CONTROL OF *ALTERNARIA* BLIGHT OF MUSTARD THROUGH FERTILIZER AND FUNGICIDE APPLICATION

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CONTROL OF *ALTERNARIA* BLIGHT OF MUSTARD THROUGH FERTILIZER AND FUNGICIDE APPLICATION

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CERTIFICATE

This is to certify that thesis entitled, "**CONTROL OF *ALTERNARIA* BLIGHT OF MUSTARD 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**, embodies the results of a piece of bona fide research work carried out by **MD. NASIM HAIDER**, Registration No. 03-01182 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in anywhere.

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

Dated: December, 2008
Place: Dhaka,Bangladesh

....
(Dr. Md. Rafiqul Islam)
Professor
Supervisor

Dedicated
to
My Beloved
Parents

SOME COMMONLY USED ABBREVIATIONS AND SYMBOLS

Abbreviations		Full word
%	=	Percent
@	=	At the rate
°C	=	Degree Celsius
AEZ	=	Agro-Ecological Zone
Agric.	=	Agriculture
Agril.	=	Agricultural
Agron.	=	Agronomy
ANOVA	=	Analysis of variance
B	=	Boron
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
BD	=	Bangladesh
BSMRAU	=	Bangabundhu Sheikh Mujibur Rahaman Agricultural University
CEC	=	Cation Exchange Capacity
cm	=	Centi-meter
CV%	=	Percentage of Coefficient of Variation
DAI	=	Days after incubation
DAS	=	Days After Sowing
df	=	Degrees of Freedom
DMRT	=	Duncan's Multiple Range Test
EC	=	Emulsifiable Concentrate
et al.	=	And others
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
hr.	=	Hour (s)
j.	=	Journal
K	=	Potassium
kg	=	kilogram (s)
Kg/ha	=	Kilograms per hectare
LAD	=	Leaf area diseased
m	=	Meter
M.P.	=	Muriate of Potash
m^2	=	Square meter
MOA	=	Ministry of Agriculture
MSE	=	Mean square of the error
N	=	Nitrogen
No.	=	Number
NS	=	Not Significant
P	=	Phosphorus
PDA	=	Potato Dextrose Agar

ppm	=	Parts Per Million
RCBD	=	Randomized Complete Block Design
Rep.	=	Replication
Res.	=	Research
S	=	Sulfur
SAU	=	Sher-e-Bangla Agricultural University
Sci.	=	Science
SE	=	Standard Error
T.S.P.	=	Triple Super Phosphate
t/ha	=	Tones per hectare
Univ.	=	University
var.	=	Variety
Viz.	=	Namely
WP	=	Wetable powder
YPP	=	Yield per plant
Zn	=	Zinc
ZnO	=	Zinc oxide

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Place: SAU, Dhaka.

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CONTROL OF *ALTERNARIA* BLIGHT OF MUSTARD THROUGH FERTILIZER AND FUNGICIDE MANAGEMENT

ABSTRACT

The efficacy of different fungicides and micronutrients alone or in combination were evaluated against *Alternaria brassicae* causing *Alternaria* blight of mustard. The experiments were conducted *in-vitro* and *in-vivo* in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during November 2007 to March 2008. Four fungicides namely Rovral 50 WP (0.2%), Dithane M-45 (0.45%), Ridomil gold MZ 72 (0.2%) and Bavistin (0.1%) and micronutrients (Zypsum, Zinc oxide and Boric acid) were tested. In *in-vitro* test, Rovral 50 WP inhibited the highest radial mycelial growth (68.91%) of *Alternaria brassicae* followed by Dithane M-45 (63.87%) Ridomil gold (55.46%) and Bavistin (50.42%) compared to control. In the field experiment, 10 treatments including spray of fungicides alone or in combination with micronutrients were evaluated against the disease. Among those treatments, Rovral 50 WP + Micronutrients showed the highest performance against *Alternaria* blight in terms of disease incidence and severity followed by application of Rovral 50 WP alone, Dithane M-45 + Micronutrients and Dithane M-45 alone. The treatment Rovral 50 WP + Micronutrients reduced the leaf infection, pod infection and leaf area diseased by 82.31%, 78.19% and 86.24%, respectively compared to control which was followed by application of Rovral 50 WP alone and Dithane M-45 + Micronutrients. The yield and yield contributing characters were influenced by the treatments where the highest yield was recorded in case of Rovral 50 WP + Micronutrients that was 39.80% higher over control. The performances of micronutrients were significantly better than control but the performances of micronutrients did not differ significantly while it was used in combination with fungicides compared to the application of fungicides alone.

Chapter 1
Introduction

CHAPTER I
INTRODUCTION

Brassica, accounting for over 16% of the world's edible oil supply is an important genus of plant kingdom consisting of over 3200 species. The primary centre of origin of *Brassica campestris* is near the Himalayan region and the secondary centre of origin is located in the European –mediterranean area and Asia (Downey and Robelen, 1989). Major producing regions of mustard are China, the Indian subcontinent, Canada and Northern Europe (Ram and Hari, 1998).

A good number of oilseed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower and soybean are being cultivated in Bangladesh. *Brassica* have great economic commercial value and play a major role in feeding the world population. They range from nutritious vegetables condiments and oil producing crops to animal fodders. According to FAO (2005) the oil yielding crop *Brassica* holds the second position in the world oilseeds in respect of production. In Bangladesh rapeseed and mustard of *Brassica* is the major source of edible oil and more than 183 thousand metric tons of rape and mustard were produced from total 316.92 thousand hectares of cultivable land in the year 2005-2006 (BBS, 2006). Its average yield per hectare was only 733 kg in Bangladesh compared to the world average of 1575 kg (FAO, 2005). Almost two-third of the edible oil consumed annually in Bangladesh is imported and foreign exchange spent for this was about 690 million US dollars (BBS, 2004). The per capita consumption of edible oil in the country is 10-12 g/head/day as compared to the need of 40 g/head/day (Kaul and Das, 1986). The seeds of *Brassica* contain 42% oil and 25% protein (Khaleque, 1985). Using local Ghani an average of 33% oil may be extracted.

In this sub-continent three species of *Brassica* are cultivated for oil purposes, viz. *Brassica campestris, Brassica juncea* and *Brassica napus* (Anonymous, 2001). Variety SAU Sarisha-1 is *Brassica campestris.*

Many factors are associated with the poor yield of rapeseed-mustard in Bangladesh. Diseases have been identified as one of the major factors (Ahmed, 1992).

Rapeseed-mustard suffers from about 14 diseases (fungus-9, virus-2, bacteria-1, nematode-1 and parasitic plant-1) in Bangladesh. Among theses diseases, leaf blight, downy mildew and the parasitic plant are important (Anonymous, 2007). Leaf blight caused by *Alternaria brassicae* is widely distributed and the most serious and devastating disease of rapeseed-mustard. The characteristic symptom is the development of circular spots on leaves and pods with concentric ring. Later on spots coalesce and ultimately the leaves become blighted. The disease may cause 25% yield reduction at severe condition of infection (Anonymous, 2002).

Gray blight (*Alternaria brassicae*) (Berk) Sacc. causes blight of leaf, pod and stem (Meah *et al.*, 1988) and seed abnormalities (Howlider *et al.*, 1991). It is endemic in Bangladesh and all the cultivated *B. campestris* and *B. napus* varieties are susceptible to the disease. This disease causes an average yield loss of 40-70% in India and 30-60% in Bangladesh (Meah *et al.*, 1988). In addition to direct yield losses the disease adversely affects the seed quality reducing seed size, seed discolouration and reduction in oil contents (Howlider *et al.*, 1991; Kaushik *et al.*, 1984). Seed cleaning before sowing has recently been proved effective in reducing infection of seed-borne pathogens and increasing production of healthy seeds (Hossain and Doullah, 1998).

There is no information available on the resistance sources. Chemicals are being successfully used in controlling the disease (Meah *et al.*, 1988 and

Howlider *et al.*, 1985). Non-chemical methods of disease control may include use of biological agents, botanicals, adjustment in cultural practices etc. Researches with these ideas have yielded good results but not better than the use of chemicals.

A good number of fungicides and cultural practices are yet remained untested against this disease. Considering the present situation of the disease in the country, further selection of fungicides against *Alternaria* blight of Mustard is urgent by necessary. Fertilizer management like use of ash, cowdung, mustard oil cake, N, P, K, Zn, S and B could be the good options for the management of *Alternaria* blight of Mustard.

Considering the above facts, the present study was undertaken with the following objectives-

1. To determine the effect of selected fungicides in controlling growth of *Alternaria brassicae in-vitro*,
2. To evaluate the effect of selected fungicides either alone or in combination with micronutrients against *Alternaria* blight of mustard and
3. To determine the effect of selected fungicides either alone or in combination with micronutrients on yield and yield attributes of mustard.

Chapter 2
Review of literature

CHAPTER II

REVIEW OF LITERATURE

Alternaria blight of mustard caused by *Alternaria brassicae,* is a common and most important disease throughout the country. It causes serious yield reduction of the crop. Moreover, it is a threat to cultivation of mustard. Researchers all over the world have carried out intensive investigation on the *Alternaria* blight of mustard. Management of the disease by using chemical fungicides, host resistance, cultural practices and biological control measures are being explored in many countries of the world as the most common options for the investigations. Literature in relation to management of *Alternaria* blight of mustard is reviewed and presented below.

2.1 Chemical control

Singh *et al.* (2008) evaluated four fungicides, namely iprodione (0.2%), mancozeb (0.2%), Ridomil MZ [mancozeb+metalaxyl] (0.25%) and Apron [metalaxyl] S.T. (0.5%), as seed treatment against *Alternaria brassicae*, causing alternaria blight of mustard, in Kausambi, Uttar Pradesh, India. All the 4 fungicides were significantly superior in reducing the disease intensity over the untreated control. Iprodione was found to be the most effective fungicide in controlling the disease and increasing the yield followed by mancozeb.

Alam (2007) evaluated the efficacy of some selected fungicides and plant extracts against *Alternaria brassicae* and *Alternaria brassicicola* causing gray blight of mustard (var. SAU Sarisha-1, *Brassica campestris*). Experiments were conducted at the Farm of Sher-e-Bangla Agricultural University, Dhaka and in the laboratory of Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Rahmatpur, Barishal during rabi season during the month of November, 2006 to February, 2007. Four fungicides viz. Rovral 50 WP (0.2%), Dithane M-45 (0.3%), Ridomil 68 WP (0.2%), Bavistin DF (0.1%) and two plant extracts viz. Garlic clove extract, Allamanda leaf extract were employed in the experiment. Among the fungicides and plant extracts tested, Rovral 50 WP (0.2 %) showed the best performance in reducing disease incidence and disease severity as well as increasing seed yield against gray blight of mustard. Seed infection by *Alternaria spp.* was reduced by 64.90% and seed yield was increased by 48.19% over control by the application of Rovral.

Girish *et al.* (2007) sprayed five fungicides (Copper oxychloride, Aluminium tris, Metalaxyl, Chlorothalonil and Mancozeb) to manage white rust (*Albugo brassicae*) and Alternaria leaf blight (*Alternaria brassicae*) of mustard during kharif season of 2005 under field conditions (Karnataka, India). Among these sprays, aluminium tris was effective against white rust with minimum mean disease severity of 9.8%, followed by Mancozeb (15.50%) compared to the control (33.2%). Spraying of Mancozeb was effective against *Alternaria* leaf blight compared to control. Maximum seed yield (313 kg/ha) was recorded when the crop was sprayed with Metalaxyl, followed by Aluminium tris (239 kg/ha) and Mancozeb (233 kg/ha) compared to control (174 kg/ha).

Khan *et al.* (2007) evaluated three systemic fungicides: Topsin-M (Thiophanate methyl, 70%WP), Ridomil MZ (Mancozeb, 64%+Metalaxyl, 8%WP), and Bavistin (Carbendazim, 50%WP) alone and in combination with four non-systemic fungicides Captaf (Captan, 50%WP), Indofil M-45 (Mancozeb, 75%WP), Indofil Z-78 (Zineb, 75%WP), and Thiram (Thiram, 75%WP) both *in vitro* and *in vivo* for their effectiveness to manage *Alternaria* blight of rapeseed-mustard caused by *Alternaria brassicae*. All the fungicides were evaluated for their efficacy at various concentrations, 50, 100, 150, 200 and 500 ppm, and were sprayed in the field at 0.2% a.i. All fungicides significantly reduced the severity of the disease but Ridomil MZ was most effective. Topsin-

M at a concentration of 500 ppm was the most effective in reducing radial growth of the pathogenic fungi (74.2%). Ridomil MZ reduced disease severity by 32% followed by the combination Bavistin+Captaf (26.5%). Maximum yield was obtained in plots sprayed with Bavistin+Captaf (1198 kg ha^{-1}) followed by Bavistin+Indofil Z-78 (1172 kg ha^{-1}). The highest net profit as well as the highest cost-benefit ratio was obtained with Bavistin+Indofil Z-78 (1:3.2), followed by Bavistin+Captaf (1:1.3).

Hossain and Mian (2006) tested the efficacy of fungicides viz. of Rovral 50 WP (Iprodione), Dithane M-45 (80 WP Mancozeb), Bavistin 70 WP (Carbendazim) and Tilt 250 EC (Propiconazole) against *Alternaria* blight (*Alternaria brassicicola*) of cabbage. All the test fungicides significantly inhibited the mycelial growth of *Alternaria brassicicola* while Rovral was the best followed by Dithane M-45. Tilt treated plots showed adverse effect on seed yield, yield component, and seed viability.

Singh and Singh (2006) conducted a field experiment in India during 2002-03 and 2003-04 to develop spray schedule(s) for the management of mustard blight caused by *Alternaria brassicae* and *A. brassicicola*, and white rust caused by *Albugo candida* using Indian mustard (*Brassica juncea*) cv. Narendra Rai sown on 15 and 30 October, and 15 November. Mancozeb and Ridomil MZ-72 were sprayed in spray schedule combinations. Sowing on 15 October resulted in the lowest incidence of leaf blight, pod blight and white rust intensity and the highest 1000-seed weight and yield. Three consecutive sprays of Mancozeb 75 WP (0.2%) at fortnightly intervals, beginning at the disease initiation resulted in the lowest leaf blight incidence and pod blight intensity. Seed yield and 1000-seed weight under all the dates of sowing were highest with 2 consecutive sprays of Mancozeb followed by a third spraying with Ridomil MZ 72 (0.25%).

Prasad (2006) conducted a field trial during rabi 2002/03 and 2003/04, in India to evaluate the efficacy of different spraying combinations of three fungicides (Ridomil [metalaxyl], Carbendazim and Mancozeb) and five plant extracts (*Datura stramonium*, *Eucalyptus globosus*, *Azadirachta indica*, *Allium sativum* and *Allium cepa*) against *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. Varuna. Comparative analysis of various spraying schedules revealed that first spray of Carbendazim (0.1%) + Mancozeb (0.2%) followed by two sprays of Mancozeb (0.2%) at early sowing (20 October) was the best combination in reducing the disease severity on leaves (18.7%) and pods (10.4%) and in increasing yield (1295.8 kg/ha), 1000-seed weight (5.12 g) and oil content (42.6%). Sowing on 20 October also gave higher seed yield and reduced disease intensity on leaves and pods in comparison to later sowing. Among the botanicals integrated with the standard fungicide (Mancozeb), 5% aqueous extract of *D. stramonium, E. globosus* and *Allium sativum* reduced the disease intensity by 21.7, 23.3 and 25.5% on leaves, respectively.

Singh *et al.* (2006) reported that six seed dressing fungicides, i.e. Metalaxyl, Carbendazim, Mancozeb, Thiophanate-methyl, Iprodione and BAS 38601 F (a seed dressing fungicide, 40% Carbendazim+32% Mancozeb), in combination with spray of Mancozeb (0.25%) were tested for the control of foliar diseases, *Alternaria* leaf spot (*Alternaria brassicae*) and white rust (blister) [*Albugo candida*] of Indian mustard. All the seed treatments improved germination and reduced disease intensity. Seed treatment with Mancozeb and spray of same fungicide was most effective against *Alternaria* leaf spot controlling up to 58.8 to 74.7% disease. The highest yield were recorded with Iprodione (16.0-17.36 q/ha) and Mancozeb (26.0-31.12 q/ha).

Singh and Singh (2005a) conducted an experiment in India for controlling *Alternaria* blight (AB) caused by *Alternaria brassicae* and *A. brassicicola* and observed that seed treatment combined with three foliar sprays of Mancozeb 75% WP (0.2%) at 15-day intervals, beginning at 45 days after sowing, resulted in the lowest *Alternaria* blight incidence and the highest seed yield and cost-benefit ratio of 1:5.2. It was followed by foliar sprays of Mancozeb 75% WP alone in all cases.

Singh and Singh (2005b) investigated on timely sown (15-20 October) mustard crops during 1995/96-2001-02 revealed *Alternaria* blight [*Alternaria brassicae*] (AB), white rust [*Albugo candida*] (WR) and downy mildew (DM, *Peronospora parasitica*) were the major mustard diseases in mid-eastern India and, together, caused 44.06% avoidable yield loss. In trials conducted in the same field during 2001-02 and 2002-03 crop seasons, 3 sprays of iprodione 50 WP (Rovral; 0.20%), followed by mancozeb 75 WP (Indofil M 45, 0.20%) and propineb 70 WP (Antracol; 0.20%), gave the most effective control of *Alternaria* blight and yield gain. Significantly superior control of white rust was obtained by 2 sprays of metalaxyl+mancozeb 72 WP (Ridomil MZ; 0.25%) followed by 3 sprays of captan 50 WP (Captaf; 0.20%) and mancozeb.

Kumar *et al.* (2004) studied that the efficacy of different fungicides (Emisan 6 [2-methoxyethylmercury chloride], wettable sulfur, Ridomil MZ-72 [mancozeb + metalaxyl], Blitox-50 [copper oxychloride], Dithane M-45 [mancozeb], Kitazin [iprobenfos], Bavistin [carbendazim] and Baynate [thiophanate-methyl]) and neem products (Furpume, Bioneem, Nimbicidine and Achook) were tested against 15 isolates of *Alternaria brassicae* collected from different locations in Haryana, India. Kitazin was highly effective against all the isolates in inhibiting spore germination. It was followed by Dithane M-45 and Ridomil MZ-72 but was statistically at par. Similarly, Achook and Bioneem were also effective compared to furpume and nimbicidine. Variations were also observed among isolates in their sensitivity against these fungicides. The isolates BHI, CHR-I and CHR-III were sensitive to all the fungicides whereas JHR was sensitive only to Dithane M-45, Kitazin and Bavistin.

Yadav (2004) conducted a field experiment in Punjab, India during 2000-01 and 2001-02 to investigate the integrated control of mustard diseases (blight, *Alternaria brassicae*; white rust, *Albugo candida*; and stem rot, *Sclerotinia sclerotiorum*) in the province. Integrated disease management was possible using the tolerant genotype PBR 91, sowing on 20 October, seed treatment with Apron 35 SD [metalaxyl] at 6 g/kg, and need based spraying with Ridomil MZ 72 WP [mancozeb + metalaxyl] at 0.25% + Indofil M-45 [mancozeb + thiophanate-methyl] at 0.2% (2 sprays at 20-day intervals).

Chattopadhyay and Bhunia (2003) studied with seven fungicides viz; mancozeb 0.2%, captan 0.2%, metalaxyl m.z 0.25%, iprodione 0.2%, bayletan 0.05% [triadimefon], copper oxychloride 0.3% and antracol 0.2% [propineb] against *Alternaria* leaf blight of rapeseed-mustard (*Brassica campestris* cv. Yellow Sarson) caused by *Alternaria brassicae*. Best control of disease was observed by iprodione followed by mancozeb. Highest seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione at post-flowering stage. But maximum economic return was obtained from two spraying of mancozeb at 45 DAS and 60 DAS.

Singh and Maheshwari (2003) conducted a study during the rabi seasons of 1993 and 1994 in Haryana, India, to determine the effect of Baycor (bitertanol), Blitox-50 (copper oxychloride), Akomin-40 (phosphoric acid salt), Contaf 5E (hexaconazole), Validicin (validamycin), Bavistin (carbendazim) and Dithane M-45 (mancozeb) sprays twice at 15-day intervals on *Alternaria* leaf spot (*Alternaria brassicae*) of *Brassica juncea* cv. PR-45 (Pusa Raya). The disease caused 71 and 44% average leaf and pod infection, respectively. Among the fungicides, Contaf exhibited the most effective control of the disease on leaves and pods. The disease index was lowest (16.08) in Contaf-sprayed plots whereas it was 59.09 in unsprayed control plots. The average yield was higher by 23, 10 and 9% in Contaf, Dithane M-45 and Blitox-50 sprayed plots, respectively, over the control. Two sprayings of 0.5% Contaf at 15-day intervals was effective for the control of the disease.

Mukherjee *et al.* (2003) studied the efficacy of iprodione against *Alternaria* blight [*Alternaria brassicae*] infecting Indian mustard cv. Pusa Bold in New Delhi, India, during 1998-2000. Iprodione was sprayed to plants at 500 g a.i./ha during the early pod stage. Iprodione was more effective than mancozeb (control) in the reduction of *Alternaria* blight incidence. The increase in Indian mustard yield in iprodione-treated plots was higher by 24-59% than that in the control plots.

Yadav (2003) conducted a field experiment on Indian mustard cv. Varuna during the 1998/99 and 1999/2000 rabi seasons in Bathinda, Indian Punjab, India, to determine the efficacy of non-systemic and systemic fungicides including mancozeb against Alternaria leaf and pod blight (*Alternaria brassicae, Alternaria brassicicola* and *Alternaria raphani*) and white rust (*Albugo candida*). The treatments comprised: Ridomil MZ (metalaxyl + mancozeb); Indofil M-45 (mancozeb); one spray Ridomil MZ + 2 sprays Indofil M-45; 2 sprays Ridomil MZ; 3 sprays Indofil M-45; 3 sprays Antracol (propineb); 3 sprays Kavach (chlorothalonil); 3 sprays Bayleton (triadimefon); 3 sprays Blitox (copper oxychloride); and control (water spray). Pooled data showed that all the fungicidal treatments were significantly superior to the control in reducing leaf or pod infection and in increasing grain yield. Two sprays of Ridomil MZ at 60 and 80 days after sowing reduced the disease indices of white rust and *Alternaria* blight on the leaves from 62.7 to 17.1% and from 57.3 to 41.4%, respectively, and increased the yield from 1052 (control) to 1842 kg/ha. The highest grain yield (1900 kg/ha) was recorded from antracol treatments.

Ferdous *et al.* (2002) conducted an experiment to investigate the effect of 3 plant extracts and one fungicide on the incidence of *Alternaria* blight (caused by *Alternaria brassicae*) of mustard (*Brassica sp.*) cv. Sonali Sarisha under natural field conditions in Gopalganj, Bihar, India, during 1997-98. Young leaves of neem [*Azadirachta indica*], mustard (*Brassica sp.*) cv. Sambal (30-35 days old) and garlic cloves were macerated in tap water and 1% spray solution was prepared using the crude extracts. The fungicide Rovral [iprodione] at 0.1% was used. All the 4 treatments were used at 1 litre/10 m^2 area. Two sprays at flowering (35-45 days) and fruiting (45-55 days) were given at 7 days interval. The fungicide treatment was the best in reducing *Alternaria* blight intensity and in increasing yield. Among the non-fungicidal treatments, the spray of garlic and neem leaf crude extracts proved promising.

Godika and Pathak (2002) studied the efficacy of 0.2% mancozeb, 0.2% Antracol [propineb], 0.25% Ridomil MZ [mancozeb+metalaxyl], 0.05% Bayleton [triadimefon] and 0.3% copper oxychloride in controlling blight disease (*Alternaria brassicae*) and white rust (*Albugo candida*) in Indian mustard in a field experiment conducted during 1997-2000. All treatments resulted in lower disease severity and higher crop yield compared to the control. Antracol spraying resulted in the lowest *Alternaria* blight severity, whereas Ridomil MZ resulted in the lowest white rust severity and the highest yield (13.47 q/ha).

Anwar and Khan (2001) conducted a study in 1997 to evaluate the most effective seed dressing fungicide for the control of leaf blight disease (*Alternaria brassicae*) and ultimately increasing the yield of Indian mustard. Indian mustard cv. RL-18 seeds were treated with four fungicides: Benlate [benomyl], Vitavax [carboxin], Ridomil [metalaxyl] or Thiovit [sulfur] at 2 g/kg seed. All the fungicides reduced the disease incidence. Benlate showed the best performance and reduced the disease incidence by 76.6%, followed by Vitavax, Ridomil and Thiovit which reduced disease incidences by 70.0, 63.3 and 53.5%, respectively. The maximum increase in yield (51.4%) was observed in plots treated with Benlate followed by Vitavax, which increased yield by

44.6%. Ridomil and Thiovit were the least effective in reducing the disease incidence and increasing the yield.

Godika *et al.* (2001) conducted a field experiment from 1994/95 to 1996/97 in Rajasthan, India to evaluate the efficacy of different fungicides namely Mancozeb, Ridomil MZ (mancozeb+metalaxyl), Captan, Rovral (iprodione), Bayletan [triadimefon], and copper oxychloride, against *Alternaria* blight (*Alternaria brassicae*) and white rust (*Albugo candida*) of Indian mustard. All the fungicides significantly controlled the diseases, but their efficacy varied among them. Rovral was the most effective in controlling of *Alternaria* blight; mean disease intensity on leaf and pod was 8.75 and 5.6%, respectively. On the other hand, Ridomil MZ was the most effective in controlling white rust; mean disease intensity in leaves and staghead were 8.5 and 0.5%, respectively. Yield was highest with Rovral (2.1 t/ha), followed by Mancozeb and Ridomil MZ, each recording a yield of 1.9 t/ha.

Panja *et al.* (2000) tested four different fungicides: Indofil M-45 [mancozeb + thiophanate-methyl], Mancozeb, 75% WP at 0.25%, Fytolan (copper oxychloride, 50% WDP) at 0.4%, Bavistin (carbendazim, 50% WP) at 0.1% and Ridomil MZ, (metalaxyl + mancozeb 72% WP) at 0.15% and their two specific combinations viz., Ridomil at 0.075% + Fytolan at 0.2% and Ridomil at 0.075% + Bavistin at 0.05% against *Alternaria*-leaf blight (*Alternaria brassicae* and/or *A. brassicicola*) and white rust diseases of mustard. Fytolan alone or in combination with Ridomil was superior to other treatments with respect to the reduction of leaf blight incidence and increase of crop yield.

Pandya *et al.* (2000) reported that four sprays of iprodione (0.2%) gave the maximum control of *Alternaria* blight. Maximum yield was obtained in the treatment Rovral 0.5% (3392 kg/ha), while it was minimum in the control (2896 kg/ha).

Meah *et al.* (1999) conducted a field experiments in Bangladesh during October 1997 to February 1998 to determine the effect of some management practices on mustard (cultivars Sampad and BINA) seed infection. Weeding treatments include: no weeding and weeding once at 30 days after sowing. Insecticide (Malathion 50EC at 0.2%) applications include no insecticides, once at 40 days after sowing, and twice at 40 and 55 days after sowing. Fungicide (Rovral 50WP, iprodione at 0.2%) applications include no fungicides, once at 40 days after sowing, and twice at 40 and 55 days after sowing. Weeding, and spraying of insecticides and fungicides, on mustard resulted in 9.5 to 7.3%, 12.7 to 3.6% and 8.3 to 4.1% reduction of infected seeds, respectively. In the control, 36-39% seed was infected by *A. brassicae*, while among the seeds under the various treatments; only 19-31% was affected. A greater percentage of healthy seeds were counted from treated crops.

Ghosh and Das (1999) reported that ten fungi were found to be associated with both mustard (*Brassica campestris*) and cauliflower (*B. oleracea* var. *botrytis*) seeds. Out of them, *Alternaria alternata* and *A. brassicicola* appeared in higher frequency on both seeds. These two pathogenic fungi are seed borne, externally and internally. Five fungicides, viz. Dithane M-45 [mancozeb], Bavistin [carbendazim], Blitox-50 [copper oxychloride], Thiram and Captan 50w, were applied on both seeds to control mycoflora. Of these, Bavistin (500 ppm) eliminated most of the fungi. Moreover, Bavistin treated seeds yielded maximum percentage of seed germination.

A field experiment was conducted at Joydebpur and Jessore in Bangladesh during Rabi 1996-97 season and reported that *Alternaria* leaf blight incidence was the lowest in the plant treated with Rovral 50 WP (0.2%) at pod formation and seed formation stage in both the locations. The highest seed yield was also recorded from the same treatment in both the locations. Thousand seed weight was higher in the seeds of treated plants (Anonymous, 1997)

Ayub *et al.* (1996) conducted an experiment to evaluate the efficacy of 7 fungicides to control *Alternaria* blight of mustard caused by *A. brassicae* and *A. brassicicola.* Carbendazim (as Bavistin) and Benomyl (as Benlate) at 0.1%, ziram (as Cuman L), mancozeb (as Dithane M-45), fentin hydroxide (as Duter), iprodione (as Rovral) and copper salts + mancozeb (as Trimiltox forte) at 0.2%, were applied 3 times to plants which were 40-, 50- and 60-days-old. Experiments were carried out in Gazipur, Bangladesh during the rabi season between 1986 and 1989. Iprodione reduced disease severity promisingly and increased seed weight and yield. Fentin hydroxide was the second best fungicide. Maximum reduction of disease severity and increased yield was achieved when the spraying was carried out on plants at 40-days-old.

Kumar and Kumar (1996) evaluated the effects of 4 fungicides on *Alternaria brassicae, Albugo candida* and *Peronospora parasitica* infection of Indian mustard in field trials. Minimum Alternaria blight infection was recorded with Rovral [iprodione, 0.2%], followed by Difolatan [captafol, 0.2%], Indofil M-45 [mancozeb + thiophanate-methyl, 0.2%] and Ridomil MZ [mancozeb + metalaxyl, 0.25%]. Maximum yield was recorded with iprodione but Indofil M-45 is recommended on the basis of the cost-benefit ratio.

Priya *et al.* (1995) stated that eight fungal species were associated with Indian mustard seeds in Haryana, India. These were: *Aspergillus niger; A. flavus; Penicillium sp.; Rhizopus sp.; Cochliobolus lunatus; C. sativus; Alternaria alternata* and *Mucor sp.* Bavistin [carbendazim] at 750 p.p.m. completely inhibited the fungi and enhanced seed germination.

Mridula *et al.* (1994) tested five fungicides, Blitox-50 [copper oxychloride], Bavistin [carbendazim], Dithane M-45 [mancozeb], Topsin-M [thiophanate-methyl] and thiram, *in vitro* against *A. brassicae*, which causes leaf blight in [Indian] mustard. Mancozeb was the most effective fungicide for inhibiting growth.

Chattopadhyay and Bagchi (1994) reported that the severity of leaf blight of mustard, caused by *Alternaria brassicae*, was negatively correlated with seed yield. The lowest severity and the highest yields were obtained following 4 foliar sprays of mancozeb (0.2%) at intervals of 15 d, starting from 30 d after sowing. Three sprays at 45, 60 and 75 d after sowing gave the highest benefit ratios.

Seed-health test was carried out after harvest of the crops at the laboratory to evaluate the seed-borne infection by standard blotter method. Seed germination on the top of the blotter was also recorded and expressed in percentage. The experiment with cv. SS-75 (HYV) was conducted at ORC, BARI, Joydebpur. In the laboratory test it was observed that the Rovral spray reduced the seed-borne pathogen infection and increased the germination percentage of mustard seeds. Seed-borne *Alternaria* spp. infection was reduced above 90% and germination increase was above 9% over the control. Seed infection was reduced up to 18.8% with three times Rovral spray (Anonymous, 1992).

Babadoost (1993) tested three systemic fungicides to evaluate their potentiality to control *Alternaria* disease of *Brassica* seed crops. It was observed that two applications of Iprodione (Rovral), Analyzine or Chlorothalonil significantly reduced the severity of diseases of Brussels sprouts seed plants infected by *A. brassicae* and *A. brassicicola*. Three applications of Iprodione (Rovral) @ 1.12 kg a.i./ha was proved to be the most effective in cabbage seed field in controlling the diseases and it minimized the incidence of the pathogens in seeds.

Meah *et al.* (1992) observed the effects of frequencies, doses and time of application and their combination in controlling *Alternaria* blight of mustard in two consecutive cropping seasons under natural conditions. They found Rovral (1.0 Lit/ha) significantly reduced disease severities and increased seed yield by

147% over control when applied two times commencing from fruiting stage (50 days age) at 10 days intervals.

An experiment was conducted at BARI, Joydebpur, RARS, Ishurdi and Jessore during the Rabi season of 1991-92 using mustard variety Tori-7. Rovral 50wp @ 0.2% was sprayed at an interval of 10 days starting from initiation of leaf blight disease. It was observed from the field test that the increase in number of Rovral spray had significant effect in reduction of *Alternaria* leaf blight disease and increases seed yield and 1000 grain weight. The disease reduction was observed from 37.5 to 74.3% over control at the three locations for three times sprayed that influenced the increase in yield from 40.5 to 60.3%. But the maximum yield increase 62.8% observed in case of four time spray at Joydebpur. The 1000 grain weight was also increased 21.9 to 44.9% over control at three times spray and maximum increase of 1000 grain weight (47.8%) was found in four times spray at Ishurdi (Anonymous, 1992).

In a field trial, Howlider *et al.* (1991) used 5 fungicides (Dithane M-45, Thiovit, Delan, Topsin M and Cupravit) at 3 doses in controlling *Alternaria* blight of mustard. Five sprays were applied with first spray at 40 days growth stage maintaining an interval of 8 days. Dithane M-45 was proved the best. A reduction of 73 and 72% in leaf spot severity and siliqua spotting corresponding to an increase of 30% seed yield -was obtained. Some 92% apparently healthy seeds, 3 and 5%, respectively deformed and discoloured seeds were produced as against 78, 4 and 18%, respectively apparently healthy, deformed and discoloured seeds in untreated plot. The benefit of increase in dose of fungicide in reducing disease severity and decreasing abnormal seeds was not significant.

Singh *et al.* (1990) investigated the economic efficacy of different fungicides for control of leaf spot of cauliflower. Seven fungicides, namely Emisan-6, Bavistin, Captafol, Cuman-L, Difolatan, Dithane M-45 and Dithane Z-78 were tested under field conditions for suggesting economically viable control measures acceptable to farmers. Their findings concluded that three sprays with Difolatan (0.2%) distinctly scored higher performance over other fungicides, both in terms of additional crop yield and net profit with a benefit cost ratio of 13: 2.

Ferdous (1990) evaluated extracts of garlic, neem and Shambal Sarisha against *Alternaria* blight of mustard. Garlic extract proved promising when 64.3% reduction in leaf area disease (%) and an increase in yield by 28.7% were obtained.

Saha (1989) observed that *in vitro* growth of *A. brassicae* and *A. brassicicola* isolated from rape and Indian mustard was reduced by each of 10 fungicides tested. Ziram and Ceresan Wet [phenylmercury acetate] completely inhibited growth of those fungi. The second highest growth reductions occurred when *A. brassicae* was treated with Dithane M-45 [mancozeb] or Difolatan [captafol] and when *A. brassicicola* was treated with Dithane Z-78 [zineb] or mancozeb. In all cases, growth inhibition increased with the increase of concentration of fungicides.

In India, Sinha and Prasad (1989) evaluated five fungicides in field trials over three years to control *Alternaria* blight of cauliflower. The best control of *Alternaria brassicae* and/or *A. brassicicola* and highest yield was achieved by captafol, followed by Dithane M-45 (Mancozeb), applied when the disease appeared and subsequently five times at 15-days interval.

Shivpuri *et al.* (1988) conducted an experiment during 1986-87, six fungicide treatments Rovral (iprodione), Captafol, Dithane M-45 [mancozeb], Thiram, Blitox-50 [copper oxychloride], Bavistin [carbendazim]) were applied to Indian mustard infected by *A. brassicae* in field trials in Rajasthan, India. All of the fungicides controlled the disease but copper oxychloride was phytotoxic. The best treatment was iprodione followed by captafol and mancozeb; iprodione caused minimum defoliation.

Tripathi *et al.* (1987) reported that *A. brassicae* caused severe yield reduction and quantitative differences in oil contents of rape and mustard crops. A captafol spray followed after 15 d by a mancozeb spray gave effective disease control. A spray schedule involving 4 sprays of captafol starting 30 d after sowing at 15-d intervals was the best combination for maintaining a disease free crop.

Meah *et al.* (1985) studied the effect of 5 foliar fungicides viz. Dithane M-45, Duter, Benlate, Tri-Miltox Forte and Cupravit at single dose against *Alternaria brassicae* on mustard in two cropping seasons under natural condition. All the fungicides sprayed at 8 days intervals starting from 30 days of sprouting and continued up to crop maturity significantly reduced defoliation, leaf spot severity and number of spot on siliqua and increased the yield.

Sharma (1984) recommended that out of 6 fungicides [Diconil (Chlorothalonil) (0.1%), Difolatan (Captafol) (0.3%) and Dithane Z-78 (Zineb) (0.2%) were found effective.], 4 sprays of Dithane M-45 (Mancozeb) at 10 days interval starting with onset of *Alternaria* blight of mustard caused by *Alternaria* spp.

Humpherson and Maude (1983) suggested that three sprays of Rovral at 0.5-10.0 kg a.i. per ha applied in *Brassica oleracea* seed crops at three-week intervals from the young green siliqua stage to control pod infection caused by *A. brassicicola*. Their findings demonstrated that seed yield was increased and spray improved the seed germination. They reported that Bordeaux mixture was also as effective as Rovral when disease levels were low but ineffective when infection pressure was severe.

The best and effective control of *A. brassicicola* of mustard was obtained in the field when Maude and Humperson (1980) applied 2-3 sprays of Rovral from early siliqua stage of mustard onwards.

Gupta *et al.* (1978) evaluated the effectiveness of different fungicides against white rust and *Alternaria* leaf spot of mustard. The fungicides used were Difoltan, Cis 4, Benlate Brestan, BAS-305, Dithane M-45, Miltox, Thiovit and Aureofungin. They reported that Brestan (0.18%) performed best against *Alternaria* leaf spot followed by Miltox (0.3%). They used only two sprays fortnightly starting at the age of two months of the crop.

Kolte and Tewari (1978) evaluated the efficacy of some chemicals for the control of *Alternaria* leaf blight of yellow sarson (mustard). In their experiments they used Mancozeb (Dithane M-45), Zineb (0.2%) (Dithane Z-78), TPTH (0.2%) (Fenti hydroxyl triphenyl tin hydroxide 20 WP), Captan (0.2%) Bavistin (0.5%) and Thiophanate methyl (0.05%). But in 1975-76 trials Bavistin, Thiophanate methyl, Zineb and Captan were avoided for their poor performance. At the end of their study the suggested Difolatan (0.2%) as the most effective fungicide based on raising of disease free mustard crop.

2.2 Effect of Fertilizer

Hossain and Mian (2005) conducted an experiment during 2001-2002 crop season to study the effect of five essential nutrient elements on the management of *Alternaria* blight (*Alternaria brassicicola*) of cabbage seed crop. The nutrient elements S-Zn-Mg-Mo-B were applied in 10 different combinations. All the treatments except T_1 (S_0-Zn_5-Mg_1-Mo_1-B_1) significantly reduced disease severity and increased seed yield and yield contributing characters. The significantly lowest disease severity (PDI) of leaf, pod spotting and percent pod area diseased) and the highest seed yield and yield contributing characters were recorded in the treatment T_8 (S_{30}-Zn_5-Mg_1-Mo_1-B_1) followed by the treatment T_6 (S_{20}-Zn_5-Mg_1-Mo_1-B_1). The nutrients S, Zn and Mg were found very important in increasing seed yield and yield component with the reduction of *Alternaria* blight severity.

Pot experiments were conducted by Singh (2004) under greenhouse conditions to investigate the effect of inorganic fertilizers on the development of *Alternaria* blight incited by *Alternaria brassicae* on rapeseed-mustard (a group of crops consisting of Indian mustard, yellow and brown sarson [*Brassica campestris* var. sarson], toria and taramina [*Eruca vesicaria*]). The inorganic fertilizers (N, P and K) were applied individually as well as in different combinations (24). Observations on periodical and cumulative progression of spot size on leaves and pods revealed that it increased with increase in the application of N fertilizer, but it decreased with the increased application of P and K fertilizers. N application with P increased the average spot size, whereas it decreased with P and K application and further decreased with N and P application. Application of all the fertilizers in combination (NPK) decreased the lesion size but statistically non-significant. The growth of Alternaria blight spot size with respect to all the weather variables showed that relative humidity above 90% and temperature around 20 degrees C played significant and positive role in disease progression.

A field experiment was conducted by Kurowski and Jankowski (2003) on health status of white mustard [Sinapis alba] (cv. Nakielska) and Indian mustard (cv. Maopolska) during 1997-99 in Bacyny, Poland. Oilseed crops were supplied with NPK before sowing (161 kg ha-1)+30 kg S ha-1 or 6 kg Mg ha-1 and N (as urea) top dressing at 0, 30, 25+5 and 60 kg ha-1. Alternaria blight (caused by *Alternaria alternata*, *A. brassicae* and *A. brassicicola*) intensity was ranged from 2-23% and 1-61%, respectively, on leaves of both crops during the 3 years. S application before sowing did not affect disease development whereas foliar nutrition with urea improved health status of the plants.

Godika *et al.* (2001) conducted a field experiment in Rajasthan, India, during the winter seasons of 1998-99 and 1999-2000 to study the integrated management of *Alternaria* blight (*Alternaria brassicae*) and white rust (*Albugo candida*) diseases on Indian mustard cv. Varuna. Treatments included: sowing on 15 October, 30 October or 14 November; K fertilizer at 0 or 40 kg/ha; and three applications or no application of 0.2% mancozeb at 60, 80 and 100 days after sowing. Incidence of *Alternaria* blight on leaves and white rust on leaves and flowers was lower in the earliest sown crop, but the incidence of *Alternaria* blight on pods decreased with later sowing dates. Crop yields were higher in the earliest sown crops. Basal application of K at 40 kg/ha, and mancozeb application controlled both diseases, and increased yield and 1000-seed weight of Indian mustard.

Chapter 3

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Laboratory experiment

The experiment was conducted at the Seed Health Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.1.1 Preparation of fungicidal suspension

Recommended doses of fungicidal solution were prepared by mixing thoroughly with requisite quantity of chemical and normal water. It was required 2 gm/liter of Rovral 50 WP, 4.5 gm/liter of Dithane M-45, 2gm/liter of Ridomil gold MZ-72 and 1 gm/liter of Bavistin DF for preparation of solution with respective concentration. Details of the fungicides used are given in Table 1.

Table 1: Details of Fungicides used

Common name	Chemical name	Active ingredients
1. Bavistin DF	Carbendazim	Carbendazim (50%)
2. Dithane M-45	Manganous ethylene bisdithio carbamate-ion ($C_4H_6N_2S_4$)	Mancozeb (80%)
3. Ridomil gold MZ-72	N-(2,6 dimethyl phenyl)- N-(methoxyacetyl)-alanine methyl ester ($C_{14}H_{21}NO_4$)	Mancozeb + Metalaxyl (72%)
4. Rovral 50 WP	3-(3,5dichlorophenyl)-N-(Imethylethyl)-2,4 dioxoimidazolidene carboxamide ($(C_{13}H_{13})_3N_3CI_2$)	Iprodione (50%)

3.1.2 Bioassay of fungicides against *Alternaria brassicae*

3.1.2.1 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 culture block of *Alternaria brassicae* was cut and placed at the centre of the treated PDA plate. Each treatment was replicated five times. For control treatment, only sterile water was used instead of fungicides. The plates were then placed at 25±1°C for 15 days. The linear growth (cm) of mycelium of *Alternaria brassicae* was recorded at 3 days interval until the control plates were filled in.

3.2 Field experiment

3.2.1 Experimental period

A field experiment with different fungicides and micronutrients (Gypsum, Zinc oxides and Boric acids) was conducted in the Rabi season from November 2007 to February 2008. Seeds were sown on 11, November 2007 and were harvested on 23, February 2008.

3.2.2 Experimental site

The present research work was carried out in the experimental farm, Sher-e-Bangla Agricultural University (SAU), Dhaka. The location of the site was 23° 74′ N latitude and 90° 35′ E longitude with an elevation of 8.2 meter from sea level.

3.2.3 Climate and Soil

The experimental site was situated in the subtropical zone. The soil of the experimental site lies in Agro-ecologocal regions of "Madhupur Tract" (AEZ No. 28). Its top soil is clay loam in texture and olive gray with common fine to medium distinct dark yellowish brown mottles. The pH is 4.47 to 5.63 and organic carbon content is 0.82% (Appendix IV). The records of air temperature, humidity and rainfall during the period of experiment were noted from the Bangladesh Meteorological Department, Agargaon, Dhaka (Appendix III).

3.2.4 Land preparation

The experimental plot was prepared by several ploughing and cross ploughing followed by laddering and harrowing with power tiller and country plough to bring about good tilth. Weeds and other stubbles were removed carefully from the experimental plot and leveled properly.

3.2.5 Fertilizer application

The experimental field 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), Gypsum, ZnO and Boric acid. Urea was applied by two installments. Total amount of TSP, MP, gypsum, ZnO and boric acid along with half of the urea were applied at the time of final land preparation as a basal dose. The second half of the urea was top-dressed at the time of flower initiation. But micronutrients (Gypsum, ZnO, Boric acid) were applied only in the desired plots which are selected for micronutrient application.

Manure and fertilizers were applied as per standard recommendation. The following doses were used for carrying out the field study (Anonymous, 2001).

Manures /Fertilizers	Rate /ha
Cow dung	10000 kg
Urea	250 kg
TSP	170 kg
MP	85 kg
Gypsum	150 kg
Zinc oxide	5 kg
Boric acid	10 kg

3.2.6 Treatments of experiment

Altogether there 10 different treatments were employed in the experiment. The treatments were applied into the assigned plots as per design of the experiment:

T_1= Control

T_2= Rovral 50 WP (0.2%)

T_3= Ridomil Gold MZ-72WP (0.2%)

T_4= Dithane M-45 (0.3%)

T_5= Bavistin DF (0.1%)

T_6= Rovral 50 WP (0.2%) + Micronutrients

T_7= Ridomil Gold MZ-72 WP (0.2%) + Micronutrients

T_8= Dithane M-45 (0.3%) + Micronutrients

T_9= Bavistin DF (0.1%) + Micronutrients

T_{10}= Micronutrients (Gypsum + Zinc Oxide + Boric acid)

3.2.7 Variety used

The mustard (*Brassica campestries*) variety SAU Sarisha-1 released from Sher-e-Bangla Agricultural University was used in the experiment. Seeds were collected from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka.

3.2.8 Experimental design

Field lay out was done after final land preparation. The materials were laid out in Randomized Complete Block Design (RCBD) with three replications. The whole plot was divided into three blocks each containing ten (10) plots of 2m x 1.5m size, giving 30 units plots. The space was kept 0.75m between the blocks and 0.5m between the plots, 30 cm from row to row and 10 cm from plant to plant were maintained. Seeds were sown in lines in the experimental plots. The seeds were placed at about 1.5 cm depth in the soil.

3.2.9 Seed treatment

Seeds were divided into two treatment groups. Among these two treatment groups one group of seeds were treated by using concerned fungicides with recommended concentration. Solutions were made in different petridishes. Then seeds were dipped in the solution for five minutes. The treated seeds were then taken off the solution and kept in blotting paper to remove excess moisture from the seed surface. Another group of seeds were treated with water as a control.

3.2.10 Intercultural operation

Intercultural operations, such as weeding, thinning, irrigation, pest management, etc. were done uniformly in all the plots. One post sowing irrigation was given by sprinkler after sowing of seeds to bring proper moisture condition of the soil to ensure uniform germination of the seeds. A good drainage system was maintained for immediate release of rainwater from the experimental plot during the growing period. The first weeding was done after 15 days of sowing. During the same time, thinning was done for maintaining a distance of 10 cm from plant to plant in rows of 30 cm. apart. Second weeding was done after 35 days of sowing. The crop was protected from the attack of aphids by spraying Ektara@ 2 ml/litre of water. The insecticide was applied for the first time approximately before one week of flower initiation and it was applied for another two times at an interval of 15 days. The insecticide was applied in the evening.

3.2.11 Preparation and application of spray solution

The fungicidal solutions were prepared by mixing with required amount of fungicides (Rovral 50 WP @ 0.2%, Ridomil gold MZ-72 @ 0.2%, Dithane M-45 @ 0.3% and Bavistin DF @ 0.1%) with tap water. Four fungicidal solutions and plain water was sprayed with compressed hand sprayer. Four sprays were done at 30, 40, 50, 60 days after sowing. Every time the fungicide was freshly prepared prior to application and the spray tank was thoroughly cleaned before filling with new spray materials. Special attention was given to complete coverage of the growing plants with the fungicides. Adequate precaution was taken to avoid drifting of spray materials from one plot to the neighboring ones.

3.2.12 Isolation and identification of pathogens from leaf tissue

The diseased leaves were cut into pieces (4 mm diameter) and surface sterilized with $HgCl_2$ (1:1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and then placed onto acidified PDA in petridish. The plates containing leaf pieces were placed at room temperature for seven days. When the fungus grew well, and sporulated, then the slide was prepared from the PDA and was identified under microscope with the help of relevant literature (CMI description, Plate 1).

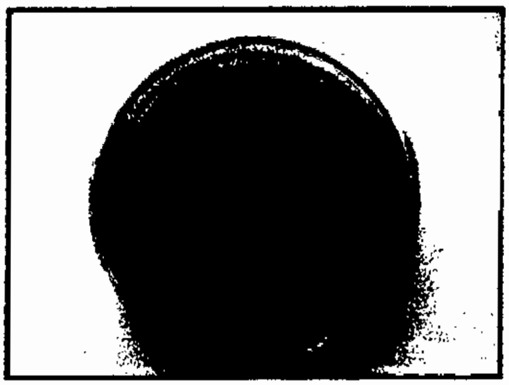

Plate 1. Pure culture of *Alternaria brassicae*

3.2.13 Harvesting of crop

When 80% of the plants showed symptoms of maturity i.e. straw colour of siliquae, leaves, stem and desirable seed colour in the matured siliquae, the crop was assessed to attain maturity. At maturity, ten plants were selected at random from all lines in each plot. The sample plants were harvested by uprooting and then they were tagged properly. Data were recorded on different parameters from these harvested plants.

3.2.14 Collection of data

The following parameters were considered for data collection.

On disease incidence and severity

a. Percent leaf infection
b. Percent leaf area diseased (% LAD)
c. Percent pod infection
d. Number of spots/pod

On growth parameters

a. Number of leaf/plant
b. Number of branches/plant
c. Plant height (cm)

On yield and yield contributing characters

a. Number of pods/plant
b. 1000-seed weight
c. Yield/plant (gm)
d. Yield (Kg/ha)

On harvested seed

a. Percent seed germination
b. Percent seed infection

3.2.15 Procedure of data collection

Ten plants per plot were selected and tagged for collection of data. Data on percent leaf infection were recorded at 65, 75 and 85 days after sowing by visual observation of symptoms. Percent leaf infection was calculated by the following formula.

$$\% \text{ Leaf infection} = \frac{\text{Number of infected leaf}}{\text{Number of total inspected leaf}} \times 100$$

Data on percent leaf area diseased were recorded at 65, 75 and 85 days after sowing by visual observation of symptoms. Percent leaf area diseased was calculated by the following formula.

$$\% \text{ Leaf area diseased} = \frac{\text{Infected leaf area}}{\text{Total leaf area}} \times 100$$

Data on percent pod infection were recorded at 70, 80 and 90 days after sowing by visual observation of symptoms. Percent pod infection was calculated by the following formula.

$$\% \text{ Pod infection} = \frac{\text{Number of infected pod}}{\text{Number of total pod inspected}} \times 100$$

3.2.16 Seed health testing

For health test and detecting the incidence of *Alternaria brassicae* in the harvested mustard seed, 200 seeds randomly drawn from each sample were tested in standard blotter technique (ISTA, 2000). In each petridish, 25 seeds were placed in equidistance. All the plates with seeds were incubated at room temperature (25 ± 2^0 C). After 7-10 days of incubation, each seed was observed under stereo-binocular microscope to detect the presence of *Alternaria brassicae*. Observations were made for seed germination and infection by *Alternaria brassicae*.

3.2.17 Analysis of data

The data were statistically analyzed using computer package program (MSTAT-C). Treatment means were compared by DMRT (Duncan's Multiple Range Test). ANOVA table was shown in appendix-I and appendix-II.

Chapter 4

Results

CHAPTER IV
RESULTS

4.1. Laboratory Experiment

Efficacy of the treatments in inhibiting radial mycelial growth of *Alternaria brassicae in-vitro* was determined.

4.1.1. Bioassay of fungicides against *Alternaria brassicae* using poison food technique (cup method)

The bioassay of fungicides against the mycelial growth of *Alternaria brassicae*, the causal fungus of *Alternaria* blight of mustard is presented in (Table 2). Among the four fungicides tested in the present study, Rovral 50 WP was appeared better followed by Dithane M-45 in controlling the mycelia growth of *Alternaria brassicae* at 15 days after inoculation (DAI). Result of the *in-vitro* test showed that Rovral 50 WP was the most effective fungicide against growth of *A. brassicae* that reduced 68.91% mycelial growth, followed by Dithane M-45 (63.87%). The least effective fungicide to reduce colony growth of the pathogen was Bavistin (50.42%) followed by Ridomil gold (55.46%). The maximum mycelial growth of the fungus i.e., colony diameter was recorded in control (5.95 cm) plate, where only sterile water was used instead of fungicides.

Table 2. Effect of different fungicides on mycelial growth of *A. brassicae* at different days after incubation (DAI) (cup method)

Fungicides	Growth (cm)				% Inhibition of mycelial growth over control at 15 DAI
	6 DAI	9 DAI	12 DAI	15 DAI	
Rovral 50 WP	1.05 e	1.21 e	1.45 e	1.85 e	68.91
Ridomil gold MZ-72 WP	1.77 c	1.97 c	2.35 c	2.65 c	55.46
Dithane M-45	1.38 d	1.58 d	1.85 d	2.15 d	63.87
Bavistin DF	2.16 b	2.50 b	2.60 b	2.95 b	50.42
Control	2.51 a	3.95 a	5.45 a	5.95 a	0
CV (%)	2.10	4.05	4.23	3.68	
$S_{\bar{x}}$	0.01414	0.04000	0.05099	0.05099	

Means within the same column having common letter (s) do not differ significantly at 1% level of significance.

4.2. Field experiment

Efficacy of fungicides viz. Rovral 50 WP, Dithane M-45, Ridomil gold MZ-72 and Bavistin DF and micronutrients alone or in combination in controlling Alternaria blight of mustard caused by *Alternaria brassicae* were assessed based on the result of percent leaf infection, percent leaf area diseased (LAD), percent pod infection, number of spots per pod, growth parameters, yield contributing characters and average yield (t/ha).

Plate 2: Field view of fruiting stage of mustard

4.2.1. Effect of treatments on percent leaf infection

All the fungicides either alone or in combination with micronutrients gave significant reduction of percent leaf infection of *Alternaria* blight. The effect of different treatments on percent leaf infection of mustard at different days after sowing (DAS) is presented in Table 3. Different fungicides had significant influence on percent leaf infection of mustard (SAU Sarisha-1) at different days after sowing (DAS). Percent leaf infection of mustard increased gradually with the advancement of crop growth. At 85 days after sowing (DAS), the highest percent leaf infection (56.13%) was found in control plots and the lowest percent leaf infection (9.93%) was recorded in Rovral + Micronutrients treated plots followed by Rovral alone (10.57%). The moderate leaf infection (27.85%) was observed in the treatment Dithane M-45 + Micronutrients. There is no significant difference between the application of fungicides alone or in combination with micronutrients but application of micronutrients alone showed significantly better performance in comparison to control.

Table 3. Effect of different treatment on percent leaf infection of mustard at different days after sowing (DAS)

Treatments	% Leaf infection			% Inhibition of leaf infection over control at 85 DAS
	65 DAS	75 DAS	85 DAS	
T_1	29.16 a	46.71 a	56.13 a	0
T_2	2.93 f	4.73 f	10.57 f	81.17
T_3	15.13 d	22.71 d	37.18 d	33.76
T_4	11.73 e	19.43 e	28.54 e	49.15
T_5	19.34 c	28.27 c	43.12 c	23.18
T_6	2.73 f	3.69 f	9.93 f	82.31
T_7	16.07 d	21.91 d	36.29 d	35.35
T_8	11.12 e	18.62 e	27.85 e	50.38
T_9	20.17 c	27.55 c	42.84 c	23.68
T_{10}	22.67 b	38.37 b	47.31 b	15.71
CV (%)	7.71	4.10	6.08	
$S_{\bar{x}}$	0.6721	0.5489	1.192	

Means within the same column having common letter (s) do not differ significantly at 5% level of significance.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

4.2.2. Percent leaf area diseased (% LAD)

The effect of different treatments on percent leaf area diseased (% LAD) of mustard at different days after sowing (DAS) is presented in Table 4. Percent leaf area diseased (% LAD) of mustard was found to be significant at different days after sowing (DAS) in response to the application of different treatments. Percent leaf area diseased (LAD) of mustard increased gradually with the advancement of crop growth. At 85 days after sowing the highest percent leaf area diseased (18.31%) was found in control plot and the lowest percent leaf area diseased (2.52%) was recorded from the Rovral + Micronutrients treated plot followed by Rovral alone (2.67%). The rest of the treatments showed significantly similar performance against *Alternaria* blight of mustard in terms of % LAD. The result showed that application of Rovral 50 WP alone caused 85.41% reduction of % LAD while Rovral 50 WP combined with micronutrients caused 86.24% reduction of % LAD.

4.2.3. Percent pod infection

Percent pod infection of mustard increased gradually with the increase of crop age. Very little pod infection (0.00%-1.52%) was recorded at 70 DAS while it increased to the range from 9.31 - 42.69% at 90 DAS in response to applying different treatments. At 90 days after sowing (DAS) the highest percent pod infection (42.69%) was obtained from control plot (Plate 3) and the lowest percent pod infection (9.31%) was recorded from Rovral + Micronutrients treatment (Plate 4) that reduced 77.58% pod infection over control followed by Rovral 50 WP alone. The least effective treatment to reduce percent pod infection was micronutrients alone (33.85%), which was followed by Bavistin alone and Bavistin + Micronutrients treated plots and there was no significant difference between these plots (Table 5).

4.2.4. Number of spots per pod

Number of spots per pod of mustard varied significantly with the application of different treatments at different days after sowing (DAS). The number of spots

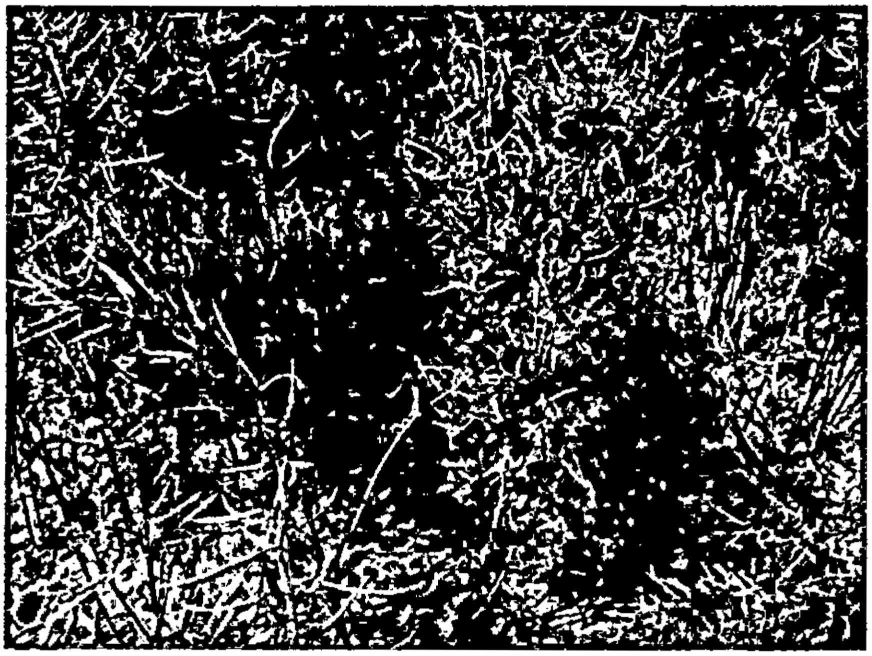

Plate 3: A view of untreated plot showing pod infection of mustard

Plate 4: **A view of Rovral treated plot of mustard showing healthy pods**

per pod was increased gradually from 70 DAS to 90 DAS. Very little number of spots per pod was recorded at 70 DAS. At 90 DAS, maximum of 1.87 spots per pod was recorded in control plot and minimum number of spots per pod (0.61) was obtained from the plot applying with Rovral + Micronutrients followed by Rovral (0.65). Moderate number of spots per pod was found in case of Ridomil + Micronutrients treated plot (1.20) followed by Ridomil (Table 6).

Table 4. Effect of different treatment on percent leaf area diseased (% LAD) of mustard recorded at different days after sowing (DAS)

Treatments	% Leaf area diseased (LAD)			% Inhibition of LAD over control at 85 DAS
	65 DAS	75 DAS	85 DAS	
T_1	5.09 a	12.17 a	18.31 a	0
T_2	0.51 e	1.53 f	2.67 d	85.41
T_3	2.16 c	4.25 c	6.96 c	61.99
T_4	1.59 d	2.62 e	6.70 c	63.41
T_5	2.24 c	3.78 cd	7.37 c	59.75
T_6	0.43 e	1.57 f	2.52 d	86.24
T_7	2.11 c	4.18 cd	6.91 c	62.26
T_8	1.48 d	2.45 e	6.63 c	63.79
T_9	2.07 c	3.72 d	7.32 c	60.02
T_{10}	3.86 b	8.40 b	15.28 b	16.55
CV (%)	8.38	6.09	7.21	
$S_{\bar{x}}$	0.1049	0.1571	0.3357	

Means within the same column having common letter (s) do not differ significantly at 5% level of significance.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

Table 5. Effect of different treatments on percent pod infection of mustard recorded at different days after sowing (DAS)

Treatments	% Pod infection			% Inhibition of pod infection over control at 90 DAS
	70 DAS	80 DAS	90 DAS	
T_1	1.52 a	28.87 a	42.69 a	0
T_2	0.00 f	4.15 d	9.57 d	77.58
T_3	0.43 d	13.18 c	20.56 c	51.84
T_4	0.15 e	12.65 c	19.13 c	55.19
T_5	0.71 c	24.89 b	30.71 b	28.06
T_6	0.00 f	4.030 d	9.31 d	78.19
T_7	0.36 d	12.95 c	20.14 c	52.82
T_8	0.00 f	12.17 c	19.98 c	53.20
T_9	0.67 c	24.63 b	30.10 b	29.49
T_{10}	1.21 b	24.59 b	33.85 b	20.71
CV (%)	13.27	8.60	8.95	
$S_{\bar{x}}$	0.03651	0.8046	1.219	

Means within the same column having common letter (s) do not differ significantly at 5% level of significance.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,

T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,

T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,

T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,

T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

Table 6. Effect of different treatments on number of spots/pod of mustard at different days after sowing (DAS)

Treatments	Number of spots/pod			% Reduction of number of spots/pod over control at 90 DAS
	70 DAS	80 DAS	90 DAS	
T_1	0.31 a	1.34 a	1.87 a	0
T_2	0.00 f	0.35 f	0.65 f	65.24
T_3	0.17 cd	0.78 d	1.25 d	33.16
T_4	0.08 e	0.64 e	1.02 e	45.45
T_5	0.22 bc	0.93 c	1.49 c	20.32
T_6	0.00 f	0.32 f	0.61 f	67.38
T_7	0.16 d	0.75 d	1.20 d	35.83
T_8	0.00 f	0.60 e	0.97 e	48.13
T_9	0.21 cd	0.90 c	1.46 c	21.93
T_{10}	0.27 ab	1.16 b	1.67 b	10.70
CV (%)	9.42	2.37	1.89	
$S_{\bar{x}}$	0.01826	0.01826	0.01826	

Means within the same column having common letter (s) do not differ significantly at 5% level of significance.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,

T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,

T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,

T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,

T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

4.2.5. Number of leaves/plant

Number of leaves per plant varied due to the application of different treatments. The highest number of leaves per plant (8.58) was recorded in case of Rovral + Micronutrients treated plot followed by the lowest number of leaves per plant (6.25) obtained from Control plot (Table 7).

4.2.6. Number of branches/plant

Number of branches per plant differed due to application of different treatments. Significantly higher number of branches per plant was found in fungicide treated plots alone or in combination with micronutrients compared to control plots. The maximum branches per plant (5.13) was recorded in case of Rovral + Micronutrients treated plots whereas control plots produced the lowest number of branches per plant (4.12) (Table 7).

4.2.7 Plant height (cm)

Different fungicides and micronutrients had influence on plant height (cm) of mustard. The tallest plant was obtained from Rovral + Micronutrients treatment (103.3 cm) which was statistically identical with Ridomil + Micronutrients. The lowest plant height (85.71cm) was recorded in case of control plot (Table 7).

4.2.8. Number of pods/plant

Number of pods per plant of mustard differed due to the application of different fungicides and micronutrients. The control plot yielded 35.82-pod/plant which was statistically identical with Bavistin. The pod numbers per plant ranged 37.23-47.25 in all fungicides treated plots either applied alone or in combination with micronutrients. The highest number of pod per plant (47.25) was recorded in case of Rovral + Micronutrients (Table 8).

Table 7. Effect of different treatments on growth parameters of mustard

Treatments	Growth parameters		
	No. of leaf/plant	No. of branches/plant	Plant height (cm)
T_1	6.25 e	4.12 c	85.71 c
T_2	8.37 ab	4.80 abc	91.82 bc
T_3	7.15 cd	4.64 abc	87.93 bc
T_4	7.62 bc	4.72 abc	91.18 bc
T_5	6.76 cde	4.49 abc	87.75 bc
T_6	8.58 a	5.13 a	103.3 a
T_7	7.56 bc	4.98 ab	95.74 ab
T_8	8.28 ab	5.05 ab	102.5 a
T_9	7.13 cd	4.86 ab	96.22 ab
T_{10}	6.66 de	4.36 bc	90.89 bc
CV (%)	6.33	7.69	5.25
$S_{\bar{x}}$	0.2714	0.2090	2.831

In a column means having same letter (s) denote no significant difference at 5% level.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

4.2.9. 1000-Seed weight

Thousand-seed weight was found to vary significantly due to application of different fungicides and micronutrients. Spraying with Rovral + Micronutrients produced the maximum 1000-seed weight (3.65g) while control plots produced the minimum 1000-seed weight (2.88g) (Table 8).

4.2.10. Yield

Yield per plant (g) and yield (Kg) per hectare were significantly higher in fungicides alone or in combination with micronutrients treated plots compared to that of control plots. Maximum yield per plant (4.07 g) (Table 8) and per hectare (1086 kg) was obtained from Rovral + Micronutrients treated plots which is statistically similar with Rovral alone followed by Dithane M-45 + Micronutrients in both the cases. The minimum yield per plant (2.45 g) and per hectare (654.2 kg) was recorded from control plots.

Table 8: Effect of different treatments on yield and yield contributing characters of mustard

Treatments	Yield and yield contributing characters				% Yield increased over control
	No. of pods/plant	1000-Seed weight (g)	Yield/plant (g)	Yield Kg/ha	
T_1	35.82 e	2.88 f	2.45 e	654.2	0
T_2	44.69 ab	3.59 ab	4.01 a	1071.0	38.92
T_3	38.87 de	3.18 cde	3.29 bc	875.6	25.29
T_4	40.54 cd	3.34 bc	3.44 b	918.7	28.79
T_5	37.23 e	2.92 ef	2.85 d	761.0	14.03
T_6	47.25 a	3.65 a	4.07 a	1086.0	39.76
T_7	41.38 cd	3.23 cd	3.35 bc	894.6	26.87
T_8	43.77 bc	3.39 abc	3.51 b	937.3	30.20
T_9	40.64 cd	2.98 def	2.98 cd	795.8	17.79
T_{10}	38.41 de	2.97 def	2.88 d	769.1	14.94
CV (%)	3.81	4.81	6.39	6.34	
$S_{\bar{x}}$	0.8819	0.08944	0.1211	32.10	

In a column means having same letter (s) denote no significant difference at 5% level.

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

4.3. Seed health test (After harvest)

4.3.1. Percent seed germination

Percent seed germination was found to be significantly higher in the seed lot harvested from the treated plot due to the application of different fungicides alone or in combination with micronutrients in comparison to control except micronutrients alone. Seed obtained from Rovral + Micronutrients treated plots showed the maximum germination percentage (99.10%) which was statistically similar (98.92%) to seed obtained from Rovral treated plots. Seed obtained from control plots showed the minimum germination percentage (82.17%) which is statistically identical with only micronutrients treated plots (Fig 1).

4.3.2. Percent seed infection

Percent seed infection by *Alternaria brassicae* of harvested seeds obtained from treated plots with different fungicides and micronutrients differed significantly. Lower seed infection was found in the seed obtained from treated plot compared to control, except seeds collected from micronutrient treated plots. Seeds obtained from control treatment showed the highest percent seed infection (18.34%) while seeds obtained from Rovral + Micronutrients treated plots showed the lowest seed infection (6.5%) preceded by Rovral (6.53) (Fig 2).

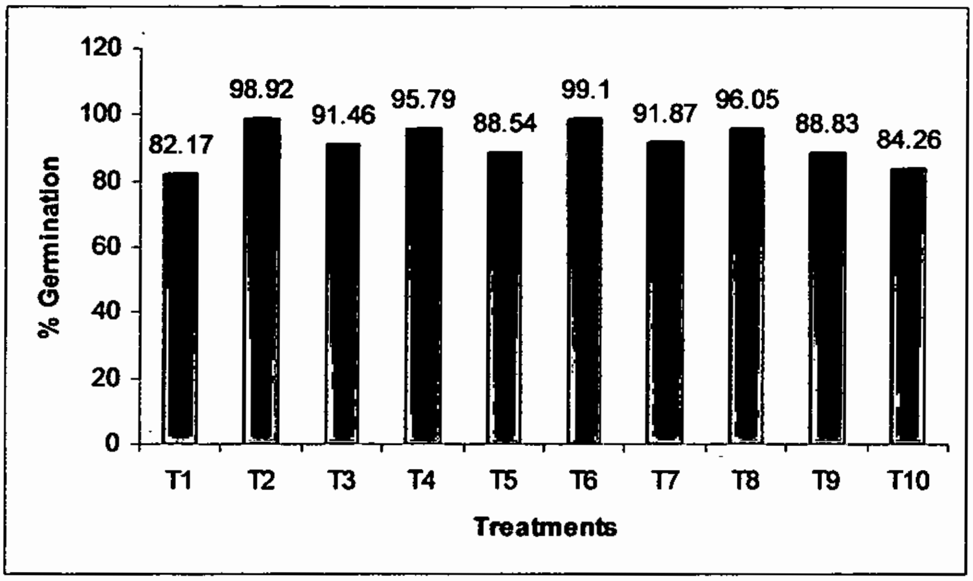

Figure 1: Effect of different treatments on percent seed germination of mustard

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

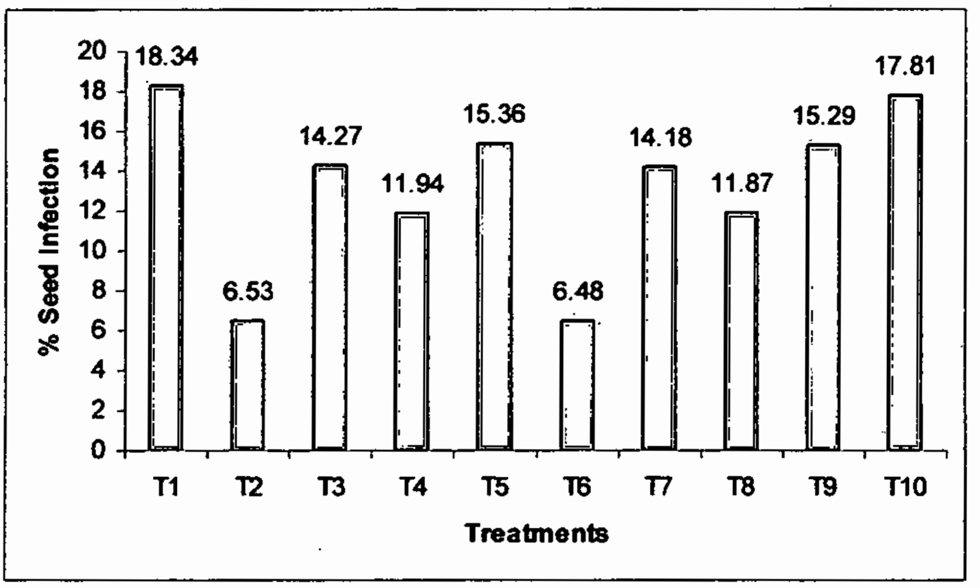

Figure 2: Effect of different treatments on percent seed infection of mustard

T_1 = Control (Foliar spray with water), T_2 = Rovral 50 WP,
T_3 = Ridomil gold MZ-72, T_4 = Dithane M-45,
T_5 = Bavistin DF, T_6 = Rovral 50 WP + Micronutrients,
T_7 = Ridomil gold MZ-72 + Micronutrients, T_8 = Dithane M-45 + Micronutrients,
T_9 = Bavistin DF + Micronutrients, T_{10} = Micronutrients

Chapter 5
Discussion

CHAPTER VI
DISCUSSION

The efficacy of fungicides against the mycelial growth of *Alternaria brassicae* was found in *in-vitro* test proved promising. Rovral 50 WP (0.2%) inhibited the mycelial growth by 68.91% followed by Dithane M-45, Ridomil gold and Bavistin. Similar results were reported by Hossain and Mian (2006) who reported that Rovral 50 WP (iprodione) significantly inhibited the growth of *Alternaria brassicicola* followed by Dithane M-45. Kumar *et al.* (2004) reported that among 6 fungicides and 1 plant extract, Dithane M-45 performed the best result against spore germination of *Alternaria brassicae*.

In the field experiment the application of fungicides either alone or in combination with micronutrients had significant effect in reducing the disease incidence and severity increasing the seed yield. Among the treatments, seed treatment followed by foliar spraying with Rovral in combination with soil application of micronutrients inhibited the leaf infection by 82.31% over control which was statistically identical with the application of Rovral 50 WP without micronutrients. The second highest performance in reducing leaf infection was recorded incase of seed treatment followed by foliar spray with Dithane M-45 combined with soil application with micronutrients. Similar trend of results were observed incase of reducing pod infection due to the application of different treatments. Incase of percent leaf area diseased, the significant reduction was observed in response to the application of Rovral in combination with micronutrients that reduced 86.24% LAD compared to control. Application of Rovral 50 WP alone caused 85.41% reduction of LAD. It was observed that percent leaf infection and percent leaf area diseased increased gradually with the advancement of crop growth but the rate of increasing varied significantly in response of the treatment application.

The effect of treatments on yield contributing characters like number of leaves per plant, number of branches per plant, plant height and 1000-seed weight was remarkably influenced increasing seed yield. Significantly the highest seed yield (1086 Kg/ha) was obtained from Rovral 50 WP + Micronutrients treated plot against the disease that increased seed yield by 39.80% compared to control. Statistically similar yield was obtained from Rovral alone treated plot (1071 Kg/ha) that increased seed yield by 38.92% and 30.20% respectively. The findings of the field experiments are well supported by the previous researchers. Alam (2007) while working with fungicides and plant extracts against the *Alternaria* blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola*, reported that Rovral 50 WP (0.2%) was the potential fungicide in controlling disease incidence and severity and increasing seed yield by 48.19% over control. Hossain and Mian (2006) reported that in field trial, Rovral 50 WP (iprodione) significantly reduced the disease incidence and severity and increased seed yield when applied alone or in combination with other fungicides followed by Dithane M-45. Singh and Singh (2005 a) reported that foliar spray of mancozeb (0.2%) at 15 days interval resulted in the lowest incidence of *Alternaria* blight and the highest seed yield providing cost benefit ratio 1:5.2. Mukherjee *et al.* (2003) studied the efficacy of iprodione against *Alternaria* blight (*Alternaria brassicae*) and found more effective than mancozeb reducing the disease incidence, increasing mustard yield by 59% over control. Ferdous *et al.* (2002) also reported that foliar spray of Rovral at 1% concentration given at 7 days interval remarkably reduced *Alternaria* blight intensity increasing seed yield.

Seed health regarding seed infection and seed germination were found to differ significantly due to the application of different treatments. The lowest seed infection (6.5%) by *Alternaria brassicae* was found in the seed lot obtained from treated plot with Rovral 50 WP + Micronutrients compared to control. Seeds obtained from Rovral + Micronutrients treated plots showed the maximum germination (99.10%). Seeds obtained from control plots showed the minimum germination (82.17%). The present findings corroborate with the findings of previous research report (Hossain and Mian, 2006; Anonymous, 1992): Hossain and Mian (2006) evaluated 6 fungicides alone or in combination in a field trial and reported that seed infection with *Alternaria* spp. was significantly lower on seed obtained from Rovral and Dithane M-45 treated plot increasing more than 90% germination of seed. Anonymous (1992) reported that foliar spray of Rovral significantly reduced the seed borne infection of *Alternaria* spp. and increased germination percentage of mustard seed. It was reported that seed born infection of *Alternaria* spp. was reduced above 90% and seed germination was increased above 9% over the control while seed infection was reduced up to 18.8% with 3 times foliar spray of Rovral.

CHAPTER VI

SUMMARY AND CONCLUSION

In order to evaluate the effect of four fungicides either alone or in combination with micronutrients in controlling *Alternaria* blight of mustard laboratory and field experiments were carried out in the Seed Health Laboratory Department of Plant Pathology and in the field of Sher-e-Bangla Agricultural University, respectively Dhaka, Bangladesh, during the period from November, 2007 to March, 2008.

Before field experiment an *in-vitro* test was conducted to determine effectiveness of the fungicides on radial mycelial growth of *A. brassicae*. For the laboratory experiment the plates were arranged on the laboratory desk following Complete Randomized Design (CRD) with 5 replications. The fungicides tested were Rovral 50 WP @ 0.2%, Dithane M-45 @ 0.45%, Ridomil gold MZ-72 @ 0.2% and Bavistin DF @ 0.1%. In the present study, all the tested chemical fungicides significantly reduced mycelial growth of fungus *in vitro*. Among the fungicides, Rovral 50 WP reduced the highest radial mycelial growth (68.91%) of *Alternaria brassicae* followed by Dithane M-45 (63.87%), Ridomil gold MZ-72 (55.46%) and Bavistin DF (50.42%) compared to control.

The field experiment was laid out in the Randomized Complete Block Design with three replications. A mustard variety SAU Sharisha-1 was used. There were ten treatments, Viz.T_1 (Control), T_2 (Rovral 50 WP), T_3 (Ridomil gold MZ-72), T_4 (Dithane M-45), T_5 (Bavistin DF), T_6 (Rovral 50 WP + Micronutrients), T_7 (Ridomil gold MZ-72 + Micronutrients), T_8 (Dithane M-45 + Micronutrients), T_9 (Bavistin DF + Micronutrients) and T_{10} (Micronutrients). The fungicidal solutions were used in recommended dose. The study revealed that application of different treatments had remarkable effect in controlling incidence and severity of *Alternaria* blight of mustard. Seed treatment with Rovral as well as foliar spraying resulted better performance in comparison to all other treatments in controlling the disease. The lowest percent leaf infection (9.93%), percent leaf area diseases (2.52%), percent pod infection (9.31%) and number of spots per pod (0.61) were recorded from the plots treated with Rovral 50 WP @ 0.2% + Micronutrients, followed by Rovral 50WP @ 0.2% and Dithane M-45 @ 0.45% + Micronutrients. The highest percent leaf infection (56.13%), percent leaf area diseased (18.31%), percent pod infection (42.69%) and number of spots per pod (1.87) were recorded from control.

The highest yield (1086 kg/ha) was obtained from the plots treated with Rovral 50 WP + Micronutrients. The highest germination percentage (99.1%) and the lowest seed infection (6.48%) of harvested seeds were obtained from the plots treated with Rovral + Micronutrients. The lowest seed germination percentage (82.17%) and the highest seed infection (18.34%) of harvested seeds were obtained from the control treatment. The lowest yield (654.2 kg/ha) was obtained from untreated plot.

From the present findings it may be concluded that seed treatment with Rovral 50 WP @ 0.2% followed by 3 (Three) foliar spray of Rovral 50 WP @ 0.2% in combination with soil application of Micronutrients was found to be the best for lowering *Alternaria* blight incidence and severity with increasing seed yield (39.76%) of mustard (SAU Sarisha-1). However, further studies need to be carried in different Agro-ecological zones taking more options to justify the present findings.

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Appendices

Appendix I. Mean sum of square from the ANOVA of 5 treatments in respect of 4 characters

Characters	d. f.		Mean sum of square	
	Treatment	Error	Treatment	Error
Mycelial growth of *A. brassicae* at 6 days after incubation (DAI).	4	20	1.698**	0.001
Mycelial growth of *A. brassicae* at 6 days after incubation (DAI).	4	20	5.701**	0.008
Mycelial growth of *A. brassicae* at 6 days after incubation (DAI).	4	20	12.465**	0.013
Mycelial growth of *A. brassicae* at 6 days after incubation (DAI).	4	20	13.515**	0.013

** = Significant at 1% level of probability

Appendix II. Mean sum of square from the ANOVA of 10 treatments in respect of 21 characters

Characters	d. f.			Mean sum of square		
	Replication	Treatment	Error	Replication	Treatment	Error
Percent leaf infection at 65 DAS	2	9	18	1.055	209.326**	1.355
Percent leaf infection at 75 DAS	2	9	18	0.011	528.784**	0.904
Percent leaf infection at 85 DAS	2	9	18	3.914	679.843**	4.264
Percent LAD at 65 DAS	2	9	18	0.068	5.998**	0.033
Percent LAD at 75 DAS	2	9	18	0.108	33.487**	0.074
Percent LAD at 85 DAS	2	9	18	0.169	74.795**	0.338
Percent Pod infection at 70 DAS	2	9	18	0.088	0.838 **	0.004
Percent Pod infection at 80 DAS	2	9	18	101.889	239.779**	1.942
Percent Pod infection at 90 DAS	2	9	18	232.228	339.212**	4.461
No. of spots/pod at 70 DAS	2	9	18	0.003	0.040**	0.001
No. of spots/pod at 80 DAS	2	9	18	0.029	0.315**	0.001
No. of spots/pod at 90 DAS	2	9	18	0.036	0.519**	0.001
No. of leaf/plant	2	9	18	73.278	1.861**	0.221
No. of branches/plant	2	9	18	2.034	0.306NS	0.131

** = Significant at 1% level of probability

NS = Not significant

Appendix II. (Contd.)

Characters	d. f.			Mean sum of square		
	Replication	Treatment	Error	Replication	Treatment	Error
Plant height (cm)	2	9	18	8.810	110.138**	24.037
No. of pods/plant	2	9	18	0.243	29.260**	2.333
1000-Seed weight	2	9	18	0.008	0.231**	0.024
Yield/plant(g)	2	9	18	0.001	0.788**	0.044
Yield (Kg/ha)	2	9	18	91.698	56120.972**	3091.719
Percent Germination	2	9	18	16.726	99.816**	0.452
Percent seed infection	2	9	18	14.185	50.605**	0.106

** = Significant at 1% level of probability

Appendix III. Monthly record of year temperature, rainfall, relative humidity and Sunshine of the experimental site during the period from October 2007 to March 2008

Year	Month	*Air temperature (°c)			*Relative humidity (%)	*Rainfall (mm)	*Sunshine (hr)
		Maximum	Minimum	Mean			
2007	October	31.4	23.8	27.6	77	320	5.7
	November	29.0	19.9	24.45	69	111	5.5
	December	25.8	15.0	20.4	73	00	5.6
2008	January	24.7	12.5	18.6	67	00	5.8
	February	27.2	16.8	22	67	31	5.8
	March	31.5	19.7	25.6	55	12	8.3

*Monthly average

Source: Bangladesh Meteorological Department (Climate division)

Agargoan, Dhaka .

Appendix IV.

Physical and chemical characteristics of initial soil (0-15 cm depth)

A. Physical composition of the soil

Soil separates	%	Methods employed
Sand	36.90	Hydrometer method (Day,1915)
Silt	26.40	Do
Clay	36.66	Do
Texture class	Clay loam	Do

B. Chemical composition of the soil

Sl. No.	Soil characteristics	Analytical data	Methods employed
1	Organic carbon (%)	0.82	Walkley and Black, 1947
2	Total N (kg/ha)	1790.00	Bremner and Mulvaney, 1965
3	Total S (ppm)	225.00	Bardsley and Lanester, 1965
4	Total P (ppm)	840.00	Olsen and Sommers, 1982
5	Available N (kg/ha)	54.00	Bremner, 1965
6	Available P (kg/ha)	69.00	Olsen and Dean, 1965
7	Exchangeable K (kg/ha)	89.50	Pratt, 1965
8	Available S (ppm)	16.00	Hunter, 1984
9	pH (1 : 2.5 soil to water)	5.55	Jackson, 1958
10	CEC	11.23	Chapman, 1965