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CONTROL OF STEMPHYLIUM BLIGHT (*Stemphylium botryosum*) OF ONION THROUGH SELECTED FUNGICIDES AND PLANT EXTRACTS FOR SEED PRODUCTION

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BY

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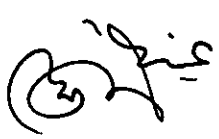

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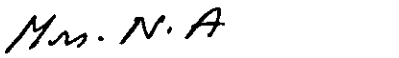

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CERTIFICATE

This is to certify that the thesis entitled, "*CONTROL OF STEMPHYLIUM BLIGHT (Stemphylium botryosum) OF ONION THROUGH SELECTED FUNGICIDES AND PLANT EXTRACTS FOR SEED PRODUCTION* " submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY*, embodies the result of a piece of bona fide research work carried out by *Ariful hoque, Registration No. 00868,* under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

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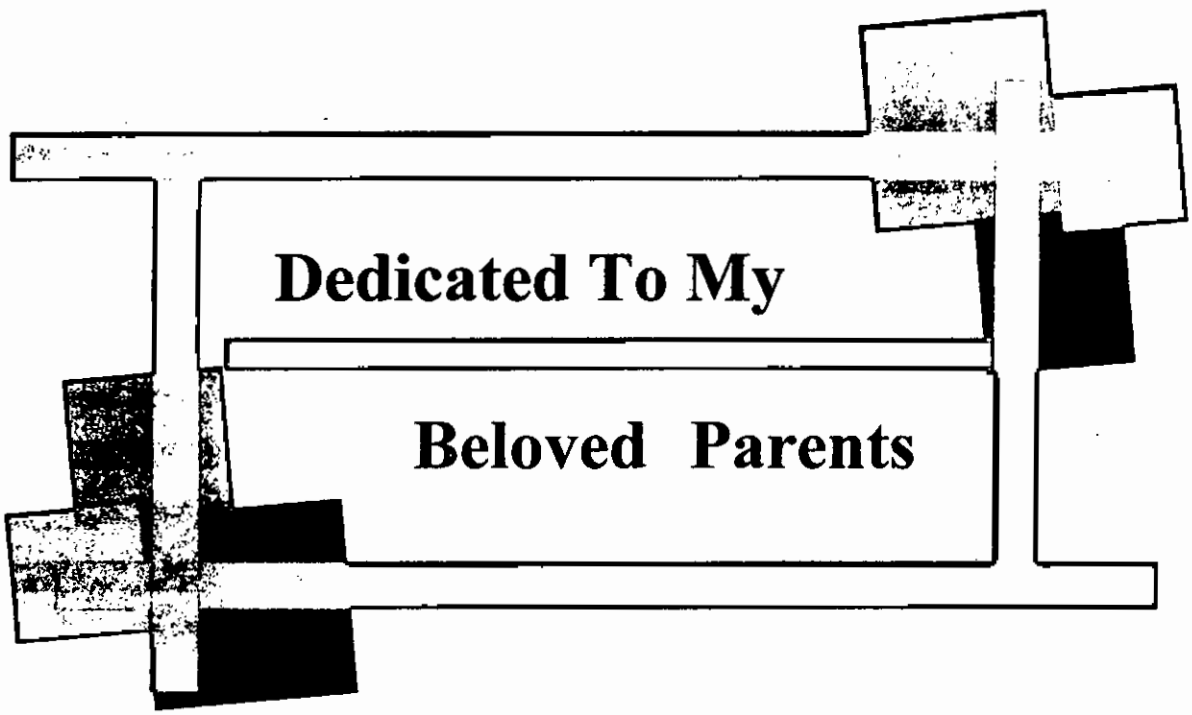
Dedicated To My
Beloved Parents

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The Author

CONTROL OF STEMPHYLIUM BLIGHT (*Stemphylium botryosum*) OF ONION THROUGH SELECTED FUNGICIDES AND PLANT EXTRACTS FOR SEED PRODUCTION

ABSTRACT

A field experiment was conducted at the research farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the rabi season of 2007-08 to study the control of stemphylium blight (*Stemphylium botryosum*) of onion for seed production through some selected fungicides, plant extracts and micronutrients. The experiment was laid out in a Randomized Complete Block Design (RCBD) with 3 (three) replications. An onion variety BARI Piaj-1 was used in the experiment. Treatment of onion bulb followed by foliar spraying with nine treatments comprising Rovral 50WP, Dithane M-45, Ridomil Gold, Bavistin 50WP, Cupravit 50WP, Neem leaf extract and Alamanda leaf extract along with micronutrients and Control were explored in the experiment. In *in vitro* experiment (cup method) Rovral 50WP proved to be the best in controlling the radial mycelium growth of *Stemphylium botryosum*. In field condition, bulb treated with Rovral 50WP (0.2%) followed by foliar spraying at 7 days interval starting from onset of the diseases minimized disease incidence, severity and increased seed yield followed by Dithane M-45. Neem leaf extract showed better performance than Alamanda leaf extract in controlling Stemphylium blight of onion. The micronutrient along without spraying of fungicides and plant extracts had significant effect compared to control.

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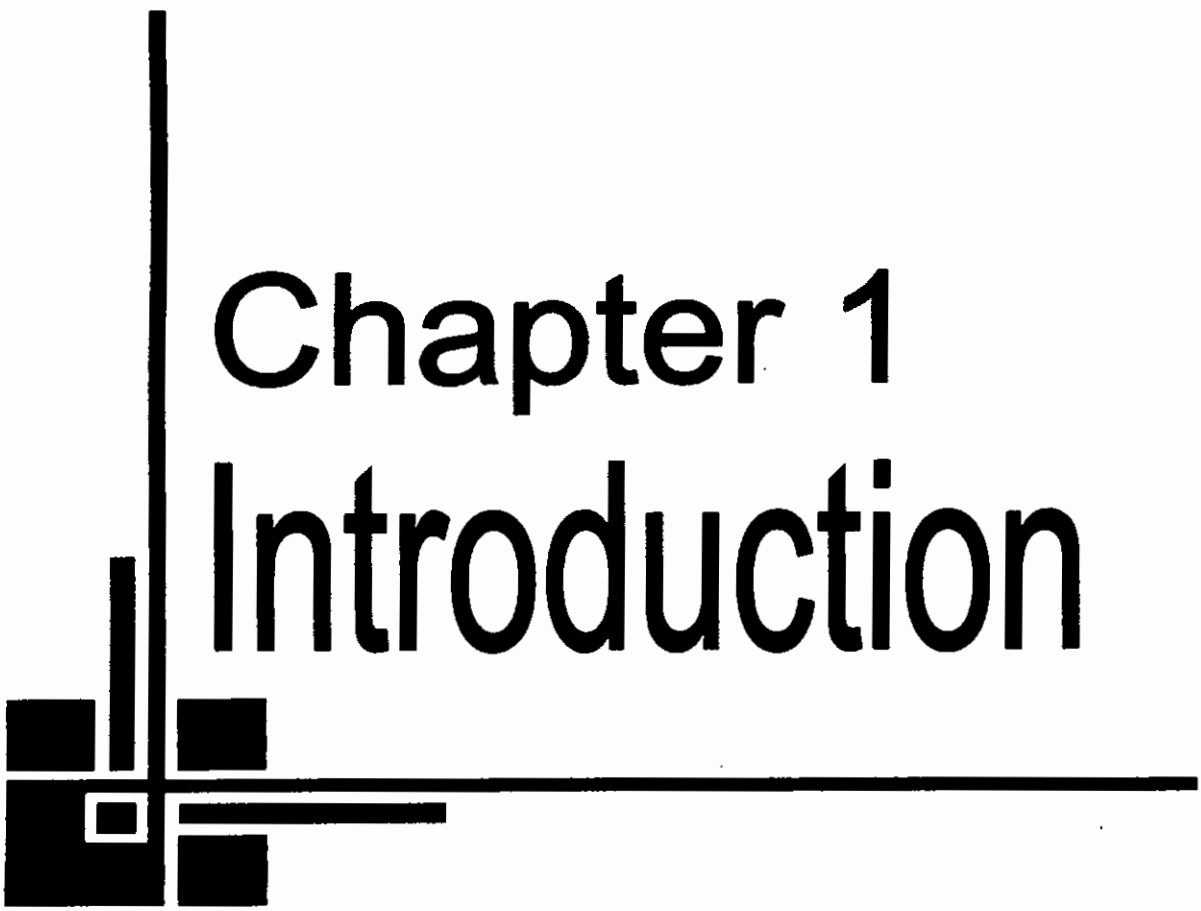
ABBREVIATIONS USED

AEZ	=	Agro-Ecological Zone
@	=	At the rate
Anon.	=	Anonymous
BARI	=	Bangladesh Agricultural Research Institute
cm	=	Centimeter
CT	=	Conventional tillage
cv.	=	Cultivar (s)
DAS	=	Days after sowing
DMRT	=	Duncan's Multiple Range Test
e.g.	=	Example
gm	=	Gram
FAO	=	Food and Agriculture Organization
GY	=	Grain yield
ha	=	Hectare
HLB	=	*Helminthosporium* leaf blight
hr	=	Hour
i.e.	=	That is
K	=	Potassium
Kg	=	Kilogram
lb	=	Pound
LSD	=	Least significant difference
m	=	Meter
mm	=	Millimeter
MP	=	Muriate of potash
N	=	Nitrogen
P	=	Phosphorus
PDA	=	Potato Dextrose Agar

RCBD	=	Randomized Complete Block Design
S	=	Sulphur
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t / ha	=	Ton per hectare
TSP	=	Triple Super Phosphate
UNDP	=	United Nation Development Program
wt.	=	Weight
w/v	=	weight/ volume
Zn	=	Zinc
^0C	=	Degree Centigrade
%	=	Percent



Chapter 1
Introduction

Chapter 1

INTRODUCTION

Onion (*Allium cepa*) is a popular vegetable grown for its pungent bulbs and flavorful leaves. Onion is an important spice as well as vegetable crop, commercially grown in many countries of the world including Bangladesh. This is the member of the family Alliaceae.

The 23 districts of onion growing areas of the country are Faridpur, Comilla, Manikgonj, Dinajpur, Jessore, Pabna, Rajshahi, Mymensingh, Jamalpur ,Patuakali, Kisorganj, Tangail, Borisal, Bandarban, Khagracori, Sylhet, Bogra, Rangamati, Kustia, Dhaka, Chittagong and Rangpur (BBS, 2007). The highest yield 208935 thousand metric ton was in Faridpur in 78695 thousand acre of land. (BBS, 2007).

Onion has manifold uses; such as spice, vegetable, salad dressing etc. It is also used as condiments for flavoring a number of foods and medicines (Vohora *et al.*, 1974). On the other hand, raw onion is being used and given protection to human beings from sun stroke, normally is consumed green as well as in mature stages almost by every one, by different means. Recently, Bunching onion (*Allium fistulosum*) is coming up as a popular vegetable. It does not form bulbs but grows in clusters with long white stems (Benoit and Coustermans, 1987).

The onion has a great importance in earning foreign currency through export and in case of shortages onion is imported by spending huge currency. In terms of global weight of vegetable produced, nearly

28 million tons onion bulbs per annum next to tomatoes and cabbages bears importance (FAO, 1991). In Bangladesh, the production of onion is nearly 1, 43,000 tons from 34,000 hectares of land (FAO, 1993).

In Bangladesh, the production of onion is nearly 1, 50,000 metric tons from 36,800 ha of land (BBS, 2005). The national annual yield is only 4.07 t/ha (BBS, 2005). In the year 2005-06 the production of onion is nearly 768655 thousand metric tons from 285655 thousand acre of land (BBS, 2006). And in the year 2006-07 the production of onion is nearly 894255 thousand metric tons from 318085 thousand acre of land (BBS, 2007) which is quite low compared to other onion growing countries of the world.

In Bangladesh, the local varieties namely Faridpuri and Taherpuri are commonly grown. The high yielding variety, such as- BARI 1, BARI 2, and BARI 3 also are now famous for cultivation in Bangladesh. In Bangladesh, the demand of bulb onion as well as the onion seeds is increasing every year and the price of the true seeds remains fairly high in each season.

As per the world literatures, onion is attacked by about 66 diseases including 38 fungal, 10 bacterial, 6 nematode, 3 viral, 1 mycoplasmal, 1 parasitic plant and 7 miscellaneous diseases and disorders.

In case of seed production of onion in Bangladesh, several major diseases become widespread and serious enough to limit production. Among the major diseases, stemphylium blight (*Stemphylium botryosum*), purple blotch (*Alternaria porri*), downy mildew (*Peronospora destructor*) grey mold (*Botrytis sp.*) and basal/pink rot (*Fusarium sp.*) etc are the most destructive diseases, damage the crop,

& sometimes reduced seed yield up to 100%. Among those diseases stemphyllium blight commonly known as white blotch, caused by *Stemphylium botryosum*, is noted as an important disease throughout the world including Bangladesh (Bose and Som 1986; Meah and Khan, 1987 and Castellanos-Linares *et al.* 1988).

Stemphylium leaf blight of onion caused by *Stemphylium vesicarium* was first reported in Egypt (Hassan, *et al.* 2006). Stemphylium blight has been recorded on onion and garlic from many parts of the world viz , Europe, Africa, North and west America (Elis,1971). In India the disease was first recorded by Rao and pavgi (1975). The disease has now become serious in northern parts of the country. About 90% losses in seed yield were recorded (Anonymous, 1982). Severity of disease was also reported from other parts of the world.

Stemphylium blight has become more widespread in Bangladesh in the onion growing region during recent years. Disease cycle and epidemiology of Stemphylium leaf blight are similar to purple botch. The conidia have up to six transverse septa, besides several vertical septa. Wet and warm conditions favor the disease spread.

Under tropical conditions, the disease is a limiting factor for yield of onion. Onion seed production is severely affected because the disease causes breaking of floral stalks (Munoz *et al.* 1984).

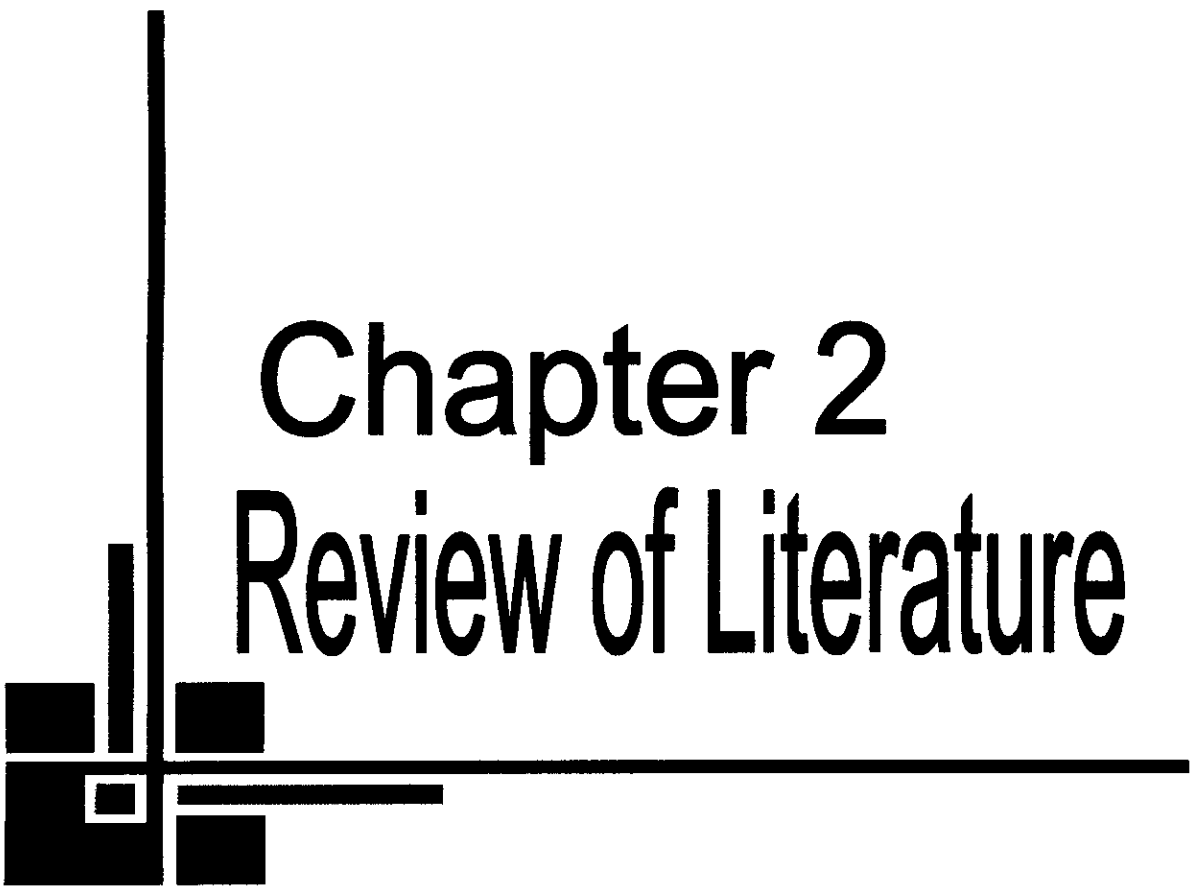
The first symptoms of disease appear on the radical leaves at 3-4 leaf stage. The disease symptoms are developed in the middle of the leaf as small, yellow to orange flecks or streaks, which soon develop into elongated, spindle shaped to ovate elongate diffusate spots surrounded by characteristic pinkish margin. These spots turn gray at the centre, later brown to dark olive brown with the development of conidiophores and conidia of the pathogen (Miller *et al.,* 1978). The spots frequently coalesce into extended patches, blighting the leaves and gradually cover entire foliage. The infection is usually confined to the leaves and do not extend down to the scales of the bulb. Similar symptoms are developed on the inflorescence stalk (peduncles) of the onion. The symptoms develop only on the dorsal side of the leaf/stalk (Gupta and Pandey,1986a).

Damage of foliage and breaking of floral stalks due to Stemphylium blight resulting failure of seed production of onion are common. Onion production in Bangladesh is gradually decreasing due to the disease (BBS, 2001). For its less production, Bangladesh are being depended to the neighboring countries like India, Barma, Pakistan for importing onion bulb and onion seed to meet up the consumption and cultivation demand.

Control of plant diseases becomes successful and economical when management approach involving several methods are employed including chemical means (Bakr and Ahamed, 1992) cultural practices (Rahman *et al.,* 1988) and use of resistant varieties (Ahmed *et al.,* 1949 and Ahamed,1986).

People globally are conscious about environmental hazards due to use of costly and toxic spray chemicals. So, to save the nature and escape polluting the environment, a judicial use of fungicide and ecofriendly alternatives of chemical fungicides are to be employed. Thus present study was undertaken to achieve the following objectives:

1. To determine the effect of plant extract and fungicide against *Stemphylium botryosum* *in vitro* and *in vivo*.
2. To determine the effect of micronutrient in controlling the Stemphylium blight of onion in the field.



Chapter 2
Review of Literature

Chapter 2

REVIEW OF LITERATURE

Stemphylium blight caused by *Stemphylium botryosum*, is a common disease of onion in Bangladesh. The disease is considered as serious one as it has profound effect in reducing the yield of onion. Now it is an acute problem in the country both for the researchers and the onion growers. Management of the disease by using chemicals, botanicals and biological control measures are being explored in many countries of the world. Literatures in relation to Stemphylium blight of onion are presented below:

2.1 Epidemiology

Verwoperd & Du Plessis (1931) reported that the pathogen *Stemphylium* spp are widespread in Asia and Europe and has been recorded previously on onion plants in South Africa.

Simmons (1969) conducted an experiment and reported that the telemorph of *Stemphylium vesicarium* as *Pleospora allii.*

Wu (1979) surveyed the seed-borne diseases of vegetables. Results of the survey on onion showed that *Alternaria porri* and *Stemphylium botryosum (Pleospora herbgrum)* reduced germination of onion seeds

Miller (1983) reported that measurements of infected leaves were taken weekly from bulb initiation to bulb maturity. They observed that the leaf damage levels were significantly lower on younger than older leaves. Leaves emerging 9, 8, 7, 6 and 5 week before bulbing maturity

required $5^1/_2$, 5, $4^1/_2$, $3^1/_2$ and $2^1/_2$ weeks respectively to reach 50% damage within 2 weeks.

Tomaz & Lima (1988) stated that *Stemphylium vesicarium* can cause severe damage especially to the onion seed crop and losses of 80-85% on onion by affecting leaves and seed stalk. This was the first report for Egypt.

Gupta *et al.*, (1996) stated that Stemphylium Blight [*Stemphylium vesicarium*] and purple blotch [*Alternaria porri*] are important diseases causing considerable damage to onion crops in India. Diseases are severe during the rainy season especially when thrips are also associated with the crop.

Lakra (1999) conducted an experiment at the Choudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India, found that numerous blotches were observed on older leaves and scapes when fortnightly dew fall was >1. 0 mm, mean maximum relative humidity > 75% and mean maximum temperature 20-30^{0}C with> 18 hr favourable temperature (10-30 ^{0}C) duration. Exposure of leaf and/or scape to wetness for 8 hr was a pre-requisite for conidial germination with increasing disease intensity, every yield component was adversely affected; the most severe infection reduced the number of scapes/ plant, the height of scape, the number of umblets/umbel, the number of seeds/ umblet, 1000-grain weight, number of seeds/ plant and the seed yield/ plant by 28.7, 74.5, 89.9, 41.7, 35.7, 95.7 and 97.3%, respectively, compared with healthy plants.

Sharma *et al.* (2002) reported that onion seed production in Punjab was reduced by 60% - 70% due to the severe downy mildew (*Peronospora destructor*) disease outbreak on seed stalks resulting in low seed recovery and poor seed health and vigor. They detected *Fusarium, Alternaria, Stemphylium* and *Aspergillus spp.* in the onion seeds of N-53, ADR, and PRR, Punjab selection, Punjab white, Punjab naroya and Punjab 48 cultivars.

Hassan and Hossein *et al.* (2004) reported that onion plants (*Allium cepa* cv. 'Giza 6') in several commercial fields in upper Egypt, exhibited symptoms of blight on the leaves and seed-stalk. Initial symptoms on leaves consisted of tip necrosis followed by small white and/or large purple spots. A fungus was consistently isolated from diseased tissue and identified as *Stemphylium vesicarium* .

2.2 Morphology of *Stemphylium botryosum*

Ellis (1971) conducted an experiment and described that the conidiophores were pale to medium brown with dark bands, smooth or minutely verruculose; conidia oblong to ovoid, densely verrucose with 1-5 transverse and several longitudinal septa, 13-21 x 25-40 µm. Ascomata forming in culture contained hyaline, bitunicate, clavate asci with 8 ascospores that were light to medium brown, ellipsoidal, verrucose with 5-7 transverse and several longitudinal septa, usually in incomplete series, 9-17 x 17-46 µm.

2.3 Chemical control

Patil *et al.*, (1976) evaluated different fungicides against leaf blight of onion. In culture media the fungus was inhibited by Kitazin, Cuman, Difolatan, Vitavax, Captan, Hinosan, Dutex, Miltox, and Aureofungin. As a prophylactic spray, Kitazin was proved to be superior to all the other fungicides tried.

Joi and Sonone (1978) evaluated nine fungicides for the control of leaf blight of onion in three experiments over three years and found that Dithane M-45 reduced the disease by 23.6% and increased the yield by 35%, whereas miltox reduced the disease by 22.6% and increased the yield by 26%.

Anonymous (1984) reported that a large number of fungicides have been tested in controlling Stemphylium blight and found that spraying of mancozeb @ 0.25% alongwith sticker triton or sandvit was found effective and economical method of control of disease, if the spraying is started just after appearance of disease

Rahman *et al.*, (1988) tested six fungicides *in-vitro* in different concentrations viz., 250 ppm, 500 ppm, 1000 ppm, 2000 ppm and 10000 ppm to study their effect on mycelial growth and sporulation of a necrotrophic pathogen like *Stemphylium botryosum* using paper disc method. They found that the diameter of the inhibition zone increased significantly and gradually with increasing concentration of Rovral 50WP and Dithane M-45. Inhibition zone developed at the lowest concentration of Rovral 50 WP was significantly wider than that of the highest concentration of the other fungicides under trial.

Rahman *et at.,* (1988) evaluated six fungicides in the field to control leaf blotch of onion caused by *Stemphylium botryosum* and *Alternaria porri.* They found that Rovral 50WP as the best fungicide to reduce severity (PDI) and increase yield to the maximum.

Barnoczki-stoilova *et al.,* (1989) conducted trails with onion cv. Makoi Brons to determine the efficacy of several treatments (2 insecticides and 4 fungicides) for pest and disease control during flowering. At the initiation of flowering (10-15% open flowers), spraying had a beneficial effect on seed yield and plant health. Spraying at full bloom (50-60% open flowers) should be avoided for harmful effect. At the end of flowering (5-10% open flowers), spraying improved seed health. Ridomil plus 50 WP (methyl + copper oxychloride) and Rovral 25 FW were the most effective fungicides.

Bakr and Ahmed (1992) studied the efficacy of four fungicides viz., Rovral 50WP (0.2%), Antracol 7OWP (0.2%), Dithane M-45. (0.2%), and Uniflow TM sulfer (0.4%), to control Stemphylium blight of lentil caused by *Stemphylium sarcinifortnae* L. Among the fungicides, Rovral 50WP (0.2%) was the best fungicides to reduce disease severity and increase yield.

Gupta *et al.,* (1992) observed that *Alternaria porri* and *Stemphylium vesicarium* cause the most important disease of onion in India. Both the diseases were successfully controlled by 4 sprays of Dithane M-45 (mancozeb) at 0.25% applied at weekly intervals.

Aveling *et al.*, (1993) reported that during surveys in the Cape Province of South Africa, *Alternaria Porri* and *Stemphylium vesicarium* were found to be very destructive seed borne pathogens of onion. Six fungicides (Anilazine, Benomyl, Carbendazim/ Flusilazole Mixture, Procymidone, Tebuconazole and Thirum) were tested for their efficacy to control the diseases on the seed and in culture. None of the treatments eradicated *Alternaria porri* and *Stemphylium vesicarium* from onion seeds.

Kamalesh *et al.*, (1993) worked with some systemic and non-systemic fungicides to control the gray leaf spot of tomato caused by *Stemphylium botryosum* for two successive years. They stated that Chlothanil was the best fungicide to control the disease and give the highest yield, which was at par with Captafol (0.2%). They further reported that systemic fungicides were less effective than non-systemic fungicides. It is also revealed that the systemic fungicides though reduced disease severity to some extent but were not at all economical as compared to non-systemic fungicides.

Jakher *et. al.*, (1994a) evaluated 5 different fungicides and 4 sprays of mancozeb @ 0.2% was found most effective followed by Zineb, Captafol and Copper oxychloride.

Srivastava *et. al.*, (1995) conducducted an experiment on control of onion diseases and reported that fortnightly spray of 0.25% Mancozeb or 0.25% Iprodione controlled stemphylium blight disease in onion seed crop. During 1992-93 and 1993-94, in Haryana, India, total failure of onion seed crop was occurred due to stemphylium blight (*Stemphylium vesicarium*) and purple blotch (*Alternaria Porri).* To overcome this

alarming situation Srivastava *et al.* (1995) conducted trials with Iprobenfos (Kitazin), Iprodione (Rovral), Fosetile (Aliette), Kavatch, Thiophanate-methyl (Topsin M), Benomyl, Metalaxyl (Ridomil) and Mancozeb. Observations on disease intensity / PDI were recorded at fortnightly intervals, just before each spray, and a total of 5 sprays were applied. They recommended that seed growers in North India should apply fortnightly sprays of 0.25% Mancozeb or 0.25% Iprodione to control onion seed diseases caused by *Stemphylium vesicarium* and *Alternaria porri.*

Gupta *et al.,* (1996 a) reported that to control Stemphylium blight of onion caused by *Stemphylium botryosum,* Indofil M-45 (0.25) as the best fungicide which was at par with Rovral 50WP (0.25). Rovral 50WP (0.25) was not as economical as Idofil M- 45 (0.25) because of its high cost. Gupta *et at. (*1996*)* and Srivastava *et al.* (1993) obtained similar results.

Gupta *et al.,* (1996 b) undertaken studies in Karnal, Haryana, India, during kharif, 1994 and 1995 to control blight of onion. Treatment comprised of 5, 4 or 3 sprays of mancozeb, chlorathalonil and fosetyl (as Aliette) starting at 40 DAP at intervals of 10 days. It was observed that 3-4 sprays of 0.25% Mancozeb at 10 days intervals starting at 50 days after transplanting reduced infection caused by *Stemphyllium vesicarium* and *Alternaria porri.* Three sprays of 0.25% kavatch at 10 days intervals starting 60 days after transplanting was also effective.

Gupta *et al.,* (1996 b) conducted an experiment on the effect of foliar spray of different fungicides on the control of Stemphylium blight diseases and yield of onion bulb and resulted that Rovral 50WP as the best fungicide to reduce PDI (percent diseases index) and increase yield to the maximum.

Gupta et. al., (1996 b) reported that among the eight fungicides (Chlorothalonil, Iprodione, Carbendazim, Triadimefon, Metalaxyl, Ziram, Dinocap and Copper oxychloride) tested and compared with mancozeb, none of the fungicide performed better than mancozeb which gave the highest cost benefit ratio also.

Gupta *et. al.,* (1996 c) reported 3- 4 sprays of 0.25% Mancozeb at 20 days intervals starting at 50 days of transplanting or 3 spray of 0.25`% chlorothalonil at 10 days intervals starting from 60 days after transplanting was effective in controlling purple blotch and Stemphylium blight disease.

Rahman *et. al.,* (2000) reported that control of leaf blight disease caused *by Alternaria porri, Colletotrichum sp., Stemphyliurn* sp., and *Cercospora* sp., singly or combined can be controlled by giving four sprays of Mancozeb © 0.3% from 45 days after transplanting.

Huq (2003) conducted an experiment on epidemiology and management of stempylium blight of lentil with seven fungicides (Rovral 50wp, Dithane M 45, Tilt 250EC, Cupravit, Macuprax, Ridojmil MZ 72 and Bavistin) and reported that, In *in vitro* condition Rovral 50wp (2000 ppm) was the most effective showing 90.6% inhibition of mycelium growth.

Anon. (2004) conducted an experiment and reported that for onion seed production six sprays of Rovral (0.2%) with Ridomil (0.2%) found promising to combat the diseases and gave more than 70% seed yield over untreated control.

Nizam (2005) conducted an experiment on the management of purple blotch complex of onion (*Alternaria porri & Stemphylium botryosum*) with five fungicides viz., Dithane M-45 (Mancozeb), Bavistin (Carbendazim), Ridomil (Metalaxyl + Mancozeb), Rovral (Iprodione), Tilt (Propiconazole) were employed in the field experiment. Bulb treatment with Dithane M-45 (0.45%) or Rovral (0.2%) followed by foliar spraying at 10 days interval minimized disease incidence & severity and increased seed yield.

Khatun (2007) conducted an experiment on management of stemphylium blight of onion through some selected treatments. A local onion variety Taherpuri was used in the experiment. Treatment of onion bulb followed by foliar spraying with eight fungicides viz., Score, Tilt-250 EC (Propiconazole), Dithane M-45 (Mancozeb), Champion, Cupravit, Ridomil MZ-72 (Metalaxyl + Mancozeb), Rovral 50 WP (Iprodione), Bavistin (Carbendazim) and one botanical treatment viz. Neem leaf extract (1:5) were employed in the experiment. Rovral (0.2%) or Dithane M-45 (0.45%) minimized disease incidence & severity and increased seed yield. The least leaf infection by *Stemphylium vesicarium* and the highest bulb yield was recorded in the Rovral and Dithane M – 45 treated plot.

2.4 Botanical Control

No literatures are available in relation to control stemphylium blight of onion through plant extracts. Thus some other literatures regarding control of other plant diseases by plant extracts are presented here.

Mohanty *et al.* (1995) investigated allelopathic control of *Phomopsis vexans*, causal agent of *Phomopsis* fruit rot of brinjal by aqueous leaf extracts of five plants. Fungal growth was inhibited to a maximum by leaf extracts of *Allamanda cathertica* (93.75%) followed by *Aegle mermelos* (85.34 %). Leaf extracts of *Catheranthus roseus*, *Polyalthia longifolia* and *Azadirachta indica* were equally effective, but that of *Ocimum sanctum* was the least effective causing 52.23% growth inhibition.

Panda *et al.* (1996) tested the efficacy of leaf extracts from *Polyalthia longifolia, Aegle mermelos, Azadirachta indica, Catheranthus roseus, Ocimum sanctum* and *Allamanda cathertica* for control of *Phomopsis* blight (caused by *Phomopsis vexans*). Leaf extracts of *Allamanda cathertica* had excellent potential as a fungus.

Khan (1999) studies the effect of plant extracts (Allamanda, Bael and Neem) for the management of *Phomopsis* blight / fruit rot of egg plant in field condition. Among the 3 plant extracts, Allamanda spray was the most effective.

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

Islam *et al.*, (2004) 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. TLC studies showed the presence of a number of compounds having very low to high polarity in garlic bulbs and allamanda leaves extracts.

2.5 Cultural Practices

Awad *et al.*, (1978) found that transplanting onion seedlings on 1 December gave the highest percentage of healthy plants and disease severity decreased significantly with decreased plant density. Applications of nitrogen fertilizer at twice the normal dose increased the severity of infection, while a double dose of calcium super phosphate reduced infection. Disease incidence decreased by adding potassium chloride at twice the normal dose.

2.6 Other information regarding the pathogen and its management

Jouan *et al.*, (1972), besides chemical treatments, treated infected plants with a spore suspension of a phylloplane fungi, *Stemphylium allii.* This raised seed yield, although control was not as effective as chemical treatments. The results of laboratory and field trials with various fungicides were inconsistent, sufficient protection was afforded by dithiocarbamate and iprodione fungicides.

Srivastava *et al.*, (1996) conducted an *in vitro* studies to determine the role of infected plant debris and soil in the perpetuation of disease and air borne spore of purple blotch (*Alternaria porri*) and Stemphylium blight (*Stemphylium vesicarium*) on onions in Harayana, India, in order to establish a forecasting system for effective control measures. The pathogens remained viable for 4 months on diseased plant debris, 3 months at soil in depths of 2.5, 5.0 and 7.5 cm and for 2 months at soil in depths of 10.0 and 15.0 cm. It was suggested that the inoculum load of *Alternaria porri* and *Stemphylium vesicarium* during ploughing of infected soil was higher during the winter.

Chapter 3

Materials and Methods

Chapter 3

MATERIALS AND METHODS

3.1. Laboratory Experiment

3.1.1 Isolation and Identification of Pathogens

Isolation and identification pathogen were made in two ways-

a) By direct observation

b) By inoculating sample tissues on Potato Dextrose Agar (PDA) medium.

a) By direct observation

The diseased leaves of onion plants were collected and kept in polythene bags and tagged (Plate 1). The samples were then taken to the laboratory. Then slides were prepared from the diseased samples, observed under microscope and identified the pathogen according to CMI Description.

b) By inoculating sample tissues on Potato Dextrose Agar (PDA) medium.

The diseased leaves were cut into pieces (5mm diameter) and surface sterilized with Hg Cl_2 (1: 1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and were placed on to acidified PDA medium in Petridish. The plates containing leaf pieces were incubated at room temperature for seven days. When the fungus grew well and sporulated, the organism was re-cultured by single spore or tip culture method to obtain pure culture. Then slides were prepared from pathogenic structures and was observed under microscope and identified with the help of relevant literature (CMI Description).(Plate 2 and Plate 3).

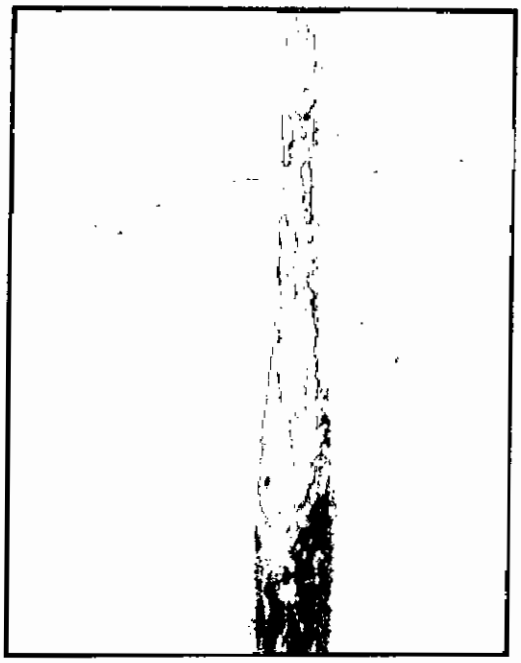


Plate 1.A stalk infected by Stemphylium blihgt

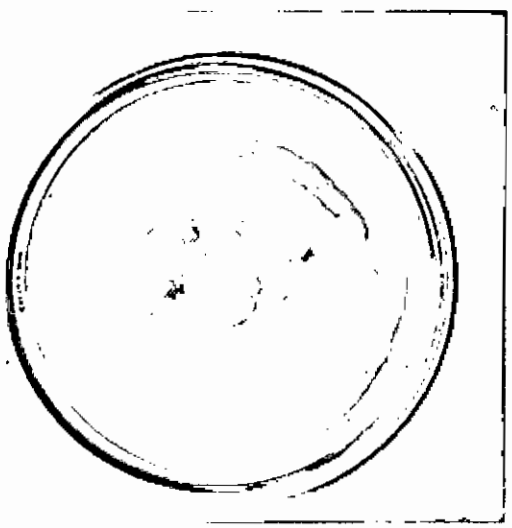


Plate 2. Pure culture of *Stemphylium botryosum*

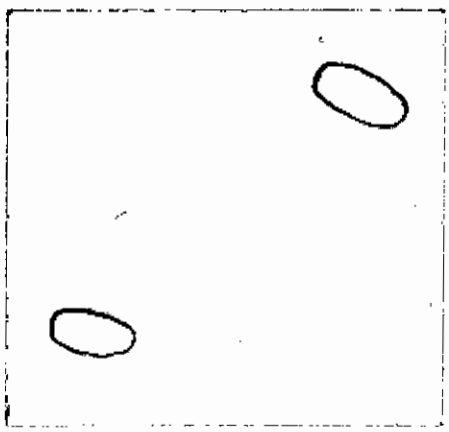


Plate 3.Conidia of *Stemphylium botryosum*

3.1.2 Treatment of the Laboratory Experiment

Treatment-1: Rovral-50wp

Treatment-2: Dithane M-45

Treatment-3: Ridomil Gold

Treatment-4: Bavistin 50wp

Treatment-5: Cupravit 50wp

Treatment-6: Neem leaf extract

Treatment-7: Allamanda leaf extract

Treatment-8: Control

3.1.3 Preparation of plant extract

The extracts were prepared by using the method of Ashrafuzzaman and Hossain, 1992. For preparation of extracts, collected leaves were weighted in an electric balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:2 (w/v) ratio 200 ml of distilled water was added with 100 g plant parts. This solution was used in laboratory trial and field spray.

3.1.4 Preparation of fungicidal solution

In laboratory, fungicide formulation was prepared by recommended dose. Each formulation was prepared by adding 1000 ml of water with required amount of fungicide viz 3 gm, 4.5gm, 2gm, 1 gm and 7 gm for Rovral-50wp, Dithane M-45, Ridomil MZ-72, Bavistin 50wp, & Cupravit 50wp, respectively.

3.1.5 In vitro test of plant extracts and fungicides against *Stmphylium botryosum*

The effectiveness of the extracts was evaluated by following method:

Cup Method

In this method, petriplates of 15 ml acidified PDA media were prepared. After solidification, three 5mm disc of the medium were scooped from three places by a disc cutter. Three drops of extract or fungicides solutions were put into each hole and the plates were stored overnight at room temperature for allowing diffusion of the extract in the medium around the hole. Next day, a 5mm culture block of *Stemphylium botryosum* was cut and placed at the middle of the PDA plate. Each treatment was replicated thrice. For control treatment, only sterile water was used instead of plant extracts. The plates were then placed at $25\pm1^{0}C$ for 15 days and the radial mycelial growth of the fungus was recorded. (Plate 4, Plate 5 and Plate 6).

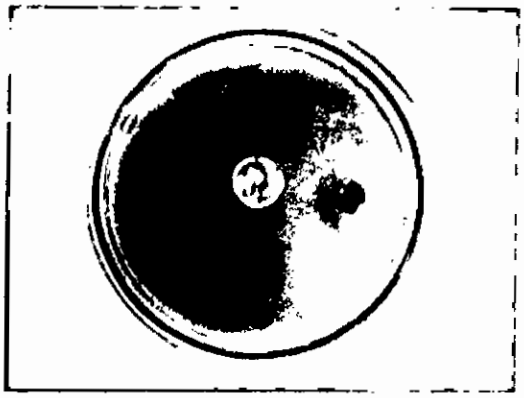


Plate 4. Radial mycelial growth of fungus against Rovral 50WP after 15days of incubation.

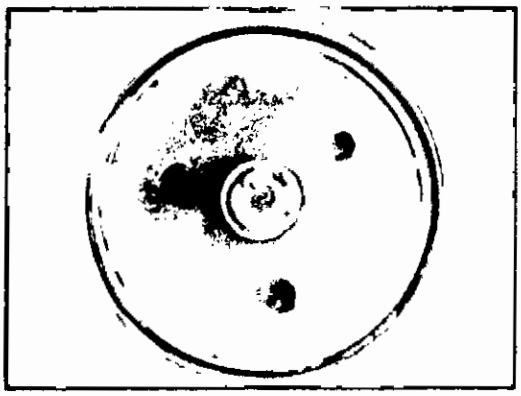


Plate 5. Radial mycelial growth of fungus against Dithane M-45 after 15 days of incubation.

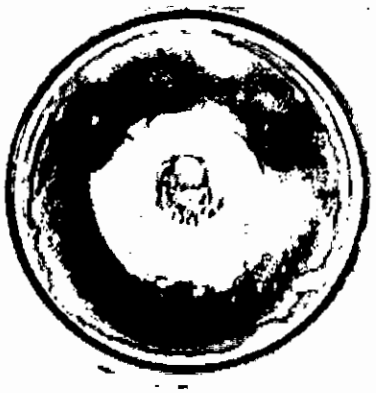


Plate 6. Radial mycelial growth of fungus against allamanda leaf extract after 15 days of incubation.

3.2 Field experiment

3.2.1 Experimental Site

The experiment was conducted at the Research farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, during winter season from 1st November ' 2007 to March 2008 for the management of Stemphylium Blight of onion through chemicals, plant extracts and micronutriants.

3.2.2 Climate of Experimental Site

The experimental site was located at 23077' N latitude and 9003' E longitude with an elevation of 1.0 meter from sea level (Fig. 1). The soil of the experimental site belongs to Tejgaon series under the Agro-ecological zone, Madhupur Tract (AEZ-28). The experimental area was under the sub tropical climate which characterized with the comparatively high rainfall, high humidity, high temperature, relatively long day during April to September and scantly rainfall, low humidity, low temperature and short day period during October to March. The later period is favorable for onion cultivation. The soil of the experimental field was clay loam.

3.3.3 Soil type

The soil of the experimental plots was a medium high land with clay loam in texture belonging to Modhupur tract under the Agro-Ecological Zone (AEZ) 28.

The information about AEZ 28 is given below:

Land Type	Medium high land
General soil type	**Non-Calcareous Darkgray floodplain soil**
Soil series	**Tejgaon**
Topography	**Upland**
Elevation	**8.45 m from the sea level**
Location	**SAU Farm, Dhaka.**
Field level	**Above flood level**
Drainage	**Fairly good**
Firmness (consistency)	**Compact to friable when dry**

Fig.1. Map showing the experimental site under study

3.2.4 Preparation of Soil

The soil of the experimental plot was clay loam. Land was prepared using a disc plough and harrow. Ploughed soil was brought into desirable tilth condition by four operations of ploughing and harrowing with country plough and ladder. Then a wooden hammer broke the soil clods. After ploughing, the field was left to nature for 10 days for sun and nature to work upon. After 10 days when the weeds were sufficiently dried off, the residues and stubbles of the previous crops and weeds were removed, fertilizers applied and the land was prepared finally after a light irrigation to ensure the optimum condition of the soil moisture for transplanting.

3.2.5 Fertility Status of the Field Soil

The soil of experimental site was analyzed in Soil Resource Development Institute (SRDI), Dhaka and the physical and chemical properties of the experimental field are shown in Table 1.

Table 1. Physical and chemical properties of the experimental soil

Soil properties	Value
1. Soil texture	clay loam
2. Soil pH	5.8
3. Organic matter (%)	1.35
4. Total N (%)	0.08
5. C : N ratio	10 : 1
6. Available P (ppm)	35
7. Exchangable K (me/100g soil)	0.18
8. Available S (ppm)	40

3.2.6 Fertilizer Application

Soil was fertilized with Nitrogen (in the form of urea), Phosphorus (in the form of Triple Super Phosphate -TSP), Potassium (in the form of Muriate of Potash- MP), Boric acid Zypsum and Cowdung was applied during final land preparation as per treatment necessity. Whole quantity of TSP, Zypsum, Boric acid , half MP and one third of urea was applied at final land preparation. The rest half urea and half MP was applied 30 days after bulb splanting. And two third of the urea was applied 30 days & 60 days respectively. Cow dung was applied at the rate of 10 tons/ ha. Doses of fertilizer were used considering existing nutrient of the field soil mentioned below as BARC fertilizer recommendation guide.

Fertilizer	**Doses**
Cow dung	10 tons/ha
TSP	415 Kg/ ha
Mp	170 Kg/ha
Urea	320 Kg/ha
Gypsum	100 Kg/ha
ZnO	5 kg / ha
Boric acid	5 kg / ha

3.2.7 Treatment of the Field Experiment

Nine treatments were evaluated in controlling Stemphyllium blight of onion and one untreated control was done to compare. The treatments were-

- **Treatments** : 9

Treatment-1 (T1) : Rovral 50WP + Gypsum + ZnO + Boric acid

Treatment-2 (T2) : Dithane M-45 + Gypsum + ZnO + Boric acid

Treatment-3 (T3) : Ridomil Gold + Gypsum + ZnO + Boric acid

Treatment-4 (T4) : Bavistin 50 WP + Gypsum + ZnO + Boric acid

Treatment-5 (T5) : Cupravit + Gypsum + ZnO + Boric acid

Treatment-6 (T6) : Neem leaf extract + Gypsum + ZnO + Boric acid

Treatment-7 (T7) : Allamanda extract + Gypsum + ZnO + Boric acid

Treatment-8 (T8) : Gypsum + ZnO + Boric acid (micronutrient)

Treatment-9 (T9) : Untreated (Control)

Treatment applied;

1. Soil treated with normal fertilization (Cowdung + Urea + TSP + MP) + Micronutrients (Gypsum + ZnO + Boric acid) and bulb treated with Rovral 50WP followed by foliar spraying
2. Soil treated with normal fertilization + Micronutrients and bulb treated with Dithane M-45 (0.45%) followed by foliar spraying
3. Soil treated with normal fertilization + Micronutrients and bulb treated with Ridomil MZ-72 (0.2%) followed by foliar spraying
4. Soil treated with normal fertilization + Micronutrients and bulb treated with Bavistin (0.1%) followed by foliar spraying
5. Soil treated with normal fertilization + Micronutrients and bulb treated with Cupravit (0.7%) followed by foliar spraying
6. Soil treated with normal fertilization + Micronutrients and bulb treated with Neem leaf extract followed by foliar spraying

7. Soil treated with normal fertilization + Micronutrients and bulb treated with Allamanda leaf extract followed by foliar spraying
8. Soil treated with normal fertilization + Micronutrients and no foliar spraying.
9. Soil treated with normal fertilization followed by no foliar spraying.

3.2.8. Chemical and Botanical Ingredients

a) Chemical Ingredients

The fungicides used in the experiment were presented below with their active ingredient (Table-2).

Table 2 : Selected fungicides and their active ingredient used in the experiment.

Fungicide	Active ingredient
1. Cupravit	50% Copperoxychloride
2. Rovral 50 WP	Iprodione 50%
3. Dithane M-45	Mancozeb 80% + manganese ethylene
4. Ridomil MZ-72	Metalaxyl 80g a.i/Kg+ Mamcozeb 640 g a.i/ Kg
5. Bavistin	Carbendazin 50%

b) Botanical ingredients

1. Neem leaf extract (1:2) was used as botanical treatment.
2. Allamanda leaf extract (1:2) was used as botanical treatment.

3.2.9 Experimental Design

The experimental units arranged in Randomized Complete Block Design (RCBD) with three (3) replications. The unit plot size was 2m x 1.5m. One block is separated from other by 0.75 m and within a block each unit plot separated from each other by 0.5 m.

3.2.10 Variety Used

BARI Piaj-1 onion variety was used in this experiment.The onion bulbs were collected from BARI (Banglades Agricultural Research Institute), Joydebpur, Gazipur.

3.2.11 Unit plot and spacing

The unit plot size was 2 m x 1.5 m and experimental field size was (20 x 10) m. The row to row and bulb to bulb distance was maintained 25 cm and 15 cm, respectively.

3.2.12 Date of sowing

Bulbs were planted on 1st November, 2007

3.2.13 Gap filling

The dead or sick bulbs were replaced by healthy bulbs within 15 days after transplanting. The damaged plants in the subsequent period were also replaced by the bulbs maintained as border plants. Gap filling was done on 15th november, 2008.

3.2.14. Collection of Treatment materials

Dithane M-45, Cupravit, Ridomil MZ-72, Rovral 50 WP, and Bavistin were procured from the farm store of Sher-e-Bangla Agricultural University. Neem leaf extract & Allamanda leaf extract were obtained from Neem leaves Allamanda leaves respectively, collected from Sher-e-Bangla Agricultural University campus.

3.2.15 Preparation of Chemical Suspension/ Solution

At recommended doses suspension/solution of fungicides were prepared by mixing thoroughly with requisite quantity of chemical with normal clean water. It was required 45g Dithane M-45/ liter water, 7g Cupravit/ liter water, 2 g Ridomil MZ-72/ liter water, 2 g Rovral 50 WP/ liter water, and 1g of Bavistin / liter water for preparation respective solution.

3.2.16 Bulb treatment

According to the spacing of sowing bulb in the experiment, 234 bulbs were required for each treatment 3 unit plots. So, 234 bulbs were treated for each of the treatment with the respective fungicidal solution by dipping the bulbs in the suspension for half an hour. The bulbs were then drained off and sown in the field without delay.

3.2.17 Inter cultural operations

3.2.17.1 Irrigation

Irrigation was given as per requirement of the soil with regular intervals. First irrigation was given just after planting. Then irrigation was done with regular interval and continued up to harvesting of the crops. Water cane with perforated mouth piece was used for soft discharged of water. Irrigation was followed the each weeding of the crops.

3.2.17.2. Weeding and mulching

Weeding and mulching were done as and when required to keep the crop free from weeds and for better soil aeration and conservation of soil moisture. The common weed were *Cynodon dactylon* L (Durba grass), *Cyperus rotundus* L. (Mutha) and *Chenopodum album* L. (Bathua). Weeding was done carefully keeping the delicate young plants undisturbed.

3.2.17.3. Application of Insecticide

The field was infected by cutwarm and mites. In order to overcome those problem, Diazpum and Actara was applied in recommended dose.

3.2.17.4. Application of Treatments

Spraying was started from 14 days after planting. Totally 10 spraying were done at 7 days intervals with a hand sprayer. One liter of suspension of each spray material was used to spray the plants under each treatment. Control plots were spraying with plain water only.

3.2.18. Data Collection

3.2.18. 1. *In vitro* data collection

In lab condition data were collected on mycelium growth. Radial growth of mycelium was measured by mm scale. Data were recorded three days of interval. Data were recorded for 15 days from first inoculation (by cup method).

3.2.18. 2. Data Collection in the field

Two plants were selected randomly from each row of the unit plot and tagged, thus every row was considered for data collection. In all, 10 plants were considered for data collection for each unit plot. Data collection was started after the onset of the disease and continued up to maturity with 7 days intervals. Data were collected on the following parameters.

3.2.18. 2.1 Percent infected plant

Number of plant infected per unit plot were recorded and used for calculation of disease incidence. The leaf with characteristic spot or blighted tip was denoted as diseased leaf.

3.2.18. 2.2 Calculation of disease incidence

The percent disease incidence was calculated using the following formula.

$$\text{Disease incidence} = \frac{\text{No. of infected plant}}{\text{Total no. of inspected plants in unit plot}} \times 100$$

3.2.18. 2.3 Percent leaf infection per treatment

Numbers of leaf infected per plant were recorded and used for calculation of percent leaf infection The leaf with characteristic spot of blotch or blighted tip was denoted as diseased leaf.

$$\% \text{ Leaf infection} = \frac{\text{Number of infected leaf x 100}}{\text{Total number of observation (leaf)}}$$

3.2.18. 2. 4 Leaf area diseased (LAD)

Leaf area diseased of the selected plants under each treatment were measured and recorded by conversion to percentage. Mean percentage of leaf area diseased was calculated by dividing number of total observation and used for PDI (Percent disease index) estimation. (Plate 7)

3.2.18.2. 5 Stalk area diseased (SAD) per plant

Stalk area diseased of the selected plants under each treatment were measured and recorded by conversion to percentage. Mean percentage of Stalk area diseased was calculated by dividing number of total observation and used for PDI (Percent disease index) estimation.(Plate 8)

3.2.18. 2.6. Estimation of PDI (Percent disease index)

The following diseased scoring scale "0 – 5" scale was used to estimate the disease severity (PDI) for each unit plot under each treatment. (Plate 7 and Plate 8)

0 = no disease symptoms

1 = a few spots towards the tip, covering less than 10% leaf area

2 = several white patches covering less than 20% leaf area

3 = several patches covering up to 40% leaf area

4 = long streaks covering up to 75% leaf area or breaking of Leaves/ stems from the centre

5 = complete drying of the leaves/ stems or breaking of the leaves/stems from the base.

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

$$PDI = \frac{\text{Total sum of numerical ratings}}{\text{Number of observation} \times \text{Maximum disease rating grade in the scale}} \times 100$$

Plate 7. Infected leaf of onion showing 0-5 scale.

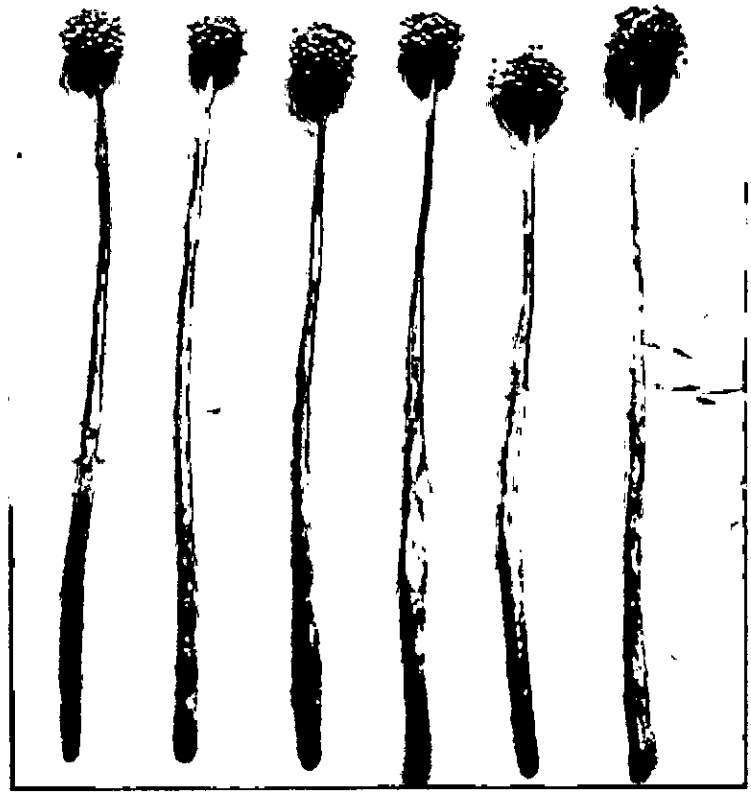


Plate 8. Infected stalk of onion showing 0-5 scale.

3.2.18. 2.7 Plant height

Plant height of selected plants under each treatment were measured and recorded by conversion to percentage. Data were collected in every 7 days.

3.2.18. 2.8 Umbel per hill

Umbel number was recorded for each unit plot. For every treatment data were recorded separately.

3.2.18. 2.9 Seed per umbel

For every treatment individual umbel and seeds were counted.

3.2.18. 2.10. Weight of thousand seeds

After drying of seed 1000 seed weight was recorded by digital balance (0.001 g) for each treatment.

3.2.18. 2.11 Yield per plot

Seed yield of onion per plot were recorded individually by digital balance (0.001 g) for each treatment.

3.2.19 Analysis of Data

Data were analyzed statistically using MSTAT Computer Program. Data were transformed, following Arcsine transformation whenever necessary. Means were compared using Duncan's Multiple Range Test (DMRT).

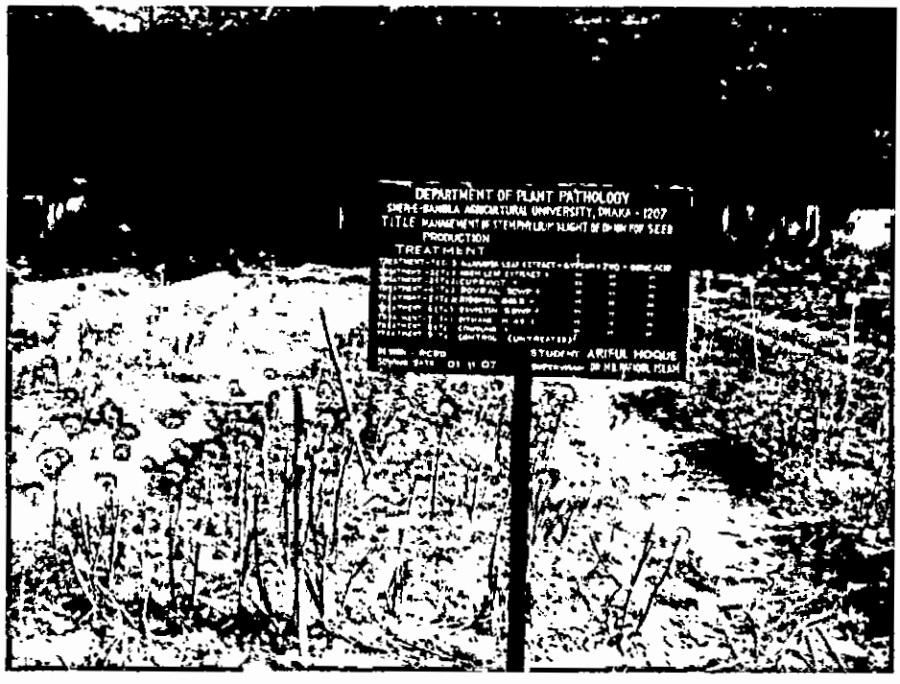


Plate 9. Showing the field view of the whole experimental plot of onion.

Plate 10. Showing onion plants affected by Stemphylium blight under treatment T_9 (control).

Plate11. Showing healthy onion plants treated with Rovral (T_1)

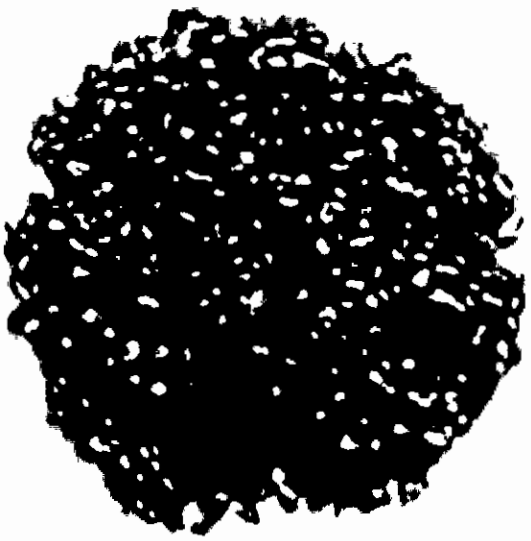
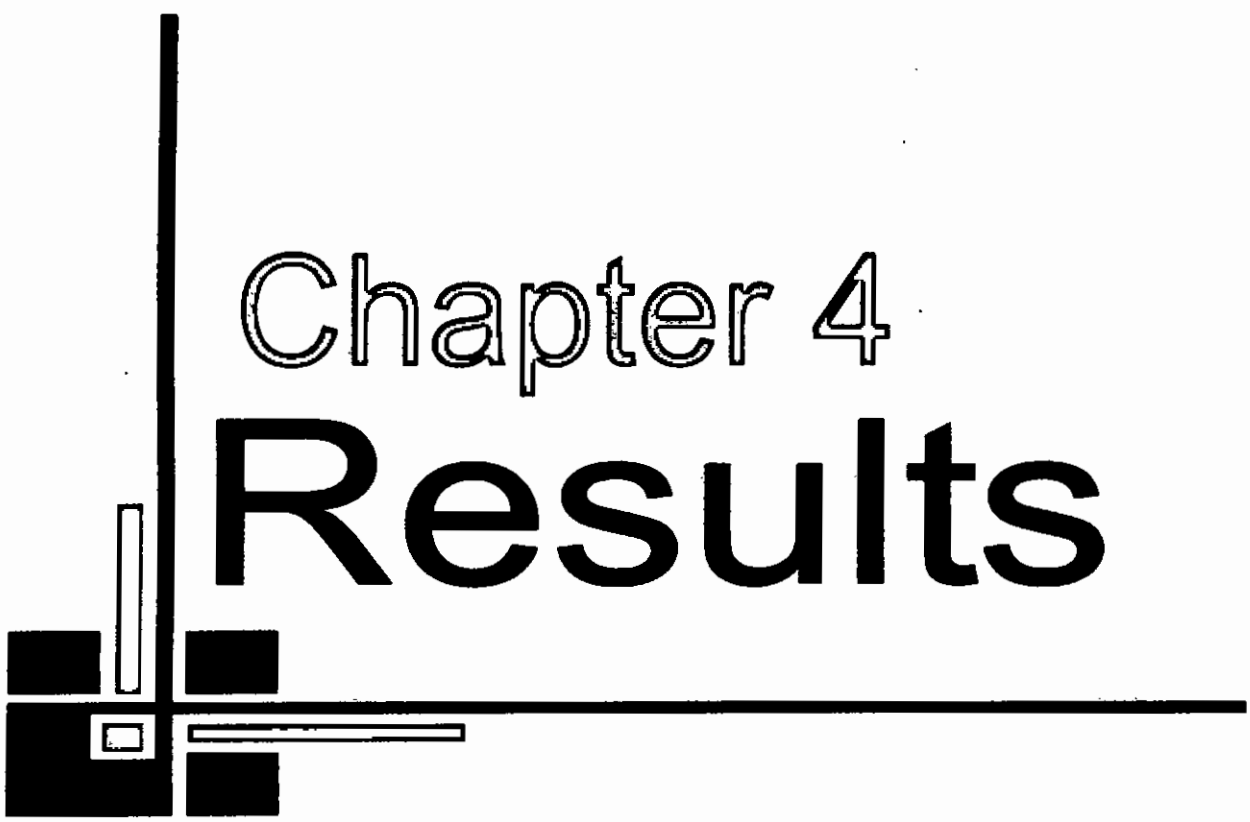


Plate12. Thousand seeds collected from T_1 treatment (Rovral 50 WP)



Chapter 4
Results

Chapter 4

RESULTS

This Chapter includes the experimental results. Effect of the treatments in controlling Stemphylium blight of onion caused by *Stemphylium botryosum* was assessed *in vitro* and *in vivo* method. The results were compiled based on the inhibition of radial mycelium growth, percent plant infection, percent leaf infection, percent leaf area diseased (% LAD), percent disease index (PDI), plant height, percent stalk area diseased (%SAD), percent disease index (Stalk PDI), umbel per plant, 1000 seed weight, seed per umbel and yield per treatment. (Table. 3-8, plates 9-12).

4.1. Laboratory experiment

4.1.1 Efficacy of fungicides and botanicals on radial mycelia growth of *Stemphylium botryosum in* vitro (Poisoned food technique, Cup method)

Efficacy of fungicides and plant extracts on mycelial growth of *Stemphylium botryosum* is shown in (Table 3). Fungicides have profound effect on reduction of mycelial growth of the fungus. Tested fungicides significantly reduced radial mycelial growth of the fungus. Radial mycelial growth for all the tested fungicides & botanicals ranged from 1.70 cm to 6.50 cm recorded after incubation of 15 days. The lowest radial mycelium growth (0.60cm, 0.90 cm, 1.20 cm, 1.50 cm and 1.70 cm) of *Stemphylium botryosum* was recorded in case of Rovral 50WP at 3 day, 6 day, 9 day , 12 day and 15 day respectively. The performance of Rovral 50WP in reduction of radial mycelium growth was the best followed by Dithane M-45, Ridomil gold, Bavistin 50 WP and Cupravit irrespective of days after inoculation. The highest radial mycelium growth (6.50 cm) was recorded in untreated control preceded by allamanda leaf extract (5.60 cm) and Neem leaf extract (5.10

cm) at 15 days. Rorval 50 WP found promising in reducing the growth of the fungus in the laboratory followed by Dithane M-45 (Table 3).

Table 3. Efficacy of fungicides and botanicals on radial mycelia growth of *Stemphylium botryosum in vitro.*

Treatment	Radial Mycelium Growth (cm)				
	3 day	**6 day**	**9 day**	**12 day**	**15 day**
T1= Rovral 50WP	0.60 e	0.90 f	1.20 g	1.50 h	1.70 g
T2= Dithane M-45	0.70 e	1.30 e	1.60 f	1.70 g	2.10 f
T3= Ridomil Gold	0.90 d	1.70 d	2.50 e	3.10 f	3.60 e
T4= Bavistin	1.10 c	2.40 c	3.50 e	4.20 e	4.50 d
T5= Cupravit	1.20 bc	2.50 c	3.70 c	4.50 d	5.0 c
T6=Neem leaf extract	1.30 b	2.50 c	3.90 b	4.70 c	5.10 c
T7=Allamanda leaf extract	1.60 b	2.90 b	4.20 a	5.033 b	5.60 b
T8= Control	1.70 a	3.10 a	4.30 a	5.60 a	6.50 a
CV(%)	8.87	4.05	3.07	2.07	2.48
LSD(0.05)	0.1751	1.1566	0.1661	0.1356	0.1837

Figure in column, having same letter(s) do not differ significantly at 5% level of significance

4.2 Field Experiment

4.2.1 Percent (%) Plant Infection

The effects of the treatments on plant infection differed significantly among themselves with some extents (Table 4 and Plate11). At 28 Days after Transplanting (DAT) the lowest plant infection (8.310 %) was observed in the plot spraying with Rovral 50WP followed by Dithane M-45 (8.791 %), Ridomil Gold (11.52 %), Bavistin 50WP (16.30 %), Cupravit 50WP (17.82 %) and Neem extract (20.14 %). The highest plant infection (27.50 %) was recorded in control treatment preceded by treatment-8 (micronutrient) (25.11 %), Allamanda leaf extract (21.50%) and Neem leaf extract (20.14 %).

Further at 35 DAT, 42 DAT, 49 DAT, 56 DAT and 63 DAT the trend of plant infection was more or less similar with that of plant infection observed at 28 DAT. The lowest plant infection was recorded in case of spraying with Rovral 50WP that were 11.12 % , 17.44 % , 20.65 %, 25.84 % and 35.24 % at 35 DAT, 42 DAT, 49 DAT, 56 DAT and 63 DAT, respectively followed by Dithane M- 45. Similarly the highest plant infection was recorded in control plot that were 39.67 % , 49.23 %, 55.83 %, 77.48 % and 100.0 %, respectively at 35 DAT, 42 DAT, 49 DAT, 56 DAT and 63 DAT (Plate 10). It was noted that the plant infection was gradually increased with the increase of the age of the plant. But the increasing rate of plant infection of the treated plot with fungicides or plant extracts was quiet slow then the plots, those were not treated with fungicides or plant extracts.

Table 4. Effect of fungicides and plant extracts on % plant infection of Stemphylium blight of onion at different days after transplanting (DAT)

Treatment	%Plant infection					
	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT	63 DAT
T1	8.310 f	11.12 g	17.44 f	20.65 i	25.84 g	35.24 f
T2	8.791 f	16.54 f	20.37 f	25.09 h	31.48 f	37.42 f
T3	11.52 e	19.02 ef	25.05 e	31.52 g	39.52 e	50.09 e
T4	16.30 d	20.92 e	29.95 d	36.18 f	41.58 e	49.11 e
T5	17.82 d	31.12 c	37.82 c	43.02 e	52.61 d	59.96 d
T6	20.14 c	28.14 d	35.35 c	45.58 d	52.36 d	60.35 d
T7	21.50 c	33.31bc	40.96 b	48.20 c	57.27 c	63.34 c
T8	25.11 b	35.61 b	47.88 a	52.92 b	60.11 b	66.05 b
T9	27.50 a	39.67 a	49.23 a	55.83 a	77.48 a	100.0 a
CV(%)	5.85	5.69	5.18	3.09	2.65	2.83
LSD(0.05)	1.766	2.575	3.028	2.133	2.185	2.688

Figure in column, having same letter(s) do not differ significantly at 5% level of significance

Here,

T1= Rovral 50WP + micronutrient
T2= Dithane M45+ micronutrient
T3= Ridomil Gold + micronutrient
T4= Bavistin + micronutrient
T5= Cupravit + micronutrient
T6= Neem leaf extract + micronutrient
T7=Allamenda leaf extract + micronutrient
T8= Micronutrient
T9=Control

4.2.2 Percent (%) leaf infection

Data recorded on percent leaf infection as affected by the application of different fungicides, plant extracts and micronutrients were summarized and presented in (Table 5). The effects of different treatments recorded at different days after transplanting (DAT) differed significantly as compared to control. The results showed that the spraying of Rovral 50WP gave the lowest leaf infection irrespective of different days after transplanting that were 4.19 % , 11.86 % , 14.16 %, 23.37 %, 28.11 % and 32.78 % , respectively at 28 DAT, 35 DAT, 42 DAT, 49 DAT, 56 DAT and 63 DAT followed by Dithane M-45. The highest leaf infection was recorded in control treatment which were 27.14 % , 41.20 % , 49.26 %, 60.79 %, 81.67 % and 100.00 % , respectively at 28 DAT, 35 DAT, 42 DAT, 49 DAT, 56 DAT and 63 DAT preceded by treatment-8 (micronutrient), Allamanda leaf extract and Neem leaf extract. It was noted that the percent leaf infection was gradually increased with the age of the crop and increasing rate was much slower in Rovral 50WP and Dithane M-45 treated plot compared to control.

Table 5. Effect of fungicides and plant extracts on % leaf infection of Stemphylium blight of onion at different days after transplanting (DAT)

Treatment	% Leaf Infection					
	28 DAT	**35 DAT**	**42 DAT**	**49 DAT**	**56 DAT**	**63 DAT**
T1	**4.19 e**	**11.86 g**	**14.16 g**	**23.37 d**	**28.11 f**	**32.78 g**
T2	5.49 e	13.56 f	19.52 f	32.91 f	35.42 ef	37.15 f
T3	16.14 c	21.56 e	24.48 e	35.46 ef	43.11 cd	47.01 e
T4	12.83 d	22.87 e	28.53 d	38.25 e	40.00 de	48.91 e
T5	14.01 cd	26.69 d	32.64 c	48.64 d	50.35 bc	57.67 d
T6	21.50 b	33.15 c	35.15 bc	52.01 c	51.48 bc	58.26 cd
T7	22.53 b	34.21 c	36.83 b	50.77cd	58.65 ab	60.61 c
T8	22.20 b	36.53 b	47.10 a	56.94	58.43 ab	66.21 b
T9	27.14 a	41.20 a	49.26 a	60.79 a	81.67 a	100.00a
CV(%)	9.77	2.91	5.20	13.29	3.53	2.77
LSD(0.05)	2.744	1.354	2.878	2.868	10.20	2.552

Figure in column, having same letter(s) do not differ significantly at 5 % level of significance

Here,

T1= Rovral 50WP + micronutrient
T2= Dithane M45+ micronutrient
T3= Ridomil Gold + micronutrient
T4= Bavistin + micronutrient
T5= Cupravit + micronutrient
T6= Neem leaf extract + micronutrient
T7= Allamanda leaf extract + micronutrient
T8= Micronutrient
T9= Control

4.2.3 Percent Disease Index (PDI) of Leaf

The experiment results showed that there were significant differences among the effect of fungicides and plant extracts on Percent Disease Index (PDI-leaf) of Stemphylium blight of onion compared to control (Table 6). On the basis of the effectiveness of the fungicides and plant extracts in reducing PDI-leaf of Stemphylium blight, Rovral 50WP showed the highest performance followed by Dithane M-45, Ridomil Gold, Bavistin 50WP, Cupravit. The results showed that at 28 DAT, the lowest PDI-leaf (1.197 %) was found in treatment T_1 (Rovral 50WP), which was statistically same as T_2 (Dithane M-45) and T_3 (Ridomil Gold). Similarly the lowest PDI-leaf were recorded against Stemphylium blight in case of Rovral 50WP followed by Dithane M-45, Ridomil Gold, Bavistin 50WP and Cupravit at 35 DAT , 42 DAT, 49 DAT, 56DAT and 60 DAT . The highest PDI-leaf was observed in control treatment, where only plain water was sprayed, preceded by treatment-8 (micronutrient), Allamanda leaf extract and Neem leaf extract. The result showed that with increasing age of onion the disease index was increased. But in every case, control treatment showed the highest disease severity. Between two plant extract, Neem extract showed better performance as compared to Allamanda extract for reducing disease severity with some extent. Among all the treatments, Rovral 50WP was the best for reducing Percent Disease Index (PDI-leaf) followed by Dithane M-45 (Table 6).

Table 6. Effect of fungicides, plant extracts and micronutrients on the severity of stemphylium blight disease of onion

Treatment	Percent disease index (PDI)					
	28 DAT	35 DAT	42 DAT	49 DAT	56DAT	63 DAT
T1	1.19 f	2.56 h	3.89 i	5.22 f	6.49 i	8.78 i
T2	1.520 f	3.97 g	5.630 h	7.880ef	10.86 h	14.38 h
T3	1.707 ef	4.01 g	6.820 g	11.00 de	14.34 g	26.26 g
T4	2.247 e	5.570 f	10.78 f	14.27 cd	19.85 f	30.37 f
T5	4.547 d	7.630 e	11.61 e	16.56 cd	20.37 e	33.78 e
T6	7.928 c	11.07 d	14.25 d	20.63 bc	28.38 d	36.26 d
T7	8.237 c	13.56 c	18.61 c	24.63 ab	34.64 c	41.76 c
T8	8.890 b	15.35 b	20.65 b	26.46 ab	39.76 b	45.60 b
T9	9.790 a	16.27 a	23.29 a	28.39a	42.74a	58.38 a
CV (%)	7.74	3.74	2.59	22.32	4.11	0.23
LSD(0.05)	0.6858	0.5767	0.5767	6.655	0.17	0.13

Figure in column, having same letter(s) do not differ significantly at 5 % level of significance.

Here,

T1= Rovral 50WP + micronutrient
T2= Dithane M45+ micronutrient
T3= Ridomil Gold + micronutrient
T4= Bavistin + micronutrient
T5= Cupravit + micronutrient
T6= Neem leaf extract + micronutrient
T7= Allamanda leaf extract + micronutrient
T8= Micronutrient
T9= Control

4.2.4 Plant height

Results showed that The effect of fungicides and plant extracts on plant height of onion was slightly different among the treatments and that ranged from 60.47 cm to 35.50 cm (Table 7). The highest plant height (60.47 cm) was recorded in plants where Rovral 50WP was applied, followed by Dithane M-45 (55.47cm), Ridomil Gold (51.40 cm), Bavistin 50WP (48.50 cm) and Cupravit (46.50cm). Shortest plant (35.50 cm) was found in control treatment, where plain only water was sprayed. The plant height for application of Neem leaf extract (43.60 cm), Allamanda leaf extract (41.60 cm) and Treatment-8 (38.50 cm) did not show any significant effect on plant height compared to control (35.50 cm).

4.2.5 Percent Disease Index (PDI) of Stalk

Results obtained from the experiment showed that there were significant differences among the effect of treatments on Percent Disease Index (PDI-stalk) of Stemphylium blight of onion compared to control (Table 7). The highest PDI- stalk was observed in control treatment (38.40) where only plain water was sprayed, preceded by the Treatment-8, Allamanda leaf extract, Neem leaf extract. Among the fungicides and plant extracts in reducing PDI of Stemphylium blight, Rovral 50WP showed the highest performance (9.63%) which was statistically similar with Dithane M-45 (10.97 %) and followed by Ridomil Gold (19.43%), Bavistin 50WP (22.98 %) and Cupravit (28.28%). Between two plant extract, Neem extract (30.31 %) showed better performance as compared to Allamanda extract (34.80 %) for reducing disease severity. Among all the treatments, Rovral 50WP was the best for reducing Percent Disease Index (PDI- stalk) followed by Dithane M-45 (Table 7).

Table7. Effect of fungicides and plant extracts on plant height and disease severity of stalk.

Treatment	Plant height (cm)	Percent disease index (PDI) of Stalk
T1	60.47 a	9.630 f
T2	55.47 b	10.97 f
T3	51.40 c	19.43 e
T4	48.50 cd	22.98 d
T5	46.50 de	28.28 c
T6	43.60 ef	30.31 c
T7	41.60 fg	34.80 b
T8	38.50 gh	36.36 ab
T9	35.50 h	38.40 a
CV(%)	4.44	4.96
LSD(0.05)	3.601	2.204

Figure in column, having same letter(s) do not differ significantly at 5 % level of significance.

Here,

T1= Rovral 50WP + micronutrient

T2= Dithane M45+ micronutrient

T3= Ridomil Gold + micronutrient

T4= Bavistin + micronutrient

T5= Cupravit + micronutrient

T6= Neem leaf extract + micronutrient

T7= Allamanda leaf extract + micronutrient

T8= Micronutrient

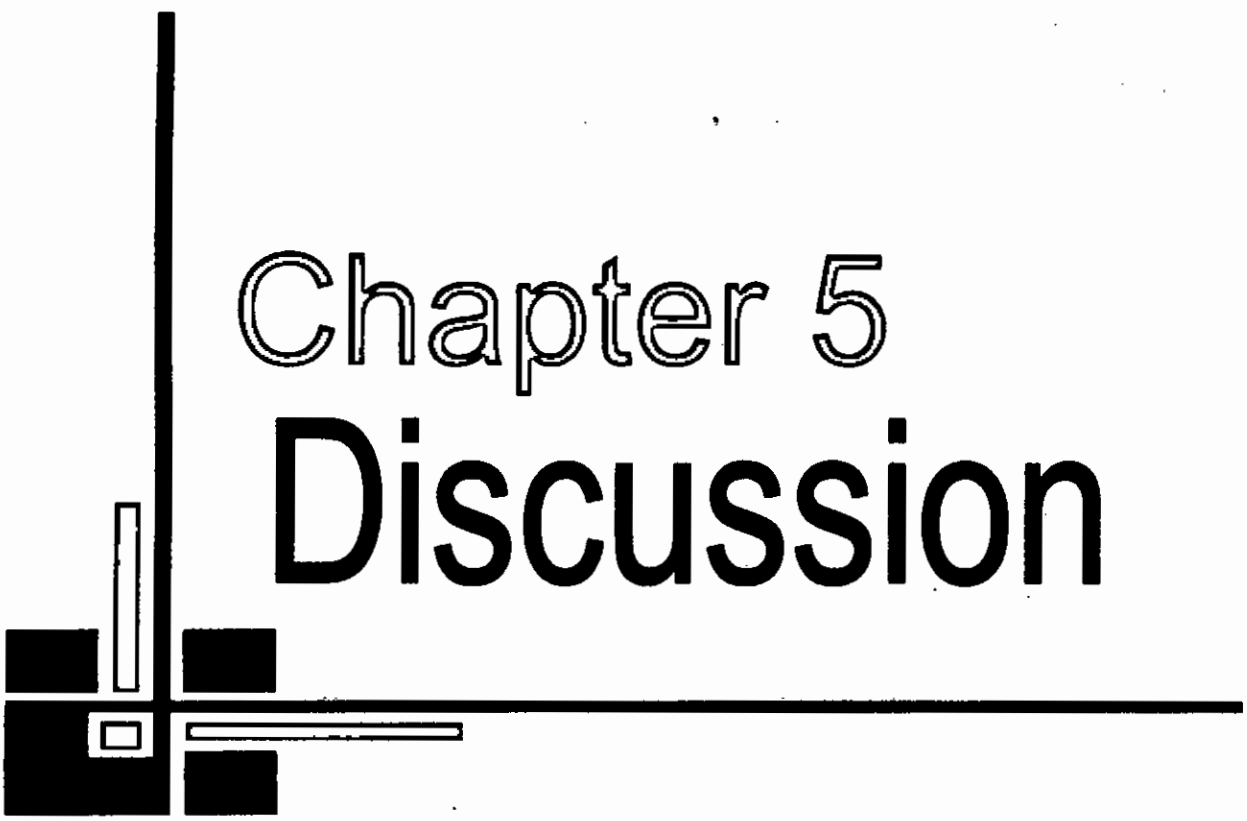
T9= Control

4.2.6. Umbel per hill

From the result it was revealed that the effect of fungicides and plant extracts on umbel number of onion was different among the treatments and that ranged from 1.800 to 1.030 (Table 8). The highest umbel number (1.80) was recorded in plants where Rovral 50WP was applied followed by Dithane M-45 (1.730), Ridomil Gold (1.60), Bavistin 50WP (1.55) and Cupravit (1.50). The lowest umbel number (1.03) was found in control treatment preceded by treatment-8 (micronutrient) (1.080), Allamanda leaf extract (1.28) and Neem leaf extract (1.41).

4.2.7. Seed per umbel

Number of seeds per umbel differed significantly based on the effect of fungicides and plant extracts. The highest seed number per umbel was recorded in case of Rovral 50WP (540.0) which is statistically similar with Dithane M-45(509.7) and followed by Ridomil Gold (490.0), Bavistin 50WP (480.0) and Cupravit (450.0). The lowest seed number per umbel (348.0) was found in control treatment preceded by Treatment-8 (370.0), Allamanda leaf extract (400.0) and Neem leaf extract (420.0) (Table 8).



Chapter 5
Discussion

Chapter 5
DISCUSSIONS

This Chapter includes the discussions of experimental results. Effect of the treatments in controlling white blotch of onion caused by *Stemphylium botryosum* was assessed based on the result of laboratory experiment and field experiment. Discussions on laboratory and field experiment are presented in this chapter.

5.1. Laboratory experiment

Among the fungicides and plant extracts assayed in the laboratory showed significant effect in reducing radial mycelial growth of *Stemphylium botryosum.* It has been also found that Rovral 50WP and Dithane M-45 have strong effect to inhibit mycelium growth of *Stemphylium botryosum* in culture media. The present findings were well supported by the reports of Rahman *et al.* (1988) and Huq (2003). Rahman *et al.* (1988) while conducting an in vitro experiment with 6 fungicides reported that Rovral 50WP and Dithane M-45 produced the largest inhibition zone of mycelium at lower concentration against *Stemphylium botryosum*. Huq (2003) reported that Rovral 50WP reduced 90.6% mycelial growth of *Stemphylium botryosum.*

5.2. Field experiment

In the field condition , the performance of the treatments applied were evaluated based on the results of diseases incidence and severity and yield contributory characters of onion against Stemphylium blight .

The effect of fungicides, plant extracts and micronutrients in term of percent plant infection and percent leaf infection were found remarkable in the field experiment. Data on diseases incidence recorded at different DAT showed that spraying of Rovral 50WP @ 0.2% proved to be the most effective in reducing the diseases incidence. Dithane M-45 also found effective next to Rovral 50WP. Between the two plant extracts, neem leaf extract showed better performance in reducing diseases incidence than Allamanda leaf extract. The effect of micronutrient alone (without foliar spraying with fungicides or plant extracts) was not so promising but significantly better than control.

In case of disease severity (PDI- leaf), the result showed that Rovral 50 WP proved to be the best potential among the fungicides used in the experiment followed by Dithane M-45 irrespective of days after transplanting (DAT). At 63 DAT, Rovral 50WP scored only 8.78 % (PDI- leaf) while in control treatment the percent disease index (PDI) of leaf was 58.38%. Dithane M-45 scored only 14.38 percent disease index (PDI) of leaf next to Rovral 50 WP.

In case of disease severity, (PDI-Stalk) Rovral 50 WP also proved to be the superior among the fungicides used in the experiment followed by Dithane M-45. Rovral 50 WP have only (9.63) percent disease index (PDI-Stalk) while the PDI-Stalk of control plot was 38.40. The performance of Rovral 50WP in reducing PDI-stalk was followed by Dithane M-45 which have only 10.97 percent disease index (PDI-Stalk).

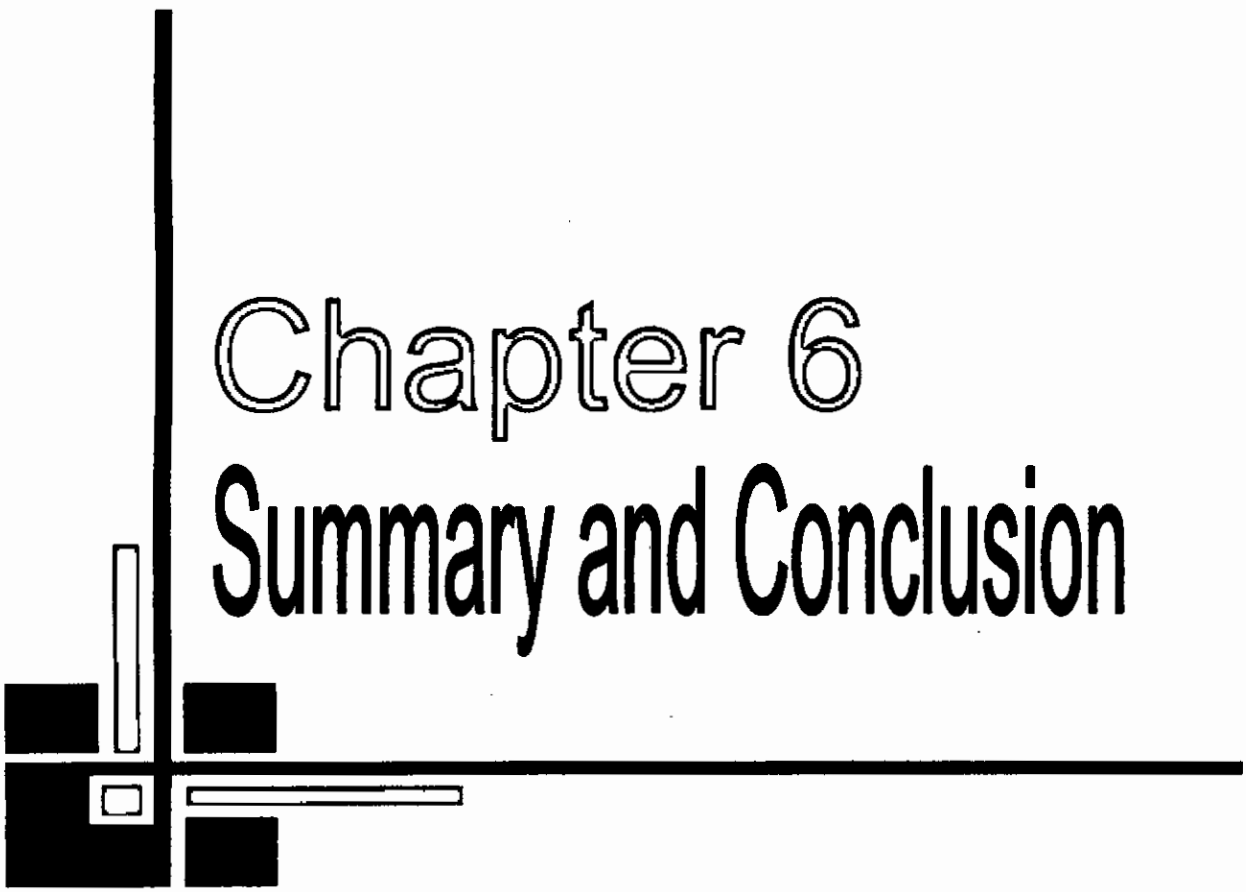
In respect of umbel per plant, 1000 seed weight, number seed per umbel and yield per treatment kg/ha, Rovral 50WP contributed the height effect against stempylium blight of onion followed by Dithane M-45. Rovral 50WP treated plot yielded the maximum number umbel per plant (1.80), maximum 1000 seed weight (3.251gm) and maximum number seed per umbel (540.0 seed). In respect of seed yield Rovral 50WP showed the maximum yield 821.0kg/ ha followed by Dithane M-45 (734.0 kg/Ha) while the seed yield of control was 247.0 kg/ha.

In this experiment data recorded on disease incidence and disease severity at different days after transplanting of bulbs, it was noticed that the first onset of infection and preliminary diseased development was more or less similar for all the treatments but the disease incidence and severity appeared to be distinct among the treatments in comparison to control with the progress of time due to the consecutive spraying with the fungicides and plant extracts. It indicated the inhibitory effect of the treatments applied on the field for controlling the disease.

The findings of the present investigation are supported by reports of the previous researchers (Patil *et al.*, 1976; Rahman *et al.*, 1988; Barnoczki-stoilova *et al.*, 1989; Bakr and Ahmed 1992 ; Srivastava *et al.*, 1995; Gupta *et al.*, 1996 b ; Anon. 2004 ; Nizam 2005 and Khatun, 2007). Rahman *et al.*, (1988) evaluated six fungicides in the field to control leaf blotch of onion caused by *Stemphylium botryosum* and *Alternaria porri.* They found Rovral 50WP as the best fungicide to reduce PDI and increase yield to the maximum.

Six sprays of Rovral (0.2%) with Ridomil (0.2%) found promising to combat the diseases for onion seed production that gave more than 70% seed yield over untreated control Anon. (2004). Nizam, (2005) conducted an experiment on the management of purple blotch complex of onion (*Alternaria porri* and *Stemphylium botryosum*) with five fungicides and reported that bulb treatment with Dithane M-45 (0.45%) or Rovral (0.2%) followed by foliar spraying at 10 days interval minimized disease incidence & severity and increased seed yield. Khatun, (2007) reported that among eight selected treatments ,treatment of onion bulb followed by six (6) foliar spraying at 10 days interval starting from 20 days after planting with Rovral (0.2%) or Dithane M-45 (0.45%) minimized disease incidence & severity and increased seed yield.

Gupta *et al.* (1996) reported that 3-4 sprays of Mancozeb (0.25%) with 10 days intervals starting from 50 DAT reduced infection of *Stemphylium vesicarium* and *Alternaria porri*. Srivastava *et al.* (1995) while conducted an experiment in India recommended to use Mancozeb (0.25%) and Iprodion (0.25%) to control stemphylium blight of onion for seed production. Bakr and Ahmed (1992) also reported that stemphylium blight of lentil caused by *Stemphylium sarcinifortrae* could be controlled by Rovral 50WP (0.2%).



Chapter 6
Summary and Conclusion

Chapter 6

SUMMARY AND CONCLUSION

An experiment was conducted at the research farm of Sher-e-Bangla Agricultural University, Dhaka, during 1st November 2007 to March 2008 to study the control of stemphylium blight of onion caused by *Stemphylium botryosum*. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications of each treatment. Onion variety BARI Piaj-1 was used in the experiment. The unit plot size was 3 m^2 (2.0 m x 1.5 m). Soil treatment with micronutrients and bulb treatment with Rovral 50WP, Dithane M-45, Bavistin 50WP, Cupravit 50WP, Ridomil Gold, Neem leaf extract, Allamanda leaf extract, followed by foliar spray were explored in the experiment. The *in vitro* effect of the fungicides and plant extracts were compiled based on inhibition of mycelium growth and *in vitro* effect of the treatments were compiled based on percent plant infection, percent leaf infection, percent leaf area diseased (%LAD), percent disease index (PDI), plant height, percent stalk area diseased (%SAD), percent disease index (PDI) of stalk, umbel per hill, 1000 seed weight, number of seed per umbel and seed yield per treatment.

In *in vitro* assay, Rovral 50WP performed the best result in inhibition of mycelial growth of *Stemphylium botryosum* followed by Dithane M-45. Between the two plant extracts, Neem leaf extracts showed better performance than Allamanda leaf extracts in inhibition of mycelial growth of *Stemphylium botryosum*.

In case of field experiment, the disease incidence, the percent plant infection and leaf infection were observed at different DAT. The minimum plant infection was observed in case of Rovral 50 WP treated plot irrespective of days after transplanting (DAT). The lowest percent plant infection (35.24 %) was recorded in case of Rovral 50 WP at 63 days. The highest plant infection (100.00 %) was recorded in control treatment while Allamanda leaf extract and Neem leaf extract treated plots showed 63.34% and 60.35% plant infection respectively at 63 DAT. The plant infection gradually increased with the increase of age of onion and increasing rate was sharp in control plot but very slower in treated plot.

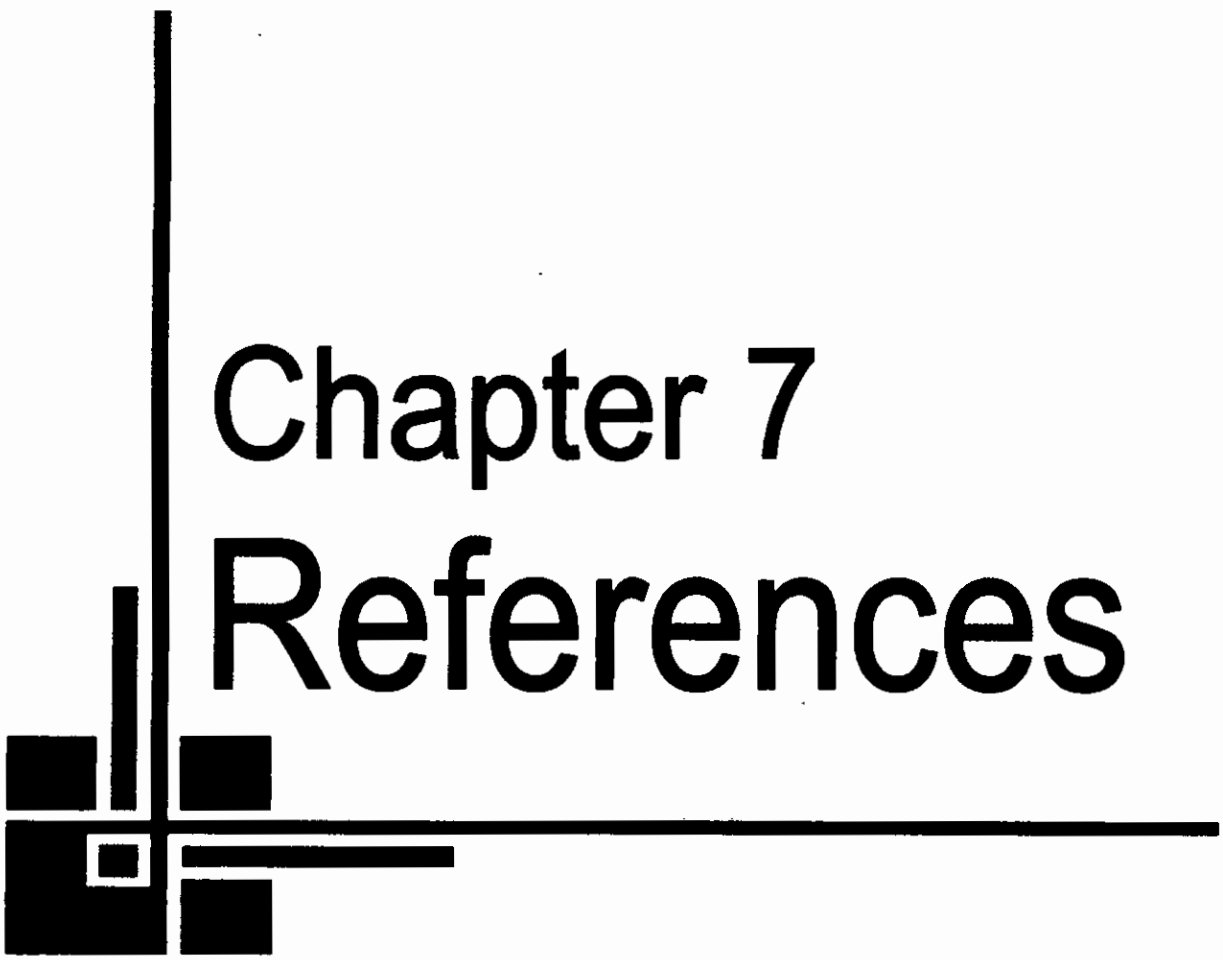
In case of leaf infection, spraying of Rovral 50WP showed the lowest infection irrespective of DAT followed by Dithane M-45. At 63 DAT, the leaf infections respectively were 32.78% and 37.17% while the leaf infection of control was 100 %. The neem extracts scored 58.26% while the Allamanda leaf extract scored 60.61%.

Regarding diseases severity, the lowest percent diseases index (PDI) of leaf and stalk were recorded in case of Rovral 50WP (8.78%, 9.63%) followed by Dithane M-45 (14.38%, 10.97%) while control plot yielded 58.38% and 38.40% PDI, respectively. The PDI-leaf and PDI-stalk, respectively were 36.26% and 30.31% in case of neem leaf extract while it was 41.76% and 38.40% in case of Allamanda leaf extract.

The highest plant height (60.47 cm) was recorded in treatment T_1, where Rovral 50WP was sprayed, which was statistically similar with T_2 (55.47 cm), where Dithane M-45 was sprayed. The shortest plant (35.50 cm) was recorded in control treatment.

In terms of yield and yield contributing characters, the maximum umbel number per plant (1.80), the maximum number of seeds per umbel (540), the maximum 1000 seed weight (3.25 gm) and the highest yield (821 Kg/ha) were achieved by applying Rovral 50WP.The second highest performances regarding the yield contributing characters and seed yield were achieved by Dithane M-45. The performance of neem leaf extracts was better than Allamanda leaf extracts.

From the findings of the present investigation it may be conclude that Rovral 50WP had a promising effect in reducing the incidence and severity of Stemphylium blight of onion and increasing seed yield. Dithane M-45 also showed the second highest performance in suppressing the disease and increasing seed yield. So, micronutrient, Neem leaf extract, Allamanda leaf extract and micronutrient alone were not effective against the disease but showed significantly better performances. Thus, the farmers may be suggested to use Rovral 50WP or Dithane M-45 for the control of Stemphylium blight of onion.



Chapter 7
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Chapter 7

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APPENDICES

Appendix 1. Layout of the experimental field (RCBD)

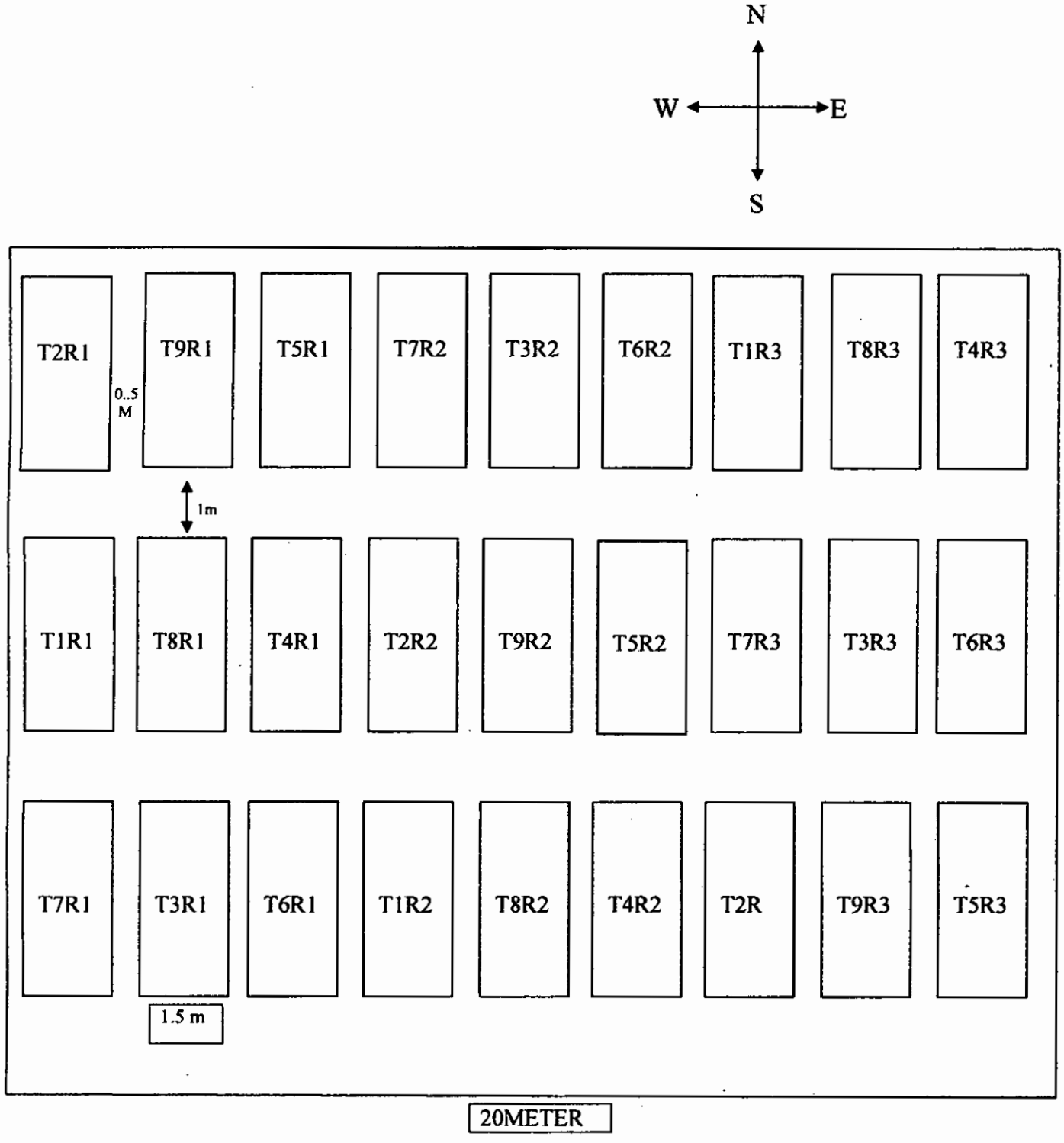

Fig. 2 . Layout of the field experiment showing treatment assigned.

Appendix 2. ANOVA for effect of different fungicides and plant extracts on radial mycelium growth of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	0.031	0.015	0.2826
Treatment	7	59.772	8.539	767.1235
Error	14	0.156	0.011	--

Appendix 3. ANOVA for effect of different fungicides and plant extracts on % plant infection of onion at 63 DAT

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F value
Replication	2	15.182	7.591	3.1475
Treatment	8	3991.096	498.887	206.8635
Error	16	38.587	2.412	--

Appendix 4. ANOVA for effect of different fungicides and plant extracts on % leaf infection of onion at 63 DAT

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	7.930	3.965	1.8246
Treatment	8	3934.522	491.815	226.3149
Error	16	34.770	2.173	--

Appendix 5. ANOVA for effect of different fungicides and plant extracts on Percent Disease Index (leaf) of onion at 63 DAT

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	0.005	0.003	0.4709
Treatment	8	5000.036	666.629	117076.24
Error	16	0.091		--

Appendix 6. ANOVA for effect of different fungicides and plant extracts on plant height of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	8.623	4.311	00.9961
Treatment	8	1559.630	194.954	45.0432
Error	16	69.250	4.328	--

Appendix 7. ANOVA for effect of different fungicides and plant extracts on Percent Disease Index (stalk) of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	1.644	0.822	0.5067
Treatment	8	2723.002	340.375	209.8293
Error	16	25.954	1.622	--

Appendix 8. ANOVA for effect of different fungicides and plant extracts on umbel per treatment of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	0.001	0.001	12.2500
Treatment	8	1.737	0.217	4886.6341
Error	16	0.001	0.000	--

Appendix 9. ANOVA for effect of different fungicides and plant extracts on seed per umbel per treatment of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	272.519	136.259	0.3336
Treatment	8	87848.296	10981.037	26.8808
Error	16	6536.148	408.509	--

Appendix 10. ANOVA for effect of different fungicides and plant extracts on thousand seed weight per treatment of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	0.001	0.000	0.1069
Treatment	8	1.099	0.137	49.8047
Error	16	0.044	0.003	--

Appendix 11. ANOVA for effect of different fungicides and plant extracts on seed yield in hectare per treatment of onion

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	f value
Replication	2	314.889	157.444	5.4395
Treatment	8	935160.000	116895.000	4038.5988
Error	16	463.111	28.944	--

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