BIO-SAFETY MANAGEMENT AND CHEMICAL INVESTIGATION OF RADISH ROOT TOWARDS ALTERNARIA BLIGHT OF MUSTARD (*Brassica napus*)

A THESIS

BY

MD. MIZANUR RAHMAN
Student No. 0805022
Session: 2008-2009

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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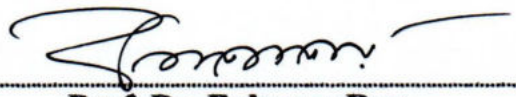

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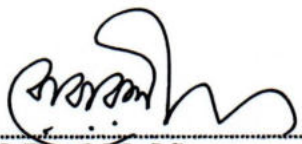

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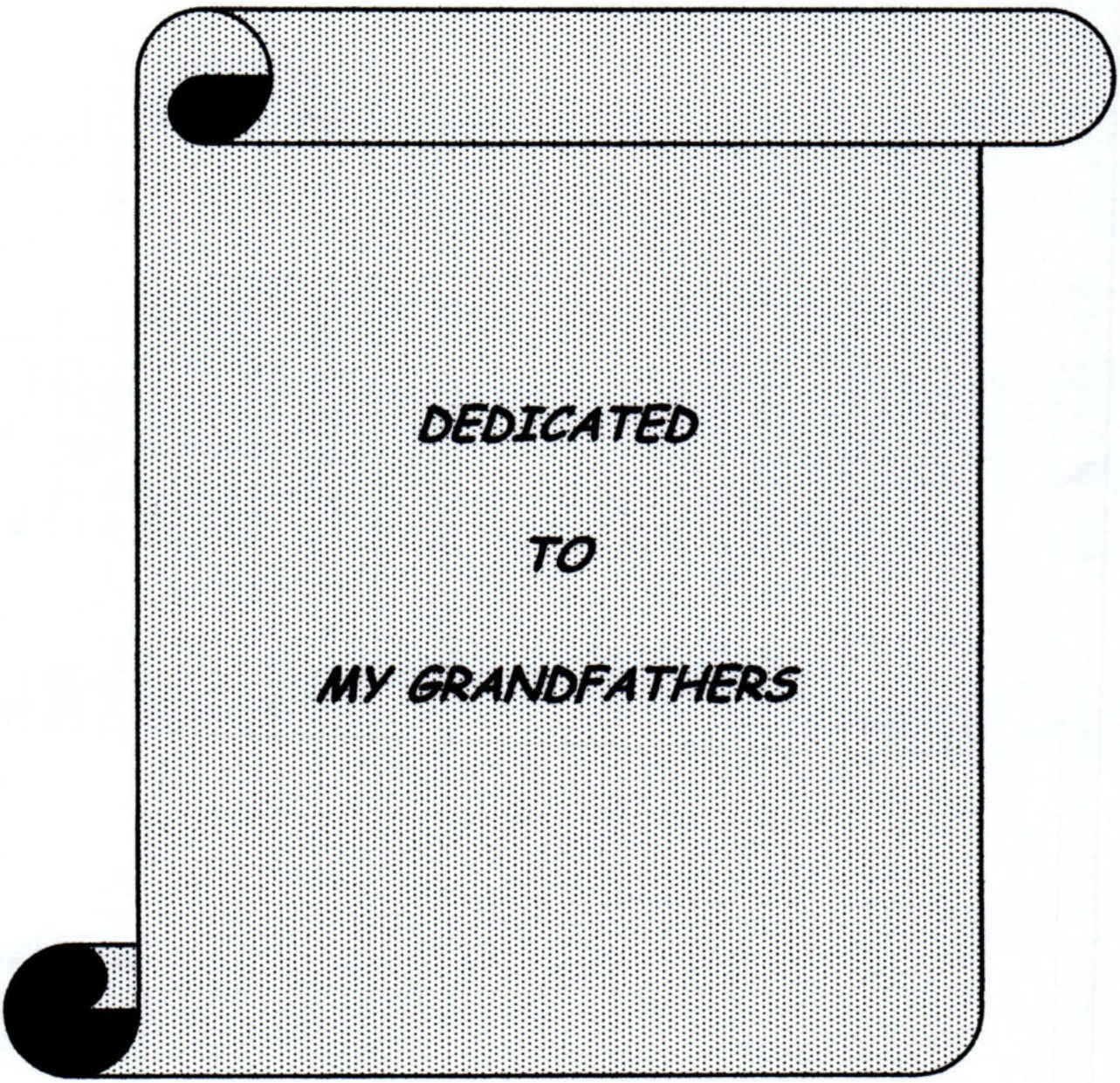
DEDICATED
TO
MY GRANDFATHERS

ABSTRACT

An investigation was carried out at Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of 2008-09 to explore the possibility of controlling Alternaria blight of mustard caused by *Alternaria brassicae* and *A. brassicicola*. A promising mustard variety BARI-9 was used in this investigation. A total of eight management practices viz., aqueous radish root extract (1:1), aqueous radish root extract (1:2), petroleum ether radish root extract (1:1), petroleum ether radish root extract (1:2), BAU-Biofungicide(0.2%), Iprodione-2 spary (0.25%)and Iprodione-3 (0.25%)spary including a control (plain water spray) were applied as foliar spray. The Alternaria symptom wass appeared on lower leaves at 27-days old plants and spraying were started from the time of disease initiation with 7 to 14-day interval. Among the treatments, Iprodione-3-spray showed superior performance in controlling Alternaria blight of mustard. Iprodione-3 spray reduced leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection, percent siliqua infection and number of spot per siliqua by 69.92%, 66.41%, 35.08%, 65.17%, 80.13% and 57.23%, respectively and increased seed yield by 55.68% over control. The second best performance against Alternaria blight was obtained from Iprodione-2 spray. Radish root extracts was sprayed with 7-day interval. Other than Iprodione, petroleum ether radish root extract (1:1) performed best in controlling the disease. Spray of petroleum ether radish root extract (1:1) reduced leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection, percent siliqua infection and number of spot per siliqua by 34.87%, 44.39%, 20.07%, 27.33%, 34.07% and 39.13%, respectively and increased seed yield by 35.93% over control. BAU-Biofungicide was sprayed twice with 14-day intervals and also showed significant result next to radish root petroleum ether extract (1:1) and increased seed yield by 25.64% over control. Regarding the economic consideration, iprodione-2 spray gave the maximum return (BCR=3.57) while the control treatment showed the lowest return (BCR=0.82). Petroleum ether radish root extract (1:1) as eco-friendly management practice exhibited good performance next to Iprodione (BCR=1.96). In laboratory investigation with Thin Layer Chromatography (TLC) of petroleum ether radish root extract towards antifungal compounds showed one distinct compound and mixture of compounds. The distinct compound showed high R_f value indicate non-polar type. Therefore, it may be concluded that Alternaria blight of mustard can be controlled with petroleum ether radish root extract (1:1) as foliar spray reducing maximum disease incidence and providing satisfactory higher seed yield in the sense of environmental pathology.

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The Author

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CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Mustard (*Brassica napus* L. 2n=4x=36) belonging to the family Cruciferae is the first important oil seed crops and one of the most important industrial crop in Bangladesh. Mustard and rapeseed are widely cultivated throughout the world as condiments, spices and oil seed crops. Out of the total cropped area of oil seed production approximately 74% is covered by mustard, 9% by groundnut and 17% by sesame (Anon., 1997). The amount of edible oil from mustard seed does not meet the current requirements of the 140 millions people of Bangladesh. Most of the common people of this country are traditionally fond of mustard oil. This country is in deficit of about 90.000 metric tons of edible oil annually (Rahman *et al.*, 1993) which is imported from other countries. About 2.17 lakh hectares land are occupied by mustard cultivation. Based on consumption requirement in Bangladesh, mustard hold the second position next to soybean. It contributes significantly to the national economy of the country. Mustard shared 183 thousand tons, which constitutes 70 percent of the total edible oilseeds in Bangladesh (Anon, 2006). Mustard fleshy leaves are used as a very popular green vegetable in the country. As compared to the developed countries, the yield of mustard is too low. The yield of this crop in Bangladesh is only 0.84 t/ha (Anon, 2006) which is poor compared to that of advanced countries like Japan, U.K., French and Poland producing 1.73, 3.43, 2.68, and 2.04 t/ha, respectively.

Many factors are responsible for such low yield of mustard in Bangladesh. One of the major constraints is incidence of various diseases. There are about fourteen diseases occurring on mustard in our country (Hossain *et al.*, 1992). Among the diseases, Alternaria blight caused by *Alternaria brassicae* (Bark) Sacc. and *A. brassicicola* are the most destructive which is responsible for low oil-seed production and this organisms are seed borne in nature (Talukder, 1974; Ahmed, 1985 and Meah *et al.,* 1985, Fakir, 1976 b; Anon., 1988 and Mridha, 1985). The disease 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 varieties are susceptible to this disease. This disease causes an average yield loss of 40-70% in India (Vishwanath and Kolt, 1997) 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). There is no information available on the resistance source, although sort of tolerance to the disease has been reported (Rai *et al.*, 1976). There have been multifarious efforts to control the disease, both success and failures have been achieved. Chemicals have been successfully used in controlling the disease (Meah *et al.*, 1988; Howlider *et al.*, 1985 and Kolte and Tewari, 1978). Single spray of Iprodione in early sown mustard significantly reduced leaf area infection by 24% and pod infection by 288% over untreated (control) late sown mustard (Rashid *et al.*, 1995). However, the issue of environmental pollution is linked with indiscriminate and unplanned use of

pesticides, which causes health hazards. People are now very conscious about environmental deterioration due to excessive use of spray chemicals.

To combat the harmful effect of chemicals and to get desirable yield, scientists have been thinking to reduce chemical pesticide use through biological agent. The use of specific microorganisms that interfere with plant pathogens, is nature-friendly and ecological approach to overcome the problems. The fungi (*Trichoderma harzianum*) is known as antagonist and very potential bio-control agents of several plant pathogenic fungi. Moreover, it is beneficial microbes to contribute the plant health and vigor. At present few elementary studies of *Trichoderma harzianum* as BAU-Biofungicide is going on as seed treating as well as foliar application in Bangladesh. This antagonistic fungus had antifungal effect on Alternaria species as seed born as well as cause of leaf spot disease of cruciferous (Hossain, 2007 and Shultana, 2007). Plant extracts are also now using in controlling yhe plkant diseases. Radish root have some antifungal protein which inhibit the growth of *Alternaria* pathogen (Terras *et al.*, 1993). The above ground part of radish plant is very susceptible to *Alternaria* where the root has been found resistant to the *Alternaria* species (Rashid, 2008).

Considering all the factors as the most important disease of mustard it is necessary to control the disease by incorporating different eco-friendly management practices. Therefore, an experiment was conducted to investigate the effect of iprodione, one biocontrol agent (*Trichoderma harzianum)* and root extract of

radish in controlling root extract on Alternaria blight and increasing the yield of mustard. Hence the objectives of the present investigation were:

(i) To compare the effectiveness of iprodione, root extract of radish and BAU-Biofungicide in controlling the Alternaria blight of mustard cv. BARI-9

(ii) Chemical investigation of radish root

(iii) To isolate the biologically active compounds from the root extracts of radish

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mustard (*Brassica napus* L.) suffers from many diseases. Rangaswami (1984) listed 11 diseases of mustard in India. Alternaria blight is a major and endemic disease of mustard. The disease is seed-borne and caused by *Alternaria brassicae* and *Alternaria brassicicola*. In mustard growing areas in the world, this disease is potential threat to successful cultivation of the crop for the last few decades. Many workers and researchers are trying to control Alternaria blight with fungicides. But the possibilities of controlling the disease through Biological means along with chemical have been explored in many mustard growing countries. So far, a review of various works relevant to Alternaria blight management in mustard seed production is furnished below:

2.1 Effect of Iprodione

Rashid (2008) conducted an experiment to investigate the efficacy of iprodione (0.25%) in controlling Alternaria blight of radish seed crop. In field study, he found maximum control of *Alternaria* blight and increased seed yield significantly higher with foliar spray of iprodione (0.25%).

Jyoti-Singh *et al.* (2006) tested six seed dressing fungicides, i.e. metalaxyl, carbendazim, mancozeb, thiophanate-methyl, iprodione and BAS 38601 F (40% carbendazim+32% mancozeb) in combination with spray of mancozeb (0.25%)

for the control of foliar diseases of Indian mustard. All seed treatments reduced *Alternaria* leaf spot (*Alternaria brassicae*). The highest yields were recorded with iprodione (16.0-17.36 q/ha) and. recommended for management of foliar diseases of Indian mustard.

Singh and Singh (2005) studied on Alternaria blight of mustard crops during 1995/96-2001-02 and revealed that Alternaria blight *(Alternaria brassicae)* was the major diseases in mid-eastern India (Uttar Pradesh, Bihar and Jharkhand) and caused 44.06% yield loss. In field they found best effect from three sprays of Iprodione during 2001-02 and 2002-03 cropping season in the same field.

Chattopadhyay and Bhunia (2003) used 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] in controlling the Alternaria-leaf blight of rapeseed-mustard (*Brassica campestris* cv. Yellow Sarson) caused by *Alternaria brassicae*. Highest seed yield and significant increase of 1000-seed weight were recorded from single spray of iprodione at post-flowering stage.

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 in the reduction

of Alternaria blight incidence. Iprodione-treated plots increased the yield by 24-59% than control plots.

Kumar *et al.* (2003) conducted a pot experiment to determine the efficacy of 0.2% Captan, Iprodione, Ridomil MZ [Mancozeb + Metalaxy1], Achook [*Azadirachta indica*], Zineb, Ziram and Blitox-50 [Copper oxychloride] applied at recommended rates against Alternaria blight (Caused by *Alternaria brassicae*) infecting radishes (cv. Kalyanpur No. 1). The fungicides were sprayed immediately after the appearance of initial symptoms and 10 and 20 days later. Observations on disease intensity and percentage disease reduction were recorded 15 days after the last spray. All fungicides significantly reduced disease incidence; the best control of the disease was obtained by spraying plants with Iprodione.

Khoda *et al.* (2003) conducted a field experiment during October 2002 to October 2003 in Gazipur, Bangladesh to evaluate the effectiveness of 5 fungicides to control Alternaria blight (*A. brassicae* and *A brassicicola*) of cauliflower seed crop. They sprayed Rovral 50WP (0.2% iprodione), Dithane M-45 (0.25% mancozeb), Ridomil (0.10% metalaxy1). Bavistin (0.25% carbendazim) and knowin (0.25% carbendazim). The fungicides were applied as foliar sprays for 4 times with 10-day intervals starting from the development of curd. The maximum reduction in severity of stalk rot and pod blight and the highest increase in seed yield over control were achieved with Rovral 50WP (0.20%).

Anonymous (2002) conducted an experiment with a susceptible variety Tori-7 of mustard and sprayed Rovral 50W.P. @ 0.2% for three times. The lowest leaf blight severity (3.3) was recorded from Rovral sprayed plot in respect of disease severity. The highest seed yield (1152 kg/ha) was obtained from Rovral sprayed plot than control (867 kg/ha).

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. The fungicide Rovral [iprodione] at 0.1% was used. All the 4 treatments were used at 1 litre/10 m2 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 seed yield.

Godika *et al.* (2001) carried out a field experiment was conducted from 1994/95 to 1996/97 in Rajasthan, India to evaluate the efficacy of different fungicides, mancozeb, Ridomil MZ (mancozeb+metalaxyl), captan, Rovral (iprodione), Bayleton [triadimefon], and copper oxychloride, against alternaria blight (*Alternaria brassicae*) of Indian mustard. Rovral was the most effective in controlling Alternaria blight and reducing mean disease intensity on leaf and pod was 8.75 and 5.6%, respectively. Yield (2.1 t/ha) was highest with Rovral spray.

Pandya *et al.* (2000) studied that Alternaria blight (*Alternaria brassicae, A. brassicicola*) is the major diseases of Indian mustard (*Brassica juncea*) causing heavy losses in Madhya Pradesh of India. Four sprays of iprodione (0.2%) gave the maximum control of Alternaria blight.

Ashok-Kumar (1996) studied that the effects of 4 fungicides on *Alternaria brassicae* of Indian mustard in field level. 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 spray.

Ayub *et al.* (1996) conducted an experiment to evaluate the efficacy of 7 fungicides to control Alternaria blight of mustard caused by *Alternaria brassicae* and *Alternaria 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 at 40, 50 and 60 days old. Iprodione reduced disease severity the most and increased seed weight and yield. Maximum reduction of disease severity and significantly higher yield was achieved when spraying was carried out on plants at 40-days-old.

Rashid *et al.* (1995) reported that single spray of Iprodione in early sown mustard crop significantly reduced leaf area infection by 24% and pod infection by 288%

over untreated (control) late sowing. This treatment also increased yield by 62% with a cost benefit ratio of 1:3.

2.2 Effect of BAU-Biofungicide (*Trichoderma harzianum*)

Rashid (2008) conducted an experiment to investigate the efficacy of biological agent (BAU-Biofungicide) and iprodione (0.25%) as foliar spray to control Alternaria blight of radish .He found significant effect in reducing Alternaria blight and increased seed yield by 120.45% and 97.73%, respectively over non-sprayed control.

Hossain (2007) found significantly minimum incidence of seed borne pathogen of *Alternaria brassicae* , *A brassisicicola* and *A alternata* in case of Vitavax -200 treted seed of radish followed by BAU-Biofungicide. He reported that this biofungicide had a significant effect in controlling the Alternaria leaf spot disease of radish.

Meena *et al.* (2004) conducted an experiment to investigate the efficacy of biological agent (*Trichoderma viride* isolates H-1, H-2, SI-1, GR, SI-2, B. P and T) and fungicides (Mancozeb and Carbendazim), alone or in combination, in controlling alternaria blight (*Alternaria brassicae*) of Indian mustard. In dual culture, GR isolate of *T. viride* performed best among the test isolates of *Trichoderma*, causing 81 and 82% reduction in mycelial growth of *Alternaria brassicae* over the control.

Anonymous (2003) reported a new product of BAU-Biofungicide and found to be effective in controlling the seed borne disease of vegetables and increase the seed yield. About 25-80% yield increase was reported from the use of BAU-Biofungicide.

Hossain (2003) reported that the yield of the crops can be increased by 25-80% over the untreated (control) by using BAU-Biofungicide and can be applied for controlling seed-borne diseases of vegetables by treating seeds.

Sivapalan (1993) surveyed to determine the effect of *T. harzianum* as seed-treating and reported that it has been found to be reduced leaf infection of Broccoli caused by A. *brassicicola.*

Vannacci and Harman (1987) conducted an experiment of 2 strains of *Trichoderma harzianum and T. koningii* and foundon radish crop increased numbers of healthy radish seedlings and also decreased pod infection by *A. raphani*

2.3 Effect of radish extract

Terras *et al.* (1993) found some new antifungal protein from radish that inhabits the growth of fungus. Seven antifungal proteins, which was near identical to 2 previously characterized proteins isolated from radish seeds. These basic

proteins, multimers of a 5-kDa polypeptide, specially inhibit the growth of *Alternaria brassicicola.*

Rashid (2008), personal observation on Development of healthy seed production technology of radish during PhD study the root has been found resistant to the *Alternaria* species.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 FIELD EXPERIMENT

3.1.1 Experimental site

The field experiment was carried out in the field of Plant Pathology Department and the chemical investigations were done at the laboratory of Agricultural Chemistry and Biochemistry Department during the period of December 2008 to May 2009.

3.1.2 Land topography and soil

The place of the field experiment falls under the AEZ-1 (Agro-Ecological-Zone) of Bangladesh and situated at latitude of 25.38 °N, longitude of 88.41 °E. The soil type and other characteristics are given below (UNDP and FAO, 1988):

Characteristics of AEZ-1

Name	:	Old Himalayan Piedmont Plain
Land type	:	Medium high land
Textural class	:	Sandy loam
Soil pH	:	Above 5.5
Organic matter (%)	:	About 1.0
Field level	:	Above flood level

3.1.3 Seed collection

Seeds of mustard cv. BARI-9 of Bangladesh Agricultural Development Corporation (BADC) was collected from local market of Dinajpur.

3.1.4 Land preparation and fertilizer application

The land was first plowed in late October. Later four-plowings and cross plowings followed by 3 laddering were done during the courses of land preparations. The following doses of manures and fertilizers were applied in the fields as recommended by BARC (Anonymous, 2004).

Manures and fertilizers	**Rate / hectare**
Cow dung	25t
Urea	200-250 Kg
TSP	150-170 Kg
MOP	70-85 Kg
Gypsum	120-150 Kg
Zinc Sulphate	4-5 Kg
Boric Acid	10-15Kg

The well-decomposed cowdung was incorporated with soil during first plowing. Half of the urea and all other fertilizers were incorporated with during final land preparation. Rest half of the urea was top dressed at 25 days after sowing.

3.1.5 Seed sowing

Seeds were sown in rows at the rate 8kg/ha with a distance of 4 cm and 15 cm plant to plant, row to row respectively. Date of sowing was 2nd December, 2008.

3.1.6 Intercultural operations

Two irritations were given at 25 days and 30 days age of the crop. One weeding was done at 14 days age of the crops to keep the plots free from weeds. Asataf 75 SP (Acephate) @ 0.2% was applied twice at 40 and 50 days age of the crops to control Aphids.

3.1.7 Treatments of the experiment

T_0 = Control (plain water).

T_1 = Aqueous radish root extract (1:1)

T_2 = Aqueous radish root extract (1:1)

T_3 = Petroleum ether radish root extract (1:1)

T_4 = Petroleum ether radish root extract (1:2)

T_5 = BAU-Biofungicide -2 spary

T_6 = Iprodione-2 spary

T7 = Iprodione-3 spary

3.1.8 Design and layout of the experiment:

The experiment was laid out in Randomized Block Design (RCBD) with three replications. The whole plot was divided in to three blocks containing 8 plots of 4m x 2.5m size giving total 24 plots. Each of the treatment was set in each block. The space kept between the block was 1.0m and plot to plot was 0.5m.

3.1.9 Preparation and application of treatments

3.1.9.1 Radish root extract

3.1.9.1.1 Aqueous extract

First the root was cleaned and makes them chop with sterilized knife. Then 200g of fresh root chop with 200 ml water was blended in an electric blender for 1:1 stock solution and sieved with cheese cloth. For 1:2 stock solution water was taken 400 ml. First spray was applied at the time of disease symptoms appearance followed by six more sprays at 7 days interval. The control plots were sprayed with same volume of plain water.

3.1.9.1.2 Petroleum ether extract

The root was cleaned first and made them chop with sterilized knife. Then 200g of fresh root chop and 200 ml petroleum ether was blended in an electric blender for 1:1 stock solution and sieved with cheese cloth .For 1:2 solution petroleum ether was taken 400 ml. Spray of the petroleum ether applied was given same as aqueous form.

3.1.9.2 Iprodione

Fungicidal solution of Iprodione was prepared by dissolving 2.5g of Rovral 50WP per 1000 ml water (0.25%). Spray was stored at the time of disease initiation and next applications were given at 14 days of interval by means of pneumatic knapsack hand sprayer while the control plots were sprayed with plain water. The spray time was 3:00pm to 4:00pm. The details of Rovral 50WP is given below:

Fungicide	Trade name	Chemical name	Active ingradient	Conc. (%)
Iprodione	Rovral 50WP	3- (3,5 dichloropheny) -2,4 dioxuimidazolidene $(C_{12}H_{13})3\ O_{12}\ N_3Cl_{12}$	50%	0.25

3.1.9.3 BAU-Biofungicide

BAU-Bio fungicide was taken in a 500ml beaker and added required amount of water to wet for 24 hours. Then, they were sieved with cheese cloth. First spray of BAU-Bio fungicide @ 2% was applied at the time of disease symptoms appearance followed by one more spray at 14 days interval. The control plots were sprayed with same volume of water. The details of BAU-Bio fungicide is given below:

Trade Name	Bio-agent	Conc (%).
BAU-Biofungicide	*Trichoderma harzianum*	2.0

3.1.10. Parameters studied and data collection procedure:

Data were collected on following parameters

1) % LAD (Leaf area diseased)
2) %SAD (Siliqua area diseased)
3) % Plant infection
4) %Leaf infection
5) % Siliqua infection
6) Number of spot per siliqua
7) 1000-seed weight (g)
8) Yield per plant (g)
9) Yield (t/ha)

There were 7 lines of mustard plants in each plot. Five plants were selected from 5 middle lines out of seven. The outer two lines were avoided during data collection. Percent leaf area diseased (LAD) and siliqua area diseased (SAD) were measured following eye estimation. Area of a single leaf or siliqua was considered as 100%. Percent LAD was recorded from all leaves within 30cm above from the soil level and percent pod area infection was recorded from 30 cm from the base of the older branch of main stalk considering all pods. Leaf area and pod area infection data were recorded at an interval of 7 days from first date of disease initiation for 5 and 4 times, respectively. Percent plant infection, number of spots

per siliqua was recorded at 68 days after sowing and percent leaf infection, percent siliqua infection was recorded at 54 and 61 days after sowing.

3.1.11 Harvesting, threshing and weighting:

Crop was harvested at fully mature stage at100 day's old crops. The five tagged plants were harvested and made bundled separately. Remaining plants of each plot were also harvested and made bundles. The all bundled were carried to farm office and dried under sunlight for 7 days. The fully dried bundles were threshed carefully and have been taken weigh of 1000 seed and yield per plant from 5 tagged plants. Yield per plot was taken adding the seed weight of five tagged plants to the of rest of the plant in a specific plot. The yield per plot was converted in the yield per plot to the yield per hectare.

3.1.12 Analysis of the data

Data were subjected to statistical analysis to find out the levels of significance of the experimental results. The mean of all the treatments were calculated and analysis of variance was performed. The significance of differences among pairs of treatment means were evaluated by Duncan's New Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984). All the data were calculated carefully and analyzed with the help of a personal computer in MSTAT-C computer package program (Russell, 1986).

3.1.13 Benefit cost analysis

Costing of application of treatment against Alternaria blight of mustard was done based on the current market price of input, rate of hiring labour and agricultural machineries. Price of field product was determined on the basis of the current market value (was shown Appendix I and II) estimation of the Benefit Cost Ratio (BCR) was done according to Gettinger (1982) and Islam *et al.* (2004) using the following formula:

$$\text{Benefit cost ratio (BCR)} = \frac{Gross\ return\ (Tk / ha)}{Total\ cost\ of\ production\ (Tk / ha)}$$

3.2 LABORATORY EXPERIMENT

3.2.1 Isolation of crude compound from effective a radish root extract using petroleum ether

For isolation of antifungal compound radish root was grinding in blender. Then 100g of crude compound was taken in 500ml conical flask and 400ml of Petroleum ether was added in to it. It was kept 72 hours with regular interval of shaking. After 72 hours it was filtrated with whatman no. 1 filter paper. The process was repeated for three times. The petroleum ether extract of individual flask were collected combined together. The solvent was evaporated by using Thin Film Rotary Evaporated under reduced pressure. Then the root extract was stored in refrigerator in 0^{o} C for further investigation.

The plate was then placed vertically with the spotted end downward in a solvent tank .crude extract checked by TLC in the following way:

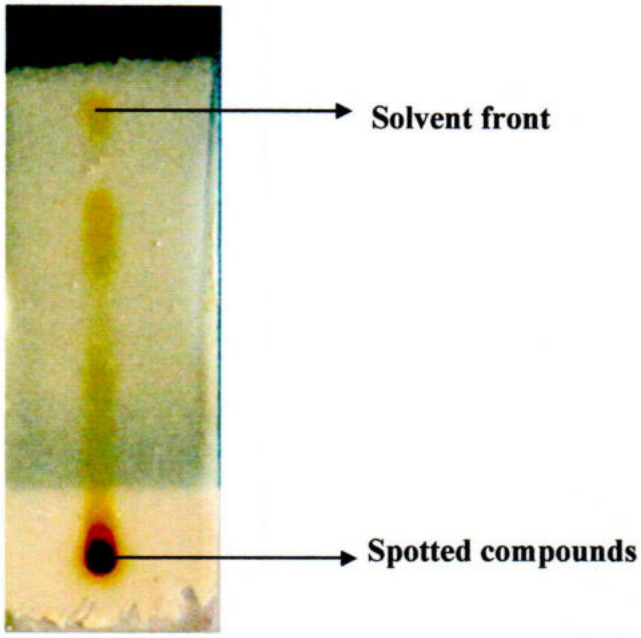

Fig. 1 Thin Layer Chromatographic plate

3.2.2.5 Preparation of solvent tanks and its working principles

A 250 ml wide mouth reagents bottle was used as solvent tanks, containing desired single solvent or mixture of solvents as the mobile phase, so that the spots did not immerse in the solvent. After running the solvent to appropriate distances (which were designed as solvent front) he plates were taken out and allowed to dry in air for 10-20 minutes. Subsequently, the plates were developed in iodine vapour in an iodine tank. The mixture of the polar and the non-polar solvent were used in a solvent tank as mobile phase.

3.2.2.6 Preparation of iodine tank and its working principles

A 250 ml wide mouth reagent bottle containing little amount of iodine crystal was used as iodine tank. After drying the TLC plate from the solvent tank, it was then placed with the spotted end downward in the iodine tank. Where the desired compound reacts with iodine vapour forming a complex compound and was indicated as yellowish spot for compound.

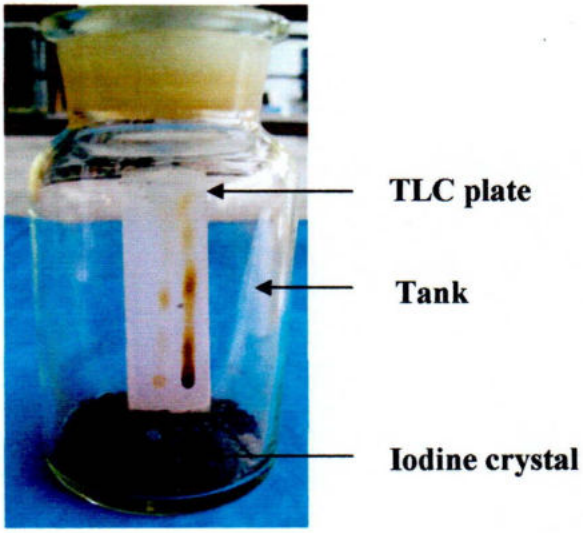

Fig. 2 An iodine tank

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 FIELD EXPERIMENT

4.1.1 Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent leaf area diseased (LAD) at different days after sowing (DAS) in 2008-2009

The foliar application of Iprodione, BAU-Biofungicide and **radish root extracts** were evaluated in controlling Alternaria blight disease of mustard and the findings are presented in Table 1. It was revealed that foliar application of treatments had significant effect in reducing disease severity. The highest leaf area diseased (LAD) at 26 days after sowing (DAS) was observed in the control plots (7.70%) and the lowest was obtained in Iprodione three sprayed plots (5.97%). At 33 DAS, the LAD ranged from 6.10% to 21.83%, where the significantly highest was obtained in the control plots and the lowest was recoded in Iprodione three sprayed plots. The lowest LAD at 40 DAS was recorded in Iprodione three sprayed plots (12.27%) which were followed by Iprodione two sprayed plots (13.80%) and the highest was obtained in the control plots (66.03%). At 47 DAS, the LAD ranged from 20.80% to 85.53%, where the significantly highest LAD (85.53%) were obtained in the control plots and the lowest (20.80%) was recoded in Iprodione three sprayed plots. The lowest LAD at 54 DAS was observed in Iprodione three sprayed plots (27.47%) which was followed by Iprodione two

sprayed plots (28.80%) and the highest was obtained in the control plots (91.33%).Regard the leaf area diseased Iprodione three spray plots ranked first, Iprodione two spray plots ranked second and Petroleum ether radish root extract (1:1) plots ranked third.

Table 1. Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent leaf area diseased (LAD) at different days after sowing (DAS) in 2008-2009

Treatment	26 DAS	33 DAS	40 DAS	47 DAS	54 DAS	% LAD decrease over control at 54 DAS
Control	7.70	21.83	66.03	83.53	91.33	-
Aqueous radish root extract (1:1)	6.80	14.93	59.67	78.00	89.93	1.59
Aqueous radish root extract (1:2)	6.80	17.20	59.13	79.07	88.87	2.46
Petroleum ether radish root extract (1:1)	7.63	11.03	23.17	37.40	59.50	34.87
Petroleum ether radish root extract (1:2)	6.00	11.33	23.13	38.40	63.53	30.43
BAU-Biofungicide	7.57	19.93	29.00	37.57	66.23	27.48
Iprodione 2 spray	6.20	7.23	13.80	22.07	28.80	64.47
Iprodione 3 spray	5.97	6.10	12.27	20.80	27.47	69.92
LSD (P=0.05)	1.08	2.36	3.15	4.06	2.69	

4.1.2 Effects of Iprodione, BAU Biofungicide and radish root extracts on percent siliqua area diseased (SAD) at different days after sowing (DAS) in 2008-2009

All the treatments (Iprodione, BAU-Biofungicide and radish root extract) started spraying at 26 days of the crops which brought down the infection and significantly reduced the siliqua infection .The findings are presented in Table 2. The result revealed that foliar application of all treatments had significant effect in reducing siliqua disease severity. The highest siliqua area diseased at40 DAS was found in the control plots (3.03%) and the lowest was obtained in Iprodione three sprayed plots (1.20%). At 47 DAS, the SAD ranged from 13.20% to 5.20%, where the significantly highest were obtained in the control plots and the lowest were recoded in Iprodione three sprayed plots. The lowest SAD at 54 DAS was recorded in Iprodione, three sprayed plots (11.93%) which was followed by Iprodione two sprayed plots (12.87%) and the highest was obtained in the control plots (22.40%). At 61 DAS, the SAD ranged from 14.60% to 43.47%, where the significantly highest SAD (43.47%) was obtained in the control plots and the lowest (14.60%) was recorded in Iprodione, three sprayed plots which was followed by petroleum ether radish root extract (1:1) sprayed plots (34.13%).

Table 2. Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent siliqua area diseased (SAD) at different days after sowing (DAS) in 2008-2009

Treatment	40 DAS	47 DAS	54 DAS	61 DAS	% SAD decrease over control at 61 DAS
Control	3.03	13.20	22.40	43.47	-
Aqueous radish root extract (1:1)	2.97	10.73	19.53	39.67	8.74
Aqueous radish root extract (1:2)	2.77	11.40	17.93	34.13	21.48
Petroleum ether radish root extract (1:1)	1.33	10.40	15.23	24.17	44.39
Petroleum ether radish root extract (1:2)	1.95	9.40	14.97	25.23	41.95
BAU-Biofungicide	2.93	11.20	18.30	34.63	20.33
Iprodione 2 spray	1.48	5.87	12.87	19.40	55.37
Iprodione 3 spray	1.20	5.20 d	11.93	14.60	66.41
LSD (P=0.05)	0.53	1.60	2.30	2.58	

4.1.3 Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent plant infection in 2008-2009

Percent plant infection was recorded at 68 DAS and is presented at Table 4.There were significantly variations among the treatments in respect of percent plant infection. The lowest percent plant infection was obtained in Iprodione three sprayed plots (64.92%) which were followed by Iprodione two sprayed plots (75.80). Among the non-chemical treatments, petroleum ether radish root extract (1:1) performed best (79.93%). The highest percent plant infection (100.00%) was found in BAU-Biofungicide, aqueous radish root extract (1:2) and control plots.

Table 3. Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent plant infection in 2008-2009

Treatment	% plant infection	% plant infection decrease over control at 68 DAS
Control	100.00	-
Aqueous radish root extract (1:1)	98.38	1.62
Aqueous radish root extract (1:2)	100.00	-
Petroleum ether radish root extract (1:1)	79.93	20.07
Petroleum ether radish root extract (1:2)	85.98	14.02
BAU-Biofungicide	100.00	-
Iprodione 2 spray	75.80	24.2
Iprodione 3 spray	64.92	35.08
LSD (P=0.05)	0.6265	

4.1.4 Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent leaf infection in 2008-2009

The effects of Iprodione, BAU-Biofungicide and radish root extracts on percent leaf infection were recorded at 68 DAS and is presented in Table 5. It was revealed that the maximum percent leaf infection was obtained in aqueous radish root extract (1:2), aqueous radish root extract (1:1) and control treatment. The minimum percent leaf infection was recorded in Iprodione three sprayed (34.83%) plots which were followed by Iprodione two sprayed (42.57%) plots.

Table 4. Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent leaf infection in 2008-2009

Treatment	% leaf infection 48 DAS	% Leaf infection decrease over control at 68 DAS
Control	100.00	-
Aqueous radish root extract (1:1)	100.00	-
Aqueous radish root extract (1:2)	100.00	-
Petroleum ether radish root extract (1:1)	72.67	27.33
Petroleum ether radish root extract (1:2)	75.50	24.5
BAU-Biofungicide	95.83	4.17
Iprodione 2 spray	42.57	57.43
Iprodione 3 spray	34.83	65.17
LSD (P=0.05)	0.6265	

4.1.5 Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent siliqua infection in 2008-2009

Iprodione, BAU-Biofungicide and radish root extracts had a significant influence on percent siliqua infection (Table 6). The lowest percent siliqua infection (19.87%) was found in Iprodione three sprayed plots which was followed by iprodione-2 sprayed (25.77%). Except chemical treatments, Petroleum ether radish root extract (1:1) performed best (65.93%) in respect of percent siliqua infection which was followed by petroleum ether radish root extract (1:2).

Table 5. Effects of Iprodione, BAU-Biofungicide and radish root extracts on percent siliqua infection in 2008-2009

Treatment	Percent siliqua infection 60 DAS	% Siliqua infection decrease over control at 68 DAS
Control	100.00	-
Aqueous radish root extract (1:1)	88.03	11.97
Aqueous radish root extract (1:2)	94.73	5.27
Petroleum ether radish root extract (1:1)	65.93	34.07
Petroleum ether radish root extract (1:2)	71.07	28.93
BAU-Biofungicide	87.17	12.83
Iprodione 2 spray	25.77	74.23
Iprodione 3 spray	19.87	80.13
LSD (P=0.05)	0.4828	

4.1.6 Effects of Iprodione, BAU-Biofungicide and radish root extracts on number of spot per siliqua in 2008-2009

The effects of Iprodione, BAU-Biofungicide and radish root extracts were evaluated against number of spot per siliqua at 68 DAS. There were significant effects on the treatments on the number of spot per siliqua (Table 7). The minimum number of spot per siliqua was recorded in iprodion-3 sprayed plots (9.62) and the maximum was observed in the untreated control plots (22.51). Other than Iprodione, petroleum ether radish root extract (1:1) exhibited superior performance in respect of number of spot per siliqua which was followed by petroleum ether radish root extract (1:2).

Table 6. Effects of Iprodione, BAU-Biofungicide and radish root extracts on number of spot per plant in 2008-2009

Treatment	No. spot/ siliqua	% No. of spot per siliqua decrease over control at 68 DAS
Control	22.51	-
Aqueous radish root extract (1:1)	18.10	19.59
Aqueous radish root extract (1:2)	20.10	10.70
Petroleum ether radish root extract (1:1)	13.70	39.13
Petroleum ether radish root extract (1:2)	15.57	30.83
BAU-Biofungicide	13.75	38.91
Iprodione 2 spray	11.07	50.82
Iprodione 3 spray	9.627	57.23
LSD (P=0.05)	0.5454	

4.1.7 Effects of Iprodione, BAU-Biofungicide and radish root extracts on 1000-seed weight, yield per plant and yield per hectare in 2008-2009

In case of seed yields the lowest 1000 seed weight was obtained in the control plots (1.91g) and the highest yield was recorded from Iprodione three sprayed plots (3.13g). Yield per plant and yield per hectare were significantly influenced by different spray materials. Seed yield per plant was significantly different among the sprays. The lowest yield per plant (2.90g) was recorded in control plots where the highest (6.10g) was obtained in Iprodione three sprayed plot which was followed by Iprodione two sprayed plots (5.80g).The lowest yield per hectare (0.39t\ha) was recoded from control plots and the highest (0.88t\ha) is recorded from Iprodione three sprayed plot which was followed by Iprodione two sprayed plots (0.87t\ha).

Table 7. Effects of Iprodione, BAU-Biofungicide and radish root extracts on seed yield of Mustard cv. BARI- 9

Treatment	1000-seed weight (g)	Yield/Plant (g)	Yield (t/ha)	% Yield increase over control
Control	1.91	2.90	0.39	-
Aqueous radish root extract (1:1)	2.38	3.20	0.47	20.51
Aqueous radish root extract (1:2)	2.43	3.41	0.46	17.94
Petroleum ether radish root extract (1:1)	2.84	5.33	0.64	35.93
Petroleum ether radish root extract (1:2)	2.71	5.21	0.62	37.09
BAU-Biofungicide	2.50	5.09	0.49	25.64
Iprodione 2 spray	2.95	5.80	0.87	55.17
Iprodione 3 spray	3.13	6.10	0.88	55.68
LSD (P=0.05)	0.15	0.33	0.06	

Plate 1. Control Plot (non treated)

Plate 2. Iprodione (0.25%) three sprayed plot

Plate 3. Petroleum ether radish root extract (1:1) sprayed plot

4.1.8 Benefit Cost Analysis

The benefit cost ratio analysis regarding the use of Iprodione, BAU-Biofungicide, radish root extracts was estimated and the results are presented in the Table 8. Estimation showed that application of Iprodione-2 spray yielded the highest return (BCR=3.57) followed by Iprodione-3 spray (BCR=3.09) where the lowest return (BCR=0.82) was obtained from the control treatment in mustard seed crop. Other than Iprodione, petroleum ether radish root extract (1:1) achieved highest return (BCR=1.96) where TK. 1.96 could be earned investing TK. 1.00 (Appendices I and II)

Table 8. Benefit Cost Ratio (BCR) of different management practices in controlling *Alternaria* blight of musard seed crop

Treatment	Average yield (t/ha)	Price (Kg)	Total cost (TK.)	Gross margin (TK.)	BCR
Control	0.39+2.0	30/-+1.0/-	16531/-	13700/-	0.82
Aqueous radish root extract (1:1)	0.47+2.0	30/-+1.0/-	18397/-	16100/-	0.87
Aqueous radish root extract (1:2)	0.46+2.5	30/-+1.0/-	17463/-	15800/-	0.90
Petroleum ether radish root extract (1:1)	0.64+2.5	100/-+1.0/-	33864/-	66500/-	1.96
Petroleum ether radish root extract (1:2)	0.62+2.0	100/-+1.0/-	50531/-	64000/-	1.26
BAU-Biofungicide	0.49+2.0	50/-+1.0/-	25864/-	26500/-	1.02
Iprodione 2 spray	0..87+2.5	100/-+1.0/-	25031/-	89500/-	3.57
Iprodione 3 spray	0.88+2.5	100/-+1.0/-	29281/-	90500/-	3.09

Details shown in Appendices I and II

4.2 LABORATORY EXPERIMENT

Thin Layer Chromatography (TLC)

The Thin Layer Chromatography (TLC) of petroleum ether crude extract of radish root was performed to isolate the compound which retained the inhibitory properties against Alternaria blight pathogens. The TLC of petroleum ether radish root extract showed distinctly one compound which is designated as R_1 (Fig. 3) and mixture of compounds at Hexane and ethyl acetate as R2 (15:1v/v). The compound showed high R_f value (0.965 cm) in iodine tank indicate most non-polar compound (Table 9).

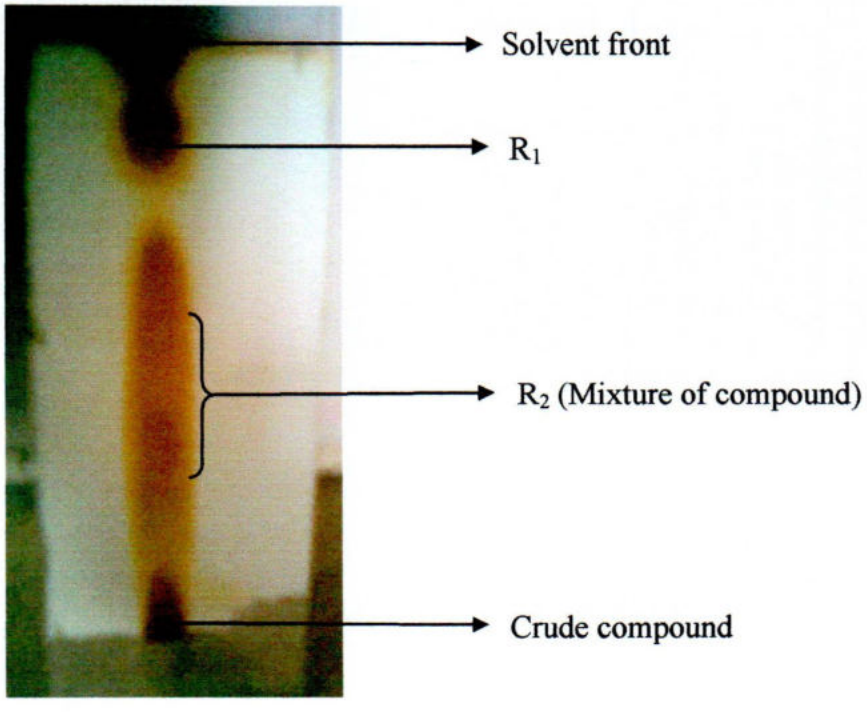

Fig. 3. TLC of petroleum ether extract of radish root with Hexane: Ethylacetate (15:1)

Table 9. R_f values of detected component of petroleum ether radish root extract

Name of plant species	Ratio of Hexane and Ethyl acetate	Detected compound	Rf value (cm)
Radish (*Raphanus sativus)*	15:1	R1\C1	0.964

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

Alternaria blight caused by *Alternaria brassicae* and *A. brassicicola* is the most devastating disease of mustard in Bangladesh. The present investigation has been carried out to find out the effects of Iprodione, Radish root extract and BAU-Biofungicide on the severity of Alternaria blight of mustard cv. BRRI-9. The disease symptoms appeared on lower leaves at 27- day old plants during 2008-2009. To avoid yield loss, application of treatments was commenced at the beginning of disease symptoms appearance in the growing plants for later progress. Plants sprayed with plain water served as the untreated control. Observation on the disease severity as leaf area diseased (LAD) and siliqua area diseased (SAD) were recorded at 7 day intervals and disease intensity as percent plant infection at 68 DAS, percent leaf infection at 48 DAS and percent siliqua infection were recoded at 68 DAS, respectively.

Among the treatments, Iprodione (0.25%)-3 spray showed superior performance in controlling Alternaria blight of mustard seed crop. Iprodione-3 spray reduced leaf area diseased, pod area diseased by 69.92% and 66.41% over control at last observation. This treatment also reduced percent plant infection, percent leaf infection, percent siliqua infection and number of spot per siliqua by 35.08%, 65.17% 80.13% and 57.23% over control, respectively and increased yield per hectare by 55.68%. The performance of Iprodione against Alternaria blight of

mustard as found in this study is supported by Jyoti-Singh *et al.* (2006). They reported that Iprodione-3 spray (0.25%) performed best against Alternaria blight of mustard with less disease incidence and increased 1000-seed weight, yield per plant and yield per hectare. Similar results were also found by Rashid (2008). He mentioned that Alternaria blight of radish seed crop cane be effectively controlled by Iprodione (0.25%) with maximum BCR (3.90). The findings of the present study are also corroborated by the finding of Ayub *et al.* (1996) demonstrating that Iprodione (as Rovral 50 WP) @ 0.25%, three spray at 40, 50 and 60 days old significantly reduced disease severity and increased seed yield. The present finding is also in line with many authors such as Kumar et al. (2003) demonstrating that foliar application of 0.2% Rovral 50 WP as effective against Alternaria blight. Rovral 50 WP (Iprodione) proved most effective spraying immediate after the appearance of initial symptoms and 10 and 20 days later which is in agreement with the present findings.

The results of Iprodione-2 spray showed the second best next to Iprodione-3 spray in controlling the Alternaria blight. Two time sprays of Iprodione brought a tremendous reduction of leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection and percent siliqua infection by 64.47%, 55.37%, 24.20%, 57.43% and 74.23% over control. This treatment increased seed yield by 55.17% over control. This finding is supported by the reports of Meah *et al.* (1992). He cited that Rovral 50 WP (Iprodione) significantly reduced disease severities and increased seed yield when applied for 2 times. Anonymous (1996)

found that foliar spray of Iprodione (0.2%) applied at disease initiation and siliqua formation stage achieved the highest yield with lowest disease severity. It is also indicated that lower frequency of chemical sprays Alternaria blight can be brought under control and highest seed yield can be obtained. Since chemical spray involves cost and handling troubles, therefore minimum chemical spray should be involved to ensure maximum output. However, other manual of disease management may be tagged with it.

Other than Iprodione, petroleum ether radish root extract (1:1) performed best in controlling Alternaria blight of mustard. Spray of petroleum ether radish root extract (1:1) reduced leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection, percent siliqua infection and number of spot per siliqua by 34.87%, 44.39%, 20.07%, 27.33%, 34.07 and 39.13% and increased seed yield 35.93% over control, respectively. This is an interesting finding that radish root extract can be used to control Alternaria blight. This idea is in agreement with the finding of Terras *et al.* (1995) who found some new antifungal protein from radish that inhabits the growth of fungus. Seven antifungal proteins, which was near identical to 2 previously characterized proteins isolated from radish seeds. These basic proteins designated *Raphanus sativus*- antifungal protein- 1 (Rs-AFP1) and Rs-AFP2, multimers of a 5-kDa polypeptide, specially inhibit the growth of *Alternaria brassicicola.*

BAU-Biofungicide was sprayed at 36 DAS and 51 DAS which showed significant effect next to petroleum ether radish root extract in reducing disease intensity as well as severity. This treatment also increased seed yield (25.64%) over control. This finding is in agreement with the findings of Rashid (2008) who reported that BAU-Biofungicide (*Trichoderma harzianum*) had a significant effect against Alternaria blight and increased seed yield. This idea is also in line with Hossain (2007) who demonstrating that BAU-Biofungicide had significant effect in reducing seed borne Alternaria blight of radish in the field. Bhuyan *et al.* (2005) observed that BAU-Biofungicide play a significant role in controlling Alternaria blight of radish with vigorous plant growth and maximum seed yield. This result is also corroborated with Shultana (2007). She reported that BAU-Biofungicide (*Trichoderma harzianum*) showed remarkable reduction of leaf blight severity of wheat in the field condition. Considering the Bio-safety measures petroleum ether radish root extract advisable as a control measure of Alternaria blight of mustard in order to avoid the use of chemical hazards.

Cost of analysis of management practices applied for controlling Alternaria blight of mustard revealed that Iprodione-2 spray provided maximum return (BCR=1.81) due to effective management of the disease, higher seed yield and low management cost. Other than Iprodione, petroleum ether radish root extract (1:1) obtained the highest return (BCR=1.20). The benefit cost ratio indicated that eco-friendly management practice, petroleum ether radish root extract which gave a good performance next to Iprodione.

Petroleum ether radish root extract gave a good result in controlling Alternaria blight of mustard. So it is important to identify that which compound is responsible for this type of inhibitory activity against Alternaria blight pathogens. Keeping this view in mind, Thin Layer Chromatography (TLC) of petroleum ether radish root extract was performed which showed one distinct compound and mixture of compounds at Hexane and ethyl acetate (15:1v/v). The compound exhibited high R_f value which indicate non-polar in nature. This laboratory experiment is still continuing for purification of above crude by coloum chromatography. After purification further investigation is to be done for the field experiment against Alternaria blight for detection of effective component(s). The structure of the effective component will be determined by HPLC or spectroscopic studies, i.e., by ^{1}H-NMR, 13 C-NMR, IR and Mass spectroscopy. The ultimate goal of this investigation is to formulate a noble botanical fungicide that can be used in the field against Alternaria blight disease. But due to lack of time after completion of the field experiment availability of radish root and proper laboratory facilities only TLC have been done. Similar type of chemical investigation were performed by Mitra *et al.,* (1987) demonstrating chloroform and ethyl acetate extracts of *Clerodendron inermi* showed antifungal activity against two spore forming filamentous fungi, *Drechslera oryzae* and *Aspergillus niger.*

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

An investigation was carried out at Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period 2008-09 to explore the possibilities of controlling Alternaria blight of mustard caused by *Alternaria brassicae* and *A. brassicicola* with minimal management practices. The promising variety mustard cv. BARI-9 of Bangladesh Agricultural Development Corporation (BADC) was used in this experiment. A total of eight management practices viz., aqueous radish root extract (1:1), aqueous radish root extract (1:2), petroleum ether radish root extract (1:1), petroleum ether radish root extract (1:2), BAU-Biofungicide, Iprodione-2 spary and Iprodione-3 spary including a control where only plain water was usedin this study. Observation on the disease severity as leaf area diseased (LAD) and siliqua area diseased (SAD) were recorded at 7 day intervals and disease intensity as percent plant infection at 68 DAS, percent leaf infection at 48 DAS and percent siliqua infection were recoded at 68 DAS, respectively. The Alternaria symptom wass appeared on lower leaves at 27-days old plants. Spray of Iprodione (0.25%) was initiated at the time of diseased symptom appeared and next applications are given at 14 days interval. Among the treatments, Iprodione (0.25%)-3 spray showed superior performance in controlling Alternaria blight of mustard seed crop. Iprodione-3 spray reduced leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection,

percent siliqua infection and number of spot per siliqua by 69.92%, 66.41%, 35.08%, 65.17%, 80.13% and 57.23% and increased seed yield by 55.68% over control. The second best performance against Alternaria blight was obtained from Iprodione-2 spray which increased seed yield by 55.17% over control. It is indicated that lower frequency of Iprodine spray can brought the disease under control and higher seed can also be obtained. Radish root extracts was sprayed at the time of diseased initiation followed by six more sprays at 7 days interval. Other than Iprodione, petroleum ether radish root extract (1:1) performed best in controlling Alternaria blight of mustard. Spray of petroleum ether radish root extract (1:1) reduced leaf area diseased, siliqua area diseased, percent plant infection, percent leaf infection, percent siliqua infection and number of spot per siliqua by 34.87%, 44.39%, 20.07%, 27.33%, 34.07 and 39.13% and increased seed yield 35.93% over control, respectively. This is an interesting finding that radish root extract can be used to control Alternaria blight of mustrard as radish root contains antifungal compounds which inhibit the growth of *Alternaria.* BAU-Biofungicide was sprayed at 36 DAS and 51 DAS which also showed significant effect next to radish root petroleum ether extract (1:1) and increased seed yield (25.64%) over control. Considering the Bio-safety measures, radish root petroleum ether extract (1:1) can be advisable as a control measure of Alternaria blight of mustard in order to avoid the chemical hazards. Regarding the economic consideration, iprodione-2 spray gave the maximum return (BCR=3.57) while the control treatment showed the lowest return (BCR=0.82). The eco-friendly management practice petroleum ether radish root extract (1:1) exhibit a good

performance next to Iprodione (BCR=1.96). The Thin Layer Chromatography (TLC) of petroleum ether radish root extract was performed to isolate the crude compound which retained the inhibitory properties against Alternaria blight pathogens. The test showed one distinct compound and mixture of compound. The distinct compound showed high R_f value indicate non-polar type nature. Therefore, it may be concluded that Alternaria blight of mustard can be controlled with eco-friendly management practice by using petroleum ether radish root extract (1:1) as foliar spray for minimum disease incidence and satisfactory seed yield with sound environment. Before making any final recommendation to use this management practice at field level, this research work especially laboratory study should be repeated with high laboratory facilities.

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APPENDIX I

Appendix: Analysis of cost of application of common cultural practices in production of radish seed

Cost items	Per hectare cost in TK.				
	Unit	Quantity	Cost/unit*	Times	Total cost
Seed	Kg.	8.0kg	70/kg	-	560/-
Land preparation, Ploughing (tractor, power tiller, spade)	Tractor, power tiller	1	500	-	500/-
Human labor	Man^{-1}	1	120	-	120/-
Seed sowing in line	Man^{-1}	2	120	-	240/-
Fertilization and manures					
Urea	Kg	200	6/-	-	1200/-
TSP	Kg	150	16/-	-	2400/-
MP	Kg	70	16/-	-	1120/-
Gypsum	Kg	120	5/-	-	600/-
Zinc sulphate	Kg	4	10/-	-	40/-
Boric acid	Kg	14	10/-	-	140/-
Cow dung	Ton	25	200/-	-	5,000/-
Labor	Man^{-1}	2	120/-	-	240/-
Weeding(Seedbed+Main plot) Irrigation + Human labor	- Man^{-1}	4	120/-	2	960/-
Insecticide spraying					
Acephate	Man^{-1}	.70	1000/-	2	1400/-
Human labor	Sprayer	2	120/-	2	240/-
Harvesting, threshing, cleaning and drying etc					
Labor	Man^{-1}	8	120/-	-	960/-
Miscellaneous cost	-	-	-	-	831/-
Total (a)					**16531/-**

*Calculated on the basis of market price of 2008-2009

APPENDIX II

Appendix: Analysis of cost of application of management practices in production of radish seed

Cost items Application of Iprodione	Per hectare cost in TK.				
	Unit	Quantity	Cost/unit	Times	Total cost
Control	L/Kg	-	-	-	-
Aqueous radish root extract (1:1)	L/Kg	446	4/-	seven	1866/-
Aqueous radish root extract (1:2)	L/Kg	233	4/-	seven	932/-
Petroleum ether radish root extract (1:1)	L/Kg	167+167	100/-+4/-	seven	17333/-
Petroleum ether radish root extract (1:2)	L/Kg	334+167	100/-+4/-	seven	34000/-
BAU-Biofungicide	L/Kg	93	100/-	Two	9333/-
Iprodione two sprays	L/Kg	5	1700/-	Two	8500/-
Iprodione three sprays	L/Kg	7.5	1700/-	Three	12750/-