CONTROL OF PHOMOPSIS BLIGHT OF EGG PLANT THROUGH FERTILIZER AND FUNGICIDE MANAGEMENT

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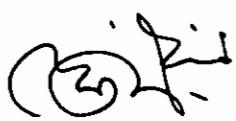

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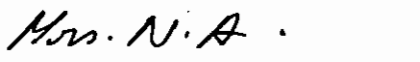

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CERTIFICATE

This is to certify that the research work entitled, "**CONTROL OF PHOMOPSIS BLIGHT OF EGG PLANT THROUGH FERTILIZER AND FUNGICIDE MANAGEMENT**" 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**, was successfully carried out by **Muhammad Iqbal Hossain,** Registration No. **07-02584** under my supervision and my guidance No part of the thesis has been submitted for any other degree or diploma.

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

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Dedicated to
My
Beloved Parents
&
Younger Sister

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The Author

CONTROL OF PHOMOPSIS BLIGHT OF EGG PLANT THROUGH FERTILIZER AND FUNGICIDE MANAGEMENT

Abstract

Four fungicides viz. Bavistin 50 WP (Carbendazim), Tilt 250 EC (Propiconazole), Cupravit 50 WP (Copperoxychloride) and Dithane M-45 (Mancozeb) and micronputrients (Gypsum, ZnO and Boric acid) were evaluated against *Phomopsis vexans* causing Phomopsis blight and fruit rot of eggplant. The fungicides and micronutrients applied either individually or in combination showed significant effect in terms of percent leaf infection (%LI), fruit infection (%FI), leaf area diseased (%LAD) and fruit area diseased (%FAD) in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance in reducing disease incidence and disease severity than the fungicides applied alone. Among the fungicides, Bavistin 50WP (0.1%) proved to be effective arresting the spore germination and mycelia growth of *Phomopsis vexans* assayed in *in vitro* test. Reduction of leaf area diseased (%LAD) caused by Bavistin 50 WP (0.1%) in combination with micronutrients were 58.17%, 67.37%, 78.41% and 85.25%, respectively at pre-flowering, post-flowering, fruiting and fruit ripening stages while Bavistin 50 WP (0.1%) alone reduced (% LAD) by 52.22%, 58.67%, 74.19% and 83.09%, respectively at those stages. Similarly reduction of fruit area diseased (%FAD) caused by Bavistin 50 WP (0.1%) in combination with micronutrients were 57.93% and 79.79%, respectively at fruiting and fruit ripening stages while Bavistin 50WP (0.1%) alone reduced (%FAD) by 56.93% and 76.14%, respectively at those stages. Micronutrients had little effect against the disease but significantly better than control.

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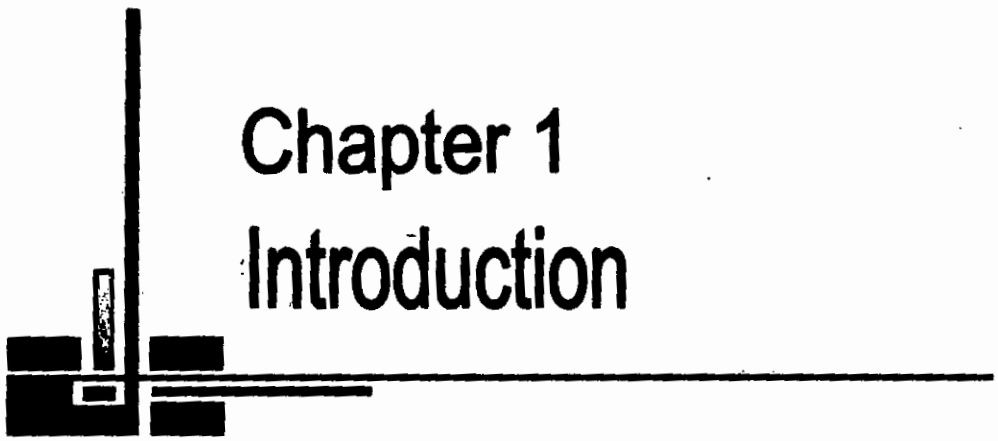


Chapter 1
Introduction

INTRODUCTION

Eggplant (*Solanum melongena*) is an important vegetable in Bangladesh cultivated round the year in all districts of the country (Anonymous, 2001). The eggplant is of much important in the warm areas of Far East and grown extensively in India, Bangladesh, Pakistan, China and Philippines. It is thought to be originated in India sub-continent with the secondary centre of origin in China (Zeven and Zhukovsky, 1975). Eggplant is locally known as "Begun" and its early European name is Aubergine or Egplant. Eggplant is the species of *Solanum* also known as Guinea squash and garden egg (Nonnecke, 1989).

Eggplant is nutritious and widely grown vegetable in Bangladesh as well as in the world and has got multifarious use as a dish item (Bose and Som, 1986; Rashid, 1993). About 8 million farm families are involved in eggplant cultivation. Its position in terms of acreage production is second in vegetable crops (BBS, 2003). It is grown round the year both as winter (Rabi) and summer (Kharif) crops. Eggplant is thus regarded as a cash crop. The total acreage of eggplant is 60,065 hectares with total annual production of 3, 58,370 tones (BBS, 2005). A large number of cultivars are grown in Bangladesh, which show a wide range of variation in yield performance. This gives small, marginal and landless farmers a continuous source of income and provides employment facilities for the rural people. For most of the time, except peak production period, market price of eggplant compared to other vegetables remains high which is in favor of the farmer's solvency. So it plays a vital role to boost our national economy.

Eggplant suffers from 12 diseases of which Phomopsis blight and fruit rot caused by *Phomopsis vexans* has been treated as major constraints of its

cultivation in our country (Das, 1998; Khan *et al.*, 2002). The causal organism of the disease, *Phomopsis vexans* viable for about 14 months in soil debries and in the seed from infected fruits. The pathogen is reported both externally and internally seed borne. The disease was first reported from Gujrat in 1914 and since then from many parts of India. Occurrence of the disease in Bangladesh has been reported by Fakir (1983) and Ahmad (1987). The disease has become a major constraint in case of intensive cultivation of eggplant. The disease affects the crops from seedling to maturity (Singh, 1992).

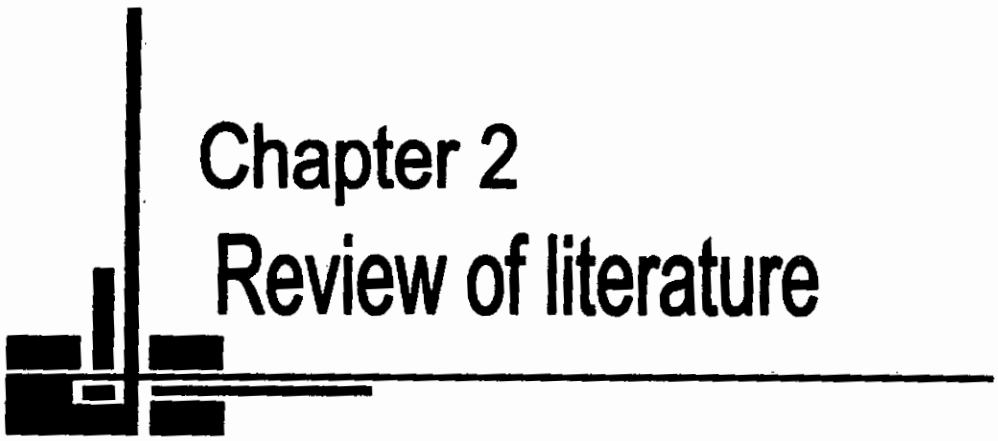
Crop losses due to this disease are evident, loss ranges from 15-20% in general but 30-50% in severe case which estimated as a losses equivalent to TK. 1255 million ($20 million) per annum (BBS, 2003; Das, 1998). It is a serious disease which may cause damping off symptoms if attacked at seedling stage. When the leaves are infected, small circular spots appear which become grey to brown with a light color centre. The infected leaves may turn yellow and die. Lesion may also develop on petiole and stem cause blighting of affected portions. In course of time, the spot enlarges and produces concentric circular area. Ultimately, the fruits become mummified and rotten (Kumar *et al.*, 1986). There is no recognized resistant variety of eggplant against fruit rot disease. A very few works have been made by different workers to control this disease (Khan, 1999; Hawlader, 2003; Nazimuddin, 2004).

Therefore, the present research work was undertaken to achieve the following objectives-

i) To evaluate some selected fungicides against *Phomopsis vexans* in the laboratory.

ii) To determine the efficacy of selected fungicides in controlling Phomopsis blight of eggplant in the field condition.

iii) To determine the effect of micronutrient in controlling Phomopsis blight of eggplant in the field.



Chapter 2
Review of literature

REVIEW OF LITERATURE

Eggplant (*Solanum melongena*) is a popular Solanaceous vegetable crop of Bangladesh. It is cultivated throughout the year and consumed by the people from all strata of the country (Rashid, 1993).

Brinjal is the only economic host of *Phomopsis vexans* (CMI description of pathogenic fungi and bacteria no. 338). Evidence of research work regarding Phomopsis blight and fruit rot is very dearth. However, it is known from literatures that emphasis have been given to control the disease through different means. Literatures available on Phomopsis fruit rot disease are presented below:

2.1 Phomopsis blight/fruit rot disease and its pathogen *Phomopsis vexans*

Eggplant suffers from many diseases caused by fungi, bacteria, virus, nematode and mycoplasma. Of them Phomopsis fruit rot of eggplant caused by the fungus *Phomopsis vexans* (Sacc. and Syd) Harter is a serious disease which attacks all above ground parts of the plant. It is damaging to the crop and is a threat particularly in Kharif season and late crop in winter season.

Halsted first described the organism in the United States in 1892 as *Phoma solani* Halst. Since the name had been used for another fungus it was changed to *Phoma vexans* by Saccardo and Sydow in 1899. Harter transfered it to *Phomopsis vexans* (Sacc. and Syd.) Harter (Walker, 1952). The disease was first reported from Gujrat in 1914 by Harter and since then from many parts of India (Bose and Som, 1986). Now a day it is common throughout the world in most tropical and sub-tropical areas.

2.2 Incidence and Severity of Phomopsis blight/fruit rot

Meah (2003) made an epidemiological survey on the incidence and severity of Phomopsis blight and fruit rot of eggplant in 18 major growing areas of Bangladesh and reported that sandy loam soil, soil moisture in the range of 50-60%, moderate air temperature (20-28°C) and high atmospheric moisture (65-78% RH) significantly influenced the prevalence of Phomopsis blight and fruit rot of eggplant. The prevalence was higher in the south eastern part of Bangladesh with moderate weather than the middle and northern part of the country with severe weather.

Khan *et al.* (2002) analyzed 22 seed samples collected from farmers of different eggplant growing areas of Bangladesh and recorded 0.5 – 7.0% infection of *Phomopsis vexans.*

Meah *et al.* (2002) recorded up to 25% disease incidence and 9.8% disease severity in eggplant (cultivar Dohazari). Meah *et al.* (2002) also reported a loss equivalent to Taka 808 million (US$ 134 million) per annum due to Phomopsis blight and fruit rot of eggplant in Bangladesh.

Khan (1999) reported that Phomopsis blight and fruit rot of eggplant causes about 21% fruit rot and 7% seed rot in eggplant.

Gangadharaswamy *et al.* (1997) studied the impact of *Phomopsis vexans* on seed quality of brinjal and reported that 13% seed infection was found in variety collected from farmers and no seed infection was found in variety Pusa purple collected from National Seed Corporation. *Phomopsis vexans* even at low incidence caused failure of emergence of seedlings, ultimately rotting of seeds.

Pan and Acharya (1995) studied the seed borne nature of *Phomopsis vexans* and reported that *Phomopsis vexans* was present on seed coat and

on the cotyledons of aubergine seeds collected from disease fruit in West Bengal, India. It was suggested that the seeds were an infection source and might served as a substrate for pathogen survival.

Karuna *et al.* (1994) studied on seed borne inocula of *Phomopsis vexans* and the effect of infection on seed quality in egg plant and found that seed borne infection of varieties PBR-7, PBR-5, MHB-1 and Pant rituraj were 29%, 36%, 35% and 56%, respectively. Seed infection caused various degrees of seed discoloration. On the surface of dry seed, the fungus was observed as black pycnidial bodies. Pre-treatment of seeds with 0.1% $HgCl_2$ solution reduced seed infection. *Phomopsis vexans* infection adversely affected seed quality.

Karuna *et al.* (1994) also studied locations of infection of *Phomopsis vexans* in brinjal seeds and observed that component plating of seed of the aubergine cultivar PBR-5 yielded 22% and 12% infection in seed coats and cotyledons, respectively. Pycnidia and mycelium were seen on seed coats and in the embryo.

Singh (1992) stated that *Phomopsis vexans* subsists between crop seasons on infected plant debris in the field.

Phomopsis vexans is an important fungus isolated from surface sterilized eggplant seeds and stated to be seed borne by Walker (1952) in USA.

Pawar (1957) reported that the causal organism of the disease remained viable for about 14 months in soil debris and in the seeds from infected fruits, which were poor in germination.

2.3 Symptoms of Phomopsis blight and fruit rot disease

Ashrafuzzaman (1986) has stated that, due to this disease, damping off takes place at seedling stage. Leaf may be attacked at any time.

Generally, first symptom appears on the lower leaves. Spots are clear, circular and grayish. Numerous pycnidia are formed on aged spots. Infected leaves become yellowish and eventually may die. Canker is observed at the base of the stem, bark is cracked and woody portion opened. Light coloured, sunken spots are observed on fruit. Black pycnidia are formed in spots and the fruits become mummified.

Kumar *et al.* (1986) stated that *Phomopsis vexans* caused fruit rot of eggplant which appeared as minute, circular, water soaked, sunken, grayish spots with brownish halo and have a light coloured center which later enlarged to produce concentric rings and brownish zones. Spots increased in size and formed larger rotten areas on which pycnidia developed causing blackening of the affected area.

Punithalingam and Holliday (1972) described the disease as damping off, tip over, seedling blight, stem blight or canker, leaf blight or spot and fruit rot. Leaf spots are conspicuous, irregular in outline and may coalesce; lower leaves may be affected first. In stem lesions, the cortex dries and cracks, plants become stunted and cankers cause death. Fruit spots are pale, sunken, and conspicuous and may affect the whole fruit; fruit may drop or remain attached, becoming mummified after a soft decay. Pycnidia are abundant in the old lesions.

2.4 Disease cycle of Phomopsis blight/fruit rot disease

Meah (2003) reported that the *Phomopsis vexans* exists in infected seeds and also in the infected leaves, twigs or fruits on the ground. Under favorable conditions (temperature 25 ^{0}C, R.H. 50%, pH 5.5, wet soil), seeds are infected and rotten, seedlings are damped-off or blighted. The fungus produces pycnidia on the infected seeds and seedlings where conidia are produced. Conidia are carried to stem, twig and leaves by rain

splash, insects and wind. Conidia are produced which upon dissemination cause infection on flowers and fruits. Seeds are infected through fruit infection. The fungus thereby perpetuates in seed coat, cotyledons and embryo.

Singh (1992) reports that the disease is present in several forms from the seedling stage of the plants to its maturity. In seedbed, it appears as damping off. After transplanting the leaves coming in contact with soil may get infected and show clearly defined circular, gray to brown spots with light coloured center. The old spots show numerous black pycnidia. The affected leaves turn brown and ultimately die. Sometimes petioles and stems are attacked and show cankers. The lesions on the stem are dark brown, becoming gray in center as the black pycnidia develop. Mostly the stem base is attacked and is characterized by the constriction of the base or a grayish dry rot. The bark peels off and the inner tissues are exposed. In strong wind, plants topple down due to breaking of the main stem. Pale, sunken spots develop on the fruits covering entire fruit surface and may cause dropping. The spots are also distinct by the presence of black pycnidia. The whole fruit is mummified due to dry rot. Sometimes soft rot is observed due to complication of other fungi.

2.5 Morphology of *Phomopsis vexans*

Sugha *et al.* (2002) have reported that alpha and beta are two forms of the same conidium. *Phomopsis vexans* produces only one type of conidia in its pycnidia, which are hyaline, one celled, sub-cylindrical and 5-9 x 2-2.8 μ in size during summer months, which gradually changed into the beta form. Inoculation of host plants with beta conidia caused intraveinal necrosis, which progressed towards the leaf base and resulted in premature defoliation, thus indicating their role in pathogenesis.

Gratz (1942) described the ascigerous stage in culture; this stage has not been observed in nature. The pycnidium, often with a well-defined beak, is at first buried in the tissue but later speck extending slightly above the surface. On the leaves pycindia are 60-200μ in dia; on the fruit they are 120-350μ. Pyenidiospores are hyaline, continuous, subcylindrical, 2 to 2.8 by 5 to 8μ. Stylospores are curved, filiform, hyaline, continuous, 13 to 28μ long. Conidia are hyaline, narrowly ellipsoid to bluntly fusoid. One septate and 3 to 4.4 by 9 to 12μ.

2.6 Physiology of *Phomopsis vexans*

Islam *et al.* (2004) reported that α and ß conidia varied in size among the isolates of *Phomopsis vexans* which exhibited the presence of different physiological races in the studied areas. He also observed that 25^{0}C temperature under 12/12 cycle light and 5.5 pH level of PDA medium supported the growth and sporulation of most of the *Phomopsis vexans* isolates.

Islam *et al.* (2004) also reported existence of variations in the DNA banding patterns of *Phomopsis vexans* isolates while worked on molecular characterization of 44 different isolates of *Phomopsis vexans* through analyzing of genomic DNA at molecular lab of BRRI, Gazipur, Bangladesh.

Meah (2003) worked with 32 isolates of *Phomopsis vexans* isolated from diseased samples of 29 eggplant cultivars collected from 17 growing areas in Bangladesh and observed that the isolates varied in terms of mycelial growth, colony consistency and sporulation behaviour in the culture media. On cross inoculation of 29 eggplant cultivars with the 5 groups of isolates of *Phomopsis vexans*, he recorded deferential disease reactions.

Kumar and Sugha (1999) stated that *Phomopsis vexans* (perfect stage: *Diaporthe vexans*), an incitant of leaf blight and fruit rot of brinjal (aubergine) is reported to produce alpha and beta conidia. To date no role of beta conidia in pathogenesis has been described in the phytopathological literature. Various studies reveal that formation of conidia in pycnidia of *P. vexans* is temperature dependent. At 10-16^{0}C temperature, the pathogen produces beta conidia and at 25-28^{0}C, the alpha conidia. These two forms of conidia get inter-converted when subjected to a specific temperature. It was proved from these studies that alpha and beta are two forms of the same conidium; *P. vexans* produces only one type of conidia in its pycnidia which are hyaline, one celled, sub-cylindrical and 5-9x2-2.8µm in size during summer months which gradually changed into the beta form. Inoculation of host plants with beta conidia caused intraveinal necrosis which progressed towards the leaf base and resulted in premature defoliation, thus indicating their role in pathogenesis. Isolations from such leaves produced pycnidia with alpha conidia at 25^{0}C and beta conidia at 16^{0}C.

Islam and Pan (1990) stated that in laboratory tests, symptom of brinjal fruit rot (caused by *Phomopsis vexans*) were affected by changes in incubation temperature and RH. Maximum disease was recorded at 30^{0}C and 50% RH.

Ahmad (1987) stated that the fruits were inoculated with the test fungus (*Phomopsis vexans*) by pin-prick method and was incubated for 15 days at 25^{O} C and RH 55% which had been found to be most suitable for fruit rot.

Chowdhury and Hasija (1980) conducted an experiment to inoculate the eggplant fruits (4 cvs.), which showed differences in susceptibility.

Optimum temperature and RH for disease development were 25^{0}C and 55% respectively.

Chinenye (1974) reported that *Phomopsis vexans* produced thin, smooth, creamy-white fungal colony. After prolonged incubation beyond 10 days at 28^{0}C or in storage in a petridish, the creamy-white cirrhus turned lemon to yellow, became stiff and could be easily picked up intact with a mounted needle. When fresh cirrhus was mounted in water or lactophenol with cotton blue and examined under compound microscope, typical α and β conidia were seen in large numbers.

The optimum temperature for growth *in vitro* for *Phomopsis vexans* is 28-30^{0}C and light is required for sporulation (Punithalingam and Holliday, 1972).

Divinagracia (1972) stated that *Phomopsis vexans* produced numerous pycnidia on oatmeal, rice and wheat agars; they were small and solitary at low oatmeal concentrations, large and aggregated at higher ones. Light was needed for abundant production and hyphal tips seemed most photosensitive. Optimum temperature for growth and pycnidial production was 30^{0}C and growth was inhibited at 35^{0}C. For large quantities, inoculum of *Phomopsis vexans* should be grown on 4-7% oatmeal agar at 30^{0}C in light.

2.7 Varietal resistance

Sarker (2004) reported that presence of Trichomes on leaf surface, thickness of leaf and fruit surface had significant effect on the occurrence of Phomopsis blight and fruit rot of eggplant. He also observed that thickness of fruit surface was found maximum in Katabegun (1.4mm) and showed complete resistance against *Phomopsis vexans* while Dohazari (1.0mm) and Laffa S (1.3 mm) were susceptible to the pathogen.

Meah (2003) conducted a screening experiment in induced epiphytotic and natural conditions and found cultivar Katabegun WS and wild species resistant against *Phomopsis vexans*.

Khan *et al.* (2002) evaluated 22 eggplant cultivars against *Phomopsis vexans* in induced field condition and found that Isurdi-1 had the lowest percent area of leaf and fruit infection followed by Katabegun and Diginala.

Meah *et al.* (1998) conducted a field trial with 15 varieties and found that the inoculated leaves of all the varieties experienced infection. Lowest percent of leaf infection was observed on varieties Borka, Katabegun, Bl-116 and Jamalpuri. Fruits of 14 varieties inoculated with *Phomopsis vexans* showed significantly different reaction to disease development. Katabegun did not develop infection while intermediate fruit infections were observed in rest of the varieties.

Chowdhury and Hasija (1979) inoculated 4 cultivars of eggplant, showed differences in susceptibility. Optimum temperature and relative humidity for disease development were 25^0C and 55%, respectively.

2.8 Control through chemicals

Teo (1957) conducted a field trial and found that stem blight (*Phomopsis vexans*) infection on eggplant was reduced by a mixture of Copper oxichloride+Benlate (Benomyl) or Copper oxichloride alone, but there was no significant difference the yields of treated plants and the controls.

Singh and Chakrabarti (1982) treated Phomopsis infected seeds of brinjal with 7 chemicals and hot water at 51^0 C for 15 minutes. In the spray treatments, minimum disease incidence and maximum seed yields were obtained from Difolatan and Captan treatments.

Kaur and Bedi (1989) tested seven fungitoxicants, all inhibited spore germination to a less or greater extent at all conc. Dithane M-45 at 20 ppm concentration completely inhibited the spore germination of *Phomopsis vexans.* Under field conditions, spraying of eggplant crop with Dithane M-45 (0.2%), Derosal (0.05%), Blitox (0.02%) and Captafol (0.2%) were effective in reducing fruit rot significantly. Two spraying of crop with Dithane M-45 one before and one after anthesis was more effective as compared to spraying given after anthesis.

Islam *et al.* (1989) conducted a field trial on eggplant following in vitro screening. Bavistin (Carbendazim) at 0.1% gave the best control of Phomopsis blight and fruit rot.

Islam and Sitansu (1992) stated that in a field trial against *Phomopsis vexans* Carbendazim (0.1%) and Carboxin (0.25%) provided nearly completed control of the disease with 3 sprays at 15 days intervals. Three fortnightly sprays of Carbendazim each at 700ml/ha were the best in terms of increased yields (19.4q/ha) over the untreated control.

Suhga and Singh (1992) conducted field trials with 10 fungicides during 1980-1990 the best control of *P. nicotianae* var. *nicotianae* on aubergines was given by Metalaxyl + Mancozeb 0.2% applied 3 times at fortnightly intervals from flowering.

Nene and Thapliyal (1993) suggested that at disease symptom appearance, 3 sprays with Carbendazim 50% WP,or Benomyl 50% WP at 0.5%kg/ha or Mancozeb 75% WP at 2.5 kg/ha 10 to 15 days intervals were effective for controlling Phomopsis blight and fruit rot of eggplant.

Mohanty *et al.* (1994) tested 5 fungicides, Blitox-50 (Copperoxychliride), Bavistin (Carbendazim), Dithane M-45 (Mancozeb), Ridomil MZ-72 (Mancozeb + Metalaxyl) and Difolatan (Captafol) in vitro against

Phomopsis vexans the causal agent of fruit rot in eggplant in Orissa, India. The percent inhibition of mycelia growth was invested for 3 conc. (0.1%, 0.2% and .3%) of each fungicide. All the chemicals caused significant growth inhibition at all concentration. Carbendazim caused 100% inhibition at all concentrations.

Kaushal and Sugha (1995) conducted an experiment to ascertain the role of *Phomopsis vexans* in causing pre- emergence and post-emergence damping off of eggplant seedlings and its control with seed dressing fungicides. The pathogen caused 29.9-31.8% pre emergence and 25.6-30.9% post-emergence damping off of eggplant seedlings. Seed treatments with captan, Carbendazim, Carbendazim + Thiram, Carboxin, Thiram and Triadimenol improved the seedling stands significantly over that in the untreated control. Seed treatments with Carbendazim alone or in combination with Thiram provided better control of pre- emergence and post-emergence damping off than other fungicides.

Das (1998) stated the reaction of fourteen varieties of eggplant (*Solanum melongena L.*) to *Phomopsis vexans* (Saac. Syed). He used five fungicides namely Cupravit, Rovral, Bavistin, Dithane M-45, and Tilt 250EC. Bavistin proved as the most effective fungicide against the disease.

Meah *et al.* (1998) evaluated 3 fungicides (Rovral, Bavistin and Dithane M-45) at a single dose (0.2%) on 4 varieties of eggplant (khatkhatia, Islampuri,Katabegun and Dohazari). The fungicides applied both before and after inoculation of *Phomopsis vexans* on surface rubbed fruits. Pre-inoculation sprays of fungicides significantly reduced percent fruit infection. Bavistin treated fruits developed the least sized lesion.

Khan (1999) reported the reaction of 22 varieties of bringal (*Solanum melongena L.*) to *Phomopsis vexans* causing fruit rot and possibility of its control through use of chemicals and plant extracts. Using fungicide namely, Bavistin, Macuprax and Tilt-250EC at 3 doses, Bavistin 0.2% proved as the most effective to reduce leaf and fruit infection.

Khan *et al.*(2002) evaluated the efficacy of three fungicides Bavistin (Carbendazim), Macuprax (Cufraneb) and Tilt-250 EC at 3 concentrations (0.1%,0.2% and 0.3%) in the field and found Bavistin (0.2%) significantly reduced leaf infection of eggplant caused by *Phomopsis vexans.*

Meah *et al.* (2002) and Meah (2003) reported that among eight fungicides tested against *Phomopsis vexans* in the laboratory and in the net house, Bavistin (Carbendazim) arrested highest mycelia growth and controlled nursery diseases significantly. In the field trial, spraying of Bavistin (0.1%) significantly controlled Phomopsis blight and fruit rot of eggplant.

Islam (2005) evaluated the efficacy of different fungicides for the management of phomopsis blight and fruit rot of eggplant in the field condition reported that Bavistin 50WP @ 0.1% showed the highest performances in reducing the disease incidence (%LI and %FI) and disease severity (%PDI of leaf and fruit) among the Bavistin 50WP, Tilt 250EC, Dithane M-45 and Cupravit tested fungicides at pre-flowering, post-flowering, fruiting and fruit ripening stages and increasing fruit yield.

Chapter 3
Materials and Methods

MATERIALS AND METHODS

3.1 LABORATORY EXPERIMENT

3.1.1 Experimental Site

The experiments were conducted at M.S. Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

3.1.2 Experimental Period

The experiments were conducted during September- November' 2007

3.1.3 Preparation of Culture Media

Composition of Potato Dextrose Agar (PDA) media are as follow

Potato (Peeled and Sliced)	200g
Dextrose	20g
Agar	20g
Water	1000ml

Peeled and Sliced potato was boiled to collect the extract by sieving with a fine piece of cloth. Afterwards, the other ingredients were mixed up and then heated gently for few minutes. After preparation, the contents were poured into 250ml Erlenmeyer flasks, plugged with cotton and were sterilized in autoclave at 120°c under 15 PSI for 20 minutes. This medium was acidified with 30 drops of 50% lactic acid per 250ml medium to inhibit the growth of bacteria. These 20ml of the medium was poured into each Petri-plate (9cm diameter) and allowed to solidify.

3.1.4 Collection / Isolation and maintenance of culture of *Phomosis vexans*

The pathogen, *Phomopsis vexans* was isolated by tissue plating method from naturally infected brinjal fruit collected from the Horticulture garden of Sher-e-Bangla Agricultural University, Dhaka (Islam and Pan, 1990). The sample showed typical symptoms of Phomopsis fruit rot having pale, sunken and mummified spots on the fruit (CMI Description no. 338). The infected fruit was repeatedly washed in fresh water and surface sterilized with 10 % Clorox for 1 minute followed by three times washing in distilled water to eleminate traces of mercury. Infected tissues were cut and placed in PDA media and incubated at 22 ± 2°C for 10 days. After incubation, it was observed that white mycelia and pycnidia were formed. Several slides were prepared and examined under compound microscope. The fungus was identified according to CMI description no. 338. The pathogen was purified and multiplied subsequently through block culture on PDA. Culture was stocked in PDA slants for future use.

3.1.5 Preparation of spore suspension / inocula of *Phomosis vexans*

Phomopsis vexans was grown on PDA (Potato Dextrose Agar) medium in petridish at 20°C temperature. After formation of pycnidia (in about 15-20 days), 5ml/plate sterile water added and the spore masses was scraped away with sterile needle/scalpel. The conidial suspension was made with additional 45ml water and blended in Moulinex blender for 2 minutes in medium speed and filtered through sterile cheesecloth. The suspension was adjusted to 10^5 conidia/ml solution and stored at 5°C for future use.

3.1.6 Particulars of fungicides used in the experiment

Common name	Chemical	Active ingredient
Bavistin 50WP	Methylbenzimidasol-2-ylcarbamate	Carbendazim
Tilt 250EC	1-(2-(2,4,-dichlorophenyl)-4-propyl-1,3-dioxolas-2-ylmethyl)-1H-1,2,4-triazole	250ml/litre Propiconazole
Dithane M-45	Manganous ethylene bisdithiocarbamate-ion (C4H6N2S4)	80 % Mancozeb
Cupravit 50WP	Copper-oxychloride ($CuOcl_2$)	50% Copperoxychloride

3.1.7 Bioassay of fungicides by following growth inhibition techniques:

i) Cup / Groove method: From a PDA plate three 5 mm discs of the medium were scooped from three places maintaining an equal distance from the centre by a sterilized disc cutter. One milliliter of fungicides solution was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole before resumption of fungal growth. The next day, one 5 mm block of days old fungal culture (pathogen) cut by sterilized disc cutter was placed at the centre of the plate. The linear growth (cm) of mycelium of *Phomosis vexans* was recorded at 24 hr. interval until the control plates were filled in (Nene and Thaplial, 1993).

3.2.1 FIELD EXPERIMENT

3.2.2 Experimental site

The experiment was conducted in the farm allotted for the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. It was located in 24.09^0N latitude and 90.26^0 E longitudes.

3.2.3 Experimental period

The experiment was conducted during Rabi season of the year 2007-2008

3.2.4 Soil type:

Loam to clay loam in texture belonging to the Madhupur Tract (AEZ-28).The description of the Agro Ecological Zone of the experimental site is sited below:

Agro Ecological Zone	: Madhupur Tract (AEZ-28)
Land Type	: Medium high land
General Soil Type	: Non Calcareous dark gray flood plain soil
Soil Series	: Tejgaon
Topography	: Upland
Elevation	: 8.45m
pH	: 5.6
Location	: Sher-e-Bangla Agricultural University farm,.
Field level	: Above flood level
Drainage	: Fairly good
Firmness (Consistency)	: Compact to friable when dry

The characteristics of the soil under the experimental plot were analyzed in the Soil Testing Laboratory, Soil Resource Development Institute (SRDI), Khamarbari, Farmgate, Dhaka and are presented below (For 0-14 cm depth):

Particle size distribution:

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

3.2.5 Climate

The geographical situation of the experimental site was under the subtropical climate, characterized by three distinct seasons, the post rainy or cool season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October. Details of the metrological data of temperature (^{0}C), relative humidity (%), rainfall during the period of the experiment were collected from Bangladesh Meteorological Department (Climate Division).

3.2.6 Weather

The monthly mean of daily maximum, minimum, and average temperature (^{0}C), relative humidity (%), rainfall and sunshine hours at the experimental site during the period of the study have been collected from the surface synoptic data card, Bangladesh Meteorological Department, Sher-e-Bangla Nagar, Dhaka.

3.2.7 Treatment of the experiment

In this study ten (10) different treatments were used as designated by T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10} which were as follows:

T_1 = Normal fertilization (Cowdung + Urea + TSP + MP) - Control

T_2 = Normal fertilization + Micronutrients (Gypsum + ZnO + Boric acid)

T_3 = Normal fertilization + foliar spraying with Bavistin 50 WP

T_4 = Normal fertilization + foliar spraying with Cupravit 50 WP

T_5= Normal fertilization + foliar spraying with Tilt 250EC

T_6 = Normal fertilization + foliar spraying with Dithane M-45

T_7 = Normal fertilization + Micronutrients + foliar spraying with Bavistin 50 WP

T_8 = Normal fertilization + Micronutrients + foliar spraying with Cupravit 50 WP

T_9 = Normal fertilization + Micronutrients + foliar spraying with Dithane M-45

T_{10}= Normal fertilization + Micronutrients + foliar spraying with Tilt 250EC

3.2.8 Manures and fertilizers used in the experimental field

Well decomposed cow dung was applied at the time of final land preparation. The sources of fertilizers used for N, P and K were urea, TSP, MP respectively.

Manures and fertilizers applied in the plot

FERTILIZER	RATE (Kg/ha)
Urea	380
TSP	150
MP	250
Gypsum	150
ZnO	5
Boric acid	10
Cow dung	10000

Whole amount well decomposed cowdung, TSP and half amount of MP were applied during land preparation. Urea and remaining half of MP were applied in three installments as top dressing. Micronutrients (Gypsum + ZnO + Boric acid) were applied at the time of plot preparation as per treatment design.

3.2.9 Cultivar used

In this research work, seeds of eggplant cultivar Singnath were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.2.10 Raising eggplant seedling

Plastic trays were taken and filled with fertile soil. Weeds and rubbish were removed carefully from the soil. Then the seeds of eggplant cultivar (Singnath) were treated with Vitavax-200 (0.2%). Seeds were sown in plastic trays containing sterilized sandy soil on 18th November 2007. Seeds were sown in parallel line on the surface level on the seed bed making about 2cm line depth and then a very thin cover was made with sandy soil. Four trays were taken for raising of seedlings. Seedlings were observed regularly and watering was done as per necessary up to transplanting in the field.

3.2.11 Preparation of the main field

The selected experimental plot was opened in the last week of September 2007 with a power tiller and was exposed to the sun for a week. After one week the land was harrowed, ploughed and cross-ploughed several times followed by laddering to obtain a good tilth. Weeds and stubbles were removed and finally obtained a desirable tilth of soil for transplanting of eggplant seedlings. The experimental plot was partitioned into the unit plots in accordance with the experimental design.

3.2.12 Transplanting of seedlings

After preparation of main field, 45 days old seedlings were uprooted from the seed bed and transplanted in the experimental plots on the 6th January 2008 in the afternoon on the same day. Two hours before transplanting the seedlings were watered before removing the seedlings from the pots to minimize the root damage. Plant to plant distance was maintained 75cm and each plot contain five plants. A sufficient irrigation was given just after transplantation with the help of bucket sprinkler. For keeping seedlings upright, support with bamboo sticks were provided. One

seedling was placed in a pit. The transplanted seedlings were protected from the sunshine, shading with banana leaf sheath cuttings. Shading and watering were continued till the seedlings were established in the field.

3.2.13 Preparation of spore suspension / inocula of *Phomosis vexans*

Phomopsis vexans was grown on PDA (Potato Dextrose Agar) medium in petridish at 20°C temperature. After formation of picnidia (in about 15-20 days), 5ml/plate sterile water added and the spore masses was scraped away with sterile needle/scalpel. The conidial suspension was made with additional 45ml water and blended in Moulinex blender for 2 minutes in medium speed and filtered through sterile cheese cloth. The suspension was adjusted to 10^5 conidia/ml solution and stored at 5°C for future use.

3.2.14 Inoculation of eggplant with *Phomosis vexans*

Phomopsis vexans was inoculated by spraying the prepared spore suspension to the eggplant. After spraying, the seedlings of eggplant were covered with moistened polythene with the help of bamboo sticks so that the covered polythene would not flew away. The eggplants were covered in such a way that covered polythene were retained enough moisture for the first two days.

Photograph 1: Inoculation of eggplant by *Phomopsis vexans*

Photograph 2: Field view of experimental plot

3.2.15 Development of symptoms in brinjal plants following inoculation with *Phomopsis vexans*

Seven days after inoculation of the plants (Photograph) with previously grown pathogenic suspension, leaves showed clearly defined, circular grey to brown spots with rough color centre. The spots coalesced, affected leaves turned yellow and eventually died. The old spots showed numerous black pycnidia.

Most of the flowers dropped after 5 days of inoculation and lesions of the flowers were not visible. Fruit rot appeared as minute, globose, water soaked spots with brownish color, which later enlarged to produce concentric rings. The outer most ring got separated from the healthy fruit surface. Spots increased in size and formed large rotten area as which produced blackish pycnidia distributed throughout the rotten fruit. Fruits were mummified. Some affected fruits dropped off.

Photograph 3: Infected leaves showing symptoms

3.2.16 Preparation of fungicidal suspension:

Fungicides (Bavistin 50 WP; Tilt 250 EC; Dithane M-45 and Cupravit 50WP) solution was prepared dissolving required amount of fungicide in water in 100ml Erlenmeyer flask. Flasks were labeled appropriately and shaken thoroughly before use.

3.2.17 Spraying of fungicides

The fungicides were sprayed at flowering, fruiting, and post fruiting stages. Sprays were applied after 24 hours of inoculation. The spraying was done with the help of a Mortein Hand Sprayer to cover whole surface of leaves, flowers, and fruits. Precautions were taken to avoid drifting of spray materials to neighboring plant.

3.2.18 Intercultural operation

After emergence of seedlings, various intercultural operations were accomplished for better growth and development. After 21 days of transplantation, 1/3 urea and 1/3 of remaining Murate of Potash (MP) were applied by ring method followed by weeding and watering. Remaining 2/3 urea and Murate of Potash (MP) were applied after 35 and 60 days of transplantation. The plants were kept free from insect pest attack by spraying insecticides as required intervals.

3.2.18.1 Irrigation

Light over-head irrigation was provided with a watering cane to the plots after transplantation. Irrigation also applied considering the moisture status.

3.2.18.2 Weeding

Weeding was done several times in the experimental plots. Nirani was used to make the soil loose.

3.2.19 Data Collection

After inoculation and spraying of fungicides, data were taken at every 10 days intervals on the following parameters:

% Leaf infection

% LAD (Leaf area diseased)

% Fruit infection

% FAD (Fruit area diseased)

Percent LAD (Leaf area diseased) and percent FAD (Fruit area diseased) were measured by eye estimation. Area of single leaf/fruit was considered as 100%. Deducting the healthy area, the diseased area was estimated. Average of % LAD/FAD was then calculated dividing the total diseased areas by the investigated leaves / fruits (Islam, 2005)

3.2.20 Design and layout of the Experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The layout of the experiment was prepared for distributing the treatment combinations in each plot of each block. There were 30 unit plots altogether in the experiment. The size of the plot was 3.0 m × 1.0 m, Plant to plant distance 0.75 m, Plot to boundary distance 0.5 m, Plot to plot distance 0.5 m and Row to row distance 1.0 m, Soil in between two plots were taken over to make it raised, thereby facilitating free movement for nursing and free drainage

of excess water during rain. Four fungicides, three fertilizers and control, three replications constituted the treatments

3.2.21 Statistical analysis of Data

The data obtained for different characters were statistically analyzed to find out the significance of the treatment on eggplant. The analysis of variance was performed by using MSTAT Program. The significance of the difference among the treatment means was estimated by DMRT (Duncan's Multiple Range Test) at 5% level of probability.

Chapter 4
Results

RESULTS

4.1 ISOLATION AND IDENTIFICATION OF CAUSAL AGENT

The causal fungus was isolated from both infected leaves and fruits and studied in the laboratory. The fungus was purified and identified as *Phomopsis vexans* (Photograph 4). In PDA the fungus grew with whitish mycelia which later developed dark gray colony. On fruits, colonies developed with concentric ring zones. Conidia of *Phomopsis vexans* were hyaline and single celled. Two types of conidia-Alpha and Beta. Alpha conidia was globose and fusoid (Photograph 5).

4.2 LABORATORY EXPERIMENT

4.2.1 Effect of fungicides against *Phomopsis vexans* in the laboratory

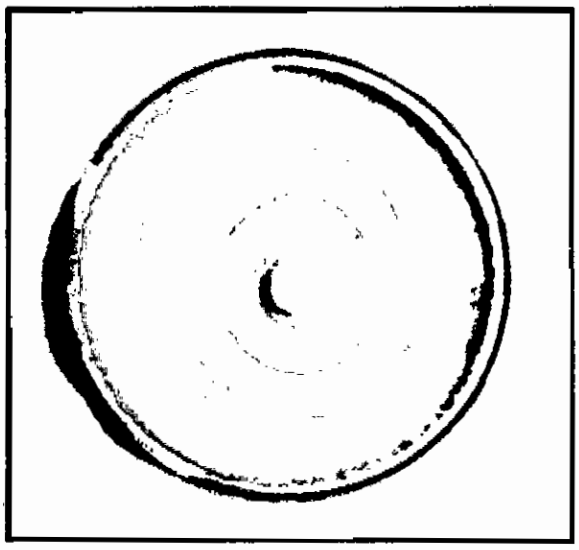
Of the four fungicides assayed against *Phomopsis vexans* by Cup method and Disc method, Bavistin 50WP and Tilt 250EC were found promising in reducing the mycelial growth of the fungus (Table 1). In Cup method, Bavistin 50 WP (0.1%) completely inhibited mycelia growth. Among the other fungicides, Tilt 250 EC, Dithane M-45 and Cupravit 50 WP showed significant inhibitory effect in reducing mycelia growth over control.

In Disc method method, the performance of the fungicides against the fungus was more or less similar with that of Cup method. The highest inhibition zone (6.29 cm) was formed by Bavistin 50 WP followed by Tilt 250EC (4.31cm) and Dithane M-45 (2.78 cm).

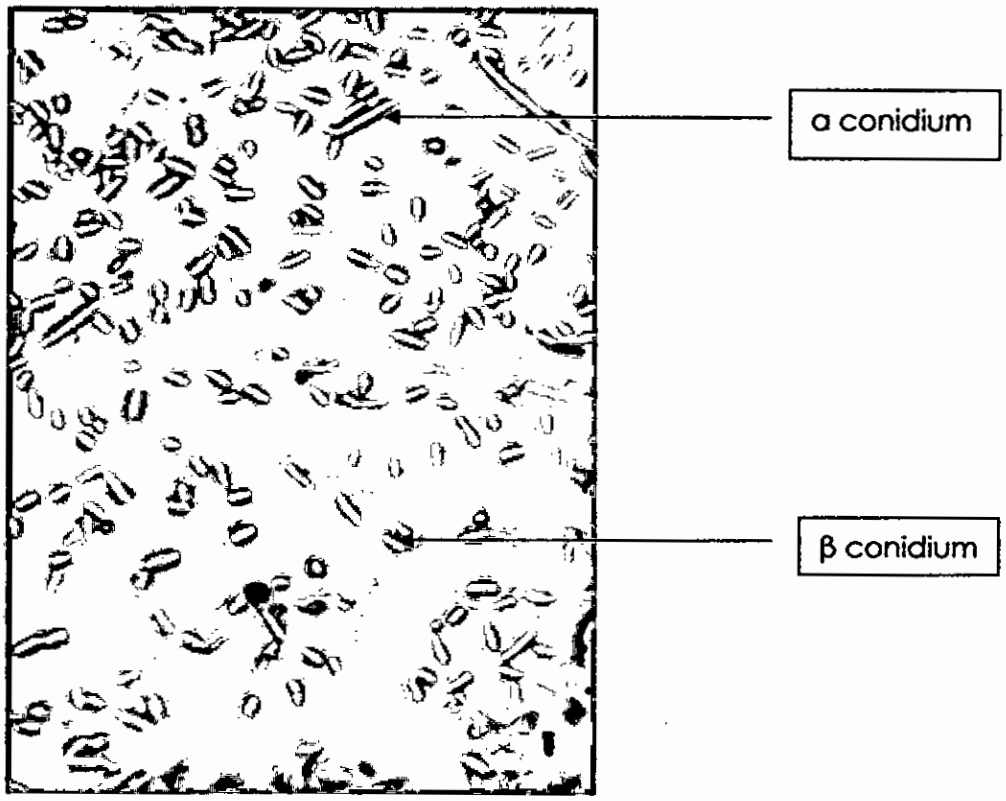
Table 1. Effect of different fungicides on inhibition of mycelial growth of *Phomopsis vexans* in the laboratory

Fungicides	Active ingredients	Cup method	Disk method
		Radial mycelial growth (cm)	Diameter of inhibition zone (cm)
Bavistin 50WP	50% Carbendazim	0.00 d	6.29 a
Tilt 250EC	25% Propiconazole	4.26 c	4.31 b
DithaneM-45	80% Mancozeb	5.43 b	2.78 c
Cupravit 50WP	50% Copperoxychloride	6.61 a	1.45 d
Control	-	7.71 a	0.00 e
LSD (P=0.05)		0.30	0.45

Figures with common letters do not differ at p=0.05



Photograph 4: Pure culture of *Phomopsis vexans*



Photograph 5: α and β conidium of *Phomopsis vexans* seen under compound microscope

4.3 FIELD EXPERIMENT

4.3.1 Effect of fungicides and micronutrients at pre-flowering stage in the field condition

The effect of fungicides and micronutrients on disease incidence (percent leaf infection) and disease severity (percent leaf area diseased) at pre flowering stage were summarized in Table-2. The fungicides and micronutrients applied either individually or in combination showed significant effect in terms of percent leaf infection (%LI) and disease severity i.e. leaf area diseased (%LAD) in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance in reducing disease incidence and disease severity than the fungicides applied alone. Significantly the lowest leaf infection (14.15%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf infection (31.09%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

Similar trend of the effect of fungicides and micronutrients was observed in case of percent leaf area diseased (%LAD). The lowest leaf area diseased (6.12%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination

with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf area diseased (14.63%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit alone (T_4) and application of Cupravit in combination with micronutrients (T_8).

Table 2. Efficacy of fungicides and micronutrients in controlling *Phomopsis vexans* causing leaf blight and fruit rot of eggplant at pre-flowering stage in the field condition

Treatments	% Leaf Infection (LI)	% Leaf Area Diseased (LAD)
T_1	31.09a	14.63 a
T_2	27.40b	12.21b
T_3	16.16e	6.99f
T_4	27.91b	12.37b
T_5	20.62d	8.94de
T_6	24.52c	10.49c
T_7	14.15e	6.12f
T_8	27.51b	11.79b
T_9	24.19c	10.16cd
T_{10}	19.31d	8.73e
LSD (0.05)	2.523	1.239

Figures with common letters do not differ at p=0.05

T_1 = Normal fertilization (Cowdung+Urea+TSP+MP) - Control

T_2 = Normal fertilization + Micronutrients (Gypsum+ZnO+Boric acid)

T_3 = Normal fertilization + foliar spraying with Bavistin 50 WP

T_4 = Normal fertilization + foliar spraying with Cupravit 50 WP

T_5= Normal fertilization + foliar spraying with Tilt 250 EC

T_6 = Normal fertilization+ foliar spraying with Dithane M-45

T_7 = Normal fertilization + Micronutrients + foliar spraying with Bavistin 50 WP

T_8 = Normal fertilization + Micronutrients + foliar spraying with Cupravit 50 WP

T_9 = Normal fertilization + Micronutrients + foliar spraying with Dithane M-45

T_{10}= Normal fertilization + Micronutrients + foliar spraying with Tilt 250 EC

4.3.2 Effect of fungicides and micronutrients at post-flowering stage in the field condition

The effect of fungicides and micronutrients on disease incidence and disease severity at post-flowering stage were summarized in Table-3. The fungicides and micronutrients applied either individually or in combination showed significant effect in terms of disease incidence (percent Leaf Infection) and disease severity (percent Leaf Area Diseased) in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance in reducing disease incidence and disease severity than the fungicides applied alone. The lowest leaf infection (13.65%), was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf infection (39.51%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit alone (T_4) and application of Cupravit in combination with micronutrients (T_8).

More or less similar trend of effects of fungicides and micronutrients were observed in case of percent leaf area diseased (%LAD). The lowest leaf area diseased (6.23%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf area diseased

(19.09%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

Table 3. Efficacy of fungicides and micronutrients in controlling *Phomopsis vexans* causing leaf blight and fruit rot of eggplant at post-flowering stage in the field condition

Treatments	% Leaf Infection (LI)	% Leaf Area Diseased (LAD)
T_1	39.51a	19.09a
T_2	30.63b	16.57b
T_3	14.49e	7.89e
T_4	31.09b	16.11b
T_5	21.87d	10.11d
T_6	28.79bc	13.50c
T_7	13.65e	6.23f
T_8	29.71b	15.15b
T_9	26.05c	12.73c
T_{10}	21.31d	9.78d
LSD (0.05)	2.977	1.407

Figures with common letters do not differ at p=0.05

T_1 = Normal fertilization (Cowdung+Urea+TSP+MP) - Control

T_2 = Normal fertilization + Micronutrients (Gypsum+ZnO+Boric acid)

T_3 = Normal fertilization + foliar spraying with Bavistin 50 WP

T_4 = Normal fertilization + foliar spraying with Cupravit 50 WP

T_5= Normal fertilization + foliar spraying with Tilt 250EC

T_6 = Normal fertilization + foliar spraying with Dithane M-45

T_7 = Normal fertilization + Micronutrients + foliar spraying with Bavistin 50 WP

T_8 = Normal fertilization + Micronutrients + foliar spraying with Cupravit 50 WP

T_9 = Normal fertilization + Micronutrients + foliar spraying with Dithane M-45

T_{10}= Normal fertilization + Micronutrients + foliar spraying with Tilt 250EC

4.3.3 Effect of fungicides and micronutrients at fruiting stage in the field condition

The efficacy of fungicides and micronutrients on disease incidence (percent Leaf Infection and percent Fruit Infection) and disease severity (percent Leaf Area Diseased, percent Fruit Area Diseased) at fruiting stage were summarized in Table-4. The fungicides and micronutrients applied either individually or in different combinations showed significant effect in terms of disease incidence and disease severity in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance than the fungicides applied alone in reducing disease incidence and disease severity. The lowest leaf infection (14.24%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) that was followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf infection (47.03%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

In case of percent Leaf Area Diseased (%LAD) the similar trend of effects of fungicides and micronutrients was observed. The lowest leaf area diseased (4.60%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) which was followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients

(T_9) and application of Dithane M-45 alone (T_6). The highest leaf area diseased (21.31%) was observed in case of control (T_1) proceeded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

The effect of fungicides and micronutrients in case of percent fruit infection (%FI) was observed more or less similar to that of leaf infection. The lowest fruit infection (16.02%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7). The second highest performance in reducing fruit infection was recorded in case of application of Bavistin 50 WP alone (T_3) followed by Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The maximum fruit infection (34.35%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

In case of percent Fruit Area Diseased (%LAD), the lowest fruit area diseased (4.89%) was recorded in case of Bavistin 50 WP applied in combination with micronutrients (T_7) which was followed by application of Bavistin 50 WP alone (T_3), Tilt 250 EC applied in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest fruit area diseased (14.00%) was noticed in case of control (T_1) proceeded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

Table 4. Efficacy of fungicides and micronutrients in controlling *Phomopsis vexans* causing leaf blight and fruit rot of eggplant at fruiting stage in the field condition

Treatments	% Leaf Infection (LI)	% Leaf Area Diseased (LAD)	% Fruit Infection (FI)	% Fruit Area Diseased (FAD)
T_1	47.03a	21.31a	34.35a	14.00a
T_2	38.47b	12.02b	29.93b	10.92b
T_3	16.50e	5.50f	17.00e	6.03f
T_4	37.65b	12.00b	28.40b	9.83b
T_5	24.21d	7.83de	20.77d	7.45de
T_6	31.03c	9.23c	24.46c	8.78c
T_7	14.24e	4.60f	16.02e	5.89f
T_8	35.51b	11.12b	27.58b	10.03b
T_9	29.18c	8.70cd	24.50c	8.19cd
T_{10}	22.50d	7.30e	20.44d	7.10e
LSD (0.05)	3.775	0.9474	2.647	0.9427

Figures with common letters do not differ at p=0.05

T_1 = Normal fertilization (Cowdung+Urea+TSP+MP) - Control

T_2 = Normal fertilization + Micronutrients (Gypsum+ZnO+Boric acid)

T_3 = Normal fertilization + foliar spraying with Bavistin 50 WP

T_4 = Normal fertilization + foliar spraying with Cupravit50 WP

T_5= Normal fertilization + foliar spraying with Tilt 250EC

T_6 = Normal fertilization + foliar spraying with Dithane M-45

T_7 = Normal fertilization + Micronutrients + foliar spraying with Bavistin 50 WP

T_8 = Normal fertilization + Micronutrients + foliar spraying with Cupravit 50 WP

T_9 = Normal fertilization + Micronutrients + foliar spraying with Dithane M-45

T_{10}= Normal fertilization + Micronutrients + foliar spraying with Tilt 250EC

4.3.4 Effect of fungicides and micronutrients at fruit ripening stage in the field condition

The effect of fungicides and micronutrients on disease incidence (percent Leaf Infection and percent Fruit Infection) and disease severity (percent Leaf Area Diseased and percent Fruit Area Diseased) at fruit ripening stage were summarized in Table-5. The fungicides and micronutrients applied either individually or in combination showed significant effect in terms of disease incidence (%LI and %FI) and disease severity (%LAD and %FAD) in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance in reducing disease incidence and disease severity than the fungicides applied alone. Significantly the lowest leaf infection (15.03%), was recorded in case of Bavistin 50 WP applied in combination with micronutrients (T_7) that was followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9), application of Dithane M-45 alone (T_6). The highest leaf infection (90.31%) was observed in case of control (T_1). The application of micronutrients alone (T_2) showed significantly lower leaf infection than control preceded by application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

The effect of fungicides and micronutrients in reducing percent fruit infection (%FI) was more or less similar to that of percent leaf infection (%LI). The lowest fruit infection (15.57%) was recorded in case of Bavistin 50 WP applied in combination with micronutrients (T_7) which was followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt

250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest fruit infection (68.89%) was observed in case of control (T_1) preceded by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

In case of percent leaf area diseased (%LAD), the lowest leaf area diseased (4.38%) was recorded in case of Bavistin 50 WP applied in combination with micronutrients (T_7) that followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest leaf area diseased (29.69%) was observed in case of control (T_1) followed by application of micronutrients (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

Similar trend of effect of fungicides and micronutrients was observed in case of % Fruit Area Diseased (%FAD). The lowest fruit area diseased (9.09%) was recorded in case of application of Bavistin 50 WP in combination with micronutrients (T_7) followed by application of Bavistin 50 WP alone (T_3), application of Tilt 250 EC in combination with micronutrients (T_{10}), application of Tilt 250 EC alone (T_5), application of Dithane M-45 in combination with micronutrients (T_9) and application of Dithane M-45 alone (T_6). The highest fruit area diseased (25.19%) was observed in case of control (T_1) followed by application of micronutrients alone (T_2), application of Cupravit 50WP alone (T_4) and application of Cupravit 50WP in combination with micronutrients (T_8).

Table 5: Efficacy of fungicides and micronutrients in controlling *Phomopsis vexans* causing leaf blight and fruit rot of eggplant at fruit ripening stage in the field condition

Treatments	% Leaf Infection (LI)	% Leaf Area Diseased (LAD)	% Fruit Infection (FI)	% Fruit Area Diseased (FAD)
T_1	90.31a	29.69a	68.89a	25.19a
T_2	52.51b	14.93b	43.21b	18.43b
T_3	17.82e	5.02e	16.82e	6.01e
T_4	52.06b	13.40b	42.31b	17.19b
T_5	28.69d	8.05d	23.39d	8.89d
T_6	37.55c	10.24c	32.13c	13.77c
T_7	15.03e	4.38e	15.57e	5.09e
T_8	48.21b	13.12b	38.63b	16.48b
T_9	35.67c	9.19cd	29.66c	11.79c
T_{10}	25.04d	7.55d	20.22de	8.05d
LSD (0.05)	4.350	1.904	4.962	2.022

Figures with common letters do not differ at p=0.05

T_1 = Normal fertilization (Cowdung+Urea+TSP+MP) - Control

T_2 = Normal fertilization + Micronutrients (Gypsum+ZnO+Boric acid)

T_3 = Normal fertilization + foliar spraying with Bavistin 50 WP

T_4 = Normal fertilization + foliar spraying with Cupravit 50 WP

T_5= Normal fertilization + foliar spraying with Tilt 250EC

T_6 = Normal fertilization + foliar spraying with Dithane M-45

T_7 = Normal fertilization + Micronutrients + foliar spraying with Bavistin 50 WP

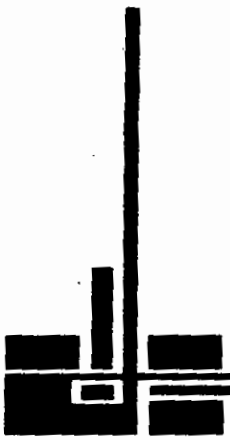
T_8 = Normal fertilization + Micronutrients + foliar spraying with Cupravit 50 WP

T_9 = Normal fertilization + Micronutrients + foliar spraying with Dithane M-45

T_{10}= Normal fertilization + Micronutrients + foliar spraying with Tilt 250EC

Photograph 6: Healthy eggplant in Bavistin 50WP treated plot

Photograph 7: Phomopsis blight & fruit rot of eggplant in untreated plot



Chapter 5
Discussion

DISCUSSION

Symptomology and causal organism

Symptoms of Phomopsis blight and fruit rot caused by *Phomopsis vexans* as observed in the experimental plot conform with those reported by Walker (1952) from United States of America; Kumar *et al.,* (1986) and Singh (1992) from India; Ahmad (1987); Das (1998) and Islam (2005) from Bangladesh. Walker (1952) described the first phase of the disease as blight of young seedlings. Singh (1992) described it as tip over, stem blight or canker and mummified fruit rot. Islam (2005) also observed the disease as seedling blight, tip over and mummified fruit rot.

The organism isolated from diseased fruits collected from farmers' field and experimental plot was resembled with those described by Islam (2005) and CMI description of pathogenic fungi and bacteria No 338. Islam (2005) observed the fungus as whitish mycelial growth in cultured media with the presence of concentric growth and sporadic pycnidia partially emerges in the media. Islam (2005) also reported that pycnidia possessed both α & β conidia.

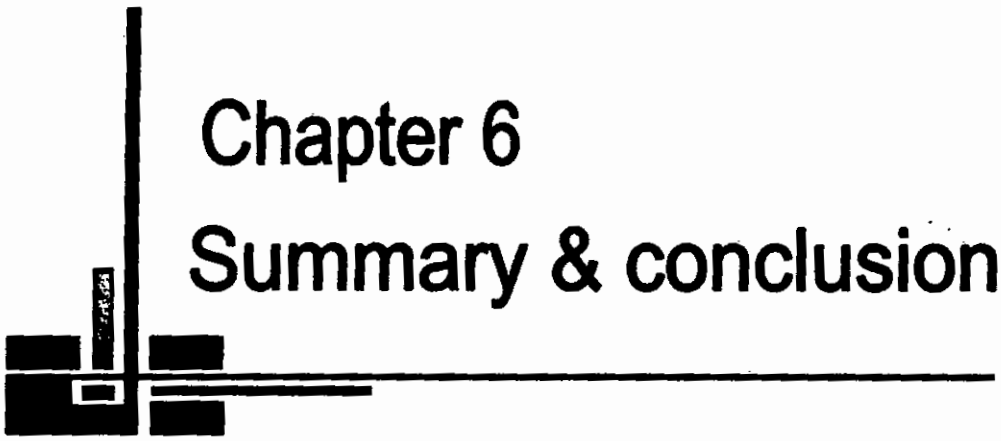
Laboratory assay of findings

The result showed that Bavistin 50WP (Carbendazim) was found promising against *Phomopsis vexans* in the laboratory assay. Bavistin (0.1%) completely arrested the mycelial growth and produced largest inhibition zones (6.29 cm) in culture media while assayed by food poisonic techniques following cup and disc method. The performance of Tilt 250 EC in controlling the mycelial growth of *Phomopsis vexans* was also promoting next to Bavistin 50WP. The performances of rest of the fungicides were less promising but significantly better than control. The results of the laboratory assay corroborate with the reports of Mohanty *et*

al., (1994) and Islam (2005). Mohanty while working with 5 fungicides in *in vitro* condition against *Phomopsis vexans* reported that Bavistin 50WP (0.1%) showed the highest performance that caused 100% inhibition of mycelial growth of *Phomopsis vexans* in the culture media. Islam (2005) assayed 4 fungicides against *Phomopsis vexans* by food poisonic method and found that Bavistin 50 WP (Carbendazim) completely arrested the mycelial growth in cup method and produce the largest inhibition zone in disc method in the culture media.

From the field study it was evident that the pathogen *Phomopsis vexans* was effectively controlled by post inoculation spray of Bavistin 50WP (0.1%) applied in combination with micronutrients at pre-flowering, post-flowering, fruiting and fruit ripening stages of eggplant in terms of leaf infection (LI), leaf area diseased (LAD), fruit infection (FI) and fruit area diseased(FAD). The performance of Tilt 250EC was next to Bavistin 50WP while applied in combination with micronutrients. The result showed that the application of micronutrients alone showed significantly better performance in controlling Phomopsis blight and fruit rot disease of eggplant compared to control. The result also showed that the fungicides while applied in combination with micronutrients always performed better compared to the fungicides applied alone. The present findings regarding the efficacy of fungicides in controlling Phomopsis blight and fruit rot of eggplant are in agreement with the findings of Islam (2005), Meah (2003), Khan *et al.*, (2002); Meah *et al.,* (1998); Kausal and Sugha (1995); Islam and Sitansu (1992) and Islam (2005) while studying the efficacy of different fungicides for the management of Phomopsis blight and fruit rot of eggplant in the field condition reported that Bavistin 50WP @ 0.1% showed the highest performances in reducing the disease incidence (%LI and %FI) and disease severity

(%PDI of leaf and fruit) among the Bavistin 50WP, Tilt 250EC, Dithane M-45 and Cupravit 50WP tested fungicides at pre-flowering, post-flowering, fruiting and fruit ripening stages and increasing fruit yield. Meah *et al.,* (1998) reported that Bavistin 50WP @ 0.2% applied both before and after inoculation of *Phomopsis vexans* on surface rough fruit and showed effective performance in reducing percent fruit infection and fruit area diseased producing the least sized lesion. Meah (2003) also reported that Bavistin 50WP (0.1%) significantly controlled Phomopsis blight and fruit rot of eggplant in the nursery as well as in the field condition. Khan *et al.,* (2002) reported that Bavistin 50WP (Carbendazim) found to be effective (0.2%) against *Phomopsis vexans* that significantly reduced the leaf infection of eggplant. Kausal and Sugha (1995) reported that seed treatment with Carbendazim alone or in combination with Thiram provided better control of pre-emergence and post-emergence damping off seedling caused by *Phomopsis vexans*. Islam and Sitansu (1992) stated in a field trial against *Phomopsis vexans*, Carbendazim (0.1%) provided nearly complete control of leaf blight and fruit rot of eggplant with 3 sprays at 15 days interval. Islam *et al.,* (1990) found in a field trial following *in vitro* screening of different fungicides, Bavistin 50 WP (Carbendazim) at 0.1% gave the best control against Phomopsis blight and fruit rot of eggplant. However, the present results do not corroborate with the report of Kaur and Bedi (1998) that Dithane M-45 better controls the fungus. They also did not include Bavistin 50WP as a candidate fungicide in their trail. In our present findings micronutrients had some positive effects in controlling leaf blight and fruit rot of eggplant applied alone or in combination with fungicides. No previous reports are available in controlling the disease by applying micronutrients.



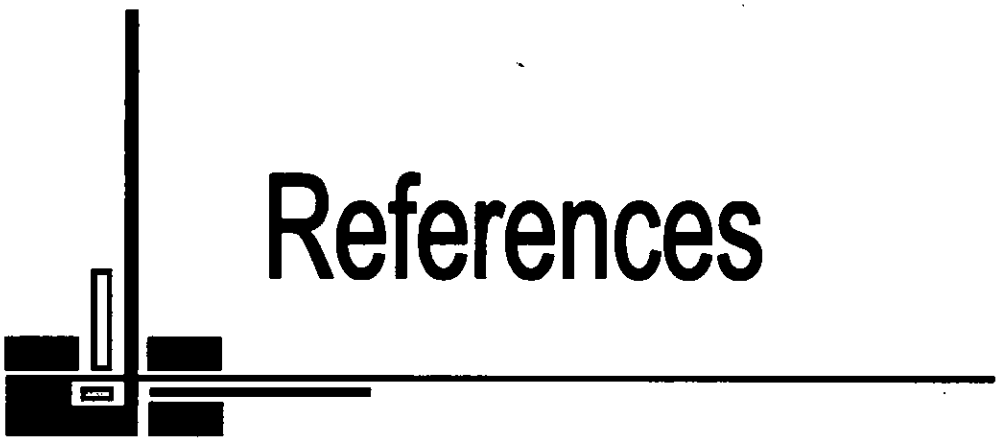
Chapter 6
Summary & conclusion

SUMMERY AND CONCLUSION

Experiments were conducted to control Phomopsis blight and fruit rot of eggplant through fungicides and micronutrients during Rabi season (November - March) of 2007-2008. Four fungicides viz. Bavistin 50 WP (Carbendazim), Tilt 250 EC (Propiconazole), Cupravit 50 WP (Copperoxychloride) and Dithane M-45 (Mancozeb) and micronutrients (Gypsum, ZnO and Boric acid) were evaluated against *Phomopsis vexans* causing Phomopsis blight and fruit rot of eggplant. The fungicides and micronutrients applied either individually or in combination showed significant effect in terms of percent leaf infection (%LI), fruit infection (%FI), leaf area diseased (%LAD) and fruit area diseased (%FAD) in comparison to control. Effect of each fungicide applied in combination with micronutrients always showed better performance in reducing disease incidence and disease severity than the fungicides applied alone. The experiment was conducted in the laboratory and field condition. Among the fungicides, Bavistin 50WP (0.1%) proved to be effective arresting the spore germination and mycelia growth of *Phomopsis vexans* assayed in *in vitro* test. In field condition, Bavistin 50 WP (0.1%) in combination with micronutrients and Bavistin 50 WP (0.1%) showed promising performance in leaf infection, fruit infection, leaf area diseased and fruit area diseased applied as post inoculation spray at pre-flowering, post flowering, fruiting and fruit ripening stages. Reduction of leaf area diseased (%LAD) caused by Bavistin 50 WP (0.1%) in combination with micronutrients were 58.17%, 67.37%, 78.41% and 85.25%, respectively at pre-flowering, post-flowering, fruiting and fruit ripening stages while Bavistin 50 WP (0.1%) alone reduced (%LAD) by 52.22%, 58.67%, 74.19% and 83.09%, respectively at those stages. Similarly reduction of fruit area diseased (%FAD) caused by Bavistin 50 WP (0.1%) in

combination with micronutrients were 57.93% and 79.79%, respectively at fruiting and fruit ripening stages while Bavistin 50WP (0.1%) alone reduced (%FAD) by 56.93% and 76.14%, respectively at those stages. Bavistin 50 WP (0.1%) in combination with micronutrients control Phomopsis blight and fruit rot of eggplant somewhat more than Bavistin 50 WP (0.1%) alone but both are statistically similar.

Considering the performance of fungicides in combination with micronutrients or fungicides alone evaluated in the experiment, it is concluded that Bavistin 50 WP (0.1%) alone or in combination with micronutrients (Gypsum, ZnO and Boric acid) could be used for management of Phomopsis blight and fruit rot of eggplant. However, the investigation needs to conduct for consecutive years in different Agro-Ecological Zones to reconfirm the findings.



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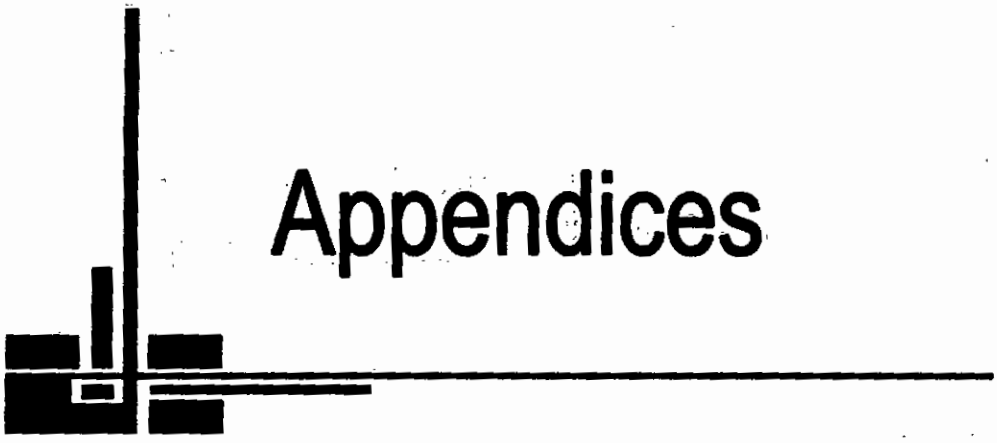

Appendices

APPENDICES

Appendix I: Monthly average air temperature, relative humidity and total rainfall of the experimental site during the period from November, 2007 to March, 2008.

Month	Relative humidity (%)	Maximum temperature (°C)	Minimum temperature (°C)	Mean	Rainfall (mm)
November	69.5	29.5	18.6	24.0	3.0
December	70.6	26.9	16.2	21.5	00
January	68.5	24.5	13.9	19.2	4.0
February	61.0	28.9	18.0	23.4	3.0
March	62.5	28.5	19.0	23.75	3.0

Source: Bangladesh Meteorological Department (Climate Division), Agargaon, Dhaka-1212.

Appendix II: Physicochemical properties of the initial soil

Characteristics	Value	Critical value
% sand	34	.....
% silt	46	.....
% clay	20	.....
Textural class	Silty-clay	.....
pH	5.6	Acidic
Organic carbon (%)	0.45	.....
Organic matter (%)	0.78	.....
Total N (%)	0.03	0.12
Available P (ppm)	20.00	27.12
Exchangeable K (me/100 g soil)	0.10	0.12
Available S (ppm)	45	.....

Source: Soil Resources Development Institute (SRDI), Dhaka-1207.

Appendix III. Food and nutritional value of eggplant

Sl. No.	Nutrition	Amount (g) per 100 g edible part
1	Protein	1.8
2	Carbohydrate	2.2
3	Fat	2.9
4	Mineral salts	0.8
5	Vitamin B-1	0.12 mg
6	Vitamin B-2	0.08 mg
7	Vitamin C	5.0 mg
8	Calcium	28.0 mg
9	Iron	0.9 mg
10	Carotin	850 I.U.

Calory = 82 K. cal

Source : Anonymous 1980. Nutritional value of native foods and vegetables. Food and Nutrition Institute, University of Dhaka, Bangladesh.

Appendix IV. Summary of analysis of variance of percent leaf infection (%LI), fruit infection (%FI), leaf area diseases (%LAD) and fruit area diseased (%FAD) at pre-flowering stage, post-flowering stage, fruiting stage and fruit ripening stage

SOURCE	df	Mean squares											
		Pre-flowering stage		Post-flowering stage		Fruiting stage				Fruit ripening stage			
		% LI	%LAD	% LI	%LAD	% LI	%LAD	%FI	%FAD	% LI	%LAD	%FI	%FAD
Replication	2	0.494	0.688	5.233	1.312	0.400	0.521	1.900	0.050	2.133	0.093	0.700	0.528
Treatments	9	105.694	25.240	226.940	69.138	516.993	100.186	127.269	22.844	2142.802	250.008	1084.045	152.215
Error	18	2.163	0.522	3.011	0.673	4.844	0.305	2.381	0.302	6.430	1.232	8.367	1.390

Appendix V. Layout of the field experiment