grown. Among them, Elachi lemon (*Citrus limon* L.) and Kagzi lime (*Citrus aurantifolia* Swing) are commonly cultivated in our country. But its production is tremendously hampered due to the attack of different pathogens. More than 100 diseases are known to occur on different species of citrus grown in the world (Klotz, 1973). In Bangladesh, twelve diseases are known to occur in different species of citrus. Among these, scab, die-back, lichen, sooty mould and canker are considered as major diseases of citrus in Bangladesh (Alam, 2003).

Scab caused by *Elsinoe fawcettii* is a serious disease of citrus in Bangladesh (Alam, 2003). It can cause severe deformation of foliage and stunting of certain citrus rootstocks (Singh *et al.*, 2000). Citrus scab (*Elsinoe fawcettii)* attacks the expanded leaves of spring shoots of lemon and stocks to attack the fruitlets in mid-late May. During the autumn, if the temperature and humidity are favorable, it attacks the young shoots and causes dropping of fruitlets up to 65.9 – 71.29% (Huang, 1999). The causal organism of scab (*Elsinoe fawcettii)* was identified as *Sphaceloma fawcettii* (Janghoong *et al.*, 1998).

Die back caused by *Colletotrichum gloeosporioides* is the most serious disease in citrus which formerly known as decline, wither tip, twig blight, anthracnose etc. The disease breaks out widely and has become limiting factor of citrus cultivation in many countries (Talukdar, 1974; Ercivan and Karaca, 1979; Ray Chaudhury *et al.*, 1980; Rawal and Saxana, 1997; Alam, 2003 and Timmer *et al.*, 2005). From a survey report, the prevalence of die back on Elachi lemon and Kagzi lime was 89.9% and 100% respectively in Bangladesh (Miah and Fakir, 1987). Thus, the urgent attention needs to concentrate on the management of citrus scab and die back.

Now-a-days use of plant extracts in controlling plant pathogens is considered as an eco-friendly approach (Fakir and Khan, 1992). But a few works have been done regarding control of citrus scab and die back with plant extracts. Though fungicides are now being treated as a concerned tool of environment pollution, but in absence of suitable alternatives, we can't do without fungicides for immediate control of plant diseases. Besides, conclusive reports are scanty in the literature for the management of the disease with fungicides.

In view of the above facts, the present study has been carried out to evaluate the selected fungicides and plant extract against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides* causing scab and die-back of citrus.



Chapter 2
Review of literature

CHAPTER-II
REVIEW OF LITERATURE

2.1.1 Symptoms of scab of citrus

Gopal and Kumar (2003) reported that only young tissues are affected by citrus scab. Leaves are most susceptible to infection just after emergence from the bed. The grown up mature leaves are immune. Fruit remain susceptible for about three months after petal fall. The main symptom is small, grayish-brown corky scabs which develop on the twigs, young leaves and fruit. The fruits are infected when they are very young; the scabs are larger and warty. These lesions are particularly large on fruits. The scabs on fruit which are infected later are slightly raised above the surface of the rind. The numbers of lesions may join together to form large scabby areas. These may develop cracks as the fruit grows.

Amador (2002) reported that citrus scab caused by the fungus *Elsinoe fawcettii,* is an important disease in Texas. The disease is more severe on lemons, sometimes troublesome on grapefruit and seldom a problem on sweet orange. Sour orange is highly susceptible, thus, nursery stocks may become infected before young sour orange trees are budded. Because citrus tissue is susceptible to scab only while young, the disease is mainly confined to new growth. The fruit remains susceptible for longer periods, but seldom is mature fruit affected. Small lesions appear as translucent dots that later become pustules. As the disease progresses, the pustules turn into warts, consisting of a mass of corky tissue pale tan in color. The leaves become twisted and distorted and the entire young branch may be affected.

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

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

Singh *et al.* (2000) reported that citrus scab caused by *Elsinoe fawcettii* is a serious disease of citrus in India. It can cause severe deformation of foliage and stunting of certain citrus root stocks.

Janghoon *et al.* (1998) reported that citrus scab caused by *Elsinoe fawcettii* cause warty and scabby lesions on the surface of leaves, twigs and fruits of mandarin cv. Satsuma. Warty lesions were mainly developed before July but scabby lesions developed during the summer season in Cheju Island, Korea Republic.

Singh *et al.* (1998) reported that scab is primarily a disease of Sastuma, orange, tongerine, grape fruit, lemon, sour orange and trifoliate orange root stock. It does not affect the sweet orange. Scab affects fruit, leaves and young shoots causing irregular, raised, corky, scabby, wart like outgrowth, severely scabbed leaves and fruits become misshapen and distorted. The rind of scabbed fruit is thick and puffy.

2.1.2 Epidemic nature of *Elsinoe fawcettii*

Alam (2003) conducted a survey in the commercially citrus growing areas at Moulavibazer, Sylhet and Chittagong in Bangladesh and listed scab (*Elsinoe fawcettii)* and die back (*Colletotrichum gloeosporioides)* are the major diseases of citrus in Bangladesh.

Huang and Huang (2002) reported that approximately 50% of fruits from Nanfengmiju trees were damaged by citrus scab (*Elsinoe fawcettii*). The main reasons were identified as unfavorable weather conditions (Much rainfall, high humidity and frost damage)

Hartmond *et al.* (2000) in Florida reported that citrus scab caused by *Elsinoe fawcettii* can occur on stems and leaves, especially those of the summer flush, provide the main source of over wintering inoculum. Older scab pustules provide relatively little inoculum compared to those pustules on summer and fall shoot growth. Apparently, scab pustules lose their capacity for spore production as they aged.

Huang (1999) reported that citrus scab (*Elsinoe fawcettii*) attacked the expanded leaves of spring shoots of satsuma mandarin and started to attack the fruitlets in mid-late May in Jiangxi, China. During the autumn, if the temperature and humidity are favourable. The young shoots and fruitlets are attacked and 65.9-72.2% fruitlets are dropped.

Singh *et al.* (1997) conducted a survey at Panjab in India and disease incidence recorded on 3 citrus species viz. rough lemon (*Citrus jambhiri*) 76.5-80.1%, sweet orange (*C. sinensis*) 10.8-20.3% and kinnow (*C. nobilus* X *C. deliciosa*) 46.25-81.07%. The highest disease incidence was recorded in the sub-mountainous zone on rough lemon (80.1%) and kinnow (80.07%) and the lowest incidence was recorded on sweet orange 10.8% in the arid irrigated zone.

Tripathi and Srivastava (1992) observed that citrus scab caused by *Elsinoe fawcettii* on leaves and fruits appeared during the summer (April-June), little progress was observed during these months. With the onset of the rainy season (July-September), the infection progressed well and was seldom exposed to severe conditions.

2.1.3 Causal organism of scab of citrus

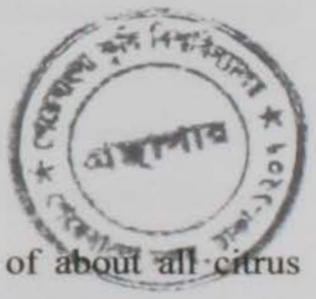
Hyun *et al.* (2001) reported that two scab diseases were recognized currently on citrus: (i) citrus scab caused by *Elsinoe fawcettii*, which has several pathotypes and (ii) Sweet orange scab caused by *E. australis*.

Janghoon *et al.* (1998) reported that the causal organism of scab was morphologically identified as *Sphaceloma fawcettii* (*Elsinoe fawcettii).*

Singh *et al.* (1997) identified the causal organism of citrus scab as *Elsinoe fawcettii* on the basis of spore morphology and pathogenicity test on rough lemon seedling.

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

Tripathi and Srivastava (1992) also reported that citrus scab caused by *Sphaceloma fawcettii* (*Elsinoe fawcettii)* in Srinagar, India.



2.2.1 Symptoms of die-back of citrus

Bobby (2003) reported that wither tip is the major disease of about all citrus species. Symptoms appear initially from top and transmit downward to bottom of infected plant/tree. Diseased twigs start drying at tips and all affected parts become silvery gray and develop black dots. Defoliation and death of the entire plant also caused under severe condition.

Benyahia *et al.* (2003) reported that citrus trees (*Citrus sinensis* L. Osbeck) with symptoms resembling wither tip on twigs and tear stain on fruits were observed in Morocco. Lime (*Citrus aurantifolia)* was not affected. The die-back often

progressed to tip wards slowly and caused wilt, turned yellow and dropped. Twigs and branches appeared to have been scorched by fire. When twigs were dry, minute brown to black, slightly raised, clumped pustules were observed. Under humid conditions, a pink slimy material appeared on dead bark and twigs. This symptom has also been observed on seedlings of citrus rootstocks in greenhouses in Morocco. Affected fruit showed tear stain symptoms. The symptoms were superficial on the unbroken peel and formed dull red to dull radish-green streaks or bands down the fruit. In some cases, these covered a large percentage of fruit surface. On late season, on overripe fruit, the streaks sometimes become brownish in colour, resembling rust mite damage.

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

Rawal and Saxana (1997) reported that anthracnose attacks the young leaves, shoots, blossoms and fruits of small acid limes. Young foliage and blossoms are blighted and distinct lesions formed on leaves and fruits. Affected fruits frequently drop prematurely. Wither tip is characterized by shedding of leaves and die-back of twigs. Leaves show light green spots which turn brown. On dead twigs, black dot like acervuli appear in concentric rings. The stem end of immature fruits results in fruit drop. In severe cases, branches show die-back and the tree dies in a few years. Symptoms of anthracnose appear on leaves, young shoots and tender fruits. On leaves, the necrotic spots show acervuli arranged in concentric rings. Dead parts of the twigs assume silvery grey appearance. Twigs show a slight gumming and a sharp line of separation between healthy and dead tissues. Affected buds fail to develop and affected fruits drop off. Often, the infected fruits develop reddish brown stain on the rind. The fungus has also been held responsible for russeting and tear staining of rind. This leads to the blight of twig. The infected hyphae produced by appresoria

remain latent even after the fruit mature and produce anthracnose in oranges and grape fruit, if the peal is injured or fruits are over matured.

2.2.2 Epidemic nature of *Colletotrichum gloeosporioides*

Bobby (2003) reported that high humidity and poor soil conditions favour infection of wither tip causing fungus *Colletotrichum gloeosporioides*, which survives on infected plant parts remain in orchards or present in trees.

2.2.3 Causal organism of die-back of citrus

Peres *et al.* (2003) reported that *Colletotrichum gloeosporioides* causes anthracnose of lime and *C. acutatum* causes post bloom fruit drop of sweet orange. This species produces conidia with at least one end of fusiform conidia.

Amador (2002) reported that twig die back can be caused by fungi, although non-pathogenic factors probably play a major important role. As a result, fungal infection is often secondary, fallowing freeze damage or damage resulting from mechanical or chemical injury. Other factors that can damage twigs are for excessive fertilization, moisture stress and damage to the root system by cultural practices or heavy nematode infestation.

Timmer *et al.* (1998) reported that *Colletotrichum gloeosporioides* (*Glomerella cingulata*), causes post harvest anthracnose of citrus fruits and a common saprobe in citrus groves, whereas *C. acutum* infects flower petals and causes post bloom fruit drop (PFD).

Rawal and Saxana (1997) reported that anthracnose attacks the young leaves, shoots, blossoms and fruits of small acid limes. The disease is caused by *Gloeosporium limetticolum* Clousen or *G. foliicolum* Nishida. Malta oranges and

grape fruits suffer much more damage. *C. gloeosporioides* remains in a dormant condition in the dead twigs and branches.

Ploetz *et al.* (1996) reported that the most commonly isolated fungi from citrus decline (dieback) were *Alternaria alternata*, *Cladosporium* sp., *Colletotrichum gloeosporioides* (*Glomerella cingulata*), *Dothiorella dominicana*, *Fusarium* spp., *Botryodiplodia theobromae*, *Penicillium* sp., *Pestaliopsis* sp. and *Phomopsis* spp.

Mourichon (1994) observed that a serious dieback caused by *Ceratocystis fimbriata* in Colombia over the last 3-4 years, which threatens overall citrus production.

Ahlawat *et al.* (1988) reported that Citrus Tristeza Closterovirus (CTV) involved in citrus dieback disease has been identified and a map showing the distribution of virus in citrus in India has been prepared.

Broadbent *et al.* (1980) reported that citrus die-back was claimed to be caused by a mycoplasma like organism in Australia

According to Ray Chaudhury *et al.* (1980) numerous agents including greening virus were involved in the causal complex of the devastating citrus die-back disease occurring in India..

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

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

2.3.1 Management of scab of citrus through fungicides

Alam (2006) conducted a research work on standing citrus plant using bio-agent, plant extracts and fungicides for controlling scab and die-back of citrus. The highest reduction of scab severity (PDI) was performed by Champion 77 WP (72.72% leaf scab and 83.98% fruit scab) followed by Bishkatali extract (67.54%, 79.99%), Bordeaux mixture (66.26%, 72.01%) and *Trichoderma harzianum* T_{22} (62.36%, 63.34%) respectively.

Timmer *et al.* (2005) reported that three applications of fungicides are needed to control the diseases, one at about ¼ expansion of the spring flush, a second at petal fall, and a third about three weeks later. If there is little carryover of disease from the previous season, the first spray can be omitted. Ferbam, Abound, Zem or Headline is good choices for the first application because they are all able to kill the fungus in old lesions and then reduce inoculum as well as protecting foliage. Copper fungicides, Abound, Gem or Headline are good choices for the third spray since they will protect fruit from scab, but copper products are less effective for scab and should not be selected where scab pressure is high.

Yesmin (2004) conducted an experiment of citrus scab. She used Capravit- 50 WP (0.4), Dithane M-45 (0.3%) and Rovral 50 WP (0.2%). All the fungicide showed significant effect in controlling scab of citrus. The highest reduction of scab incidence was recorded in case of applying Rovral 50 WP on *Citrus limon*.

Fang *et al.* (2004) conducted an experiment in South China in a Satsuma mandarin orchard. Among the materials used, 75% Menghashen (Mancozeb) and 77% Dodine found promizing that reduced the scab disease by 87.1% and 75.5%, respectively over control.

Xu *et al.* (2004) conducted an experiment in China with 15 year old satsuma trees. Fungicides used included 50% Xinling (Carbendazim + Mancozeb), 80% Bideli (Copper hydroxide), 20% Qingdaoful (of unstated composition), 50 % Carbendazim and Mancozeb. Best control of citrus scab (*Elsinoe fawcettii*) was done by 600 times solution of 80% Bideli.

Agostini *et al.* (2003) conducted an experiment in Argentina in a Greenhouse rough lemon orchard. Oxycom, Nutriphite, Messenger, Goemar H11, Serenade, Rezist, Prophyt, Aliette, Actigard and Keyplex were evaluated and compared with benomyl or strobilurin fungicides as standards. Among the fungicides the most effective products were Rezist and Actigard that contain phosphorous acid.

Huang and Huang (2002) reported that control of die-back was possible by spraying spring buds with Bordeaux mixture followed by 2 spraying of Bordeaux mixture between August and October.

Zhou *et al.* (2001) conducted an experiment to control citrus scab by chemical. They observed that the most effective chemical control for preventing attack of young leaves by scab was spraying of a copper and ammonium mixed solution (500 g Copper sulphate + 2.25 kg ammonium bicarbonate) in mid-late March.

Ran *et al.* (2001) sprayed Mancozeb M-45, Carbendazim, Pyridaben and Topsin (Thiophanatemethyl) in a citrus orchard at different concentrations to control citrus diseases and citrus rust mite. Spraying was applied at different stages. The results showed that the best control of citrus scab was achieved by spraying a 600 times solution of 80% Mancozeb M-45 when the shoots were 2 cm long, then twice more spraying at 10 days intervals (total of 3 times). Spraying a 600 times solution of 80% Mancozeb M-45 after fall and again at intervals of 15 days (total of 4 times) gave good control of citrus black spot.

Singh *et al.* (2000) conducted an experiment for management of citrus scab. They used Blitox-50 (Copper oxychloride), Bordeaux mixture, Derosal (Carbendazim), Chlorothalonil and Indofil M-45 (Mancozeb) at 1000 ppm each against the pathogen (*Elsinoe fawcettii*) *in vitro* culture resulting in 81.1, 88.0, 77.2, 76.6 and 64.4% growth inhibition of the pathogen over untreated control, respectively. Blitox-50 at 0.3%, Bordeaux mixture at 1%, Derosal at 0.2%, Chlorothalonil at 0.2% and Indofil M-45 at 0.3% reduced the disease incidence in the field by 47.8, 69.5, 46.2, 50.4 and 40.4%, respectively over control.

Bushong and Timmer (2000) reported that Benomyl was effective if applied 72 hours after inoculation and fenbuconazole and azoxystrobin were effective if applied within 16 to 48 hours after inoculation. They showed that use of post infection sprays under field conditions appears to be promising for scab control.

Huang (1999) investigated the effects of fungicide against citrus scab caused by *Elsinoe fawcettii*. They used Bordeaux mixture or thiophanate methyl (as Topisn-M) for control of citrus scab on mandarin. Thiophanate methyl controlled citrus scab by 75% over control. Spraying of Bordeaux mixture at the bud break stage gave effective disease control.

Li *et al.* (1997) conducted an experiment on four years old trees of Satsuma cv. Winzhou and Mandarin cv. Ponggan in China. They applied Pujunk (Copper hydroxide) and Bordeaux mixture as a spray 500 times in the Spring or 700 times in the summer and found that Pujunk controlled citrus scab (*Elsinoe fawcettii*) more effective than Bordeaux mixture.

Gottwald (1995) observed that Catafol significantly affected the spatiotemporal dynamics of citrus scab epidemics by reducing both inoculum production and poroviding protection to susceptible new leaves.

Whiteside (1990) conducted 3 years trial with 4 fungicides namely Dithane, Catafol and 2 sterol inhibiting fungicides, Diniconazole and Difeconazole to evaluate them against citrus scab caused by *Elsinoe fawcettii* in Florida, USA. They observed that Dithane performed better than copper fungicide treatments, recommended at the time in the field trials. However, Difeconazole usually gave better control of scab than Dithane. Where spray treatments were delayed until after some fruit had become infected, Difeconazole reduced scab severity even more than captafol.

Rawal (1990) reported that spraying of Difolatan 0.2% and Ferbam 75 WP gave effective control against scab of citrus caused by *Elsinoe fawcettii*. *Ferbam* has been found to be superior over Bordeaux mixture.

2.3.2 Management of citrus die-back through fungicides

Alam (2006) conducted a research work on the standing citrus plant raised at citrus orchard in Bangladesh. The highest reduction of die-back severity (PDI) over control was resulted by Dithane M-45 (73.46%) followed by Neem extract (67.34%), Bordeaux mixture (59.19%) and *Trichoderma harzianum* T_{22} (57.67%).

Peres *et al.* (2004) reported that post bloom fruit drop (PFD) of citrus, caused by *Colletotrichum gloeosporioides* was controlled effectively by spraying of Benomyl (Benlate 50 WP) in reasearch field and as well as in the commercial orchards.

Das *et al.* (1998) conducted an experiment to evaluate eight fungicides in controlling *Colletotrichum gloeosporioides (Glomerella cingulata) in vitro.* The fungicides tested were Carbendazim (as Bavistin), Propiconazole (as Tilt), Expoxiconazole (as Opus), Tridemorph (as Calixin), Metalaxyl + mancozeb (as Ridomil M-72), Mancozeb (as Dithane M-45), Copper oxychloride (as Biltox-

50) and Thiram at different concentrations. Expoxiconazole completely inhibited the linear mycelial growth at 50 mg/L and higher concentrations. Carbendazin and Ridomil M-72 inhibited linear mycelial growth at 250 mg/L. Tridemorph effectively checked the total growth at 500 mg/L. Mancozeb, Copper oxychloride and Thiram were moderately effective even at 500 mg/L.

Ansar *et al.* (1997) conducted an experiment in Pakistan and found *C. gloeosporioides*, the main causal agent of guava decline could be effectively controlled by combined use of Topsin-M (Thiophanate methyl) and Cupravit.

Ebenezar and Shubramanian (1996) observed the effect of chemicals to prevent die-back of acid lime caused by *Colletotrichum gloeosporioides* in India (Tamil Nadu). They used 7 fungicides like Carbendazim, Mancozeb, Copper oxychloride, Zineb, Captafol, Bordeaux mixture and Aureofungin for testing their efficacy. All the fungicides reduced die-back of acid lime. Among the treatments, Carbendazim (0.1%) and Bordeaux mixture (0.8%) showed better control.

Shayesta (1995) tested Cupravit (copper oxychloride) against leaf spot and twig blight disease of *Euchalyptus camaldutensis* caused by *Colletotrichum gloeosporioides, as foliar sprays*. He found that the disease was controlled and minimized by Cupravit sprayed at 1g/L concentration with ten days intervals.

Thakore *et al.* (1994) tested some fungicides against die-back of citrus in Rajasthan. They treated die-back affected plant with Biltox-50 (copper oxychloride), Dithane M-45 (Mancozeb) and Macuprex (Cupraneb + Boradeaux). They found all the fungicides were effective against the disease at 2000 ppm concentration.

Borros *et al.* (1993) in an *in vitro* experiment assessed the effectiveness for commercial fungicides against post-harvest decay of citrus caused by

Colletotrichum gloeosporioides (Glomerella cingulata) and *Geotrichum candidum*. Better control was obtained with 1.10 ug/ml of Captan, Folpet and Tridemorf against *Colletotrichum gloeosporioides* and *Geotrichum candidum*.

Hossain (1993) conducted several years trial with seven fungicides, namely Bordeaux mixture (4: 4: 40), Zineb (2g/L), Topsin M 70 WP (lg/L), Dithane M-45 (2 g/L), Bavistin (1g/L), Morestin (1g/L) and Daconil (1g/L) to evaluate them against anthracnose disease of Guava caused by *Colletotrichum gloeosporioides*. He observed that Topsin M and Dithane M-45 was the best effective in reducing fruit infection.

2.4.1 Management of scab and die-back of citrus through plant extracts.

Yesmin (2004) reported that Neem leaf extract was most effective in controlling canker of leaf and fruit caused by *Xanthomonas citri*. Garlic extract and Neem leaf extract were the most effective in controlling die back of twig and branch of citrus, respectively. She also recommended that citrus canker can successfully controlled by Neem leaf extract and garlic extract.

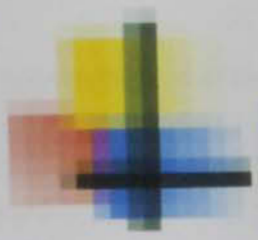
Khan *et al.* (1998) applied four neem based products, namely Nemokil, Nemokil-S, SDS and SDC and found antifungal activity against the guava wilt and the anthracnose pathogen. These neem based products however, proved less effective against *Colletotrichum gloeosporioides*.

2.4.2 Management of other plant diseases by Allamanda leaf extract

Islam (2004) found garlic bulbs and allamanda leaf extract caused 76-100% inhibition of mycelial growth of *Phomopsis vexans*. Diethyl ether, Dichloromethane and water acted as effective solvents for preparing garlic extract. TLC studies showed the presence of a number of compounds having very low to high polarity in garlic bulbs and allamanda leaves extracts.

Meah (2003) reported that garlic bulbs extract (1:10) and allamanda leaf extract efficiently controlled *Phomopsis vexans* in the laboratory, nursery house and in the field reducing severity of leaf blight and fruit rot by 71-75%.

Khan (1999) studied the effect of plant extracts (Allamanda, Bel and Neem) for the management of Phomopsis blight/fruit rot of eggplant in field condition. Among the three plant extracts, Allamanda spray was the most effective.



Chapter 3
Materials and methods

CHAPTER-III

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the citrus orchard of Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experimental period

The experiment was carried out during March, 2007 – September, 2007.

3.3 Soil type

The description of the Agro-ecological zone (UNDP and FAO, 1988) of the experimental site is as follows:

Agro-ecological region	:	Mahdupur tract (AEZ-28)
Land type	:	Medium High Land
General Soil type	:	Non-Calcarious dark-gray flood plain Soil
Soil series	:	Teigaon
Topography	:	Up land
Elevation	:	8.45
Location	:	SAU Farm, Dhaka
Field Level	:	Above flood level
Drainage	:	Fairly good
Firmness (consistency)	:	Compact of friable when dry

The physical and chemical characteristics of the soil have collected from Soil Resources Development Institute (SRDI), Farmgate, Dhaka and is presented bellow (for 0-14cm depth):

Particle size distribution:

Sand	:	34%
Silt	:	46%
Clay	:	20%
Soil texture	:	Loam to clay loam

3.4 Plant selection

Twenty seven plants were selected from the citrus orchard of Sher-e-Bangla Agricultural University (SAU) campus, Dhaka. All plants were equal aged (6 years old), which were used as the experimental unit of this study.

3.5 Treatments

Nine different treatments designated by T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 were explored in the experiment stated as fallows:

T_1 = Rovral 50 WP

T_2 = Champion 77 WP

T_3 = Dithane M-45

T_4 =Cupravit 50 WP

T_5 = Tilt 250 EC

T_6 = Proud 250 EC

T_7 = Bavistin 50 WP

T_8 = Allamanda (*Allamanda cathartica*) leaf extract

T_9 = Control

3.6 Design of experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) comprising three replications for each treatment. Thus there were altogether 9 treatments for 27 plants used for the study. Different treatments were assigned randomly to the unit plant.

3.7 Weeding

Weeding was performed five times during the experimental period with 20 days intervals starting from March 1, 2007.

3.8 Irrigation

The plants were irrigated five times. The first irrigation was done at March 3, 2007 and the fallowing irrigations were done at 20 days intervals.

3.9 Application of fertilizers and manure

Fertilizers and manure were applied to the experimental unit plant as per recommendation of BAR1 (Krishi Projukti Hat Bai). The following doses of fertilizers were applied to the unit plant.

Fertilizer /Manure	Dose/plant
Cow-dung	20 kg
Urea	450 gm
TSP	400gm
MP	400gm

Half of the urea, TSP and MP and all amount of cow dung were used at the time of 1st weeding. The rest half of the urea, TSP and MP were applied at May 1, 2007.

3.10 Application of insecticide

Insecticide Aktara was applied three times @ 0.5 gm/liter water at 15 days interval from March 7, 2007 to protect the crop from insects, pests.

3.11 Tagging and data collection

Five twigs were selected randomly from each plant and tagged for determining of scab and die back infection. Mean infection values were determined to get ratting score. But for fruit infection the whole plants were considered. Data were recorded on different parameters on individual plant basis just after onset of the disease symptom in experimental plot. As many as seven observations were taken starting from June 1, 2007 with 15 days intervals.

3.12 Preparation of spray solution

The fungicidal solutions were prepared by mixing prescribed amount of fungicides with tap water to get 0.3% solution for Dithane M-45, 0.1% solution for Bavistin 50 WP, 0.2% solution for Champion 77WP, 0.1% solution for Cupravit 50 WP, 0.1% solution for Rovral 50 WP, 0.25% solution for Tilt 250 EC and 0.25 % solution for Proud 250 EC. Details of the fungicides used as spray materials are given in Table 1.

Table 1. Particulars of Fungicides used in the experiment

Treatment	Chemical name	Active ingredient	Dose used
Rovral 50 WP	1-Isopropyl carbammoyl-3-dichlorophynyl hydration	Iprodione 50%	0.1%
Champion 77 WP	Copper hydroxide $Cu(OH)_2$	Copper hydroxide 77%	0.2 %
Dithane M- 45	Manganous ethylene bisdithio - carbamate	Dithiocarbamate 80%	0.3 %
Cupravit 50 WP	Copper Oxychloride	Copper Oxychloride 50%	0.1 %
Tilt 250 EC	Propiconazole	Propiconazole 25%	0.25%
Proud 250 EC	Propiconazole	Propiconazole 25%	0.25%
Bavistin 50WP	Methyl-2-Benzimidazole carbamate	Carbendazime 50%	0.1%

3.13. Plant extracts preparation

Allamanda leaf was used in this study (Photograph 1). The plant extract was prepared with the help of blender. Requisite amount of plant materials were dissolved in required amount of sterilized water to make the desired concentrations (1 : 4). For this concentration 25g plant materials crushed in 100ml water. The crushed materials were filtered through cheese cloth to get the fine sprays.

Table 2. Specification of the plant extract in the Experiment

Common name	Botanical name	Plant parts used	Dose used (%)
Allamanda	*Allamanda cathartica*	Leaf	1 : 4

Photograph 1. Allamanda leaves used in the experiment

3.14 Application of spray solution

All fungicides and plant extract were sprayed with compressed hand sprayer. Three plants were sprayed with each fungicides and plant extract for each treatment. First spray was done in May 15, 2007. The plants were sprayed 7 times at 15 days intervals. Required amount of spray-solution was applied per plant covering branches, twigs, leaves and fruits properly. Control plant was sprayed with plain water only. Precautions were taken to avoid drifting of spray materials to neighboring plants with polythene barrier.

3.15 Disease symptoms observed in the orchard

The plants were routinely observed from the time of spraying. Onset of new infection was recorded and symptoms of the scab and die-back were observed, recorded and photographed (Photograph 3 and 4).

3.16 Isolation of Pathogens

Infected leaves and twigs were collected for isolation of the causal fungi. Stem piece of 1 cm in length was cut out from the twigs for isolation. Four pieces of infected tissue approximately 10 mm in length and 1.5-2.5 mm width were surface sterilized in Chlorox (10%) solution for 45 seconds and washed thrice in sterile water. The inocula were then placed on acidified Potato Dextrose Agar (PDA) medium in Petri dishes aseptically. After planting, the Petridishes containing the inocula were incubated at room temperature ($26^0C \pm 2^0C$) under 12 hours light alternating with 12 hours dark. The plates were incubated for 7 days in the inoculation chamber.

3.17 Identification of fungal isolates

Fungi grown on the culture media were transferred lo fresh PDA plates. The fungal isolates were then sub cultured on 2% water agar and purified by hyphal tip culture method. The fungus was identified following the appropriate keys (Kulsherestha *et al*., 1979 and Sutton, 1980).

3.18 Data collection

Data were collected on the following parameters:

3.18.1 Total number of leaves/twig

Number of total leaves/twig was counted from randomly selected five twigs from each plant at different dates as scheduled.

3.18.2 Total number of scab infected leaves/twig

Number of scab infected leaves/twig under each treatment was counted at different observation dates as scheduled.

3.18.3 Calculation of disease incidence of leaf scab of different treatments

The percent disease incidence of leaf was calculated using the following formula

$$\text{Disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of observation (leaves)}} \text{ X } 100$$

3.18.4 Total number of fruits/plant

Numbers of total fruits were recorded at different observation dates as scheduled.

3.18.5 Total number of scab infected fruits/plant

Number of scab infected fruits/plant under each treatment was counted at different observation dates as scheduled.

3.18.6 Calculation of disease incidence of fruit scab of different treatments

The percent disease incidence of fruit was calculated using the following formula.

$$\text{Disease incidence} = \frac{\text{Number of infected fruits}}{\text{Total number of inspected fruits}} \times 100$$

3.18.7 Total number twigs/branch

Number of total twigs/branch was counted from randomly selected five branches from each plant at different dates as scheduled.

3.18.8 Total number of die back infected twigs/branch

Number of die back infected twigs/branch under each treatment was counted at different observation dates as scheduled.

3.18.9 Calculation of disease incidence of die back of different treatments

The percent disease incidence was calculated using the following formula.

$$\text{Disease incidence} = \frac{\text{Number of infected twigs}}{\text{Total number of observation (twigs)}} \times 100$$

3.18.10 Evaluation of leaf and fruit scab severity

Percent leaf area diseased (LAD) and fruit area diseased (FAD) were measured. Area of single leaf or fruit was considered as 100%. Deducting the healthy area, the diseased area was estimated. Then average of %LAD and %FAD was calculated dividing the total diseased areas by total number of investigated leaves and fruits (Islam *et al.*, 2001). The leaf and fruit scab severity was recorded following 0-5 scale with slight modification as designed by Gonzalez *et al.* (1993).

The gradation is given below.

Grade		% area infected
0	=	No infection
1	=	Up to 5% area infected
2	=	5-10% areas infected
3	=	11-20% areas infected
4	=	21-30% area infected and
5	=	above 30% area infected.

The percent disease index (PD1) was calculated using the following formula:

$$PDI = \frac{\text{Total sum of numerical rattings}}{\text{Number of observation} \times \text{Maximum grade in the diseases ratting scale}} \times 100$$

3.18.11 Evaluation of die back severity

The die back severity was recorded following '0-5' scale with slight modification as designed by Rahman and Hossain (1988). The gradation is given below:

Grade		% twig infected
0	=	No infection
1	=	Up to 10% twig area infected
2	=	11 - 20% twig area infected
3	=	21- 30% twig area infected
4	=	31 - 50% twig area infected and
5	=	above 50 % twig area infected

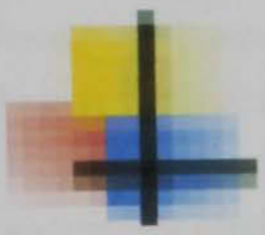
The percent disease index (PDI) was calculated using the formula as practiced in case of PDI calculation of infected leaf.

3.19 Analysis of data

The data on various parameters were analyzed using analysis of variance to find out the variation obtained from different treatments. Compilation of the experimental data and analysis were done by the computer MSTAT-C program following the statistical procedures of Gomez and Gomez (1983). Treatment means were compared by DMRT (Duncan's Multiple Range Tests).

3.20 Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity and monthly total rainfall at the experiment site during the period of the study have been collected from the surface synoptic data card, Bangladesh Meteorological Department, Dhaka (Appendix 1).



Chapter 4
Results

CHAPTER IV

RESULTS

4.1 Scab symptoms observed in the orchard

The disease first noticed as the irregular corky spot on the leaves and fruits (Photograph 2 and 3).The spots ranged from a few mm up to 1cm in diameter. They become warty and erupted with cracks and scabby on the leaves. The spots were found more often on the lower surface than on the upper. On the fruits, the warty growth is surrounded by yellowish or chlorotic halo. Several spots coalesced to form large patches of corky outgrowth. The lesions may appear on shoots. Heavily infected fruits may drop shortly after being attacked.

4.2 Die back symptoms observed in the orchard

The main characteristic symptom of the disease was drying of the shoots down wards to the stem resulting in the death of twigs or top of the branches (Photograph 4 and 5). The first visible symptom of the disease was characterized by shading of the green color of the tip of twigs and the leaves. The die beak often progressed slowly and caused wilting of leaves, turned yellow and drop off. The twigs gradually started drying from the top to downwards.

After the symptom noticed, the drying of the twig was usually rapid which extended 5 to 12 cm downwards ending in a sharp line. After wards, the disease progressed slowly or rapidly in the similar way towards the stem affecting more twigs and branches. Some times, the tip of the die back affected young branches, produce gum exudation.

Photograph 2. Scab infected leaves of citrus (*Citrus limon*)

Photograph 3. Scab infected fruit of citrus (*Citrus limon*)

Photograph 4. Die-back infected twig of citrus (*Citrus limon*)

Photograph 5. Severely die-back infected plant of citrus (*Citrus limon*)

Twigs and branches appeared to have been scorched by fire. When twigs were dried, minute brown to black, slightly raised, clumped pustules were observed. In some cases, black dot like structures (ascervuli / pycnidia of fungi) could be found to develop on the light or grayish brown to whitish ashy membranous dead twigs or branches. In severe cases, branches show die back and tree dies gradually.

4.3 Isolation and identification of *Elsinoe fawcettii*

The inocula collected from the disease leaves and fruits, plated and incubated in the inoculation chamber produced fungal colony on the PDA medium. Purified colony was subjected to identity the fungi and it was identified as *Elsinoe fawcettii (*Photograph 6*)* (Hyun *et al*., 2001).

4.4 Isolation and identification of *Colletotrichum gloeosporioides*

The inocula collected from the disease twigs, plated and incubated in the inoculation chamber produced fungal colony on the PDA medium. Purified colony was subjected to identity the fungi and it was identified as *Colletotrichum gloeosporioides* (Photograph 7) (Commonwealth Mycological Institute, Description of Pathogenic fungi and Bacteria. No 315).

4.5 Incidence and severity of scab of citrus (leaf) before commencement of spray schedule

The disease incidence and severity of scab of citrus plants (leaf) before commencement of the spray schedule was statistically similar for the citrus plant selected for each and every treatment (Table 3). The disease incidence (leaf) ranged from 12.35% to 18.19% and the disease severity (PDI-leaf) ranged from 9.04% to 14.60%.

Photograph 6. Conidia of *Elsinoe fawsettii (Sphaceloma fawcettii)*

Photograph 7. Conidia of *C. gloesporioides*

4.6 Effect of treatments in controlling leaf scab of citrus after one month of starting spray

The effect of fungicides and plant extract in controlling leaf scab of citrus recorded after one month of starting of spray was determined and presented in Table 3. The disease incidence under different treatments was differed significantly from one to another. The lowest incidence of leaf scab (10.68%) was found in T_7 (Bavistin 50WP) and the highest incidence (18.52%) in T_9 (Control). The second lowest incidence of leaf scab (11.07%) of citrus was found in T_8 (Allamanda leaf extract).

The disease severity (PDI-leaf) under different treatments was also differed significantly from one another. The lowest disease severity (7.30%) was found in T_1 (Rovral 50 WP) and the highest disease severity (18.29%) in T_9 (Control). The second lowest disease severity (8.19%) was found with T_5 (Bavistin 50 WP). It has been recorded that the effect of treatments T_1 (Rovral 50WP), T_4 (Cupravit 50 WP), T_5 (Tilt 250EC), T_7 (Bavistin 50 WP) and T_8 (Allamanda leaf extract) did not differ significantly in respect of disease severity.

The highest PDI decreased over control (60.01%) was found in T_1 (Rovral 50 WP) followed by T_7 (Bavistin 50 WP), T_8 (Allamanda leaf extract), T_5 (Tilt 250 EC) and T_4 (Cupravit 50 WP). The lowest decrease of PDI-leaf was noted in Proud 250EC (24.82%) preceded by T_3 Dithane M-45 (26.02%) and T_2 Champion 77WP (26.68%) (Table 3).

Table 3. Effect of treatments on the incidence and severity of Scab of citrus (leaf) after one month of starting spray

Treatments	Before spraying		After one month of starting of spray		
	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	PDI-leaf decreased over control (%)
T_1=Rovral 50 WP	15.69 ab	9.25 b	11.50 bcd	7.30 c	60.01
T_2= Champion 77 WP	18.19 a	14.60 a	13.95 b	13.41 b	26.68
T_3= Dithane M 45	12.94 b	14.11 a	13.72 bc	13.53 b	26.02
T_4=Cupravit 50 WP	14.81ab	9.22 b	11.25 cd	10.00 c	45.32
T_5=Tilt 250 EC	13.81 b	10.42 b	12.05 bcd	9.30 c	49.15
T_6=Proud 250 EC	15.53 ab	12.60 ab	13.90 b	13.75 b	24.82
T_7=Bavistin 50 WP	13.52 ab	12.54 ab	10.68 de	8.19 c	55.22
T_8=Allamanda leaf extract	14.35 ab	9.68b	11.07 d	8.87 c	51.50
T_9=Control	12.35 b	9.04 b	18.52 a	18.29 a	-
CV%	13.89	16.94	11.29	16.16	-

The means having same latter(s) does not significant at 5% level

4.7 Effect of treatments in controlling leaf scab of citrus after two months of starting spray

The effect of fungicides and plant extract in controlling leaf scab of citrus recorded after two months of starting of spray was determined and presented in Table 4. The disease incidence under different treatments was differed significantly from one another.

The lowest incidence of leaf scab (4.32%) was found in T_8 (Allamanda leaf extract) and the highest incidence (21.88%) in T_9 (Control). The second lowest incidence of leaf scab (5.79%) of citrus was found in T_1 (Rovral 50 WP). The effect of treatment T_1 (Rovral 50 WP), T_2 (Champion 77WP), T_7 (Bavistin 50 WP) and T_8 (Allamanda leaf extract) did not differ significantly in respect of disease incidence.

The disease severity (PDI-leaf) under different treatments was also differed significantly from one another. The lowest disease severity (4.12) was found with T_8 (Allamanda leaf extract) and the highest disease severity (22.93%) in T_9 (Control). The second lowest disease severity was found with T_2 (Champion 77 WP) (4.79%). It has been recorded that the effect of treatments T_1 (Rovral 50WP), T_2 (Champion 77 WP) and T_8 (Allamanda leaf extract) did not differ significantly in respect of disease severity.

The highest PDI decreased over control (82.03%) was found with T_8 (Allamanda leaf extract) followed by Champion 77 WP (79.11%) and Rovral 50 WP (78.32%). The lowest decrease of PDI was noted (43.00%) in T_6 (Proud 250 EC) preceded by Cupravit 50 WP (44.27%) and Dithane M-45 (45.97%) (Table 4).

Table 4. Effect of treatments on the incidence and severity of Scab of citrus (leaf) after two months of starting spray

Treatments	Before spraying		After two months of starting of spray		
	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	PDI-leaf decrea-sed over control (%)
T_1=Rovral 50 WP	15.69 ab	9.25 b	5.79 d	4.97e	78.32
T_2= Champion 77 WP	18.19 a	14.60 a	6.05 d	4.79e	79.11
T_3= Dithane M 45	12.94 b	14.11 a	14.86 b	12.39 c	45.96
T_4=Cupravit 50 WP	14.81ab	9.217 b	16.82 b	12.78 c	44.26
T_5=Tilt 250 EC	13.81 b	10.42 b	9.48 c	6.56de	71.39
T_6=Proud 250 EC	15.53 ab	12.60 ab	15.83 b	13.07 b	43.00
T_7=Bavistin 50 WP	13.52 ab	12.54 ab	9.88 c	7.56 d	67.03
T_8=Allamanda leaf extract	14.35 ab	9.68 b	4.32 d	4.12 e	82.03
T_9=Control	12.35 b	9.04 b	21.81 a	22.93 a	-
CV%	13.89	16.94	13.29	15.65	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.8. Effect of treatments in controlling leaf scab of citrus after three months of starting spray

The effect of fungicides and plant extract in controlling leaf scab of citrus recorded after three months of starting of spraying (six sprays have been given) was determined and presented in Table-5. The disease incidence under different treatments was differed significantly from one another. The lowest incidence of leaf scab (1.86%) was found in T_8 (Allamanda leaf extract) which was statistically identical with Rovral 50 WP (3.21%). The second lowest incidence of leaf scab of citrus was found with Champion 77WP (3.85%) which was statistically similar with Rovral 50WP. The highest incidence (15.61%) was recorded in T_9 (Control).

The disease severity (PDI-leaf) under different treatments was also differed significantly from one another. The lowest disease severity (2.23%) was found with T_2 (Champion 77 WP) which was statistically similar with Rovral 50WP (2.47%) and Allamanda leaf extract (2.72%). The highest disease severity was recorded (15.31%) in Control preceded by Proud 250 EC (11.26%) and Cupravit (7.25%).

The highest PDI decreased over control (85.43%) was found with T_4 (Champion 50 WP) followed by T_1 (Rovral 50 WP) (83.87%) and T_8 (Allamanda leaf extract) (82.23%). The lowest decrease of PDI was noted (26.45%) in T_6 (Proud 250EC) preceded by Cupravit 50 WP (52.64%) and Dithane M-45 (68.51%) (Table 5).

Table 5. Effect of treatments on the incidence and severity of Scab of citrus (leaf) after three months of starting spray

Treatments	Before spraying		After three months of starting of spray		
	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	Disease incidence (leaf)	Percent Disease index (PDI-leaf)	PDI-leaf decreased over control (%)
T_1=Rovral 50 WP	15.69 ab	9.25 b	3.2 1ef	2.47 e	83.87
T_2= Champion 77 WP	18.19 a	14.60 a	3.85 e	2.23 e	85.43
T_3= Dithane M 45	12.94 b	14.11 a	7.14 d	4.82 d	68.51
T_4=Cupravit 50 WP	14.81ab	9.217 b	9.11 c	7.25 c	52.64
T_5=Tilt 250 EC	13.81 b	10.42 b	6.48 d	3.35 de	78.11
T_6=Proud 250 EC	15.53 ab	12.60 ab	11.58 b	11.26 b	26.45
T_7=Bavistin 50 WP	13.52 ab	12.54 ab	6.03 d	3.51 de	77.07
T_8=Allamanda leaf extract	14.35 ab	9.680 b	1.86 f	2.72 e	82.23
T_9=Control	12.35 b	9.037 b	15.61 a	15.31 a	-
CV%	13.89	16.94	14.53	18.80	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.9 Incidence and severity of scab of citrus (fruit) before commencement of spray schedule

The disease incidence and severity of scab of citrus (fruit) plants before application of spray was statistically identical for each of the treatment (Table 6). The disease incidence (fruit) ranged from 14.18% to 24.91% and the disease severity (PDI- fruit) ranged from 12.36% to 19.37%.

4.10 Effect of treatments in controlling fruit scab of citrus after one month of starting of spray

The effect of fungicides and plant extract in controlling scab of citrus (fruit) were recorded after one month of starting of spraying (two sprays applied) summarized and presented in Table 6. The treatment effects were differed significantly in respect of disease incidence and severity.

In case of disease incidence (fruit), the lowest incidence (15.76%) was recorded in Dithane M- 45 which was statistically identical with Bavistin 50 WP (17.03%). The highest disease incidence (26.20%) was recorded in case of control treatment.

In case of disease severity (PDI- fruit), the lowest disease severity was recorded in Cupravit 50WP (12.4%), which was statistically similar with that of Dithane M-45 (13.3%). The highest disease severity was observed in control treatment (24.55%). Tilt 250 EC (18.5%) scored second highest severity in the experiment which is statistically similar with Champion 77 WP (17.3%) and Proud 250 EC (17.5%).

The highest reduction of PDI over control was observed in case of Cupravit 50 WP (49.49%) followed by Dithane M-45 (45.82%), Bavistin (45.01%) and Allamanda leaf extract (38.90%). The lowest reduction of PDI was noticed in case of Tilt 250 EC (24.64%) preceded by Proud 250 EC (28.71%) and Champion 77 WP (29.57%) over control.

Table 6. Effect of treatments on the incidence and severity of scab of citrus (fruit) after one month of starting spray

Treatments	Before spraying		After one month of starting of spray		
	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	PDI-fruit decreased over control (%)
T_1=Rovral 50 WP	24.67 ab	14.24 ab	19.31 ab	13.5 d	45.01
T_2= Champion 77 WP	17.42 bcd	18.37 ab	20.0 ab	17.3 b	29.53
T_3= Dithane M 45	15.44 cd	14.99 ab	15.76 d	13.3 de	45.82
T_4=Cupravit 50 WP	14.18 d	12.36 b	18.5 bc	12.4 e	49.49
T_5=Tilt 250 EC	24.91 a	16.30 ab	21.00 a	18.5 b	24.64
T_6=Proud 250 EC	23.12 ab	19.12 ab	19.35 ab	17.5 b	28.71
T_7=Bavistin 50 WP	17.74 bcd	17.33 ab	17.03 cd	13.5 d	45.01
T_8=Allamanda leaf extract	21.93 abc	17.32 ab	19.3 ab	15.0 c	38.90
T_9=Control	21.84 abc	19.37 a	26.20 a	24.55 a	-
CV (%)	17.39	21.36	20.17	17.62	-

Means bearing same letters within the same column do not differ significantly at 5% level.

4.11 Effect of Treatments in controlling fruit scab of citrus after two months of starting of spray

The effect of treatments in controlling scab of citrus (fruit) recorded after two months of starting of spray (four sprays applied) was summarized, determined and presented in Table 7. The treatment effects were differed significantly in respect of disease incidence and severity.

In case of disease incidence (fruit), the lowest incidence (9.33%) was recorded in Champion 77 WP. The second lowest incidence was recorded in Allamanda leaf extract (13.07%) and Dithane M-45 (13.3%) which was statistically similar with Rovral 50 WP (16.51%). The highest disease incidence (27.67%) was recorded in control treatment.

In case of disease severity (PDI- fruit), the lowest severity was recorded in Dithane M-45 (11.39 %), which was statistically similar with Cupravit 50 WP (13.90%) and Tilt 250 EC (13.90%). The highest disease severity (25.30%) was observed in control treatment. The effect of Rovral 50 WP, Allamanda leaf extract and Bavistin 50 WP were statistically similar in respect of disease severity.

The highest reduction of PDI over control was observed in case of Dithane M-45 (54.98%) followed by Allamanda leaf extract (51.38%), Rovral 50 WP (48.61%), Bavistin 50 WP (47.82%), Cupravit 50 WP (45.05%) and Tilt 250 EC (45.05%). The lowest reduction of PDI was noticed in case of Proud 250 EC (39.4%) over control.

Table 7. Effect of treatments on the incidence and severity of scab of citrus (fruit) after two months of starting spray

Treatments	Before spraying		After two months of starting of spray		
	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	PDI-fruit decreased over control (%)
T_1=Rovral 50 WP	24.67 ab	14.24 ab	16.51 d	13.00 de	48.61
T_2= Champion 77 WP	17.42 bcd	18.37 ab	9.33 e	14.35 c	43.28
T_3= Dithane M 45	15.44 cd	14.99 ab	13.3 d	11.39 f	54.98
T_4=Cupravit 50 WP	14.18 d	12.36 b	16.35 b	13.90 f	45.05
T_5=Tilt 250 EC	24.91 a	16.30 ab	17.31 b	13.90 f	45.05
T_6=Proud 250 EC	23.12 ab	19.12 ab	16.66 b	15.33b	39.4
T_7=Bavistin 50 WP	17.74 bcd	17.33 ab	15.10 c	13.2 de	47.82
T_8=Allamanda leaf extract	21.93 abc	17.32 ab	13.07 d	12.35 e	51.38
T_9=Control	21.84 abc	19.37 a	27.67 a	25.30a	
CV (%)	17.39	21.36	16.47	20.01	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.12 Effect of fungicides and plant extract in controlling fruit scab of citrus (fruit) after three months of starting spray

The effect of fungicides and plant extract in controlling scab of citrus (fruit) were recorded after three months of starting of spraying (six sprays applied) was determined and presented in Table-8. The treatment effects were differed significantly in respect of disease incidence and severity.

In case of disease incidence (fruit), the lowest incidence 5.88% was recorded in Champion 77WP followed by Dithane M-45 (10.67%), Allamanda leaf extract (10.81%) and Proud 250 EC (11.76%). The highest disease incidence (28.20%) was recorded control treatment.

In case of disease severity (PDI- fruit), the lowest disease severity was recorded in Champion 77WP (5.33%) followed by Allamanda leaf extract (9.06%), Dithane M-45 (10.67%) and Proud 250 EC (11.34%). The highest disease severity was observed in control (16.81%).

The highest reduction of PDI over control was observed in Champion 50 WP (80.12%) followed by Allamanda leaf extract (66.20%) and Dithane M-45 (60.20%). The lowest reduction of PDI over control was observed in case of Tilt 250 EC (49.01%).

Table 8. Effect of treatments on the incidence and severity of scab of citrus (fruit) after three months of starting spray

Treatments	Before spraying		After three months of starting of spray		
	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	Disease incidence (fruit)	Percent Disease index (PDI-fruit)	PDI-fruit decreased over control (%)
T_1=Rovral 50 WP	24.67 ab	14.24 ab	13.79 c	12.21 cd	54.45
T_2= Champion 77 WP	17.42 bcd	18.37 ab	5.88 f	5.33 f	80.12
T_3= Dithane M 45	15.44 cd	14.99 ab	10.67 e	10.67 e	60.20
T_4=Cupravit 50 WP	14.18 d	12.36 b	14.80 b	13.15 b	50.59
T_5=Tilt 250 EC	24.91 a	16.30 ab	14.71 b	13.67 b	49.01
T_6=Proud 250 EC	23.12 ab	19.12 ab	11.76 d	11.34 de	57.70
T_7=Bavistin 50 WP	17.74 bcd	17.33 ab	13.79 c	12.96 bc	51.65
T_8=Allamanda leaf extract	21.93 abc	17.32 ab	10.81 e	9.06 e	66.20
T_9=Control	21.84 abc	19.37 a	28.20 a	26.81a	-
CV%	17.39	21.36	11.97	24.69	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.13. Effect of treatments on number of fruits per plants

The number of fruits per plant was found to differ significantly from one treatment to another. The number of fruits per plant was ranged from 13.33 to 53.00. The highest number of fruits per plant was recorded in case of Allamanda leaf extract (53.00), which was statistically identical with Bavistin 50WP (52.66), Tilt 250EC (45.00), Cupravit 50 WP (44.00) and Dithane M-45 (42.66). The lowest number of fruits per plant (13.33) was noted in case of control treatment (Table 9) (Figure 1).

Table 9. Effect of treatments on number of fruits per plant

Treatments	No. of fruits per plant
T1= Rovral 50 WP	24.33b
T2= Champion 77 WP	31.33b
T3= Dithane M 45	42.66a
T4= Cupravit 50 WP	44.00a
T5= Tilt 250 EC	45.00a
T6= Proud 250 EC	27.33b
T7= Bavistin 50 WP	52.66a
T8= Allamanda leaf extract	53.00a
T9= Control	13.33c
CV%	20.70

Means bearing same letter within the same column do not differ significantly at 5% level.

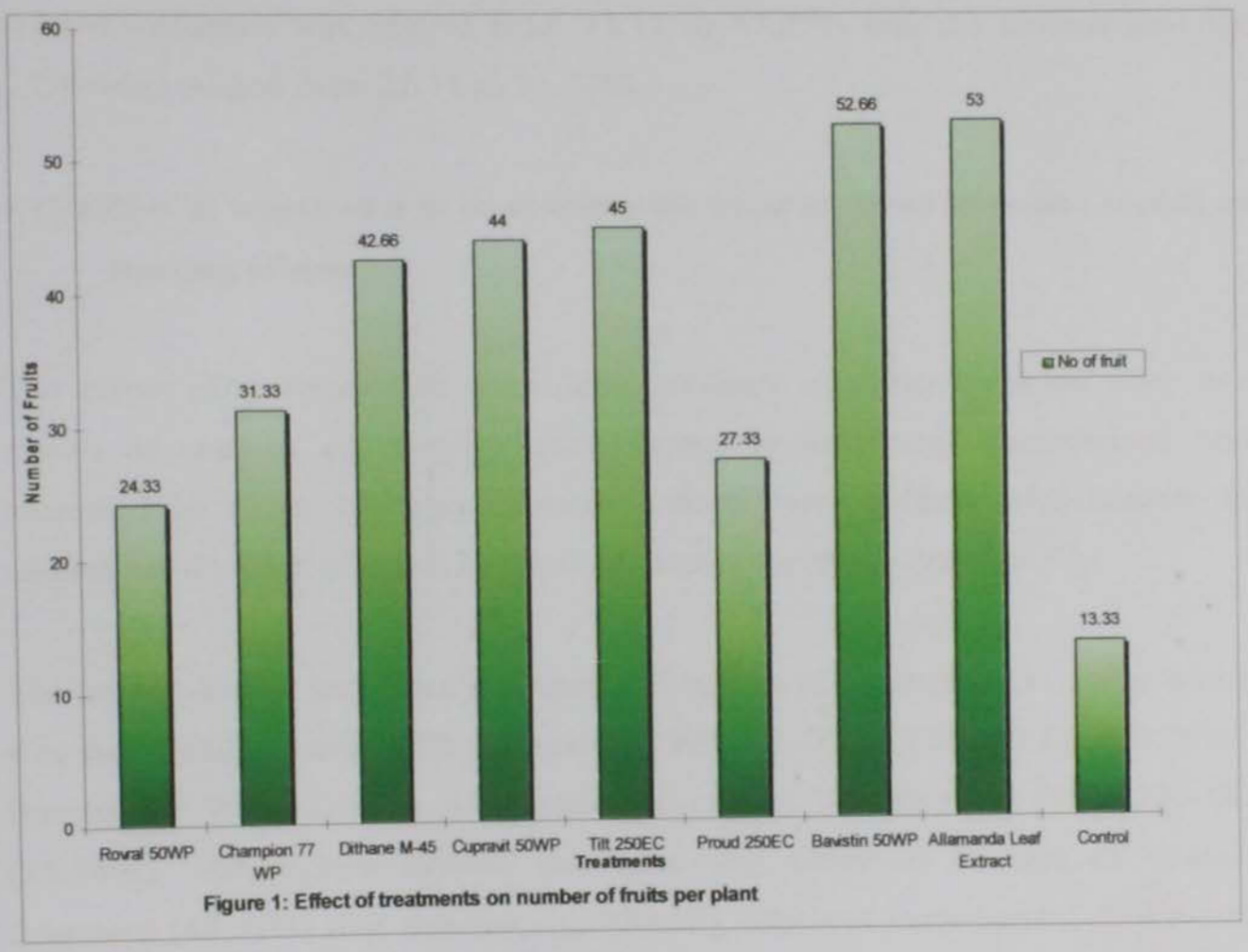


Figure 1: Effect of treatments on number of fruits per plant

4.14 Incidence and severity of die back of citrus of before commencement of spray schedule

The disease incidence and severity of die-back of citrus plants before spraying was statistically more or less similar among the treatments (Table 10). The disease incidence was ranged from 33.17 to 47.23% and the disease severity (PDI-twig) ranged from 22.71 to 31.77%.

4.15 Effect of treatments in controlling die back of citrus after one month of starting of spray

The effect of treatments in controlling die-back of citrus recorded after one month of starting of spraying (two sprays applied) was summarized and presented in Table 10. The treatment effects were differed significantly in controlling die back of citrus in terms of disease incidence and severity.

The lowest disease incidence was recorded in case of Champion (31.16%) which was statistically similar with Cupravit 50 WP (31.78%), Tilt 250 EC (32.76%), Bavistin 50 WP (33.14%), Allamanda leaf extract (34.58%) and Proud 250 EC (36.34%). The highest disease incidence was observed in case of control treatment (43.91%) that was not significantly different from Rovral 50WP and Dithane M- 45.

In case of disease severity, the lowest percent disease index (PDI-twig) was recorded in case of Champion 77 WP (21.05%) which was statistically similar with Bavistin 50 WP (21.20%), Cupravit 50 WP (21.30%), Tilt 250 EC (22.90%) and Allamanda leaf extract (23.20%). The highest PDI-twig was observed in case of control treatment (29.77%) which was not statistically differ from Rovral 50 WP (26.10%) and Dithane M-45 (25.10%).

The reduction of PDI-twig over control was recorded the highest (29.29%) sprayed with Champion 77 WP followed by Bavistin 50 WP (28.78%), Cupravit 50 WP (28.45%) , Tilt 250 EC (23.07%) and Allamanda leaf extract (22.06%) . The lowest reduction of PDI-twig over control was recorded in case of Rovral 50 WP (12.32%) proceeded by Dithane M-45 (15.68%) and Proud 250 EC (17.03%).

Table 10. Effect of treatments on the incidence and severity of die-back of citrus after one month of starting spray

Treatments	Before spraying		After one month of starting of spray		
	Disease incidence	Percent Disease index (PDI- twig)	Disease incidence	Percent Disease index (PDI-twig)	PDI-twig decreased over control (%)
T_1=Rovral 50 WP	47.23 a	28.10 ab	43.41 a	26.10ab	12.32
T_2= Champion 77 WP	38.39 bc	22.71 c	31.16 c	21.05 b	29.29
T_3= Dithane M 45	42.74 ab	27.86 b	41.27 ab	25.10ab	15.68
T_4=Cupravit 50 WP	35.36 c	23.29 c	31.78 c	21.30 b	28.45
T_5=Tilt 250 EC	35.91 c	24.87 bc	32.76 c	22.90 b	23.07
T_6=Proud 250 EC	36.56 bc	25.90 bc	36.34 bc	24.70ab	17.03
T_7=Bavistin 50 WP	33.17 c	22.73 c	33.14 c	21.20 b	28.78
T_8=Allamanda leaf extract	34.26 c	25.92 bc	34.58 c	23.20 b	22.06
T_9=Control	39.19 bc	31.77 a	43.91 a	29.77 a	-
CV (%)	8.73	8.30	8.73	13.50	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.16 Effect of treatments in controlling die-back of citrus two months after of starting spray

The effect of fungicides and plant extract in controlling die-back of citrus recorded after two months of starting of spray (four sprays applied) was summarized and presented in Table 11. The treatment effects were differed significantly in controlling die back of citrus in terms of disease incidence and severity.

The lowest disease incidence was recorded in case of Champion 77 WP (27.02%) which was statistically similar with Cupravit 50 WP (27.58%), Bavistin 50WP (29.50%) and Tilt 250 EC (31.13%). The highest disease incidence was observed in case of control treatment (39.45%) which was statistically identical with Rovral 50WP (39.00%). The effect of the rest of the treatments was moderate and statistically identical.

In case of disease severity, the lowest percent disease index (PDI-twig) was recorded in case of Cupravit 50 WP (19.00%) which was statistically similar with the effect of Champion 77WP (19.47%), Bavistin 50 WP (19.73%), Tilt 250 EC (21.20%) and Allamanda leaf extract (21.30%). The highest PDI-twig was observed in case of control treatment (35.93%).

The reduction of PDI-twig over control was recorded the highest (59.39%) while sprayed with Rovral 50WP followed by Cupravit 50 WP (47.11%), Champion 77WP (45.81%), Bavistin 50 WP (45.08%), Tilt 250 EC (40.99%) and Allamanda leaf extract (40.71%). The lowest reduction of PDI-twig over control was recorded in case of Proud 250EC (35.79%) proceeded by Dithane M-45 (35.90%).

Table 11. Effect of treatments on the incidence and severity of die-back of citrus after two months of starting of spray

Treatments	Before spraying		After two month of starting of spray		
	Disease Incidence	Percent Disease index (PDI-twig)	Disease incidence	Percent Disease index (PDI–twig)	PDI – twig decreased over control (%)
T_1=Rovral 50 WP	47.23 a	28.10 ab	39.00 ab	25.27 b	59.39
T_2= Champion 77 WP	38.39 bc	22.71 c	27.02 d	19.47 d	45.81
T_3= Dithane M 45	42.74 ab	27.86 b	32.82 bcd	23.03 bc	35.90
T_4=Cupravit 50 WP	35.36 c	23.29 c	27.58 d	19.00 d	47.11
T_5=Tilt 250 EC	35.91 c	24.87 bc	31.13 cd	21.20 cd	40.99
T_6=Proud 250 EC	36.56 bc	25.90 bc	34.21 abc	23.07 bc	35.79
T_7=Bavistin 50 WP	33.17 c	22.73 c	29.50 cd	19.73 d	45.08
T_8=Allamanda leaf extract	34.26 c	25.92 bc	31.18 cd	21.30 cd	40.71
T_9=Control	39.19 bc	31.77 a	39.45 a	35.93 a	-
CV (%)	8.73	8.30	10.57	6.92	-

Means bearing same letter within the same column do not differ significantly at 5% level.

4.17 Effect of treatments in controlling die-back of citrus after three months of starting of spray

The effect of fungicides and plant extracts in controlling die-back of citrus recorded after three months of starting of spray (six sprays applied) was summarized and presented in Table 12. The treatment effects were differed significantly in controlling die back of citrus in respect of disease incidence and severity.

The lowest disease incidence was recorded in case of Allamanda leaf extract (25.01%) which was statistically similar with rest of the treatment except Rovral 50 WP. The highest disease incidence was observed in case of control treatment (40.70%).

In case of disease severity, the lowest percent disease index (PDI-twig) was recorded in case of Dithane M-45 (15.83%) that was statistically similar with Bavistin 50 WP (16.40%), Allamanda leaf extract (17.07%), Tilt 250EC (18.63%) and Champion (18.65%). The highest PDI-twig was observed in case of control treatment (37.70%) followed by Rovral 50 WP (24.42%).

The reduction of PDI-twig over control was recorded highest (58.01%) while sprayed with Dithane M-45 followed by Bavistin 50WP (56.49%), Allamanda leaf extract (54.72%), Tilt 250 EC (50.58%) and Champion (50.53%). The lowest reduction of PDI-twig over control was recorded in case of Rovral 50 WP (35.22%) preceded by Proud 250EC (45.70%) and Cupravit 50 WP (48.19%).

Table 12. Effect of treatments on the incidence and severity of die-back of citrus after three months of starting spray

Treatments	Before spraying		After three months of starting of spray		
	Disease incidence	Percent Disease index (PDI-twig)	Disease incidence	Percent Disease index (PDI–twig)	PDI – twig decreased over control (%)
T_1=Rovral 50 WP	47.23 a	28.10 ab	35.18 b	24.42 b	35.22
T_2= Champion 77 WP	38.39 bc	22.71 c	25.44 c	18.65 cde	50.53
T_3= Dithane M 45	42.74 ab	27.86 b	29.43 bc	15.83e	58.01
T_4=Cupravit 50 WP	35.36 c	23.29 c	25.29 c	19.53cd	48.19
T_5=Tilt 250 EC	35.91 c	24.87 bc	28.98 bc	18.63 cde	50.58
T_6=Proud 250 EC	36.56 bc	25.90 bc	31.75 bc	20.47 c	45.70
T_7=Bavistin 50 WP	33.17 c	22.73 c	26.19 c	16.40 de	56.49
T_8=Allamanda leaf extract	34.26 c	25.92 bc	25.01 c	17.07 cde	54.72
T_9=Control	39.19 bc	31.77 a	44.70 a	37.70 a	-
CV (%)	8.73	8.30	12.78	8.82	_

Means bearing same letter within the same column do not differ significantly at 5% level.

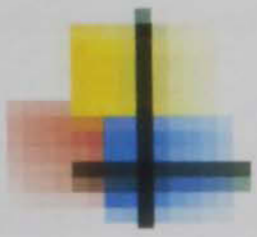
Chapter 5
Discussion

Chapter 5
Discussion

CHAPTER V
DISCUSSION

Among the different treatments applied in the experiment, all treatments in the form of foliar spray reduced the disease incidence and severity significantly compared to control (T_9). Among the treatments, Champion 77 WP was the most effective in controlling scab of citrus that reduced 85.43% leaf scab and 80.12% fruit scab after the end of the sixth spray. In controlling die-back of citrus, Dithane M-45 was found to be the most effective that reduced 58.01% die-back severity after the end of the sixth spray. It was observed that the incidence and severity of scab and die back of citrus gradually decreased with the increase of spray frequency and reached to the highest after sixth spray. But in case of control the incidence and severity gradually increased with time. The findings of the present investigation agreed with the findings of Singh *et al.* (2000), Li *et al.* (1997), Ebenezar and Subramanian. (1996), Thakore (1994), Hossain (1993), Harsh *et al.* (1992) and Rahman and Hossain (1988). Singh *et al.* (2000) who reported that copper fungicides Blitox-50 controlled citrus scab by 81.1% applied as foliar spray. Li *et al.* (1997) found that Pujunk (Copper hydroxide) controlled citrus scab (*Elsinoe fawcettii*) more effectively than others. Whiteside (1990) reported that Dithane M-45 was not so effective against scab of citrus. Thakore (1994) reported that the best control of die back of citrus was achieved by spraying Dithane M-45 at 2000 ppm.

Allamanda leaf extract showed significant effect in controlling die-back and scab of citrus. Literature on allamanda leaf extract tried against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides* was not available but evidenced that Allamanda leaf extract showed inhibitory effect against fungal pathogens (Islam, 2004; Khan, 1999 and Meah, 2003). Islam (2004) found Allamanda leaf extract caused 100% inhibition of mycelia growth of *Phomopsis vexans.* Among the three plant extracts (Allamanda, Bel and Neem), Allamanda spray was most effective for the management of Phomopsis blight/fruit rot of eggplant in field condition. (Khan, 1999). Meah (2003) reported that Allamanda leaf extract efficiently controlled *Phomopsis vexans* in laboratory, nursery house and in field reducing severity of leaf blight and fruit rot by 71-75%.



Chapter 6
Conclusion and Summary

CHAPTER VI

SUMMARY AND CONCLUSION

The research work was conducted on the citrus plants raised at the citrus orchard of Sher-e-Bangla Agricultural University, Dhaka, during the period from March to September 2007 to investigate the effect of fungicides and plant extract in controlling the scab and die back. Isolation and identification of the fungi was done in the seed pathology laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The experiment was conduced in Randomized Complete Block Design (RCBD) with three replications.

The fungicides and plant extract were sprayed 6 times; firstly May 15, 2007 followed by six sprays with 15 days intervals. Data were collected seven times and disease symptoms of the infected plants were determined compared with the control plants. The citrus plants were sprayed with Dithane M-45 (0.3%), Rovral 50WP (0.1%), Bavistin 50 WP (0.1%), Champion 77 WP (0.2%), Cupravit 50WP (0.1%), Tilt 250EC (0.25%), Proud 250 EC (0.25%) and Allamana leaf extract (1 : 4 dilution).

Among the treatments, Champion 77 WP was the most effective fungicide in controlling scab of citrus that reduced 85.43% leaf scab and 80.12% fruit scab of citrus after the sixth sprays which was closely followed by Rovral 50WP (83.87%) and Allamanda extract (82.23%) in case of leaf scab; Allamanda (66.20%) and Dithane M-45 (60.20%) in case of fruit scab.

The highest leaf incedence (15.61%) and the highest fruit incedence (28.20%) were observed in control. Among the treatments evaluated against *Colletotrichum goleosporioides,* Dithane M-45 was found the most effective in controlling die back of citrus which reduced die-back severity by 58.01% over

control after sixth sprays at three months after commencement of spray schedule. Bavistin 50WP, Allamanda leaf extract, Tilt 250EC and Champion 77WP also showed promising effect in reducing disease severity of die back that reduced PDI (twig) by 56.49%, 54.72% and 50.53% respectively.

From the present findings it may be concluded that among the treatments Champion 77 WP was found most effective for the control of scab of citrus (*Elsinoe fawcettii*) and Dithane M-45 was found the most effective against die-back of citrus (*Colletotrichum gloeosporioides*). Allamanda leaf extract was also effective for control scab and die back of citrus. As the chemicals are hazardous for human health and environment, Allamanda leaf extract is suggested for controlling scab and die back of citrus. However, further investigation for consecutive years need to carry out incorporating more option for confirmation of the present findings.

Chapter 7
Literature Cited

CHAPTER VI1
LITERATURE CITED

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Appendices

APPENDICES

Appendix 1. Monthly mean of daily maximum, minimum and average temperature, relative humidity and total rainfall during March, 2007 to September, 2007

Month	Temperature (^{0}C) **			Relative ** Humidity	Rainfall *(mm)
	Max	Min.	Ave.		
March	32.50	22.06	28.5	66	135
April	33.05	23.80	32.10	85	140
May	34	24.65	33.45	79.55	155
June	33.85	26.15	30.00	69.05	184
July	34.20	24.50	29.35	89.50	281
August	36.10	23.15	29.65	92.7	310
September	37.1	25.20	29.75	90.0	350

Source: Station name: PBO, Dhaka, station No. 41923, Surface synoptic data card, Bangladesh. Meteorological department, Sher-e-Bangla, Nagor, Dhaka-1207.

* = Monthly total

** = Monthly average

Photograph 8. Showing citrus leaves and flowers sprayed with Allamanda leaf extract

Photograph 9. Showing citrus fruit sprayed with Champion 77WP

Photograph 10.Showing citrus plant under control treatment

Photograph 11. Showing citrus plant sprayed with Dithane M-45

Photograph 12.Showing citrus plant sprayed with Bavistin 50WP