MANAGEMENT OF SCAB AND DIE-BACK OF CITRUS (*Citrus limon* L.) THROUGH BIOAGENT, PLANT EXTRACTS AND FUNGICIDES

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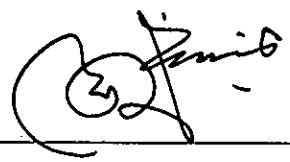

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MANAGEMENT OF SCAB AND DIE-BACK OF CITRUS (*Citrus limon* L.) THROUGH BIOAGENT, PLANT EXTRACTS AND FUNGICIDES

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 May to September 2005 to investigate the effect of bioagent, plant extracts and fungicides in controlling the scab and die back of citrus. One bioagent (*Trichoderma harzianum* T_{22}), three plant extracts viz. Bishkatali (*Polygonum hydropiper*), Garlic (*Allium sativum*) and Neem (*Azadirachta indica*) and five fungicides viz. Dithane M-45 (0.3%), Ridomil MZ-72 (0.2%), Bavistin 50 WP (0.1%), Champion 77 WP (0.25%) and Bordeaux mixture (1%) were applied for controlling scab and die back of citrus. The highest reduction of the scab severity (PDI) was performed by Champion 77 WP (72.72% leaf scab and 83.98% fruit scab) followed by, Bishkatali (67.54% and 79.99%), Bordeaux mixture (66.26%, and 72.01%) and *Trichoderma harzianum* T_{22} (62.36% and 63.34%). The highest reduction of die-back severity (PDI) was resulted by Dithane M-45 (73.46%) fallowed by Neem extract (67.34%), Bordeaux mixture (59.19%) and *Trichoderma harzianum* T_{22} (57.67%).Thus, the Champion 77 WP (0.25%) could be used for controlling scab of citrus while Dithane M-45 (0.3%) for die-back and Bordeaux mixture for both scab and die-back of citrus.

LIST OF CONTENTS

CONTENTS		Page No.
ACKNOWLEDGEMENT		
ABSTRACT		
LIST OF TABLES		xii
LIST OF FIGURES		xiii
LIST OF PHOTOGRAPH		xiv
LIST OF APPENDICES		xv
SOME COMMONLY USED ABBREVIATIONS		xvi-xvii
CHAPTER – I	**INTRODUCTION**	1-4
CHAPTER – II	**REVIEW OF LITERATURE**	5-26
2.1.1	Symptoms of scab of citrus	5
2.1.2	Epidemic nature of *Elsinoe fawcettii*	7
2.1.3	Causal organism of scab of citrus	8
2.2.1	Symptoms of Die-back of citrus	9
2.2.2	Epidemic nature of *Colletotrichum gloeosporioides*	10
2.2.3	Causal organism of die-back of citrus	11
2.3.1	Management of scab of citrus through fungicides	12
2.3.2	Management of citrus die-back through fungicides	16
2.4.1	Management of scab and die back of citrus through plant extracts	19
2.4.2	Management of other plant diseases by plant extracts and phytoncydes	20
2.5.1	Management of scab and Die back of citrus through antagonistic bioagents	23
2.5.2	Management of other diseases through antagonistic bio agents	23
CHAPTER – III	**MATERIALS AND METHODS**	27-41
3.1	Experimental site	27
3.2	Experimental period	27
3.3	Soil type	27
3.4	Plant selection	28
3.5	Treatments	29
3.6	Design of experiment	29

CONTENTS CONTD.

3.7	Weeding	30
3.8	Irrigation	30
3.9	Application of fertilizers and manure	30
3.10	Application of insecticide	31
3.11	Tagging and data collection	31
3.12	Preparation of spray solution	31
3.13	Plant extract	32
3.14	Preparation of spore suspension of bioagent	33
3.15	Application of spray solution	36
3.16	Disease symptoms observed in the orchard	36
3.17	Isolation of Pathogen	36
3.18	Identification of fungal isolates	37
3.19	Data collection	37
3.19.1	Total number of leaves/twig	37
3.19.2	Total number of scab infected leaves/twig	37
3.19.3	Calculation of disease incidence of leaf scab of different treatments	37
3.19.4	Total number of fruit/plant	38
3.19.5	Total number of scab infected fruit/plant	38
3.19.6	Calculation of disease incidence of fruit scab of different treatments	38
3.19.7	Total number twigs/branch	38
3.19.8	Total number of die back infected twigs/branch	38
3.19.9	Calculation of disease incidence of die-back of different treatments	39
3.19.10	Evaluation of leaf and fruit scab severity	39
3.19.11	Evaluation of die back severity	40
3.20	Weather	40
3.21	Analysis of data	41
CHAPTER – IV	**RESULTS**	42-78
4.1.	Scab symptoms observed in the orchard	42
4.2.	Die back symptoms observed in the orchard	42

CONTENTS CONTD.

4.3	Isolation and identification of fungi (*Elsinoe fawcettii*)	46
4.4.	Isolation and identification of fungi (*Colletotrichum gloeosporioides*)	46
4.5.	Incidence and severity of scab of citrus (leaf) before commencement of spray schedule.	49
4.6.	Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after one month of starting of spray.	49
4.7	Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after two month of starting of spray.	51
4.8	Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after three month of starting of spray.	53
4.9	Incidence and severity of scab of citrus (fruit) before commencement of spray schedule.	55
4.10.	Effect of fungicides, plant extracts and bio-agent in controlling fruit scab of citrus after one month of starting of spray.	55
9.11.	Effect of fungicides, plant extracts and bio-agent in controlling fruit scab of citrus after two month of starting of spray.	58
4.12.	Effect of fungicides, plant extracts and bio-agent in controlling fruit scab of citrus after three month of starting of spray.	60
4.13.	Incidence and severity of die back of citrus of before commencement of spray schedule.	62
4.14.	Effect of fungicides, plant extracts and bioagent in controlling die back of citrus after one month of starting of spray	62
4.15	Effect of fungicides, plant extracts and bioagent in controlling die back of citrus after two month of starting of spray	64
4.16	Effect of fungicides, plant extracts and bioagent in controlling die back of citrus after three month of starting of spray	66
4.17	Relationship between disease incidence, disease severity and time (days) of scab and die-back of citrus due to the application of plant extracts, fungicides and	68

bioagent.

4.17.1.	Relationship between disease incidence and time (days) for leaf scab of citrus.	68
4.17.2.	Relationship between disease severity (PDI) and time (days) for leaf scab of citrus.	70
4.17.3.	Relationship between disease incidence and time (days) for fruit scab of citrus.	73
4.17.4.	Relationship between disease severity (PDI) and time (days) for fruit scab of citrus.	73
4.17.5.	Relationship between disease incidence and time (days) for die-back of citrus.	75
4.17.6.	Relationship between disease severity (PDI) and time (days) for die-back of citrus.	77

CHAPTER – V **DISCUSSION** 79-81

CHAPTER – VI **SUMMARY AND CONCLUSION** 82-84

CHAPTER – VII **LITERATURES CITED** 85-98

CHAPTER – VIII **APPENDICES** 99-101

LIST OF TABLES

Table No.	Subject matter	Page
1.	Particulars of Fungicides used in the experiment	32
2.	Particulars of plant extracts used in the experiment	33
3.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (leaf)after one month of spray	50
4.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (leaf) after two month of spray	52
5.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (leaf) after three month of spray	54
6.	Effect of fungicide, plant extract and bio-agent on the incidence and severity of scab of citrus(fruit) after one month of spray	57
7.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (fruit) after two month of spray	59
8.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (fruit) after three month of spray	61
9.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of Die-back of citrus after one month of spray	63
10.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of Die-back of citrus after two month of spray	65
11.	Effect of fungicides, plant extracts and bio-agent on the incidence and severity of Die-back of citrus after three month of spray	67

LIST OF FIGURES

Fig. No.	Subject matter	Page No.
1.	Relationship between disease incidence and progress of time (days) for leaf scab of citrus due to application of treatments	69
2.	Relationship between disease severity (PDI) and progress of time (days) for leaf scab of citrus due to application of treatments	71
3.	Relationship between disease incidence and progress of time (days) for fruit scab of citrus due to application of treatments	72
4.	Relationship between disease severity (PDI) and progress of time for fruit scab of citrus due to application of treatments	74
5.	Relationship between disease incidence and progress of time (days) for die-back of citrus due to application of treatments	76
6.	Relationship between disease severity (PDI) and progress of time for die-back of citrus due to application of treatments	78

LIST OF PHOTOGRAPH

Photograph No.	Subject matter	Page No.
1.	Plant extract used against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides*	35
2.	Pure culture of *Trichoderma harzianum* on PDA plate	36
3.	Structures of *Trichoderma harzianum* T_{22} (x 400)	36
4.	Scab infected leaf of citrus	43
5.	Scab infected twig of citrus	43
6.	Severely Scab infected citrus plant (*Citrus limon*)	43
7.	Scab infected fruit of citrus(*Citrus limon*)	44
8.	Scab infected leaves, fruit and twigs of citrus(*Citrus limon*)	44
9.	Severely die-back infected citrus plant (*Citrus limon*)	45
10.	Stem lession caused by *Colletotrichum gloeosporioides* (bark removed)	45
11.	Symptom of die-back on twigs of citrus	45
12.	Pure culture of *Elsinoe fawcettii* grown on PDA plate isolated from infected citrus leaf	47
13.	Pure culture of *Colletotrichum gloeosporioides* grown on PDA plate isolated from infected citrus twig	47
14.	Acervuli of *Colletotrichum gloeosporioides* grown on PDA as seen under compound microscope (x 400)	48
15.	Conidia of *Colletotrichum gloeosporioides* seen under compound microscope (x 400)	48

LIST OF APPENDICES

SL. NO.	Subject matter	Page No.
1.	Composition of 100 gm fresh peeled fruit of citrus	99
2.	Monthly mean of daily maximum, minimum and average temperature, relative humidity and total rainfall during May, 2005 to September, 2005.	99
Photograph 16	Showing Citrus plant sprayed with Dithane M-45	100
Photograph 17	Showing Citrus plant sprayed with Ridomil MZ-72	100
Photograph 19	Showing Citrus plant sprayed with Bavistin 50 WP	100
Photograph 20	Showing Citrus plant sprayed with Champion 77 WP	100
Photograph 21	Showing Citrus plant sprayed with Bordeaux mixture	100
Photograph 22	Showing Citrus plant sprayed with Bishkatali extract	100
Photograph 23	Showing Citrus plant sprayed with Neem leaf extract	101
Photograph 24	Showing Citrus plant sprayed with Garlic bulb extract	101
Photograph 25	Showing Citrus plant sprayed with *Trichoderma harzianum*	101
Photograph 26	Showing Citrus plant under control treatment	101

Some commonly used abbreviations

Full Word	Abbreviation
Agro-ecological Zone	AEZ
Anonymous	Anon
Bangladesh Agricultural Research Institute	BARI
Bangladesh Agricultural University	BAU
Bangladesh Jute Research Institute	BJRI
Carboxeymethy1 cellulose	CMC
Centimeter	cm.
Commonwealth Mycological Institute	CMI
Citrus Tristeza Clolesterovirus	CTV
Cultivar (s)	cv.
Copper	Cu
Duncan's Multiple Range Test	DMRT
Gram	g
Food and Agricultural Organization	FAO
Figure	Fig.
Kilogram	Kg
Pound	lb
Least significant difference	LSD
Meter	m
Millimeter	mm
Not significant	NS
Potato Dextrose Agar	PDA
parts per million	ppm
Randomized Complete Block Design	RCBD
Sher-e-Bangla Agricultural University	SAU

Full Word	Abbreviation
Treatment	T
United Nations Development Programme	UNDP
Weight	wt.
Degree Centigrade	^{0}C
Percent	%

CHAPTER-I
INTRODUCTION

Citrus (*Citrus limon L.*), belongs to the family Rutaceae is one of the important fruit crops in the world as well in Bangladesh. It is thought to be originated in Indian subcontinent because of maximum genetic diversity are grown in this region (Sohi and Kapoor, 1990). It has a great demand due to its nutritive value, flavor, appealing color and taste. Many people of our country are suffering from the deficiency of some limiting vitamins like A and C and some other minerals like calcium and iron in their daily diet. It is observed that 93% people of Bangladesh are suffering from the deficiency of vitamin C (Anonymous, 1980). Vitamin C cannot be stored in human body like others and it is needed to be taken with daily diet. Thus citrus fruits play an important role in human health.

Citrus serves as a potential source of vitamins and minerals (Alam *et al.*, 2003, Appendix 1). Slices of lemon are served as a garnish on fish or meat or with iced or hot tea. Lemon juice is primarily used for flavoring cakes, cookies, cake icings, puddings, sherbet, confectionery, preserves and pharmaceutical products. It is the source of lemon oil, pectin and citric acid. It is much used as a flavoring for hard candies.

In Bangladesh citrus is cultivated in about 15,008 ha of land with a total production of 31750 metric tons per annum (BBS, 2005). Eight species of citrus fruits are grown in Bangladesh. Among them, three species viz. Elachi lemon (*Citrus limon* L.), Kagzi lime (*Citrus awrantifolia* Swing), and Pummelo (*Citrus grandis* L.) are commonly cultivated in our country. In citrus growing area, Kagzi lime and Elachi lemon occupy 6,388.66 ha of land of the country (BBS 2005). As the hilly and high land

remains fallow round the year, there is a great opportunity to extend citrus cultivating area in the country.

Various factors are responsible for lowering the yield of citrus. Among the factors, diseases play an important role. Citrus plants are prone to attack of numerous diseases. Different species of citrus grown in the world suffers from more than 100 diseases (Klotz, 1973). In Bangladesh, twelve diseases are known to occur in different species of citrus. Among the diseases occurring on citrus, scab, die-back, lichen, sooty mould and canker are considered as major diseases in Bangladesh (Alam, 2003).

Citrus scab caused by *Elsinoe fawcettii* is one of the serious diseases of citrus in Bangladesh (Alam, 2003) which is formerly known as sour orange scab and is cosmopolitan in humid citrus areas. It can cause severe deformation of folliage and stunting of certain citrus root stocks resulting in poor quality and smaller capilier of root stocks for budding (Singh *et al.*, 2000). It attacks the expanded leaves of spring shoots of lemon and stocks to attack the fruit-let in May. During the autumn, if the temperature and humidity are favorable, it attacks young shoots and fruit-lets causing up to 65.9-71.29% droppings (Huang, 1999). The causal organism of scab (*Elsinoe fawcettii*) was morphologically 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. Among the various agencies that cause decline or dieback, the disease caused by fungus, viruses and bacteria are predominant (Rawal and Saxana, 1997). The disease breaks out widely and has become limiting factor of citurs cultivation in many countries (Talukdar, 1974; Ercivan and Karaca, 1979; Raychaudhury *et al.*, 1980;

Rawal and Saxana, 1997; Alam, 2003 and Timmer *et al.*, 2005). A survey report by Miah and Fakir (1987) showed that prevalence of die-back on Elachi lemon and Kagzi lime was 89.9% and 100% respectively in Bangladesh.

The use of chemicals and the existing practices for controlling the diseases is too costly, particularly for poor farmers. In addition, their harmful effect is responsible for soil, water and air pollution. The residual effect in soil as well as in the plant products causes serious health hazards. Moreover indiscriminate use of chemicals disrupts the natural ecological balance by killing the beneficial and antagonistic soil microbes.

Use of plant extracts in controlling plant pathogens is now a day an eco-friendly approach and is successfully employed against many fungal pathogens (Mia *et al.*, 1990; Fakir and Khan, 1992; Khan, 1999; Ahmed and Islam, 2000; Meah, 2003 and Islam, 2004a). Yesmin (2004) have reported promising fungicidal effect of Neem (*Azadirachta indica*) extract on *Colletotrichum gloeosporioides*.

Biological control of plant pathogens and biologocal plant growth regulators and achievement of both the effects by a single soil borne fungus *Trichoderma harzianum* are recent discoveries that can bring revolution in agriculture. The bio-control agents are reported to have antifungal, plant growth promoting and plant defense-inducing activities. It also helps in rapid decomposition of compost and improves its nutritional quality. Singh *et al.*, 2000 also reported that *Trichoderma harzianum* reduced scab of citrus (*Elsinoe fawcettii*) in the field on spraying by 17.8%.

Use of resistant variety is the most acceptable method for controlling these diseases. But no such citrus variety is available in Bangladesh. Lack of avilabable effective eco-friendly tools such as resistant variety, plant extracts, bioagent etc, chemical fungicides is used as the last option for management of the diseases. The foliar spray with spore suspension of *Trichoderma harzianum* T_{22} (Singh *et al.*, 2000), Bishkatali extract (Ashrafuzzaman and Hossain, 1992), Neem extract (Yesmin, 2004 and Khan, 1999), Garlic extract (Islam, 2004a), Dithane M-45 (Ran *et al.* , 2001), Ridomil MZ-72 (Das *et al.*, 1998), Bavistin 50 WP (Singh *et al.*, 2000), Champion 77 WP (Xu *et al.*, 2004) and Bordeaux mixture (Huang and Huang, 2002) has been reported effective in controlling scab and dieback disease of citrus.

Considering the above facts, the present research programme has been designed with the following objectives for the management of scab and die-back diseases of citrus:

i) To find out the efficacy of selected plant extracts and bioagent against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides* causing scab and die-back diseases of citrus.

ii) To find out the efficacy of selected fungicides in controling scab and die-back diseases of citrus.

CHAPTER-II

REVIEW OF LITERATURE

Scab and die-back diseases of citrus are serious problem and limiting factor for citrus production. These diseases are widely occurring throughout the citrus growing countries in the world. Die-back disease is familiar by different names of which wither tip, decline and anthracnose are most common. Many researchers are trying to control the scab and die-back with fungicides, plant extracts and bio-agents. For precise presentation only the related literatures on disease symptoms, epidemic nature, causal organism and management of these diseases with application of fungicides, plant extracts and bio-agents are presented here.

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 bad. 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 fruit 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 fawcetti,* is an important disease in Texas. The disease is more severe on lemons, somewhat 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 roots tocks. This results in poorer quality and smaller caliper of root stocks for budding. Moreover, premature leaf and fruit drop caused by this malady result was heavy losses to orchardists.

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.

Janghoon *et al.* (1998) reported that citrus scab caused by *Elsinoe fawcettii* caused worty 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.

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 caused by *Elsinoe fawcettii* and die back caused by *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 and high humidity and frost damage)

Hartmond *et al.* (2000) in Florida reported that citrus scab caused by the fungus (*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, it attacks the young shoots and fruit lets causing up to 65.9-72.2% fruitlet drop.

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

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.

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 slowly and caused to wilt, turn yellow and drop off. Twigs and branches appeared to have been scoched 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 tearstaining of rind. This leads to the twigblight. 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 suarvives 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 postbloom fruit drop of sweet orange. This species produces conidia with at least one end of fusiforme conidia.

Amador (2002) reported that Twig dieback 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 excessive fertilization, moisture stress and damage to the root system by cultural practices or heavy nematode damage.

Timmer *et al.* (1998) reported that *Colletotrichum gloeosporioides*, causes post harvest anthracnose of citus 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* (*Glomerela 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.

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

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 as well as healthy twigs of die-back affected citrus plants. In the pathogenicity test, they found the fungus most pathogenic to Kagzi lime among several citrus species including lemon.

2.3.1 Management of scab of citrus through fungicides

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% and75.5%, 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 of 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 showed 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, Topsin (Thiophanatemethyl) and Pyridaben 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 3times). 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 hour 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 Dithianon, Catafol and 2 sterol inhibiting fungicides, Diniconazole and Difenoconazole to evaluated them against citrus scab caused by *Elsinoe fawcettii* in Florida, USA. They observed that Dithianon performed better than copper fungicide treatments, recommended at the time in the field trials. However, Difenoconazole usually gave better control of scab than Dithianon. Where spray treatments were delayed until after some fruit had

become infected, Difenoconazole 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.

Tripathi and Srivastava (1989) tested 11 fungicides against citrus scab caused by *Sphaceloma fawcettii* and found that Benomyl and Bavistin (Carbendazim)(both 0.1%) prevented growth of S. *fawcettii* in culture. In field condition spraying on Rangpur lime seedlings 4 times at 30 days intervals with Carbendazim, Benomyl or Aureofungin reduced infection by 79.09, 75.91 and 69.18%, respectively.

2.3.2 Management of citrus die-back through fungicides

Peres *et al.* (2004) repoted that postbloom 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.

Fruit detachment force (FDF) fruit dropping was observed by Hartmond *et al.* (2000) in Florida, USA when conducted an experiment to evaluate of the effect of Metsulfuron-methyl and 5-chloro-3 methyl-4 nitro—IH-pyrazole (CMM-pyrazole) on abscission of valencia orange (decline) during the harvest season. Solutions of Metsulfuron methyl at 0.5, 1 and 2 mg-L^{-1} active ingredient were applied at 10-days intervals beginning on 13 February and ending 18 May 1988. Metsulfuron- methyl significantly reduced fruit detachment force (FDF) at 1 or 2 mg/L a.i.

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. Carbendazim 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 treatment, 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.

Harsh *et al.* (1992) reported the results of laboratory and field trial to control seedling wilt or die-back of sissoo (*Colletotrichum gloeosporioides*) with 0.2% Bavistin (Carbendazim) or seed dressing with 0.2% Topsin-M. They observed that the results of laboratory and field control test indicated that a soil drench with 0.2% Bavistin or seed dressing with 0.2% Topsin M were effective.

Rahman and Hossain (1988) used 10 sprays of different fungicides for controlling guava anthracnose (*Colletotrichum gloeosporioides*). They started spray after first appearance of the disease and continued for 3 times

at an interval of 20 days. Topsin M (0.1%) followed by Dithane M-45 (0.2%) proved to be more effective. Bordeaux mixture (4:4:40) reduced 51% fruit infection over control.

Kabir and Meah (1987) sprayed Dithane M-45, Topsin M, Dithane Z-78, Cupravit and Rovral on guava plants in Horticulture base, Keyotkhali in 1986 prior to symptoms appearance. They found an excellent control of fruit infection with 5 sprays of Topsin M (0.2%), Rovral (0.2%) and Dithane Z-78 (0.3%).

Meah and Khan (1986) worked on Bavistin, Rovral 50WP, Dithane M-45 Topsin-M *in vitro* against *Colletotrichum gloeosporioides*. They observed that Bavistin (0.2%), Rovral WP (0.2%) and Topsin M (0.2%) were effective in inhibiting the mycelial growth of the test fungus.

Solel and Oren (1978) has given a description of control of anthracnose/twig blight of clementine tangerine and grape fruit caused by *Colletotrichum gloeosporioides*. Bordeaux mixture was the most effective Cu-compound; potent organic compounds were Captafol, Captan, Chlorothanil, Meneb and Macnozeb. Effective field control was achieved with one prophylactic treatment in July with Cu-containing fungicides.

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 pathgen. These neem based products however, proved less effective against *Colletotrichum gloeosporioides.*

2.4.2 Management of other plant diseases by plant extracts and phytoncydes

Islam (2004a) found garlic bulbs and allamanda leaves extract caused 76-100% inhibition of mycelial growth of *Phomopsis vexans*. Diethyl ether, Dichloromethane and water acted as effective solvents for spraying 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 leaves 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%.

Ahmed and Islam (2000) applied Neem (*Azadirachta indica*), Garlic (*Allium sativum*) and Biskatali (*Polygonum hydropiper*) and found that Neem and Garlic extracts were effective against *Bipolaris oryzae* at 1: 1 dilution.

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 3 plant extracts, Allamanda spray was the most effective.

Moniruzzaman and Ashrafuzzaman (1998) applied garlic (*Allium sativum*), neem (*Azadirachta indica*) and tobacco (*Nicotiana tabacum*) extracts against

Alternaria blight of mustard. Crude extracts of these three plants were significantly effective to reduce disease incidence and severity. In promoting plant height number of siliqua per plant, number of seeds per siliqua, thousand seed weight and yield per plant, the garlic bulb extracts (1:1) was found best among the all treatments.

Rahman (1998) reported that, the individual effect of Vitavax-200 and garlic extract (1:2) as foliar spray were less effective than the mixture of Vitavax-200 and Tilt 250 EC to control leaf blight of wheat under field condition.

Kuprashvile (1996) used extracts of garlic bulb for seed treatment of eggplant infected by *Phomopsis vexans* .The results showed that the plant extracts disinfected seeds and increased yields.

Hossain and Schlosser (1993) found Neem extract to be effetive against *Bipolaris sorokiniana*. Hossain *et al.,* (1993) further reported taht Kalojira (*Nigella sativa*) extracts completely inhibited the mycelial growth and sclerotial formation of *Rhizoctonia solani*.

Achimu and Schloesser (1992) studied the effect of neem seed extracts against downy mildew (*Plasmopara viticola*) of grapevine. They found that raw neem seed extract and commercial neem products (margo-san-O, neem oil and neem- Azal-S) had high (80-90%) antifungal properties against *P. viticola*. They concluded that the antifungal property of neem products could be attributed to an inhibition of the indirect germination of sporangia.

Ashrafuzzanan and Hossain (1992) evaluated the extract of Biskatali (*Polygonum hydropiper*) *in vitro* against *Rhizoctonia solani* in two separate experiments and obtained that the extract inhibited the mycelial growth and spore germination effectively.

Fakir and Khan (1992) reported that garlic bulb extract at different concentrations reduced the seed borne infection in jute. Both concentrated garlic extract and Vitavax-200 were more or less equally effective in controlling *M. phaseolina* reducing 90.9% and 87.9% seed borne infection of the pathogen, respectively.

Dubey and Dwivedi (1991) observed fungitoxic properties of extracts of leaves and bulb of onion, garlic bulb extract and fruit and bark of *Allium cearavica* against vegetative growth and sclerotial viability of *Macrophomina phaseolina*. They also found that all the extracts inhibited growth to very degrees but garlic bulb extract was more effective than other extracts employed in the tests.

Miah *et al.* (1990) reported the extract of eight plant species were tested against rice seed-borne fungi. Seed soaking by *Allium sativum* L. and *Curcuma longa* L. appeared to be promising.

Mia *et al.* (1990) examined the efficacy of extractof eight different plant species against seed-borne fungi of rice thhrough 8 hours seed soaking. Out of the test plant species, extract of *Allium sativum* and *curcuma longa* appeared to be promising.

Kasem and Vijai (1987) tested the effects of some medicinal plants on growth of fungi and found potential in plant disease control. Ten medicinal plants, Stemona, nux-vomica tree, derris, urging croton, staranise, clove tress, garlic and care way were tested for thir antifungal property to some fungal species namely – *Phytophthora spp., Phythium aphanidermatum, Rhizopus microsporus, Alternaria alternata and Fusarium solani.* Staranise at the concentration of 2000 ppm completely inhibited growth of all test fungi followed by caraway, lemon grass, clove tree and garlic, respectively.

2.5.1 Management of scab and die-back of citrus pathogen through antagonistic bioagents

Singh *et al.* (2000) found that *Trichoderma viride, T. ressei* and *Epicoccum purpurascens* showed antibiosis where as *Trichoderma harzianum* and *T. Koningii* showed mycoparsitism on the pathogen in *in vitro* culture. *Trichoderma harzianum* and *E. purpurasuns* reduced the disease incidence citrus scab in the field on spraying by 17.8 and 10% respectively.

Chuang and Ann (1997) investigate antagonistic bacteria and yeasts for their ability to control mango anthracnose (*Colletotrichum gloeosporioides*). Four antagonistic bacteria and five yeast isolates including *Bacillus subtilis* (isolate Tp-Tu311), *Pseudomonas fluorescens* (isolate TN-S221), *Pichia ohmeri* (isolate Y24-8), *and Sporobolomyces sp.* were individually assayed against *Colletotrichum gloeosporioides* by co-inoculating the antagonist and pathogen in artificial wounds on mango fruits. They found that post harvest treatment of mango fruit with antagonists TN-S221, Tp-Tu311 and Y24-8 decreased anthracnose development and Tp-Tu311 gave best disease control.

2.5.2 Management of other diseases through antagonistic bio agents

Bari *et al.* (2000) reported significant reduction in radial growth of *Sclerotium rolfsii* by *Trichoderma* spp. in dual culture on PDA plate.

Biswas and Sen (2000) reported, in dual culture of the 11 isolates of *T. harzianum* isolates T_8, T_{10} and T_2 were effective against *S. rolfsii*, the causal agent of stem rot of ground nut and they over grew the pathogen up to 92%, 85% and 79%, respectively *in vitro*. Both the T_8 and T_{10}

isolates reduced stem rot incidence significantly when treated as seed dressing or soil application in the pot trials. Percent disease reduction through seed dressing was 33% to 50% over control and through direct soil application it was up to 72% to 80%.

Desai and Schlosser (1999) reported that *T. harzianum*, *T. hamatum* and *T. koningii* penetrated the sclerotial wall, established and sporulated inside the sclerotium and kill the sclerotium of *S. rolfsii*.

Mukherjee *et al.* (1995) evaluated the comparative antagonistic properties of *T. harzianum* and *Gliocladium virens* in suppressing *S. roflsii (Corticium rolfsii), Rhizoctona solani in-vitro.* They observed that *T. harzianum* was less effective than *G. virens.* Only *T. harzianum* parasitized the hyphae of *S. rolfsii* and the two antagonists were comparable in respect to antibiosis on the test pathogens.

Haque *et al.* (1990) used *T. harzianum* as biocontrol agent for controlling root rot diseases of okra, sunflower, soybean and mungbean. *Trichoderma* used as seed treatments or as soil drenches for the control of root rot caused by *Macrophomina phaseolina, R. solani* of sunflower ,soybean and mungbean under field conditions. *Trichoderma* showed excellent inhibitory effect of controlling *Fusarium* and *Rhizoctonia.*

Kumar and Khare (1990) found *Trichoderma harzianum* as antagonistic to *S. rolfsii* when soybean seeds were treated with *Trichoderma harzianum, Gliocladium virens, Bacillus subtillis* and *Sterptomyces spp.* They also showed that Fusarium infection of sunflower was reduced by *T. harzianum.*

Harman *et al.* (1989) reported on combining effective strains of *T. harzianum* and solid matrix priming to improve biological seed treatments. They developed progeny strains (T_{12} and T_{95}) by fusing two strains of *T. harzianum* and two of which were selected for further study. Seeds of cotton, cucumber, pea, snap, bean maize and wheat were also planted in soil infested with Pythium ultimum and Rhizoctonia solani.In all crop pathogen combinations,seed treatments with parental and progeny *Trichoderma strs.*with or without solid matrix priming increased stands relative to the untreated control and were as effective as Vitavax-200.

Elad *et al.* (1983) studied the parasitism of *T. harzianum* to the soil borne plant pathogen, *S. rolfsii*. They observed that hyphae of the parasites either producing appressorium like bodies or coiling around the hyphae and enzymatically digested the host cell walls and the host cells become empty of cytoplasm.

Henis *et al.* (1983) reported that *Trichoderma spp.* produced volatile and non-volatile antibiotics which are active against *S. rolfsii* and inhibited the sclerotial germination.

The effectiveness of *T. viride* and *T. harzianum* were observed by Mathur and Sarbhoy (1978) under both *in-vitro* and glasshouse conditions against root rot of sugar beet caused by *S. rolfsii*. Both the species of *Trichoderma* appeared to be strongly antagonistic causing 88% and 86% inhibition of the growth of *S. rolfsii*, respectively. While tested under glasshouse condition, *S. rolfsii* caused only 13.3 and 20% infection in presence of *T. virde* and *T. harzianum*, respectively compared with 100% infection recorded in absence of any of the antagonists.

Agrawal *et al.* (1977) found that *Trichoderma harzianum* is antagonistic against *Sclerotium rolfsii*. They reported that filtrates of *Trichoderma* inhibited the growth of *Sclerotium roffsii* on PDA but effectiveness decreased with dilution. In plot experiment, the antagonist controlled seedling death. Culture was more effective when applied to seed rather than soil.

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 the kharif season (May-Sep.) of the year 2005.

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	:	Mahdupr tract (AEZ-28)
Land type	:	Medium High Land
General Soil type	:	Non-Calcarious Darkgray 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 information about physical and chemical characteristics of the soil has collected from Soil Resource 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.

Parameter		**Amount**
pH	:	5.2
Organic matter (%)	:	1.21
Ca (meq.100g soil	:	2.67
Mg (meq/100g soil)	:	0.775
K (meq/100 soil)	:	0.30
Na (meq/100 g soil)	:	0.35
N (%)	:	0.061
P (Micro gram/g Soil)	:	35.22
S (Micro gram/g Soil)	:	22.60
B (Micro gram/g Soil)	:	0.73
Fe (Micro gram/g Soil)	:	277
Mn (Micro gram/g Soil)	:	50.9
Cu (Micro gram/g Soil)	:	3.8

3.4. Plant selection

Thirty plants were selected from the citrus orchard of Sher-e-Bangla Agricultural University (SAU) campus, Dhaka. All plants were equal

aged (5 years old), which were used as the experimental unit of this study.

3.5. Treatments

There were ten different treatments designated by T_1, T_2, T_3, T_4, T5, T_6, T_7, T_8, T_9 and T_{10} which were as fallows:

T_1 = Dithane M-45 (0.3%)

T_2 = Ridomil MZ-72 (0.25%)

T_3 = Bavistin 50 WP (0.1%)

T_4 = Champion 77 WP (0.2%)

T_5 = Bordeaux mixture (1%)

T_6 = Bishkatali (*Polygonum hydropiper*) leaf extract (1:4 wt/v solution)

T_7 = Neem (*Azadirachta indica*) leaf extract ((1:4 wt/v solution)

T_8 = Garlic (*Allium sativum*) clove extract (1:4 wt/v solution)

T_9 = *Trichoderma harzianum* spore solution (10^7conidia/ml water)

T_{10} = 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 10 treatments for 30 plants used for the study. Different treatments were assigned randomly to the unit plant.

3.7. Weeding

Weeding was performed four times during the experimental period with 30 days intervals starting from 1st May, 2005.

3.8. Irrigation

The plants were irrigated three times. The first irrigation was done at May 2, 2005 and the fallowing irrigations were done at 15 days intervals.

3.9. Application of fertilizers and manure

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

Fertilizers/Manures	**Dose/Plant**
Urea	450-550 gm
TSP	375-425 gm
MP	375-425 gm
Cowdung	20 kg

Half of the urea, TSP and MP and all amount of cowdung were used at the time of 1st weeding. The rest half of the urea, TSP and MP were applied at August 1, 2005.

3.10. Application of insecticide

Insecticide Diazinon 60 EC was applied three times @ 1ml/litre water at 10 days interval from May 3, 2005.

3.11. Tagging and data collection

Five twigs were selected randomly from each plant for determining of scab and die back infection. So, 5 twigs were tagged for mean values were determined to get ratting score of the material of each score. But for fruit infection the whole plants were observed. 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 15, 2005 at intervals of 15 days.

3.12 Preparation of spray solution

The fungicidal solutions were prepared by mixing required amount of fungicides with tap water to get 0.3% solution for Dithane M-45, 0.25% solution for Ridomil MZ-72, 0.10% solution for Bavistin 50 WP, 0.2% solution for Champion 77WP and 1% solution for Bordeaux mixture (5:5:50). Details of the fungicides used as spray materials are given in table 1.

Table 1. Particulars of Fungicides used in the experiment

Common Name	Chemical name	Active ingredients	Doses used (%)
1. Dithane M-45	Manganous ethyline bisdithio - carbamate ($C_4H_6N_2S_4$)	Dithiocarbamate (80%)	0.3%
2. Ridomil MZ-72	N-(2,6 dimethyl phenyl) N- methoxy acety1)-alanine methy1 ester ($C_{14}H_2N_4$)	Metalaxyl (72%)	0.25%
3. Bavistin 50 WP	Methy1-2-Behzmidazole carbamate	Carbendazim (50%)	0.10%
4.Champion 77 WP	Copper hydroxide ($Cu(OH)_2$)	Copper hydroxide (77%)	0.2%
5. Bordeuax mixture	Copper sulphate	Copper sulphate + Calcium hydroxide + water (5:5:50)	1%

3.13 Plant extract

Three plant extracts namely Bishkatali, Rashun and Neem were used in this study (Table 2 and Photograph 1). The plant extracts were prepared with the help of blender. Requisite amount of plant materials were dissolved in required amount of sterilized water to make the solution in different concentrations (1:4 = 25 gm plant materials crushed in 100 ml

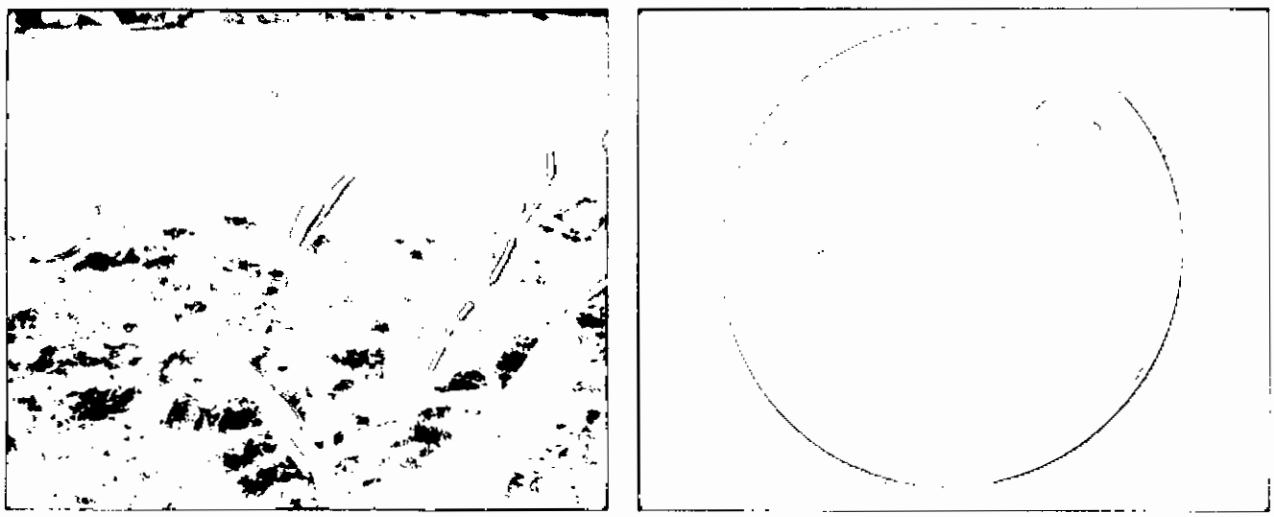
water). The crushed materials were filtered through cheese cloth to get the fine sprays.

Table 2. Particulars of plant extracts used in the experiment

Local name	English name	Botanical name	Plant parts used	Dose used (ration)
Biskatali	Biskatali	*Polygonum hydropiper*	Leaf	1:4
Rashun	Garlic	*Allium sativum*	Clove	1:4
Neem	Indian lilae	*Azadirachta indica*	Leaf	1:4

3.14 Preparation of spore suspension of bioagent

The pure culture of *Trichoderma harzianum* T_{22} was collected from the MS laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka (Photograph 2 and Photograph 3). Then the *Trichoderma harzianum* T_{22} was inoculated on PDA media inoculated at 25°C for 7-10 days for mass culture (Singh *et al.*, 2000). Then spore suspension of *Trichoderina harzianum* T_{22} (10^7 conidia/ml of water) was prepared with the help of haemocytometer to increase the efficacy of antagonist. Carboxymethyl cellulose (CMC) @ 1.2% (w/v) was added just before the use to increase the efficacy of antagonist.



A

B

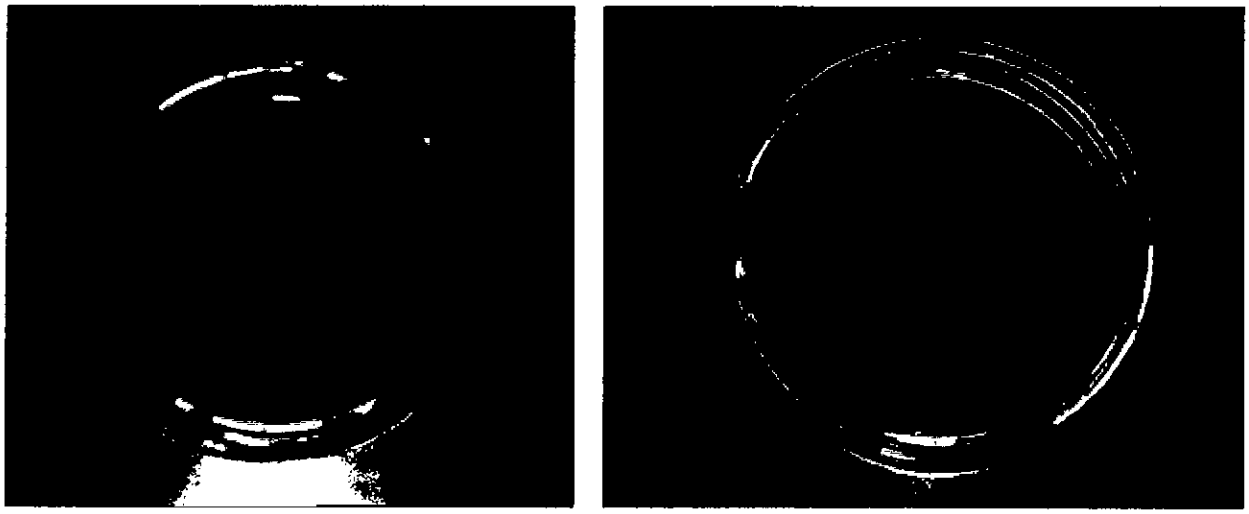
C

Phothograph 1. Plant species used against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides*

A = Bishkatali plant (*Polygonum hydropiper*)

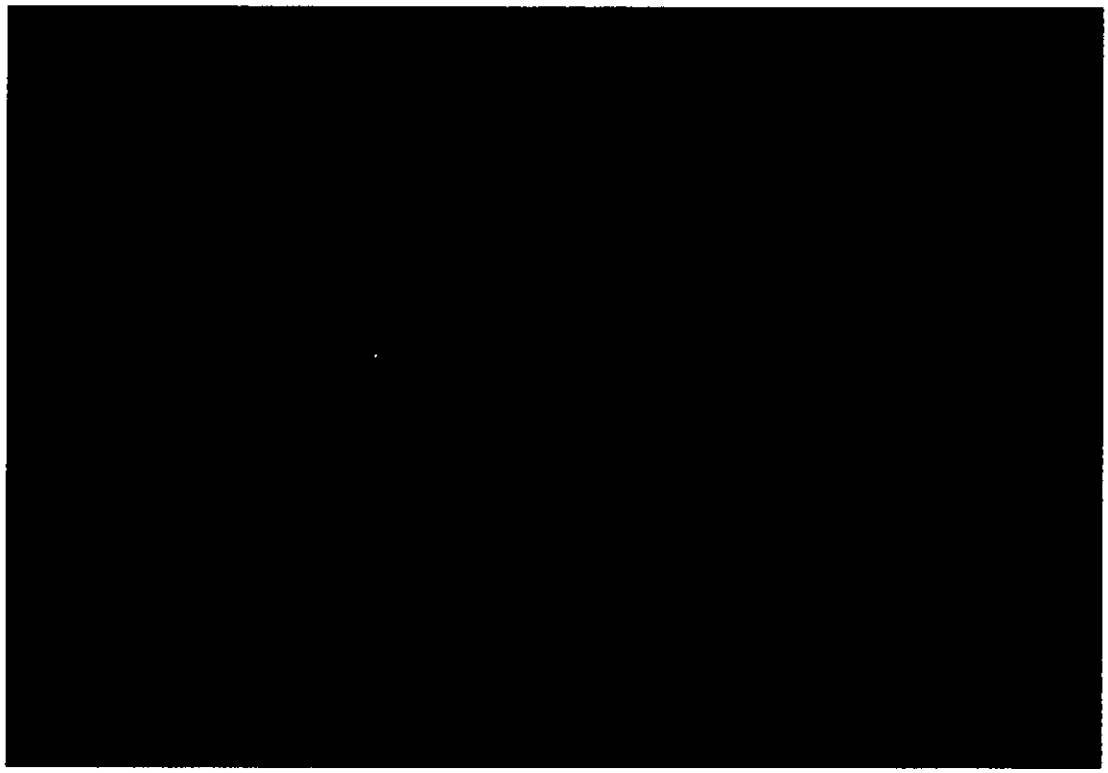
B = Garlic bulb (*Allium sativum*)

C = Neem Plant (*Azadirachta indica*)



Three days old culture Five days old culture

Photograph 2. Pure culture of *Trichoderma harzianum* on PDA plate



Photograph 3. Structures of *Trichoderma harzianum* T_{22} (x 400)

3.15 Application of spray solution

All fungicides, plant extracts and bioagent were sprayed with compressed hand sprayer. Three plants were sprayed with each fungicides, plant extracts and bioagent. First spray was done in 15 June, 2005. 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.

3.16 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.

3.17 Isolation of Pathogen

Infected leaves and twigs were collected for isolation of the fungi. Stem piece of1cm 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 pertridishes 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 in cubated for 7 days in the inoculation chamber.

3.18 Identification of fungal isolates

Fungi grown on the culture media were transferred to 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).

3.19 Data collection

Data were collected in the morning on the following parameters:

3. 19.1 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. 19.2 Total number of scab infected leaves/twig

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

3.19.3 Calculation of disease incidence of leaf scab of different treatments

The percent disease incidence of was Calculated using the following formula.

$$\textbf{Disease incidence} = \frac{\text{Number of infected leaf}}{\text{Total number of obervation (leaf)}} \times 100$$

3.19.4 Total number of fruit/plant

Number of total fruit was recorded at different observation dates as scheduled.

3.19.5 Total number of scab infected fruit/plant

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

3.19.6 Calculation of disease incidence of fruit scab of different treatments

The percent disease incidence of was Calculated using the following formula.

$$\textbf{Disease incidence} = \frac{\text{Number of infected fruit}}{\text{Total number of obervation (fruit)}} \times 100$$

3.19.7 Total number twigs/branch

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

3.19.8 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.

3.19.9 Calculation of disease incidence of die-back of different treatments

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

$$\textbf{Disease incidence} = \frac{\text{Number of infected twig}}{\text{Total number of obervation (twig)}} \times 100$$

3.19.10 Evaluation of leaf and fruit scab severity

Percent leaf area diseased (LAD) and fruit area diseased (FAD) were measured. Area of a single leaf /fruit was considered as 100%. Deducting the healthy area, the diseased was estimated. Average of % LAD and FAD was then calculated dividing the total diseased areas by total number of investigated leaves/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 (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\text{Total sum of numerical ratings} \quad \text{X } 100}{\text{Number of obervation x Maximum number of disease rattings}}$$

3.19.11 Evaluation of die back severity

The die back severity was recorded following 0-5 scale slightly 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% twigs infection
2	=	11-20% twigs infected
3	=	21-30% twigs infected
4	=	31-50% twigs infected and
5	=	above 50% twigs infected.

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

$$\text{PDI} = \frac{\text{Total sum of numerical ratings} \quad \text{X } 100}{\text{Number of obervation x Maximum number of disease rattings}}$$

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-2).

3.21 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 program fallowing the statistical procedures of Gomez and Gomez (1983). Treatment means were compared by DMRT (Duncan's Multiple Range Tests).

CHAPTER-IV

RESULTS

4.1. Scab symptoms observed in the orchard

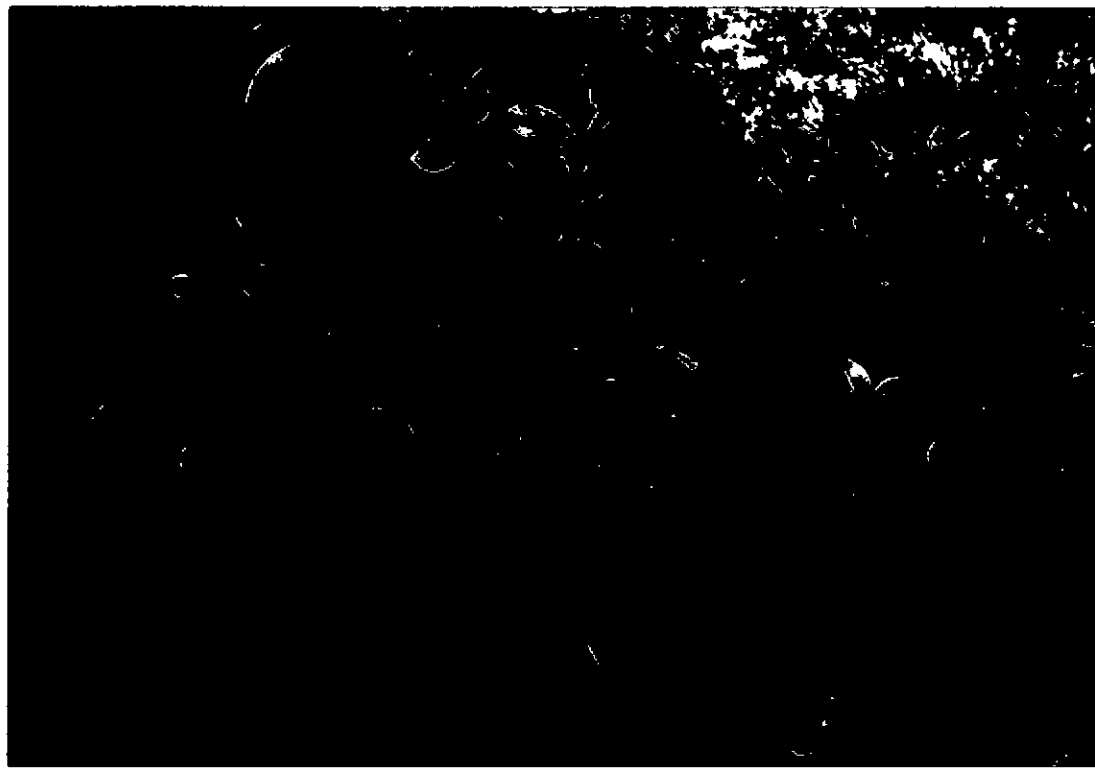
The disease symptoms first noticed on leaves. Small, semi-transluant dots appeared usually on the underside of young leaves that become well defined pustules later on. The lesions mostly appeared singly on one side of leaf. A few days later, the lesion turned to a cream to yellow orange colour at the tips (Photograph 4). The opposite surface corresponding to warty growth shows a circular depression with a pink to red colour (Photograph 5). The spots size ranged from few mm to 1 cm in diameter. Severely affected leaves become distorted and occasionally dropped prematurely (Photograph 5). The lessons appeared on shoots. The lesions on fruits have corky projections which often broke into scabs (Photograph 7). When fully formed scab lesions were raised and were buff to pink to drab in colors. Heavily infected fruits droped shortly after being attacked and those remaining on the trees may be scarred and distorted.

4.2. Die back symptoms observed in the orchard

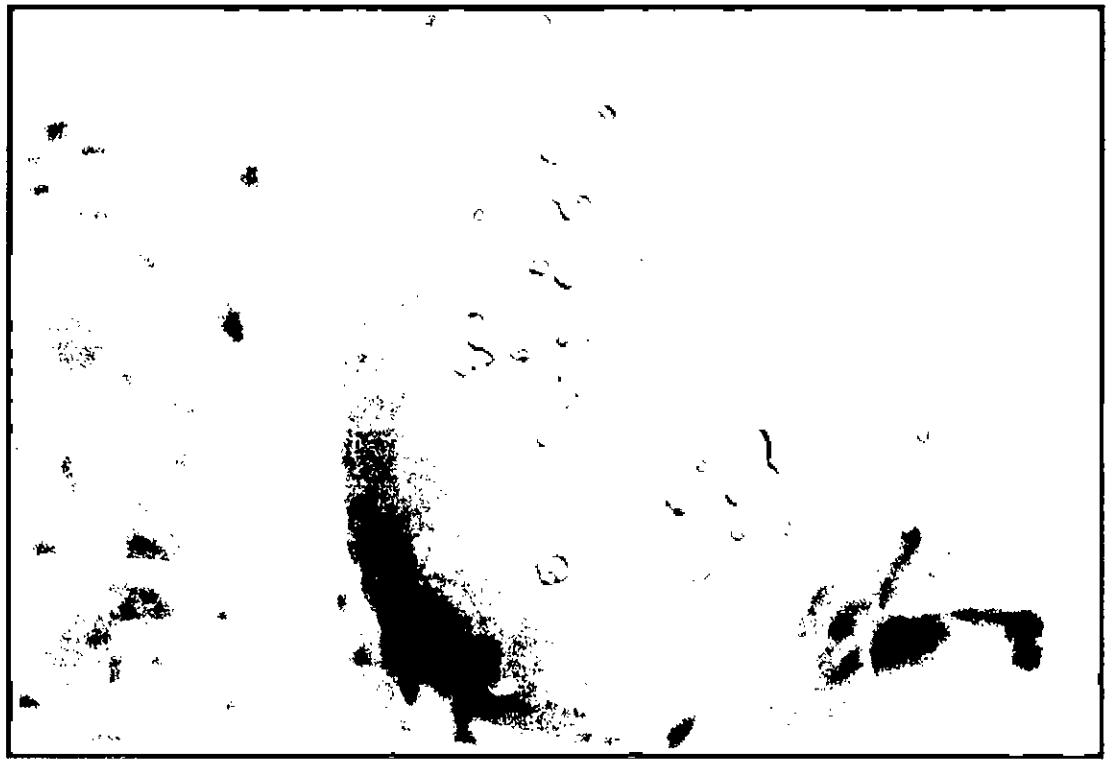
The main characteristic symptom of the disease was drying of the shoots down wards to the stem resulting in the death of twigs or top of the branches (Photograph 11). The first visible symptom of the disease was characterized by shading of the green color of the tip of twigs and the leaves. The die beak often progressed slowly and caused leaves to wilt, turn yellow and drop off. The twigs gradually started drying from the top to down wards.

Photograph 4. Scab infected leaf of citrus

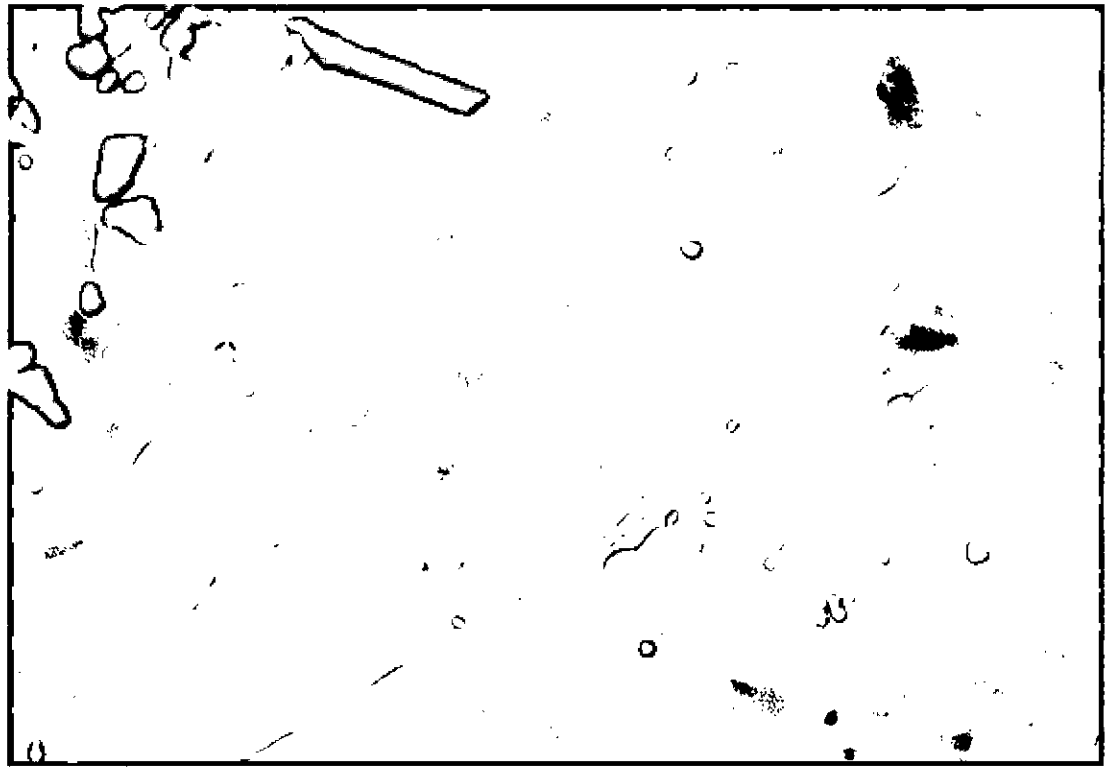
Photograph 5. Scab infected twig of citrus



Photograph 6. Severely Scab infected citrus plant (*Citrus limon*)



Photograph 7. Scab infected fruit of citrus (*Citrus limon*)



Photograph 8. Scab infected leaves, fruit and twigs of citrus (*C. limon*)

Photograph 9. Severely die-back infected citrus plant (*C. limon*)

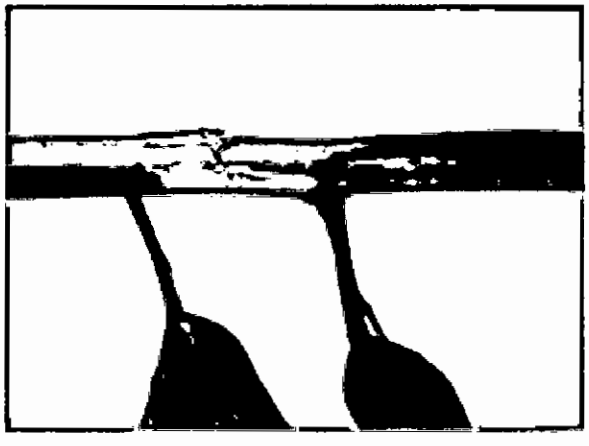


Photo.10. Stem lesion caused by *Colletotrichum gloeosporioides* (bark removed)

Photo. 11. Symptom of die-back on twigs of citrus

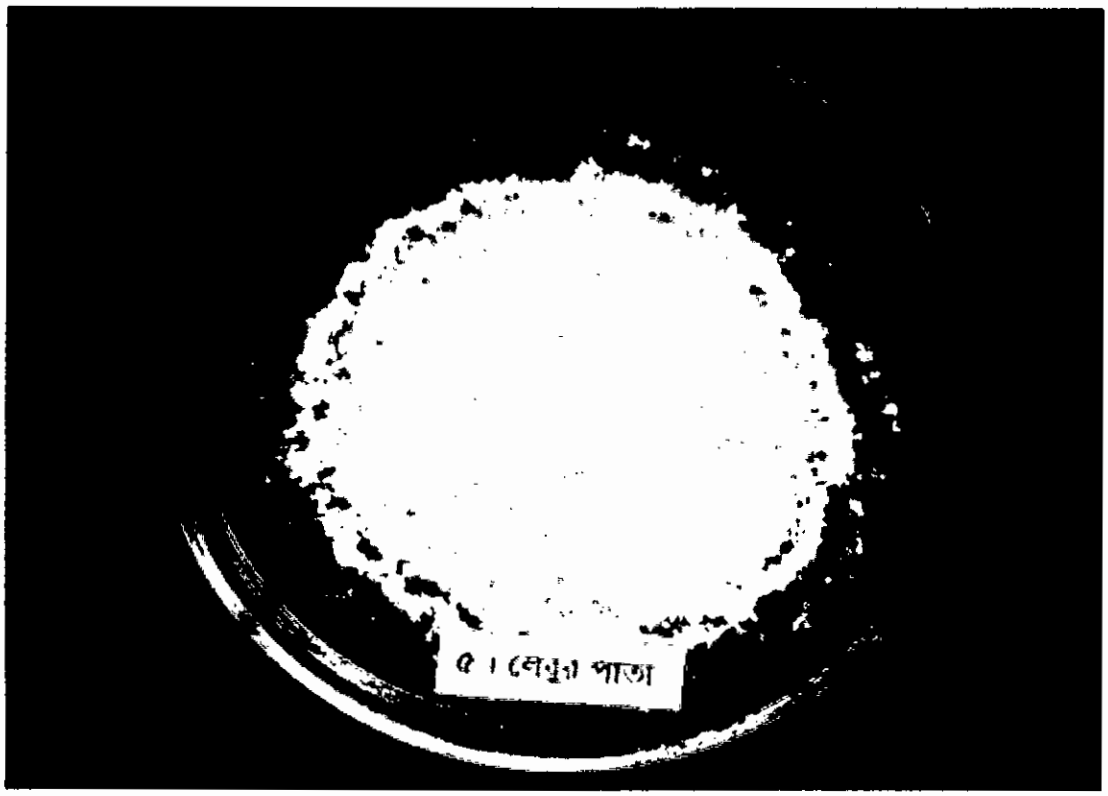
After the symptom noticed, the drying of the twig was usually rapid which extended 5 to 12 cm down wards ending in a sharp line. After wards, the disease progressed slowly or rapidly in the similar way towards the stem affecting more twigs and branches (Photograph 9). Die back affected young branches from the tip, sometimes producing gum exudation. Wood is discolored underneath the bark (Amador, 2002) (Photograph 10). Twigs and branches appeared to have been scorched by fire. When twigs were dried, minute brown to black, slightly raised, clumped pustules were observed. Under humid conditions a pink slimy material appeared on dead bark and twigs. Later on 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 in a few years (Photograph 6.1).

4.3. Isolation and identification of *Elsinoe fawcettii*

The inocula collected from the diseased 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* (Hyun *et al.*, 2001).

4.4. Isolation and identification of *Colletotrichum gloeosporioides*

The inocula collected from the diseased 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* (Commonwealth Mycological Institute. Description of Pathogenic fungi and Bacteria. No. 315).



Photograph 12. Pure culture of *Elsinoe fawcettii* grown on PDA plate isolated from infected citrus leaf

Photograph 13. Pure culture of *Colletotrichum gloeosporioides* grown on PDA plate isolated from infected citrus twig

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

The disease incidence and severity of scab of citrus (leaf) plants before commencement of the spray schedule was statistically similar for all the citrus plants selected for each and every treatment (Table 3). The disease incidence (leaf) ranged from 30.83 to 33.01% and the disease severity (PDI-leaf) ranged from 26.67 to 29.04%.

4.6. Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after one month of starting of spraying.

The effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus recorded after one month of starting of spraying (two sprays have been given) was determined and presented in Table 3. The disease incidence under different treatments differed significantly. The lowest incidence of leaf scab (18.77%) was found in T_4 (Champion 77WP) and the highest incidence (34.83%) in T_{10} (Control). The second lowest incidence of leaf scab of citrus were observed in T_5 (Bordeaux mixture), T_3 (Bavistin 50WP) and T_6 (Biskatali extract). The effect of treatment T_3 (Bavistin 50WP), T_4 (Champion 77WP), T_5 (Bordeaux mixture), T_6 (Biskatali extract) and T_9 (*Trichoderma harzianum*) did not differed significantly in respect of disease incidence.

The disease severity (PDI-leaf) under different treatments also differed significantly. The lowest disease severity (18.09%) were recorded in T_4 (Champion 77 WP) and the highest disease severity (30.00%) in T_{10} (Control). The second lowest disease severity (19.05%) was found with T_5 (Bordeaux mixture). It has been recorded that the effect of treatments

Table 3. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (leaf) after one month of starting of 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=Dithane M-45	33.10a	28.09 a	23.65 cd	23.52 bc	21.60
T_2=Ridomil MZ-72	32.71a	27.62 a	29.11 b	24.61 b	17.97
T_3=Bavistin 50 WP	31.67a	**29.04 a**	19.78 e	21.61 b-d	27.97
T_4=Champion 77 WP	33.01a	28.57 a	**18.77 e**	**18.09 e**	**39.70**
T_5=Bordeaux mixture	32.66a	**29.04 a**	19.28 e	19.05 de	36.50
T_6=Bishkatali extract	32.84a	28.09 a	19.65 e	19.25 de	35.83
T_7=Garlic extract	30.83a	27.14 a	27.48 b	24.61 b	17.96
T_8=Neem extract	32.70a	27.62 a	24.67 c	23.52 bc	21.60
T_9=Trichoderma harzianum	31.97a	28.09 a	21.68 de	20.81 c-e	30.63
T_{10}=Control	33.10a	26.67 ab	34.83 a	30.00 a	-
CV (%)	4.72	6.32	6.62	8.20	-

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

T_4 (Champion 77WP), T_6 (Biskatali extract) and T_9 (*Trichoderma harzianum*) did not differ significantly in respect of disease severity.

The highest PDI decreased over control (39.70%) was found with T_4 (Champion 50 WP) followed by T_5 (Bordeaux mixture),T_6 (Biskatali extract) and T_9 (*Trichoderma harzianum*).The lowest decrease of PDI was noted (17.96%) in T_7 (Garlic extract) proceded by Ridomil MZ-72 (17.97%) and Dithane M-45 (21.60%). The performance of Neem extract in reducing the disease severity was statistically alike with Dithane M-45 (Table 3).

4.7. Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after two month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus recorded after two month of starting of spray (four sprays have been given) was determined and presented in Table 4. The disease incidence under different treatments was differed significantly. The lowest incidence of leaf scab (15.05%) were found in T_4 (Champion 77WP) and the highest incidence (37.47%) in T_{10} (Control). The second lowest incidence of leaf scab of citrus were found with T_3 (Bavistin 50 WP), T_5 (Bordeaux mixture), and T_6 (Biskatali extract). The effect of treatment, T_4 (Champion 77 WP), T_5 (Bordeaux mixture) and T_6 (Biskatali extract) and did not differ significantly in respect of disease incidence.

Table 4. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (leaf) after two month of starting of spray

Treatmets	Before spraying		After two 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=Dithane M 45	33.10a	28.09 a	21.06 d	20.67 bc	31.10
T_2=Ridomil MZ 72	32.71a	27.62 a	28.32 b	23.52 b	21.60
T_3=Bavistin 50 WP	31.67a	**29.04 a**	16.50 e	14.28 d	52.40
T_4=Champion 77 WP	33.01a	28.57 a	**15.05 e**	**10.47 e**	**65.10**
T_5=Bordeaux mixture	32.66a	**29.04 a**	16.93 e	11.42 de	61.93
T_6=Bishkatali extract	32.84a	28.09 a	15.08 e	12.37 de	58.77
T_7=Garlic extract	30.83a	27.14 a	24.13 c	23.81 b	20.63
T_8=Neem extract	32.70a	27.62 a	22.33 cd	19.33 c	35.57
T_9=Trichoderma harzianum	31.97a	28.09 a	16.93 d	14.76 d	50.80
T_{10}=Control	33.10a	26.67 ab	37.47 a	30.00 a	-
CV (%)	4.72	6.32	5.57	10.54	-

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

The disease severity (PDI-leaf) under different treatments was also differed significantly from one another. The lowest disease severity (10.47%) were found with T_4 (Champion 77 WP) and the highest disease severity% (30.00) in T_{10} (Control). The second lowest disease severity was found with T_5 (Bordeaux mixture) (11.42%) and T_6 (Biskatali extract) (12.37%). It has been recorded that the effect of treatments T_4 (Champion 77WP), T_5 (Bordeaux mixture) and T_6 (Biskatali extract) did not differed significantly in respect of disease severity.

The highest PDI (65.10%) decreased over control was found with T_4 (Champion 50 WP) followed by T_5 (Bordeaux mixture) and T_6 (Biskatali extract).The lowest decrease of PDI was noted (20.63%) in T_7 (Garlic extract) preceeded by Ridomil MZ-72 (21.60%) and Dithane M-45 (31.10%) (Table 4).

4.8. Effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus after three month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling leaf scab of citrus recorded after three month of starting of spray (six sprays have been given) was determined and presented in Table-5. The disease incidence under different treatments differed significantly. The lowest incidence of leaf scab (8.69%) was found in T_4 (Champion 77WP) and the highest incidence (37.83%) in T_{10} (Control). The second lowest incidence of leaf scab of citrus were found with T_5 (Bordeaux mixture) and T_3 (Bavistin 50 WP). The effect of treatment T_3 (Bavistin 50 WP) and T_5 (Bordeaux mixture) did not differ significantly in respect of disease incidence.

Table 5. Effect of fungicides, plant extract and bio-agent on the incidence and severity of scab of citrus (leaf) after three month of starting of spray

Treatments	Before spraying		After three 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=Dithane M 45	33.10a	28.09 a	17.27d	14.76 d	59.73
T_2=Ridomil MZ 72	32.71a	27.62 a	23.48b	18.09 c	50.65
T_3=Bavistin 50 WP	31.67a	**29.04 a**	12.33e	13.33 de	63.64
T_4=Champion 77 WP	33.01a	28.57 a	**8.69g**	**10.00 f**	**72.72**
T_5=Bordeaux mixture	32.66a	**29.04 a**	11.79e	12.37 e	66.26
T_6=Biskatali extract	32.84a	28.09 a	10.27f	11.90 e	67.54
T_7=Garlic extract	30.83a	27.14 a	21.22c	21.61 b	41.05
T_8=Neem extract	32.70a	27.62 a	17.96d	16.66 c	54.56
T_9=Trichoderma harzianum	31.97a	28.09 a	13.30e	13.80 de	62.36
T_{10}=Control	33.10a	26.67 ab	37.83a	36.66 a	-
CV (%)	4.72	6.32	5.06	6.37	-

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

The disease severity (PDI-leaf) under different treatments was also differed significantly from one another. The lowest disease severity (10.00%) were found with T_4 (Champion 77 WP) and the highest disease severity % (36.66) in T_{10} (Control). The second lowest disease severity (11.90%) was found with T_6 (Biskatali extract). It has been recorded that the effect of treatments, T_3 (Bavistin 50 WP), T_5 (Bordeaux mixture) and T_6 (Biskatali extract) did not differ significantly in respect of disease severity.

The highest PDI decreased over control (72.72%) was found with T_4 (Champion 50 WP) followed by T_5 (Bordeaux mixture) and T_6 (Biskatali extract).The lowest decrease of PDI (41.05%) was noted in T_7 (Garlic extract) preceeded by Ridomil MZ-72 (50.65%) and Neem extract (54.56%) (Table 5).

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 36.49% to 44.28% and the disease severity (PDI- fruit) ranged from 25.56% to 30.00%.

4.10. Effect of fungicides, plant extracts and bio-agent in controlling fruit scab of citrus after one month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling scab of citrus (fruit) were recorded after one month of starting of spray (two sprays applied) 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 25.19% was recorded in Champion 77WP which was statistically identical with Bordeaux mixture (27.38%), Biskatali extract (27.58%), *Trichoderma harzianum* (35.35%) and Bavistion 50WP (36.61%). The highest disease incidence (43.91%) was recorded incase of control, which was also statistically similar with Dithane M-45 (37.50%), Ridomil MZ-72 (40.12%), Garlic extract (40.49%), and Neem extract (41.67%).

In case of disease severity (PDI- fruit), the lowest was recorded in Champion 77 WP (22.22 %), which was statistically similar with Dithane M-45 (24.44%), Bavistin 50WP (23.73%), Bordeaux mixture (22.52%), Biskatali extract (23.33%) and *Trichoderma harzianum* (24.44%). The highest disease severity was observed in control (31.11%) that was statistically similar to Garlic extract (29.11%). Ridomil MZ-72 scored second highest severity (26.67%) in the experiment.

The highest reduction of PDI over control was observed in case of Champion 77 WP (28.58%) followed by Bordeaux mixture (27.61%), Biskatali extract (25.00%), Bavistin 50WP (23.72%) and *Trichoderma harzianum* (21.44%). The lowest reduction of PDI was noticed in case of Garlic extract (6.43%) preeceded by Neem extract (10.70%) and Ridomil MZ 72 (14.77%) over control.

Table 6. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (fruit) after one month of starting of 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=Dithane M 45	43.33 ab	25.56 e	37.50 ab	24.44 c-e	21.44
T_2=Ridomil MZ 72	40.95 ab	26.67 de	40.12 a	26.67 b-d	14.27
T_3=Bavistin 50 WP	43.33 ab	28.89 b-e	36.61 a-c	23.73 de	23.72
T_4=Champion 77 WP	41.90 ab	30.00 a-e	**25.19 c**	**22.22 e**	**28.58**
T_5=Bordeaux mixture	44.28 ab	30.00 a-e	27.38 bc	22.52 e	27.61
T_6=Biskatali extract	41.90 ab	30.00 a-e	27.58 bc	23.33 de	25.00
T_7=Garlic extract	42.06 ab	30.00 a-e	40.49 a	29.11 ab	6.43
T_8=Neem extract	44.28 ab	30.00 a-e	41.67 a	27.78 bc	10.70
T_9=Trichoderma harzianum	43.33 ab	27.78 c-e	35.55 a-c	24.44 de	21.44
T_{10}=Control	36.49 b	26.67 de	43.91 a	31.11 a	-
CV (%)	10.97	8.70	17.08	7.23	-

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

4.11. Effect of fungicides, plant extracts and bioagent in controlling fruit scab of citrus after two month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling scab of citrus (fruit) were recorded after two month of starting of spray (four sprays applied) was measured 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 (23.15%) was recorded in T_4 (Champion 77 WP) which was statistically identical with Bordeaux mixture (24.07%) and Biskatali extract (24.27%). The highest disease incidence (48.15%) was recorded in case of control treatment.

In case of disease severity (PDI- fruit), the lowest disease severity was recorded in T_4 (Champion 77 WP) (15.56 %), which was statistically similar with Bordeaux mixture (16.67%), Biskatali extract (17.78%) and Bavistin 50WP (23.73%). The highest disease severity was observed in control treatment (31.21%).

The highest reduction of PDI over control was observed in case of Champion 50 WP (49.98%) followed by Bordeaux mixture (46.41%), Biskatali extract (42.84%), Bavistin 50WP (39.28%) and *Trichoderma harzianum* (32.14%). The lowest reduction of PDI was noticed in case of Ridomil MZ 72 (17.84%) peoceeded by Garlic extract (21.44%) and Neem extract (28.57%) over control.

Table 7. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (fruit) after two month of starting of spray

Treatments	Before spraying		After two 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=Dithane M 45	43.33 ab	25.56 e	34.72 c	21.11 c-e	32.14
T_2=Ridomil MZ 72	40.95 ab	26.67 de	36.94 bc	25.56 b	17.84
T_3=Bavistin 50 WP	43.33 ab	28.89 b-e	29.44 d	18.89 d-f	39.27
T_4=Champion 77 WP	41.90 ab	30.00 a-e	**23.15 e**	**15.56 f**	**49.98**
T_5=Bordeaux mixture	44.28 ab	30.00 a-e	24.07 e	16.67 f	46.41
T_6=Biskatali extract	41.90 ab	30.00 a-e	24.27 e	17.78 ef	42.84
T_7=Garlic extract	42.06 ab	30.00 a-e	40.95 b	24.44 bc	21.44
T_8=Neem extract	44.28 ab	30.00 a-e	38.33 bc	22.22 cd	28.57
T_9=Trichoderma harzianum	43.33 ab	27.78 c-e	33.61 cd	21.11 de	32.14
T_{10}=Control	36.49 b	26.67 de	48.15 a	31.21 a	-
CV (%)	10.97	8.70	8.21	8.62	-

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

4.12. Effect of fungicides, plant extracts and bio-agent in controlling fruit scab of citrus (fruit) after three month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling scab of citrus (fruit) recorded after three month of starting of spray (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 9.70% was recorded in Champion 77WP which was statistically identical with Biskatali extract (10.70%). The highest disease incidence (48.38%) was recorded in case of control treatment.

In case of disease severity (PDI- fruit), the lowest disease severity was recorded in Champion 77WP (5.34 %), which was statistically similar with Biskatali extract (6.67%). The highest disease severity was observed in control treatment (33.33%).

The highest reduction of PDI over control was observed in case of with Champion 50 WP (83.98%) followed by Biskatali extract (79.99%), Bordeaux mixture (72.01%) and Bavistin 50WP (70.00%).The lowest reduction of PDI was noticed incase of Garlic extract (43.32%) preceeded by Neem extract and Ridomil MZ 72 (49.98%) over control.

The number of fruit per plant was found to differ significantly from one treatment to another. The average number of fruit per plant was ranged from 7.67 to10.33 .The highest number of fruit per plant was recorded in

Table 8. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of scab of citrus (fruit) after three month of starting of spray.

Treatments	Before spraying		After three 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 (%)	No of fruit per plant
T_1=Dithane M 45	43.33 ab	25.56 e	24.07 b	14.44 c	56.66	8.33 bc
T_2=Ridomil MZ 72	40.95 ab	26.67 de	26.19 b	16.67 bc	49.98	7.67 c
T_3=Bavistin 50 WP	43.33 ab	28.89 b-e	24.07 b	10.00 e	70.00	8.67 bc
T_4=Champion 77 WP	41.90 ab	30.00 a-e	**9.70 d**	**5.34 f**	**83.98**	**10.33 a**
T_5=Bordeaux mixture	44.28 ab	30.00 a-e	16.93 c	9.33 e	72.01	7.67 c
T_6=Biskatali extract	41.90 ab	30.00 a-e	10.70 d	6.67 f	79.99	9.33 ab
T_7=Garlic extract	42.06 ab	30.00 a-e	26.19 b	18.89 b	43.32	7.67 c
T_8=Neem extract	44.28 ab	30.00 a-e	27.78 b	16.67 c	49.98	8.33 bc
T_9=Trichoderma harzianum	43.33 ab	27.78 c-e	24.07 b	12.22 d	63.34	8.33 bc
T_{10}=Control	36.49 b	26.67 de	48.38 a	33.33 a	-	7.67 c
CV (%)	10.97	8.70	5.48	8.94	-	8.54

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

case of Champion 77 WP (10.33), which was statistically identical with Biskatali extract (9.33). The lowest number of fruit per plant was noted in case of control treatment (7.67) which was numerically equal to the Ridomil MZ-72, Bordeaux mixture and Garlic extract (Table 8).

4.13. 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 simillar in case of each and every treatment (Table 9). The disease incidence was ranged from 40.56% to 43.91% and the disease severity (PDI) ranged from 21.33% to 26.00%.

4.14. Effect of fungicides, plant extracts and bioagent in controlling die back of citrus after one month of starting of spray

The effect of fungicides, plant extracts and bioagent in controlling die-back of citrus recorded after one month of starting of spray (two sprays applied) was summerized and presented in Table 9. 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 Dithane M45 (13.35%) which was statistically similar with Bordeaux mixture (15.82%) followed by Neem extract (18.03%). The highest disease incidence was observed in case of control treatment (43.91%). The effect of the rest of the treatments was moderate and statistically identical.

Table 9. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of die-back of citrus after one month of starting of spray

Treatments	Before spraying		After one month of starting of spray		
	Disease incidence	Percent Disease index (PDI)	Disease incidence	Percent Disease index (PDI)	PDI decreased over control (%)
T_1=Dithane M 45	43.33 a	26.00 a-c	**13.35 e**	**12.33 d**	**56.82**
T_2=Ridomil MZ 72	40.56 ab	21.33 c	28.43 b	17.33 b	39.32
T_3=Bavistin 50 WP	40.56 ab	23.33 bc	32.33 b	17.67 b	38.13
T_4=Champion 77 WP	41.48 ab	26.00 a-c	23.39 c	14.33 c	49.82
T_5=Bordeaux mixture	43.91 a	24.66 bc	15.82 de	13.33 cd	53.32
T_6=Biskatali extract	41.82 ab	26.00 a-c	29.09 b	18.33 b	35.81
T_7=Garlic extract	41.11 ab	26.00 a-c	29.09 b	17.33 b	39.32
T_8=Neem extract	40.56 ab	26.00 a-c	18.03 d	13.67 cd	52.13
T_9=Trichoderma harzianum	43.91a	24.67 bc	29.09 b	17.33 b	39.32
T_{10}=Control	40.56 ab	21.67 c	43.91 a	28.56 a	-
CV (%)	4.57	12.82	4.48	5.44	-

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

In case of disease severity, the lowest percent disease index (PDI) was recorded in case of Dithane M-45 (12.33%) which was statistically similar with Bordeaux mixture (13.33%) and Neem extract (13.67%). The highest PDI was observed in case of control treatment (28.56%). The PDI were ranged from 14.33% to 18.33% for the rest of the treatment.

The reduction of PDI over control was recorded highest (56.82%) while sprayed with Dithane M-45 followed by Bordeaux mixture (53.32%), Neem extract (52.13 %) and Champion 77 WP (49.82%). The lowest reduction of PDI over control was recorded in case of Bishkatali extract (35.81%) preceeded by Bavistin 50 WP (38.13%) and Garlic extract (39.32%).

4.15 Effect of fungicides, plant extracts and bioagent in controlling die-back of citrus two month after of starting of spraying.

The effect of fungicides, plant extracts and bioagent in controlling die-back of citrus recorded after two month of starting of spraying (four sprays applied) was summerized 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 Dithane M-45 (12.35%) which was statistically similar with Bordeaux mixture (14.95%), Neem extract (16.14%) and Ridomil MZ-72 (16.43%). The highest disease incidence was observed in case of control treatment (47.96%). The effect of the rest of the treatments was moderate and statistically identical.

Table 10. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of die-back of citrus after two month of starting of spray

Treatments	Before spraying		After two month of starting of spray		
	Disease incidence	Percent Disease index (PDI)	Disease incidence	Percent Disease index (PDI)	PDI decreased over control (%)
T_1=Dithane M 45	43.33 a	26.00 a-c	**12.35 e**	12.00 c-e	60.20
T_2=Ridomil MZ 72	40.56 ab	21.33 c	16.43 de	12.67 b-e	57.98
T_3=Bavistin 50 WP	40.56 ab	23.33 bc	29.55 b	15.33 b-e	49.15
T_4=Champion 77 WP	41.48 ab	26.00 a-c	22.09 cd	15.67 b-e	48.02
T_5=Bordeaux mixture	43.91 a	24.66 bc	14.95 e	12.97 b-e	56.98
T_6=Biskatali extract	41.82 ab	26.00 a-c	24.83 bc	16.00 bc	46.93
T_7=Garlic extract	41.11 ab	26.00 a-c	25.74 bc	16.67 bc	44.71
T_8=Neem extract	40.56 ab	26.00 a-c	16.14 de	**11.33 e**	**62.42**
T_9=Trichoderma harzianum	43.91a	24.67 bc	24.09 bc	11.33 b-d	52.47
T_{10}=Control	40.56 ab	21.67 c	47.96 a	30.15 a	-
CV (%)	4.57	12.82	4.70	17.27	-

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

In case of disease severity, the lowest percent disease index (PDI) was recorded in case of Neem extract (11.33%) which was statistically similar with Dithane M-45 (12.00%), Ridomil MZ-72 (12.67%), Bordeaux mixture (12.97%), Bavistin 50 WP (15.33%) and Champion 77 WP (15.67%). The highest PDI was observed in case of control treatment (30.15%). The PDI were ranged from 16.00% to 16.67% for the rest of the treatment.

The reduction of PDI over control was recorded highest (62.62%) while sprayed with Neem extract followed by Dithane M-45 (60.20%), Ridomil MZ-72 (57.98%), Bordeaux mixture (56.98%) and *Trichoderma harzianum* (52.47%). The lowest reduction of PDI over control was recorded in case of Garlic extract (44.71%) peeceeded by Biskatali extract (46.93%), Champion 77 WP (48.02%) and Bavistin 50 WP (49.15%).

4.16. Effect of fungicides, plant extracts and bioagent in controlling die-back of citrus after three month of starting of spray.

The effect of fungicides, plant extracts and bioagent in controlling die-back of citrus recorded after two month of starting of spray (four sprays applied) was summerized 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 Dithane M-45 (10.40%) which was statistically similar with Bordeaux mixture (12.01%) and Neem extracts (13.10%). The highest disease incidence was observed in case of control treatment (53.91%).

Table 11. Effect of fungicides, plant extracts and bio-agent on the incidence and severity of die-back of citrus after three month of starting of spray

Treatments	Before spraying		After three month of starting of spray		
	Disease incidence	Percent Disease index (PDI)	Disease incidence	Percent Disease index (PDI)	PDI decreased over control (%)
T_1=Dithane M 45	43.33 a	26.00 a-c	**10.40 d**	**8.67 d**	**73.46**
T_2=Ridomil MZ 72	40.56 ab	21.33 c	26.51 b	14.00 bc	55.56
T_3=Bavistin 50 WP	40.56 ab	23.33 bc	26.51 b	16.67 b	48.97
T_4=Champion 77 WP	41.48 ab	26.00 a-c	21.48 c	15.33 b	53.07
T_5=Bordeaux mixture	43.91 a	24.66 bc	12.01 d	13.33 bc	59.19
T_6=Bishkatali extract	41.82 ab	26.00 a-c	21.02 c	15.83 b	51.57
T_7=Garlic extract	41.11 ab	26.00 a-c	20.42 c	15.93 b	51.23
T_8=Neem extract	40.56 ab	26.00 a-c	13.10 d	10.67 cd	67.34
T_9=Trichoderma harzianum	43.91a	24.67 bc	20.74 c	13.83 bc	57.67
T_{10}=Control	40.56 ab	21.67 c	53.91 a	32.67 a	_
CV (%)	4.57	12.82	7.34	13.98	_

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

The effect of the rest of the treatments was moderate and statistically identical.

In case of disease severity, the lowest percent disease index (PDI) was recorded in case of Dithane M-45 (8.67%) which was statistically similar with Neem extract (10.67%) proceeded by Bordeaux mixture (13.33%), *Trichoderma harzianum* (13.83%), Ridomil MZ-72 (14.00%), Champion 50 WP (15.33%), Bishkatali extract (15.83%), Garlic extract (15.93%) and Bavistin 50 WP (16.67%). The highest PDI was observed in case of control treatment (32.67%).

The reduction of PDI over control was recorded highest (73.46%) while sprayed with Dithane M-45 followed by Neem extract (67.34%), Bordeaux mixture (59.19%), *Trichoderma harzianum* (57.67%) and Ridomil MZ 72 (55.56%). The lowest reduction of PDI over control was recorded in case of Bavistin 50 WP (48.97%) proceeded by Garlic extract (51.23%), Biskatali extract (51.57%) and Champion 77 WP (53.07%).

4.17. Relationship between disease incidence, disease severity and time (days) of scab and die-back of citrus due to the application of plant extracts, fungicides and bioagent.

4.17.1. Relationship between disease incidence and time (days) for leaf scab of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease incidence of leaf scab was reduced with the progress of time and the relationship between the decrease of disease incidence and time

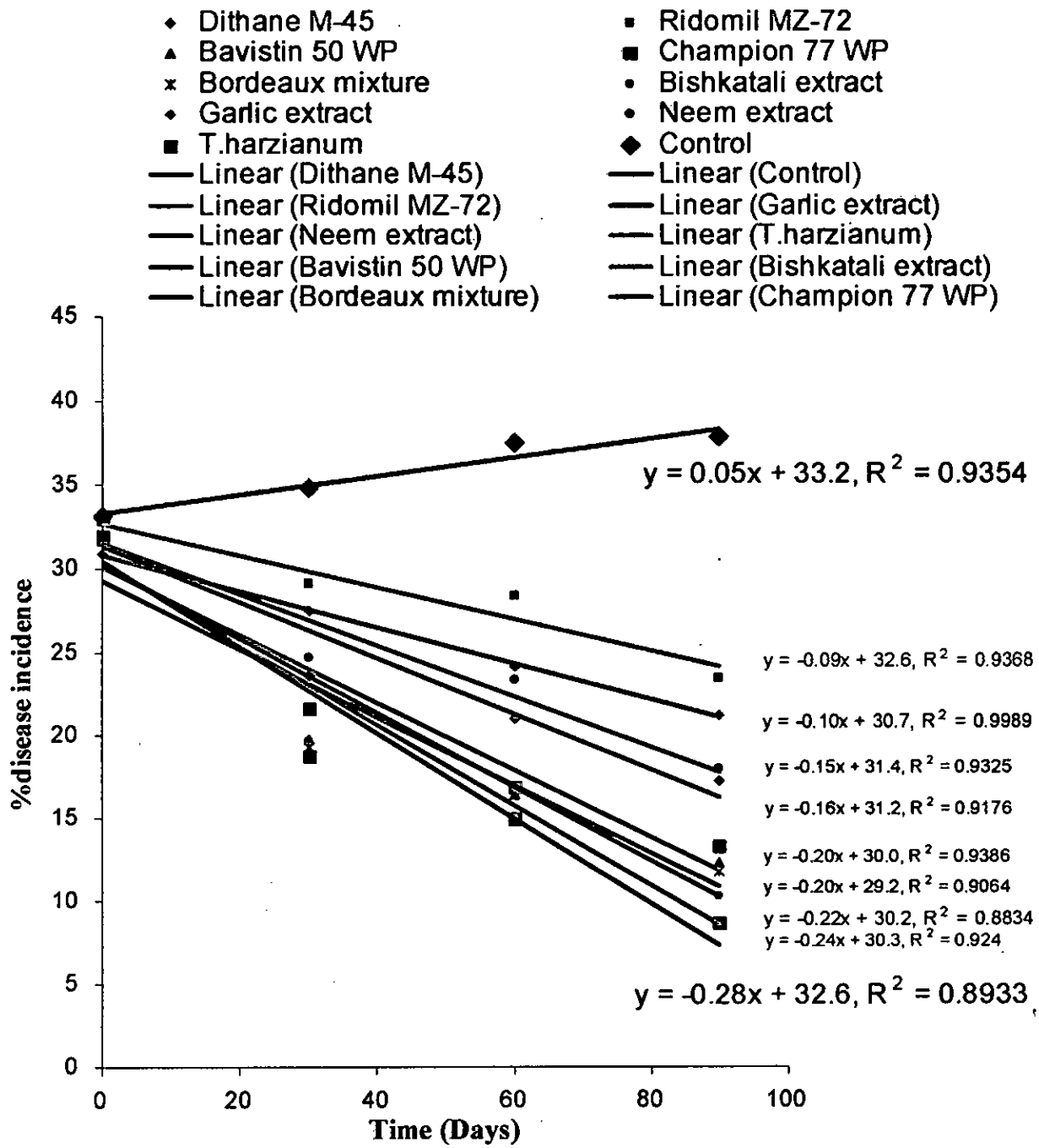


Fig.1 Relationship between disease incidence and progress of time (days) for leaf scab of citrus due to application of treatments

(days) were studied and presented in Figure 1. In case of all the treatments applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease incidence of control treatment showed positive correlation with time.

From the regression equations, it was revealed that due to the application of Champion 77 WP leaf scab incidence was decreased by 28 units while it was 24, 22, 20, 20, 16, 15, 10 and 9 units for Biskatali, Bordeaux mixture, Bavistin, *T. harzianum*, Dithane M-45, Neem, Garlic and Redomil respectively.

4.17.2. Relationship between disease severity (PDI) and time (days) for leaf scab of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease severity (PDI) of leaf scab was reduced with the progress of time and the relationship between the decrease of disease severity (PDI) and time (days) were studied and presented in Figure 2. In case of all the treatments applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease severity (PDI) of control treatment showed positive correlation with time.

From the regression equations, it was revealed that due to the application of Champion 77 WP leaf scab incidence was decreased by 21 units while it was 19, 18, 18, 16, 14, 12, 9 and 5units for Bordeaux mixture, Biskatali, Bavistin 50 WP, *T. harzianum*, Dithane M-45, Neem, Redomil MZ-72 and Garlic respectively.

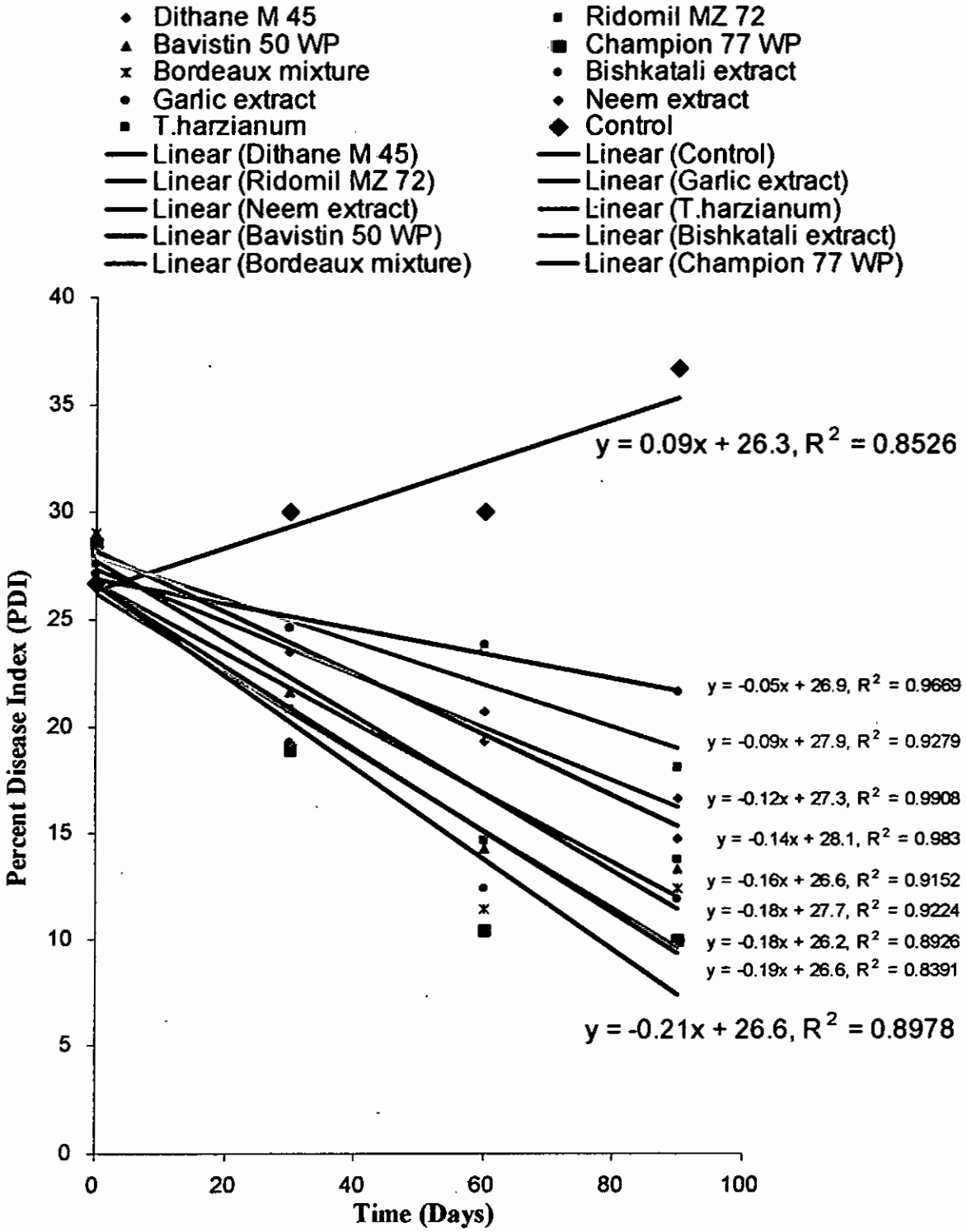


Fig.2 Relationship between disease severity (PDI) and progress of time for leaf scab of citrus due to application of treatments

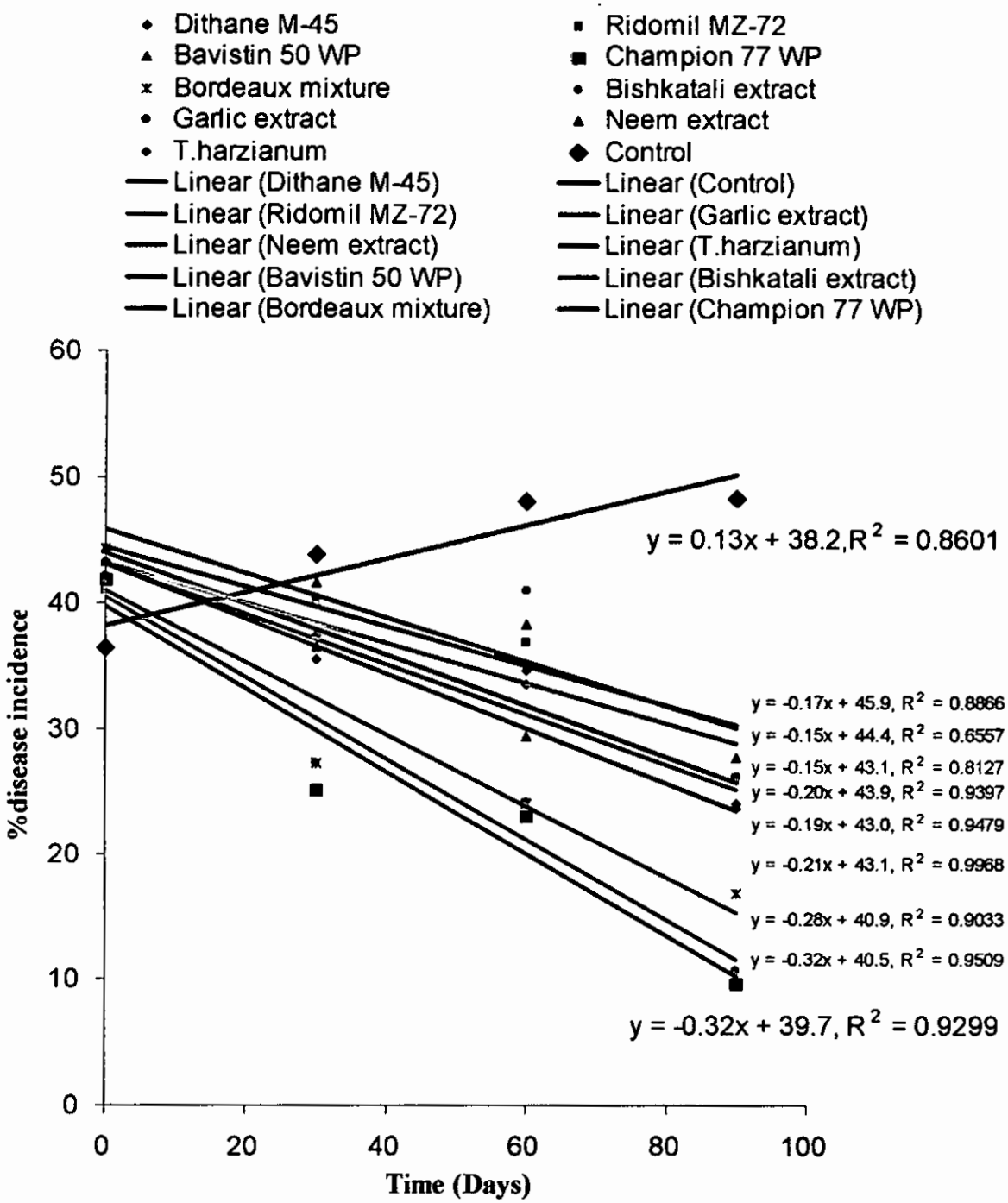


Fig. 3 Relationship between disease incidence and progress of time (days) for fruit scab of citrus due to application of treatments

4.17.3. Relationship between disease incidence and time (days) for fruit scab of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease incidence of fruit scab was reduced with the progress of time and the relationship between the decrease of disease incidence and time (days) were studied and presented in Figure 3. In case of all the treatments applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease incidence of control treatment showed positive correlation with time.

From the regression equations, it was revealed that due to the application of Champion 77 WP fruit scab incidence was decreased by 32 units while it was 32, 28, 21, 20, 19, 17, 15 and 15 units for Bishkatali, Bordeaux mixture, Bavistin 50 WP, *T. harzianum*, Dithane M-45, Neem, Garlic and Redomil MZ-72 respectively.

4.17.4. Relationship between disease severity (PDI) and time (days) for fruit scab of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease severity (PDI) of fruit scab was reduced with the progress of time and the relationship between the decrease of disease severity (PDI) and time (days) were studied and presented in Figure 4. In case of all the treatments applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease severity (PDI) of control treatment showed positive correlation with time.

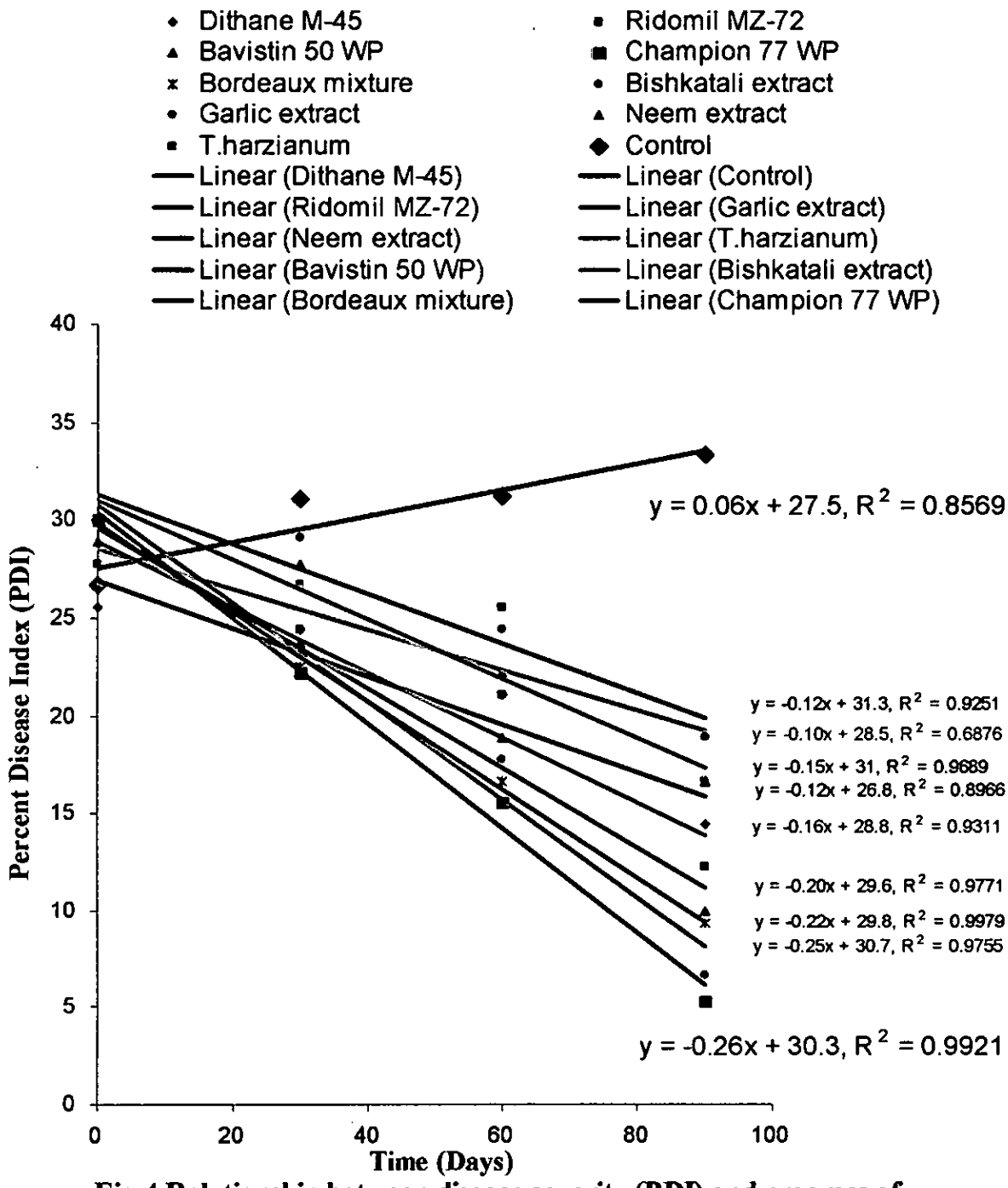


Fig.4 Relationship between disease severity (PDI) and progress of time for fruit scab of citrus due to application of treatments

From the regression equations, it was revealed that due to the application of Champion 77 WP fruit scab incidence was decreased by 26 units while it was 25, 22, 20, 16, 15, 12, 12, and 10 units for Biskatali, Bordeaux mixture, Bavistin 50 WP, *T. harzianum*, Dithane M-45, Neem, Garlic and Redomil MZ-72 respectively.

4.17.5. Relationship between disease incidence and time (days) for die-back of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease incidence of die-back was reduced with the progress of time and the relationship between the decrease of disease incidence and time (days) were studied and presented in Figure 5. In case of all the treatment applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease incidence of control treatment showed positive correlation with time.treatments applied, the disease incidences were significantly and negatively correlated with the time (days) other than control. The disease incidence of control treatment showed positive correlation with time.

From the regression equations, it was revealed that due to the application of Dithane M45 die-back incidence was decreased by 33 units while it was 32, 28, 24, 22, 21, 20and 14 units for Bordeaux mixture, Neem, *T. harzianum* , Biskatali, Bavistin, Champion 50WP, Redomil and Garlic respectively.

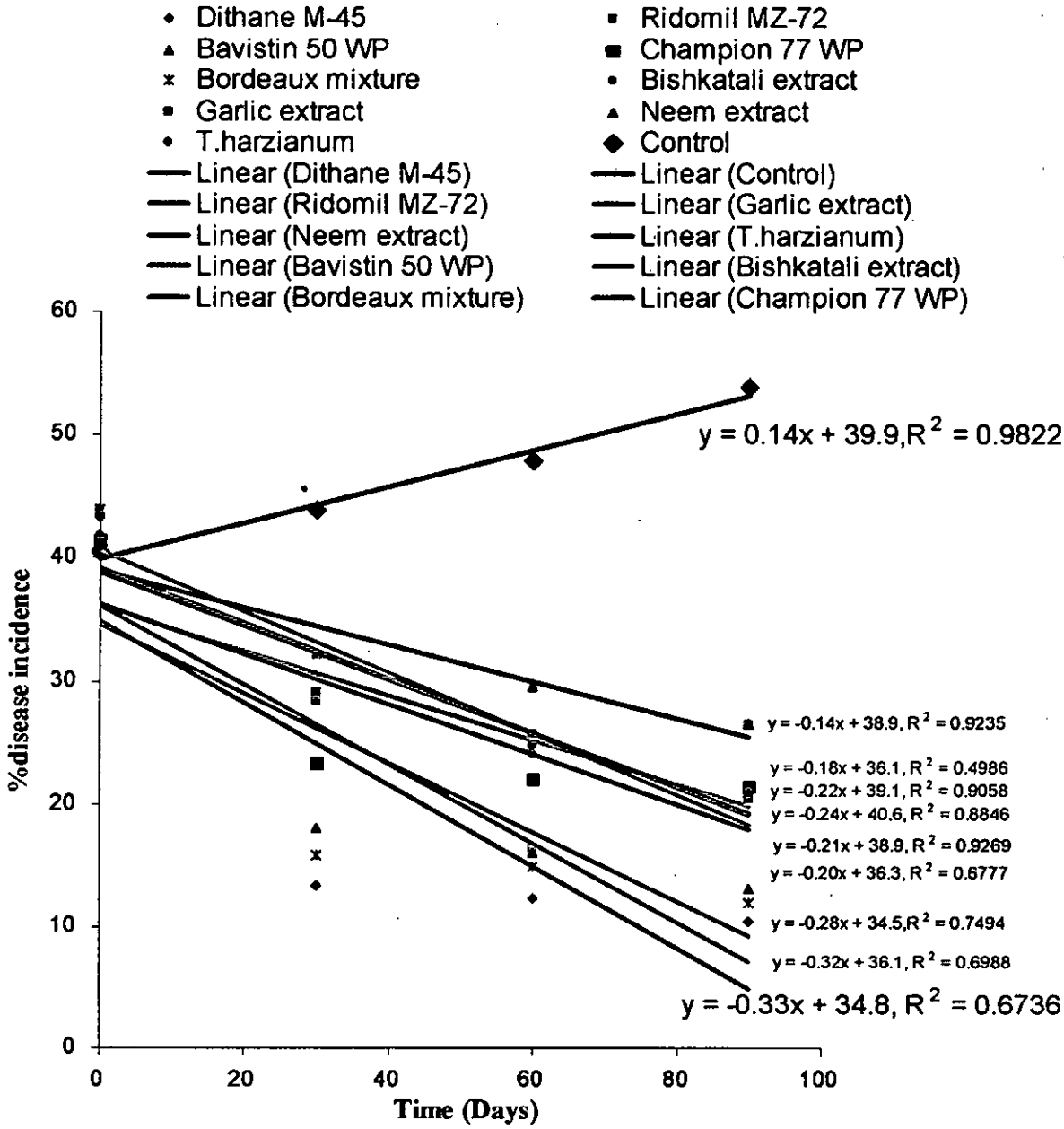


Fig. 5 Relationship between disease incidence and progress of time (days) for die-back of citrus due to application of treatments

4.17.6. Relationship between disease severity (PDI) and time (days) for die-back of citrus.

Due to the application of plant extracts, fungicides and bioagent, the disease severity (PDI) of die-back was reduced with the progress of time and the relationship between the decrease of disease severity (PDI) and time (days) were studied and presented in Figure 5. In case of all the treatments applied, the disease severity were significantly and negatively correlated with the time (days) other than control. The disease incidence of control treatment showed positive correlation with time.

From the regression equations, it was revealed that due to the application of Dithane M-45 die-back severity was decreased by 17 units while it was 16, 12, 11, 10, 10, 10, 8 and 7 units for Neem, *T. harzianum*, Bordeaux mixture, Bishkatali, Bavistin 50 WP, Champion 77 WP, Redomil MZ-72 and Garlic respectively.

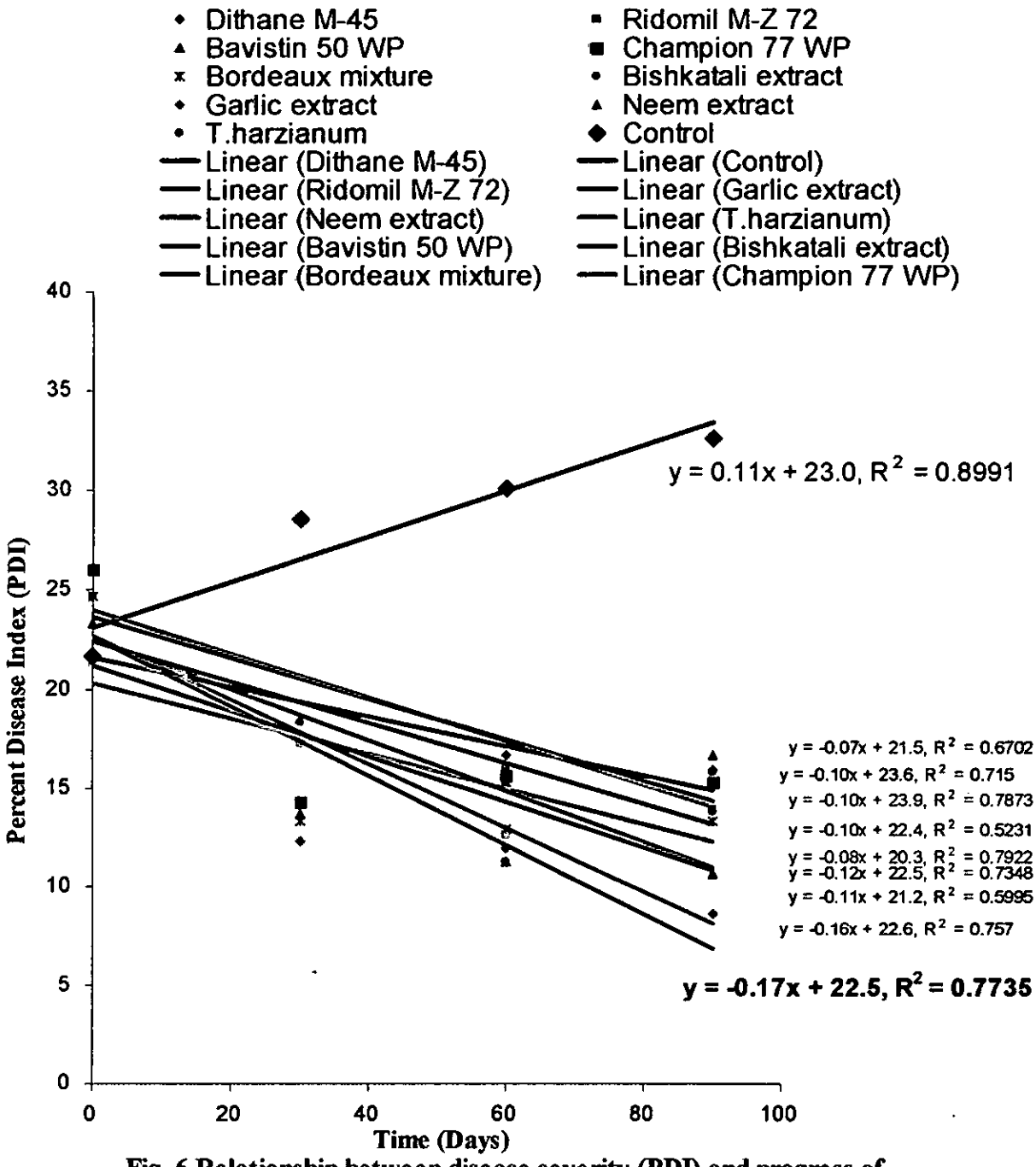


Fig. 6 Relationship between disease severity (PDI) and progress of time for die-back of citrus due to application of treatments

CHAPTER-V
DISCUSSION

Among the chemicals, Champion 77 WP was the most effective fungicide in controlling scab of citrus that reduced 72.72% leaf scab and 72.01% fruit scab. In controlling die-back of citrus, Dithane M-45 was found to be the most effective fungicide that reduced 73.46% die-back severity followed by Bordeuax mixture.The findings of the present investigation agreed with the findings of Xu *et al.* (2004), Singh *et al.* (2000), Li *et al.* (1997), Ebenezar and Shubramanian (1996), Thakore (1994), Hossain (1993), Harsh *et al.* (1992) and Rahman and Hossain (1988). Singh *et al.* (2000) reported that copper fungicides Blitox-50 controlled citrus scab by 81.1% appllied as folliar spray. Li *et al.* (1997) found that Pujunk (Copper hydroxide) controlled citrus scab (*Elsinoe fawcettii*) more effectively than Bordeaux mixture. Whiteside (1990) reported that Dithane M-45 was not so effective against scab of citrus. Ebenezar and Shubramanian (1996) reported that die- back of citrus was successfully controlled by spraying of Bordeuax mixture (1%). Thakore *et al.* (1994) reported that the best control of die back of citrus was achieved by spraying Dithane M-45 at 2000 ppm. However, Das *et al.* (1998) reported that Dithane M-45 and Ridomil MZ-72 were not found effective against die back of citrus.

Bishkatali leaf extract reduced disease severity of leaf scab, fruit scab and die-back by 67.54, 79.99 and 46.53% respectively. Evidences of using Biskatali leaf extract against plant pathogens especially against fungal pathogen are available in the literature (Asrafuzzaman and Hossain, 1992; Ahmed and Islam, 2000). Garlic extract reduced disease severity of leaf scab, fruit scab and die back by 41.05%, 43.32% and 46.53% respectively.

Garlic leaf extracts used as foliar spray were found less effective in controlling the scab and die-back of citrus than other fungicides and plant extracts used. The present findings did not agree with the findings of Yesmin (2004) who reported that garlic extract was the most effective against scab. Yesmin (2004) also reported that Neem leaf extract was the most effective in controlling die back of twig and branch of citrus. Khan *et al.* (1998) reported that four neem based products, namely Nemokil, Nemokil-S, SDS and SDC has antifungal activity against the anthracnose pathgen of guava (*Colletotrichum gloeosporioides*) and this is in accordance with supported the present findings. Neem leaf extracts reduced disease severity over control of leaf scab, fruit scab and die-back of citrus by 54.56, 49.98 and 62.78% respectively. The findings of the present investigation are well supported by the findings of Yesmin (2004) who reported that neem leaf extract controlled die back of citrus significantly. Khan *et al.* (1999) also reported that neem leaf extract have antifungal activity against anthracnose pathogen of guava (*Colletotrichum gloeosporioides*). Evidence of using neem leaf extract against plant pathogens especially against fungal pathogens is available in the literature (Ahmed and Islam, 2000; Moniruzzaman and Ashrafuzzaman, 1998; Hossain and Sehlosser, 1993 and Achimu and Schloesser, 1992).

Trichoderma harzianum were found effective against *Elsinoe fawcettii* and *Colletotrichum gloeosporioides* in the field condition. Evidences of using *Trichoderma harzianum* against plant pathogens especially against fungal pathogens are available in the literature (Islam, 2004; Meah, 2003; Bari *et al.*, 2000; Lewis and Larkin, 1997; Haque *et al.*, 1990; Harman *et al.*, 1989; Agrawal *et al.*, 1977). But work with *Trichoderma harzianum* against *Elsinoe fawcettii and Colletotrichum gloeosporioides* are few. Singh *et al.*, (2000) found that spraying of spore suspension of *Trichoderma harzianum* was effective in controlling *Elsinoe fawcettii*

causing scab of citrus in the field that reduced 17.8% disease severity over control. The account for disease reduction seemed to be the competition for space and nutrients with the pathogenic fungi (Alabouvette and Couteaudier, 1992) and the production of antibiotics by the antagonists (Larkin *et al.*, 1996). Elad *et al.*, (1982) reported the lyric activity of extra cellular enzymes of *Trichoderma harzianum* that aided to antagonize the pathogen. Reports on the specific action of *Trichoderma harzianum* against *Colletotrichum gloeosporioides* are not available. The bio control agents are usually used against soil borne pathogen. However, it has also been successfully employed for several plant pathogens infecting on foliage and other above ground parts (Singh *et al.*, 2000).

From the findings of the present investigation, it has revealed that among the fungicides, Champion 77 WP was the most effective chemical in controlling scab of citrus followed by Bordeuax mixture. Dithane M-45 was the most effective chemical in controlling die back of citrus followed by Bordeuax mixture. Thus the use of Bordeaux mixture could be a nice option in controling both scab and die-back of citrus. Among the botanicals, Biskatali extract was the most effective against scab of citrus and Neem leaf extract was effective against die-back of citrus. The bioagent *Trichoderma harzianum* also found promising in reducing both scab and die-back of citrus next to Bordeaux mixture.

CHAPTER-VI

SUMMARY AND CONCLUSION

An experiment was conducted on the standing citrus plant in Sher-e-Bangla Agricultural University campus, Dhaka, during the period of May-September'2005 to find out the effect of bioagent, plant extracts and fungicides in controling the scab and die-back of citrus. Five fungicides viz. Dithane M-45 (0.3%), Ridomil MZ-72 (0.2%) Bavistin 50 WP (0.1%), Champion 77 WP (0.25%) and Bordeaux mixture (1%); three plants extracts viz. Bishkatali (*Poligonum hydropiper*), Garlic (*Allium sativum*) and Neem (*Azadirachta indica*) and one bioagent (*Trichoderma harzianum* T_{22}) were applied in the experiment.

The effect of fungicides, plant extracts and bioagent in controling the scab and die-back of citrus were determined by recording data after different dates of spraying interms of disease incidence and severity.

After three month of starting of spraying (six sprays has been given at 15 days interval) the lowest incidence of leaf scab (8.69%) were found in T_4 (Champion 77 WP) and the highest incidence of leaf scab (37.83%) were found in T_{10} (Control). The second lowest incidence of leaf scab of citrus were found with T_5 (Bordeaux mixture) and T_3 (Bavistin 50 WP). The lowest disease severity (10.00) were found with T_4 (Champion 77 WP) and the highest disease severity (36.66%) in T_{10} (Control). The second lowest disease severity (PDI-leaf) (11.90%) was found with T_6 (Bishkatali extract).

After three month of starting of spraying the lowest incidence of fruit scab (9.70%) were recorded in T_4 (Champion 77 WP) which was

statistically identical with Biskatali extract (10.70%). The highest disease incidence (48.38%) was recorded incase of control treatment. The lowest disease severity (PDI- fruit) was recorded in Champion 77 WP (5.34 %), which was statistically similar with Biskatali extract (6.67%). The highest disease severity was observed in control treatment (33.33%).

After three month of starting of spraying the lowest disease incidence of die-back of citrus was recorded in case of Dithane M-45 (10.40%) which was statistically similar with Bordeaux mixture (12.01%) and Neem extracts (13.10%). The highest disease incidence was observed in case of control treatment (53.91%). The lowest disease severity (PDI) was recorded in case of Dithane M-45 (8.67%) which was statistically similar with Neem extract (10.67%) proceeded by Bordeaux mixture (13.33%), *Trichoderma harzianum* (13.83%), Ridomil MZ-72 (14.00%), Champion 77 WP (15.33%), Bishkatali extract (15.83%), Garlic extract (15.93%) and Bavistin 50 WP (16.67%). The highest PDI was observed in case of control treatment (32.67%).

Regreession studies between the incidence/severity of scab and die-back of citrus were significantly and negetively correlated with progress of time (days) other than control. The correlation between disease incidence/severity of scab and die-back in control treatment with time was possitive.

The study revealed that among the fungicides, Champion 77 WP was the most effective chemical in controlling scab of citrus followed by Bordeuax mixture. Dithane M-45 was the most effective chemical in controlling die back of citrus followed by Bordeaux mixture. Thus the use of Bordeaux mixture could be a nice option in controling both scab and die-back of citrus. Among the used botanicals, Biskatali extract was

found to be the most effective against scab of citrus while Neem leaf extract was found to be most effective against die-back of citrus. The bioagent *Trichoderma harzianum* also found promising in reducing both scab and die-back of citrus next to Bordeaux mixture.

CHAPTER-VII

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CHAPTER-VIII

APPENDICES

Appendix 1. Composition of 100 gm fresh peeled fruit of citrus

Ingredients	Amount
Moister	88.4%
Mineral	0.6 gm
Fiber	1.7 gm
Protein	0.3 gm
Fat	0.7 gm
Carbohydrate	10 gm
Calcium	40 mg
Iron	2.3 mg
Vitamin B-2	0.03 mg
Vitamin C	47 mg
Energy	47 kcal

Source: Alam *et al.*, 2003.

Appendix 2. Monthly mean of daily maximum, minimum and average temperature, relative humidity and total rainfall during May, 2005 to September, 2005.

Month	Temperature (^{0}C) **			Relative ** Humidity	Rainfall *(mm)
	Max	Min.	Ave.		
May	33.25	24.25	28.76	74.47	0294
June	31.05	26.84	28.94	76.10	0261
July	31.45	25.81	28.63	78.78	0542
August	32.07	26.66	29.36	80.74	0391
September	32.84	25.99	29.41	78.99	0576

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 16 Showing Citrus plant sprayed with Dithane M-45

Photograph 17 Showing Citrus plant sprayed with Ridomil MZ-72

Photograph 19 Showing Citrus plant sprayed with Bavistin 50 WP

Photograph 20 Showing Citrus plant sprayed with Champuion 77 WP

Photograph 21 Showing Citrus plant sprayed with Bordeuax mixture

Photograph 22 Showing Citrus plant sprayed with Bishkatali extract

Photograph 23 Showing Citrus plant sprayed with Neem leaf extract

Photograph 24 Showing Citrus plant sprayed with Garlic bulb extract

Photograph 25 Showing Citrus plant sprayed with *Trichoderma harzianum*

Photograph 26 Showing Citrus plant under control treatment