EFFECT OF SEED TREATING CHEMICALS AND BAU-BIOFUNGICIDE ON *ALTERNARIA* BLIGHT (*Alternaria brassicae*) OF MUSTARD

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CERTIFICATE

This is to certify that the thesis entitled, "***Effect of seed treating chemicals and BAU-Biofungicide on Alternaria blight (Alternaria brassicae) of mustard***" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN PLANT PATHOLOGY***, embodies the result of a piece of bona fide research work carried out by ***Mariam Ahmed, Registration No. 08-03172,*** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

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

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DEDICATED
TO
MY BELOVED
PARENTS

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The author

Effect of seed treating chemicals and BAU-Biofungicide on *Alternaria* blight (*Alternaria brassicae*) of mustard

ABSTRACT

The effect of three seed treating chemicals viz. Provax, Brine solution, Rovral 50WP and one Biofungicide viz. BAU- Biofungicide were employed against *Alternaria* blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola.* Three varieties viz. var. BARI-6, Tori-7 and SAU-Shorisha-1 were used in this study. The field experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka and laboratory experiment was conducted in the seed health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of November 2008 to July 2009. Among the seed treating chemicals, Rovral 50WP showed the best performance in reducing disease incidence and severity as well as increase seed yield. Again application of Rovral 50WP gave best result in terms of for reduction of number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weight (g) and 1000 seed weight (g). BAU- Biofungicide also showed promising performance in controlling *Alternaria* blight of mustard and reduced 36% and 53% disease incidence and disease severity respectively over untreated control. BAU-Biofungicide may be recommended for as an alternative means of chemical fungicide such as Rovral 50 WP for controlling *Alternaria* blight of mustard.

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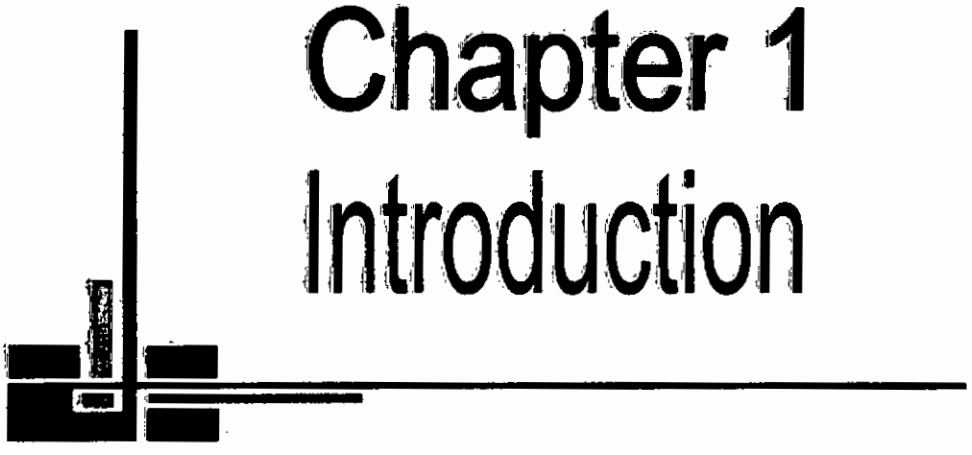


Chapter 1
Introduction

CHAPTER-I

INTRODUCTION

Mustard (*Brassica spp*) is one of the most important oil producing crops in Bangladesh. At present, mustard covered 5,77,000 acre of land area and produces 2, 28,000 metric ton of seed yield. (BBS, 2010). 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 seeds of *Brassica* contain 42% oil and 25% protein (Khaleque, 1985). Using local Ghani an average of 33% oil may be extracted. In Bangladesh three species of *Brassica* are cultivated for oil production, viz. *Brassica camprstries*, *Brassica juncea* and *Brassica napus* (Anonymous, 2001).

Many factors are associated with the poor yield of mustard in Bangladesh. Diseases have been identified as one of the major factors (Ahmed, 1992). Mustard suffers from about 14 diseases (fungus-9, virus-2, bacteria-1, nematode-1 and parasitic plant-1) in Bangladesh. Some important diseases are *Alternaria* blight, Downy mildew, powdery mildew stem rot, Damping off, wilt etc. Among these diseases, leaf blight, downy mildew and the parasitic plant are important (Anonymous, 2007). *Alternaria brassicae* is widely distributed and the most serious and devastating diseases of mustard. The diseases may cause 25% yield reduction at severe condition of infection. (Anonymous, 2007). Leaf blight caused around 30-60% losses in Bangladesh (Anonymous, 2002).

The disease causes substantial production loss to the crop resulting huge financial loss to the farmers. It has been estimated that, in an average, the disease causes up to 25% production losses (Fakir, 2008 and BARI, 2007). They also observed that the loss is around Tk. 200 crores annually.

The symptoms due to *Alternaria brassicae* first appear on the cotyledonary leaves as small light brown spots, later turning black due to sporulation of the pathogen and as necrotic streaks of hypocotyls (Vasudeva, 1958; Sharma, 1989). On leaves, the infection starts as brown to blackish point which enlarges becoming entirely black or dark bordered with a gray centre (McDonald, 1959). The spots spread rapidly on the stem and pods and become more or less circular (0.5-10 mm in diameter), slightly raised above leaf surface. Linear spots have also been reported on stem (Vasudeva, 1958; Tripathi and Kaushik, 1984). In severely infected plants, the stem is so heavily attacked that it undergoes defoliation even before the pods reach maturity. The pathogen penetrates into the pods and infects the seeds which may show gray to brown discoloration (Vasudeva, 1958). The characteristic symptom of Gray blight of mustard is the development of circular spots coalesces and ultimately the whole leaves become blighted. Gray blight (*Alternaria brassicae*) causes blight of leaf, pod and stem (Meah *et al.* 1998) and seed abnormalities (Howlider *et al.* 1991). It is endemic in Bangladesh and all the cultivated *Brassica campestris* and *Brassica 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.* 1998). In addition to direct yield losses the disease adversely affects the seed quality by reducing seed size, seed discoloration and reduction in oil contents (Howlider *et al.*, 1991and 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).

The seeds infected with *Alternaria brassicae* show gray discoloration of seed coat covering the seeds partially or wholly. (Rangel, 1945; McDonald, 1959 and Vishnuavat *et al*, 1985). With an increase in disease intensity, a reduction in fresh and dry weight, decrease in oil contents and decrease in carbohydrate level and reduction in ash content were observed (Bandyopadhya, Saha and Mukherjee, 1974), where as a reduction in oil content was reported by others (Degenhardt *et al*, 1974; Rai and Saxena, 1980; Chahal, 1981; Kadian and Saharan, 1983). Reduction in the protein content has also been reported (Rai and Saxena, 1980).

Infection of *Alternaria brassicicola* in seed resulted in discoloration, shriveling and changes in seed contents. Histopathological studies revealed that the pathogen was extra as well as intra embroyal. Weakly discolored and symptom less seeds revealed the mycelial fragments in outer seed coat layers. Heavily discolored and shriveled discolored seeds showed spores and mycelium on surface and seed coat layers and mycelial fragments distributed in all the seed components and space in between them (Sharma *et al.* 1994).

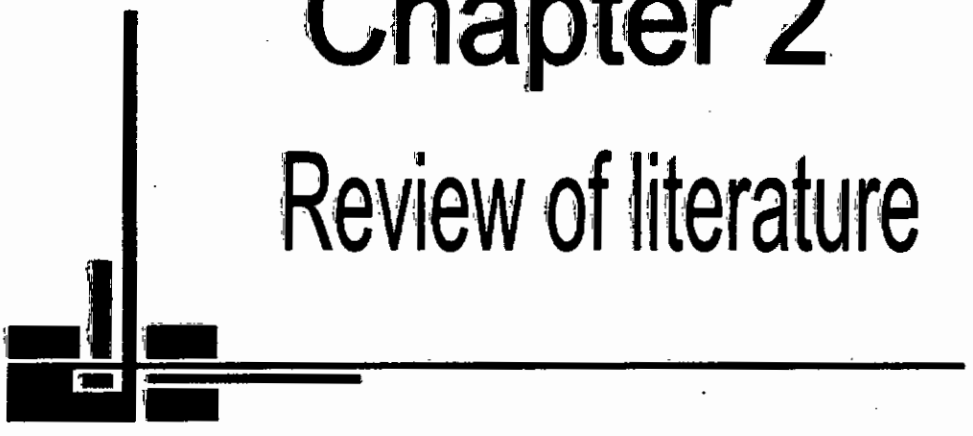
Chemicals are being successfully used in controlling the disease (Meah *et al.*, 1998). Non-chemical methods of disease control may include use of biological agents, botanicals, adjustment of cultural practices etc. Among these methods seed treatment is another important factor. Seeds can be treated with chemical fungicides to kill the spores that are present on the seed. This gives also some protection against spores present in the soil (soil-borne diseases) during the germination.

As only very small amounts of chemicals are used, this method causes little damage to the environment. Antagonistic fungi or bacteria can be used to protect seeds. An example of these biological agents is *Trichoderma* sp. (an antagonist fungus) (Lamey and McMullen 2000). An advantage of biological seed treatment is that they multiply in the soil. Therefore, even after germination, they provide protection of the root system against soil-borne pathogens

Thus the present study was undertaken to achieve the following objective

i) To evaluate comparative performance of physical seed sorting, provax-200, Brine solution, Rovral 50 wp and BAU-Biofungicide in controlling seed borne *Alternaria brassicae* and their subsequent transmission.

ii) To asses the performance of seed treatment on the quality of harvested seed.



Chapter 2

Review of literature

CHAPTER-II

REVIEW OF LITERATURE

Alternaria blight of mustard caused by *Alternaria brassicae* is a disease of world wide importance. It is a disease well studied and well investigated. Searching scientific information sources have provided large number of literature on the subject, yet some might have been left out. Presentation in a systematic position of all those literatures available has been done in this chapter.

Masum *et al.* (2009) conducted an experiment to study the effectiveness of mustard in controlling seed borne fungi associated with sorghum seeds obtained from two locations at the Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh. Five seed treatment practices used viz. hot water treatment, garlic tablet; neem leaf extract; BAU-Biofungicide and Vitavax-200 significantly reduced the total seed-borne fungal infections as well as the population of individual six target pathogenic fungi- *Agrostis tenuis*, *Bipolaris sorghicola*, *Botrytis cinerea*, *Crinum graminicola*, *Curvularia lunata*, *Fusarium moniliforme* and Vitavax-200 gave the best result in controlling seed-borne infection of all the individual target pathogenic fungi followed by garlic tablet, hot water treatment and neem leaf extract. Neem leaf extract gave over 90.0% reductions in seed-borne infection of *B. sorghicola* and *C. lunata* out of the six target pathogenic fungi. BAU-Biofungicide gave the lowest control of total seed-borne fungal infections (61.6 - 62.3%) as well as seed-borne infection of all the individual target pathogenic fungi (≤80.0%).

Panna *et al.* (2009) recorded that the highest seed germination (94.17) in apparently healthy seed treated with brine solution @ 2% for 15 minutes followed by apparently healthy seed solarized for 5 hours (99.33%), apparently

healthy seed polythene solarized for 5 hours (99.00%), apparently healthy seed treated with hot water at 52°C for 10 minutes (99.00%). Comparatively the lowest incidence of *Bipolaris sorokiniana* (1.50%) was observed in apparently healthy seeds treated with hot water at 52°C for 20 minutes followed by apparently healthy seeds polythene solarized for 15 hours (5.00%), apparently healthy seeds solarized for 15 hours (6.00%), apparently healthy seeds treated with brine solution @ 2% for 30 minutes (13.00%).

Sultana *et al.* (2009) conducted a field experiment at agricultural research station, Comilla during winter (November, 2008 to February, 2009) to identify the appropriate time of single application of Rovral in controlling *Alternaria* blight disease of mustard. Results revealed that single spray of Rovral @ 2 g / L of water at 30, 40, 50 and 60 days after sowing (DAS) control the disease severity of leaf blight compare to 70 DAS and control. These results indicate that disease severity, seed yield and yield contributing characters were significantly influenced by variety and single time of spray. Three time application of Rovral produced the lowest disease severity and produced highest seed yield.

Singh *et al.* (2008) evaluated four fungicides, namely iprodione (0.2%), mancozeb (o.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.

Yesmin *et al.* (2008) observed that pre-sowing treatment of apparently healthy seeds with hot water, brine or sun drying were noted as effective eco-friendly methods to reduce leaf blight severity and to increase grain yield and healthy seeds of wheat. Both unstored and apparently healthy seeds were treated with hot water at 50^0C for 5 minutes, brine solution (2% w/v NaCl) for 15 minutes, sun drying for 14 hours and polythene solarization for 14 hours. At all stages of data collection, the highest severity of leaf blight on flag leaf and penultimate leaf were recorded under control, where unstored and untreated seeds were planted followed by the treatment where apparently healthy seeds were planted without any treatment.

Islam *et al.* (2007) conducted an experiment to find out effective seed treated fungicides to control seed borne *Alternaria spp.* of radish crop. In the experiment seeds were treated with the six fungicides namely Rovral 50 WP (Iprodione) @ 0.20%, Dithane M-45 (Mancozeb) @ 0.45%, Ridomil MZ 68 WP (Metalaxil) @ 0.20%, Bavistin 70 WP (carbendazim) @ 0.10%, Tilt (Propiconazol) @ 0.20% and Cupravit (copperoxichloride) @ 0.70%. Their effectiveness was measured by planting the treated and untreated seeds on blotter in Petri dishes. All six fungicides significantly reduced seed borne infection of *Alternaria spp* and increased seed germination. Considering the results of the experiments, Rovral 50 WP (Iprodione) @ 0.20% was noted as the best fungicide against seed borne *Alternaria spp.*

Kabir *et al.* (2007) reported that physical and chemical seed treatments increased seed germination and suppressed the incidence of *Bipolaris sorokiniana* in the laboratory. The highest germination was counted 84.20% and 86.08% respectively at 10 and 15 DAS in the apparently healthy seed treated with Vitavax-200@ 0.4% and the lowest germination 49.84% and

55.54% at 10 and 15 DAS, respectively was recorded in the farmer's stored seed that served as control.

Bhuiyan *et al* (2006) conducted an experiment in Mymensing in novmber 2004 to evaluate the efficacy of BAU- Biofungicide for controlling seedling diseases of winter vegetables. BAU- Biofungicide was evaluated as a seed treating biocontrol means on 14 different winter vegetable crops viz. sweet gourd (*Cucurbita moschata*), snake gourd (*Trichosanthes anguina*), bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria vulgaris*), ridged gourd (*Luffa acutangula*), water melon (*Citrullus vulgaris*), tomato (*Lycopersicon esculentum*), brinjal (*Solanum melongena*), cabbage (*Brassica oleracea var. capitata*), radish (*Raphanus sativus*), amaranth (*Amaranthus gangeticus*), okra (*Hibiscus esculentus*), and spinach (*Spinacia oleracea*). BAU- Biofungicide treated seeds resulted up to 22% higher germination over the untreated control. Post emergence death of seedlings of all winter vegetables were reduced up to 20.2% due to treatment of seeds with BAU- Biofungicide. Seed treatment with BAU- Biofungicide increased vigour index of the seedlings of 14 different winter vegetables upto 57.21% over the untreated control.

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.

Shamsuzzaman *et al.* (2003) reported that BAU- Biofungicide increased germination of sweet gourd seeds up to 13% over the control (untreated). BAU- Biofungicide treated seed of sweet gourd resulted 98.62% higher vigour index over control (untreated). BAU- Biofungicide resulted vegetative proliferation of both root and shoot and thus increase vigour index of the seedlings.

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 liter 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. Spray of these 2 extracts at flowering stage suppressed disease incidence and increased 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, mancozeb, Ridomil MZ (mancozeb + metalaxyl), captan, Rovral (iprodione), Bayleten [triadimefon], and copper oxychloride, against Alternaria blight (*Alternaria brassicae*) and white rust (*Albugo candida*) of Indian mustard. All the fungicides significanlty controlled both the diseases, but their efficacy varied. Rovral was the most effective in controlling of Alternaria blight, mean disease

intensity of 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 (2.1t/ha), followed by Mancozeb and Ridomil MZ, each recording a yield of 1.9t/ha.

Ayub *et al.* (1996) conducted experiments to evaluate the efficacy of 7 fungicides of 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. Iprodione reduced disease severity the most 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 which were 40 days old.

Kumara (1996) investigated the effects of 4 fungicides on *A. brassicae*, *Albugo candida* and *Peronospora parasitica* infection of Indian mustard in field trails. 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 fungi species were annunciated with Indian mustard seeds in Haryana, India. These were: *Aspergillus niger; A flavus; Penicillium* sp; *Rhizopus* sp; *Cochliobolus lunatus; C sativus; Alternaria alternate* and *Mucor* sp.

Three varieties namely Tori-7 of *B. Campestris*, Daulat of *B. juncea* and Nap-3 of *B. Napus* were selected. The experiment was replicated 4 times in split plot design. Rovral 50 wp was sprayed at 10 days interval and continued till maturity of the pod. Scoring of disease was made on the basis of 0-8 scale. Results showed that unsprayed plot of Tori-7 scored highest disease severity while sprayed plot of Daulat and Nap -3 gave the lowest disease severity (1). Again sprayed plot of Nap -3 gave the highest (3.80g) 1000 grain wt. followed by Tori-7 (3.05 g) and Daulat (2.27g) Yield was the highest in sprayed plot of Daulat (1188.75 kg/ha) followed by Nap-3 (1172.51 kg/ha) and Tori-7 (615.16 kg/ha) while unsprayed plot of Daulat, Nap-3 and Tori-7 yielded 914.97, 982.52 and 434.78 kg/ha respectively. Depending on the yield of sprayed and unsprayed plots of Tori-7, Daluar and Nap-3 the yield loss were calculated as 29.32, 23.03 and 16.20% respectively. It may be concluded that yield reduction of 3 varieties on mustard due to leaf blight disease can be minimized by spraying Rovral 50 wp properly (BARI, 1994).

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 rice 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).

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% and 26.5% of healthy seed was found in three times Rovral spray. Therefore, three times spray of Rovral was found more effective of controlling seed-borne disease and increasing germination percentage of harvested seeds than the control. (BARI, 1992).

Meah and Hossain (1989) found that three times spraying of Rovral 50 WP (0.1%) sprayed three times from fruiting stage of mustard resulted production of disease free pods and significantly increased seed yield.

Meah and Hossain (1988) found that foliar spray of Rorval (0.2%) alone or in combination with Malathion (0.02%) proved to the most effective in controlling *Alternaria* blight of mustard. Dithane M-45 gave also good result. Four sprays of Rovral commencing at 50 days crop growth stage proved to be significanlty better than six sprays starting at 40- days' crop growth stage. Rovral increased seed yield per plot up to 217% over untreated control.

Mridha and Safa (1988) selected three seed samples having relatively high natural infection of *A. brassicae* and these were treated with three fungicides: Benlate; Granosan-M and Vitavax-200 to determine the efficacy of fungicides against the selected fungus. All the three fungicides, at both doses were effective to control the fungi and to increase germination. Treatment of seeds

with Benlate and Vitavax-200 increased germination by about 8 and 10% respectively but Granosan- M treatment reduced germination by 5.1 and 6.7 at lower and higher doses respectively as compared to germination of non -treated seeds.

Shivpuri *et al.* (1988) evaluated 6-fungicides viz. Rovral, Captafol, Dithane M-45, Thrium, Biltox-50, Bavistin were applied to Indian Mustard infected by *A. brassicae* in field trails in Rajasthan, India. All of the fungicides controlled the disease but Biltox-50 was phytotoxic. The best treatment was Rovral followed by Captafol and Mancozed; Rovral caused minimum defoliation.

Rai and saxena (1980) stated that, reduction in oil content due to fungal infection of mustard have been reported by various workers. Natural infection *of Alternaria brassicae* causes reduction of oil content varying from 14.58 to 35.97 percent in mustard seed and 14.12 to 29.07 percent in mustard. *Aspergillus flavus* caused a decrease in oil content by 21 percent in mustard seed.

Chapter 3

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

3.1. Experimental sites

Field experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka and lab experiment was conducted in the seed health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2. Experimental period

The experiment was carried out during the Rabi season from November 2008 to March 2009. Seeds were sown on 15 November 2008 and were harvested on 18 February 2009.

3.3. Soil type

The soil of the experimental plot was loam to clay loam in texture belonging to the Madhupur Tract (AEZ-28).

3.4. Climate

The climate of the experimental field area was of sub-tropical in nature characterized by high temperature associated with heavy rainfall during kharif season (April to September) and scanty rainfall with moderately low temperature during Rabi season (October to March).

3.5. Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity, monthly total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from the surface synoptic Data card, Bangladesh Meteorological Department.

3.6. Variety

Three varieties were used in the experiment as follows:

i) BARI- 6

ii) TORI- 7

iii) SAU- Shorisha

3.7. Treatments of the experiment

Five treatments were assessed in the experiment as follows:

T_1 – Provax-200

T_2 – Brine solution

T_3 – BAU-Biofungicide

T_4 – Rovral-50 WP

T_5 – Control

3.8. Design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) having three replication for each treatment. The experiment was laid out in Randomized complete Block Design (RCBD) with three replications. The whole plot was divided 45 blocks, each plot size was 1mX1m. The space was kept 1m between the blocks.

3.9 Land preparation

The land was ploughed with a power tiller in the first week of November 2008 and left exposed to sunlight for 7 days. Then the land was ploughed and cross ploughed by a country plough until the soil had a good tilth. It required different times ploughing and every ploughing was followed by laddering to level the land and break up clods. After each ploughing, weeds and rubbish were removed. Finally spade was used to prepare plots and drains.

3.10. Application of manure and fertilizers

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

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

3.11. Seed treatment

Seeds are treated with different seed treating chemicals such as provax-200, Brine solution, Rovral-50 WP and BAU-Biofungicide. Seeds were divided into four treatment groups. Among these four treatment groups of seeds were treated by using seed treating chemicals with dipping method separately in different Petri dishes. Different concentration was taken in different Petri dishes as per requirement. 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. The last group of seeds was treated with water as a control.

3.12 Seed sowing

The seeds were sown on 15th November 2008 in the field continuously in lines and were covered by soil with the help of hand.

3.13. Intercultural operations

Weeding was done when necessary followed split doze fertilizer application. After weeding and fertilizer application flood irrigation was given by filling the drains surrounding the beds by pumping water in those drains with a water

pump. After soaking the plots, excess water was allowed to be drained out. Malathion 57Ec was applied three times at 10 days intervals to control aphid.

3.14 Data collection

Data were collected on the following parameters

i) % Leaf area diseased (LAD)
ii) % siliqua infection 30 cm from the top.
iii) Number of spots per siliqua
iv) Weight of 100 siliqua (g)
v) Weight of straw per plot (g).
vi) Weight of 1000 seeds(g)
vii) Weight of seeds of 10 plants (g)
viii) Weight of seeds per plot (g)
ix) Weight of healthy seeds per plot (g)
x) Weight of diseased seeds per plot (g)
xi) % seed infection (Blotter)

3.15. Procedure of data collection

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

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

Data on percent leaf Area diseased were recorded 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 by visual observation of symptoms. Percent pod infection was calculated by the following formula.

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

3.16. Isolation and identification of the causal organisms from *Alternaria* blight infected leaves

To identify the pathogen, the diseased leaves were collected from the infected plant and taken to the laboratory. The diseased leaves were cut into small pieces (about 0.5- 1cm) and surface sterilized by dipping in 10% sodium Hypochlorite solution for 2-3 minutes or $HgCl_2$ solution (0.01%) for 30 second. The cut pieces were than washed in sterilized water at the three times and were placed into PDA media in sterilized Petri dish with the help of sterile forceps and incubated at 25±1 for 7-10 days. Then the organisms that grow freshly into the culture were isolated by means of hypal tip culture method aseptically and were cultured again in a PDA media to have pure culture is to found blackish. Then a slide was prepared from the culture and observed under compound microscope and the fungus was identified as *Alternaria brassicae.*

3.17 Grading of infected mustard seed

Meah (1994) used 0-5 scale for scoring *Alternaria* blight disease of mustard. The scale was described as:

0= leaves free from leaf spot

1= 0.1 to 6% leaf or pod area diseased

2= 6.1 to 12% leaf or pod area diseased

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 by visual observation of symptoms. Percent pod infection was calculated by the following formula.

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

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1= 0.1 to 6% leaf or pod area diseased

2= 6.1 to 12% leaf or pod area diseased

3= 12.1 to 25% leaf or pod area diseased

4= 25.1 to 50% leaf or pod area diseased and

5= above 50% leaf or pod area diseased

3.18. Germination and seed health test

Germination test of BARI-6, Tori-7 and SAU-sharisha seeds received from treated plot with different treatments was conducted in glass Petri dishes. Four hundred seeds were randomly collected from each treatment. Seed were placed on three layers of moist blotting paper (whatman No. 1) contained in plastic petrideshes. 25 seeds were placed in each petridishes and incubated at 25+1^{0}C under 12 hrs cycle of alternate Near Ultra violet (NUV) light and darkness. The experiment was laid out in CRD. Watering was done as and when required. Germination of seedling and seed infection by *Alternaria spp* was recorded. Results were expressed as percent seed germination.

Each seed was observed under stereo binocular microscope in order to record the presence of fungal colony 7 days after incubation. Temporary slides were prepared from the fungal colony and observed under compound microscope.

3.19 Detection of fungi:

3.19.1 Collection of seed sample

Seeds were collected from field of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka.

3.19.2 Blotter method

Blotter method test was done in Petri dishes following the International Rules of ISTA (2001). Two hundred seeds were taken randomly from each sample and were placed in Petri dishes (25 seeds per Petri dish according to the size of the seeds). Before plating seeds, three pieces of Whateman no. 1 filter paper dipped in sterile water and then placed on each dish. The Petri dishes with seeds were then incubated in the incubator at 22 ± 2°c under an alternate cycle of 12 hours light and darkness 7 days. Seeds were examined after 7 days of

incubation to detect the fungi. The fungi were identified by observing their growth characters on the incubated seeds in blotter method under stereomicroscope at 25 X magnification following the keys of Mathur and Cunfer (1993) and Mathur and Kongsdal (2003).

3.19.3 Agar plate technique

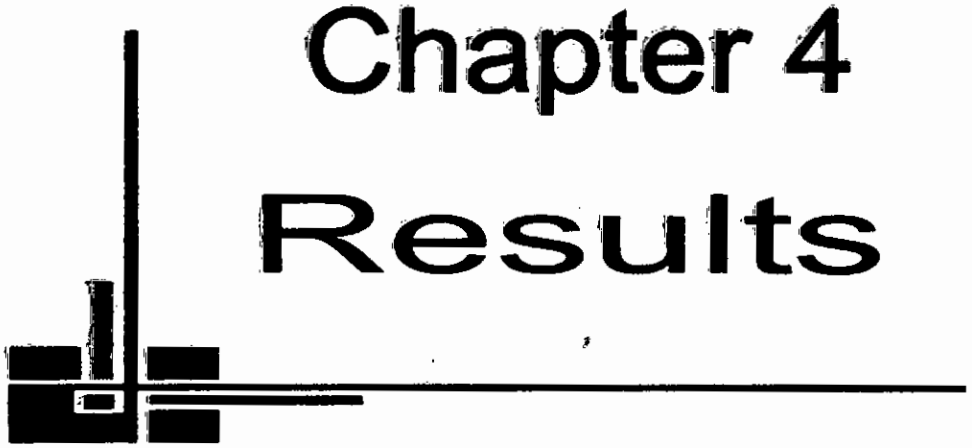
In the agar plate method surface–disinfected (0.05% $HgCl_2$ for 30 sec.) seeds was plated on an agar medium and the plated seeds was usually incubated for 5–7 days at 22–25°c under 12h alternating cycles of light and darkness. At the end of incubation period, fungi growing out from seeds on the agar medium was examined and identified. In this method, Potato Dextrose Agar (PDA) (Potato 200g , Agar 15g and H_2O–1000ml) having pH 6.5 was used. About 15ml of the media were poured in each sterilized glass Petri dishes

3.19.4 Seedling Symptom Test

In the seedling symptom test, 1% water agar was prepared in flasks. Now the water agar was poured in each test tube and the tubes were transferred into containers which can be autoclaved and the tubes were covered with aluminium foil. The containers were placed in autoclave at 121°c and 15lbs pressure per square inch for 15 minutes. After solidification of the agar medium, cotton plug was removed temporarily and one seed was dropped in each tube. It was incubated tubes at 20°c for 14 days under 12 hours alternating cycles of artificial daylight and darkness and was inspected all tubes and separated them into 3 groups;1) healthy-looking seedling, (2) Seedling showing symptoms and (3) Seeds that have not germinated.

3.20 Analysis of data

The data on various parameters were analyzed using analysis of variance to find out variation obtained from different treatments. Mean differences among the treatments were compared by Duncan's Multiple Range Test (DMRT).



Chapter 4

Results

CHAPTER-IV

RESULTS

3.1. Effect of variety and treatment on *Alternaria* blight of mustard

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against leaf infection of mustard caused by *Alternaria* blight and their significant effects were observed (Table 1 to Table 3).

Varietal effect

There is no significant effect of different mustard varieties on the percent leaf area diseased (LAD) for different date after sowing (DAS) of mustard seeds (Table 1). But considering the numerical values, percent LAD was ranged from 4.82-5.15% for 65 DAS, 10.61 to 11.71% for 75 DAS and 13.81-14.15% for 85 DAS, where the lowest percent LAD was recoded in BARI Sarisha-6 and the highest percent LAD in Tori-7.

Table 1. Varietal effect on the leaf area diseased during the management of *Alternaria* blight of Mustard

Mustard Variety		Percent Leaf Area Diseased (LAD)		
		65 DAS	75 DAS	85 DAS
V_1	BARI Sarisha-6	4.82	10.61	13.81
V_2	Tori-7	5.15	11.71	14.15
V_3	SAU Sarisha-1	5.10	11.15	13.92
$LSD_{(0.01)}$		NS	NS	NS
CV(%)		6.67	7.02	7.21

NS= Non significant

Treatment effect

The effects of different seed treating chemicals and BAU-biofungicide were significant on the percent LAD for different data recording times (Table 2). In case of 65 DAS, the percent LAD were ranged from 1.66-8.31%, where the

highest percent LAD was recorded for untreated control, which was statistically different from all other treatements followed by Brine solution (6.43%) and Provax 200 (5.88%). On the other hand the lowest percent LAD (1.66%) was recorded for Rovral 50 WP followed by BAU-biofungicide (2.85%).

Similarly at 75 DAS and 85 DAS, the percent LAD were ranged from 9.34-12.46% and 11.34-17.78%, respectively, where the highest percent LAD was recorded for untreated control, which was statistically similar with all other treatments except BAU-biofungicide for 75 DAS and statistically different form other treatments. And the lowest percent LAD was recorded for BAU-biofungicide.

Table 2. Effect of different seed treating chemicals including BAU-biofungicide on the leaf area diseased during the management of *Alternaria* blight of mustard

Treatments		Percent Leaf Area Diseased (LAD)		
		65 DAS	75 DAS	85 DAS
T_1	Provax-200	5.88 b	11.29 a	13.87 b
T_2	Brine solution	6.43 b	11.48 a	13.99 b
T_3	BAU- Biofungicide	2.85 c	9.34 b	11.34 c
T_4	Rovral-50WP	1.66 d	11.21 a	12.81 bc
T_5	Control	8.31 a	12.46 a	17.78 a
$LSD_{(0.01)}$		0.7584	1.766	2.270
CV (%)		6.67	7.02	7.21

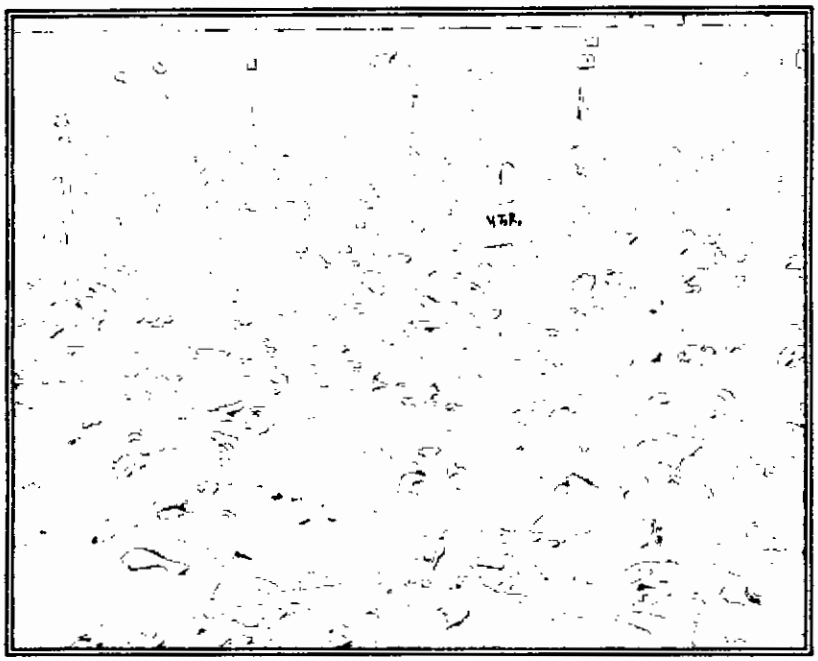
Interaction effect

Significant variations among the effects of four management practices and one untreated control treatments were observed on the percent leaf area diseased of three mustard varieties at three data recording times. (Table 3). The percent leaf area diseased ranged from 1.13 to 9.26% at 65 DAS, 7.4 to 14.00% at 75 DAS and 85 DAS, where the lowest value was recorded for Rovral-50 WP at 65 DAS, and for BAU-Biofungicide at 75 DAS and 85 DAS. On the other

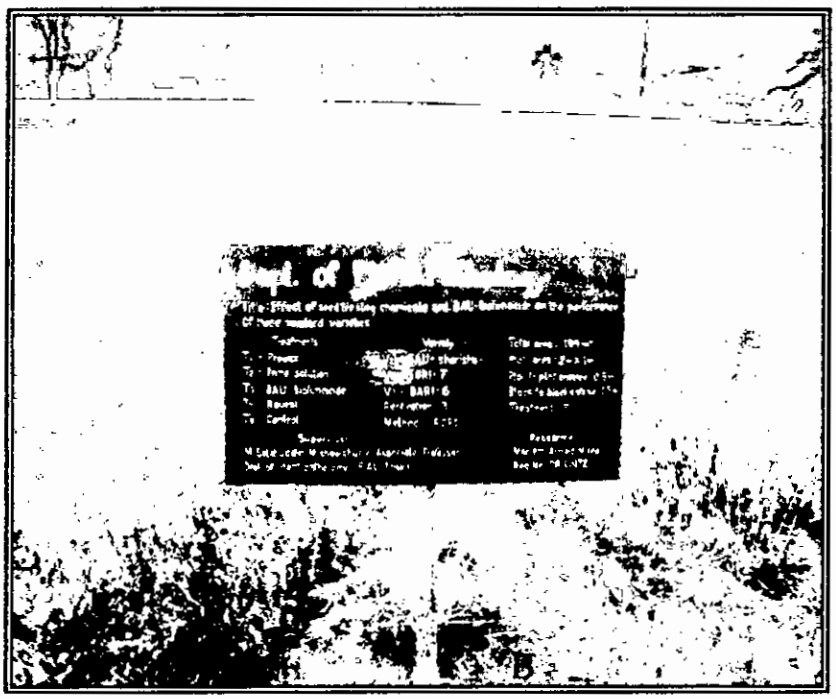
hand, the highest value was recorded for untreated control treatment at all data recording times.

Table 3. Effect of different treatments on percent leaf area diseased of mustard at different days after sowing (DAS)

Variety	Treatments	Percent Leaf Area Diseased (LAD)		
		65 DAS	75 DAS	85 DAS
BARI Sarisha-6	Provax-200	6.00 d	11.33 b	11.33 b
	Brine solution	6.19 d	11.13 b	11.13 b
	BAU- Biofungicide	2.93 ef	7.400 c	10.93 b
	Rovral-50WP	1.63 gh	10.93 b	7.40 c
	Control	7.39 c	12.23 ab	12.23 ab
Tori-7	Provax-200	6.16 d	10.93 b	10.93 b
	Brine solution	5.94 d	12.07 b	12.07 b
	BAU- Biofungicide	3.27 e	10.30 b	10.30 b
	Rovral-50WP	1.13 h	11.23 b	11.23 b
	Control	9.26 a	14.00 a	14.00 a
SAU Sarisha-1	Provax-200	5.48 d	11.60 b	11.60 b
	Brine solution	7.16 c	11.23 b	11.23 b
	BAU- Biofungicide	2.36 fg	10.34 b	10.34 b
	Rovral-50WP	2.22 fg	11.47 b	11.47 b
	Control	8.28 b	11.13 b	18.30 b
$LSD_{(0.01)}$		0.7584	1.766	2.270
CV (%)		6.67	7.02	7.21



A



B

Fig. 1 A view of the experimental field
A) Seedling stage B) mature stage

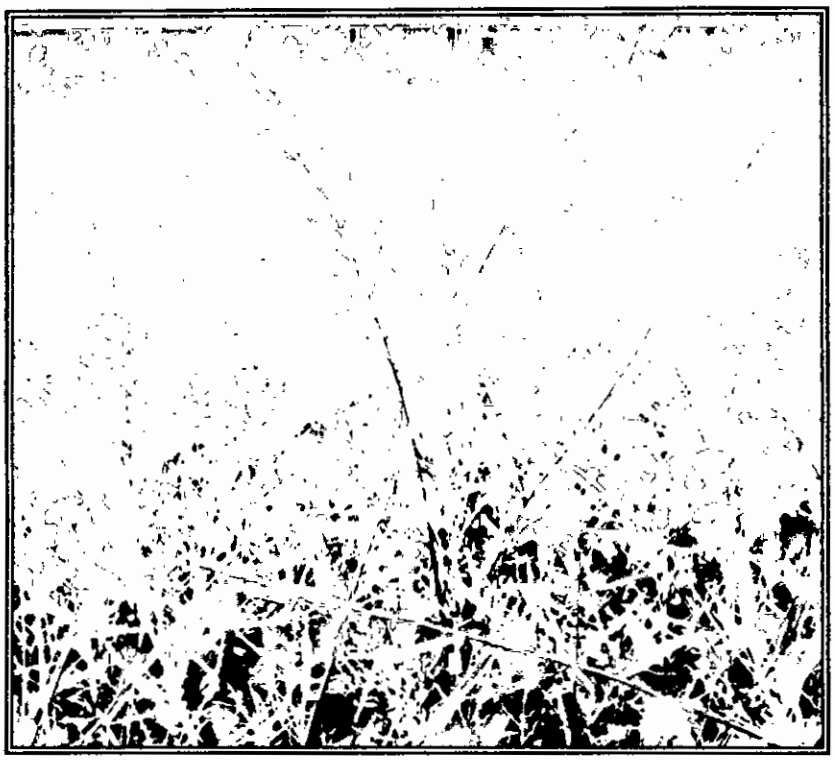


Fig.2 Flowering stage of BARI-6 (Rovral 50WP treated plot)

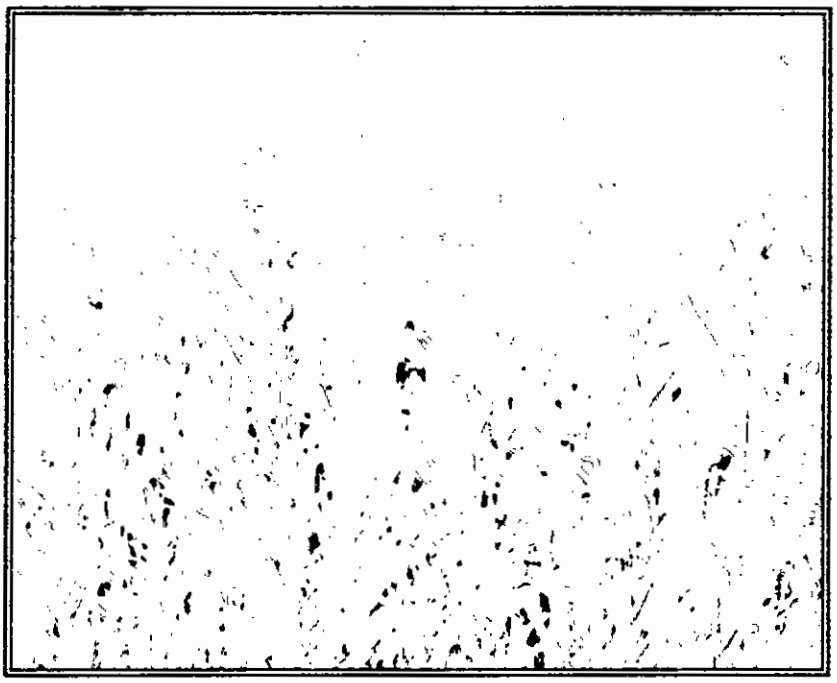


Fig.3 Healthy pod of SAU-Sharisha (Rovral 50 WP treated plot)

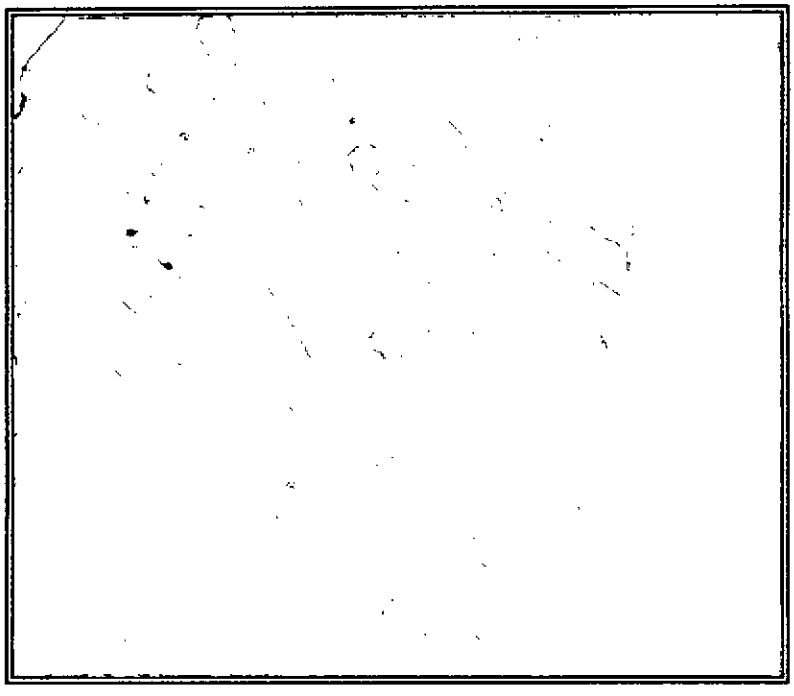


Fig.4 Infected leaf of Mustard

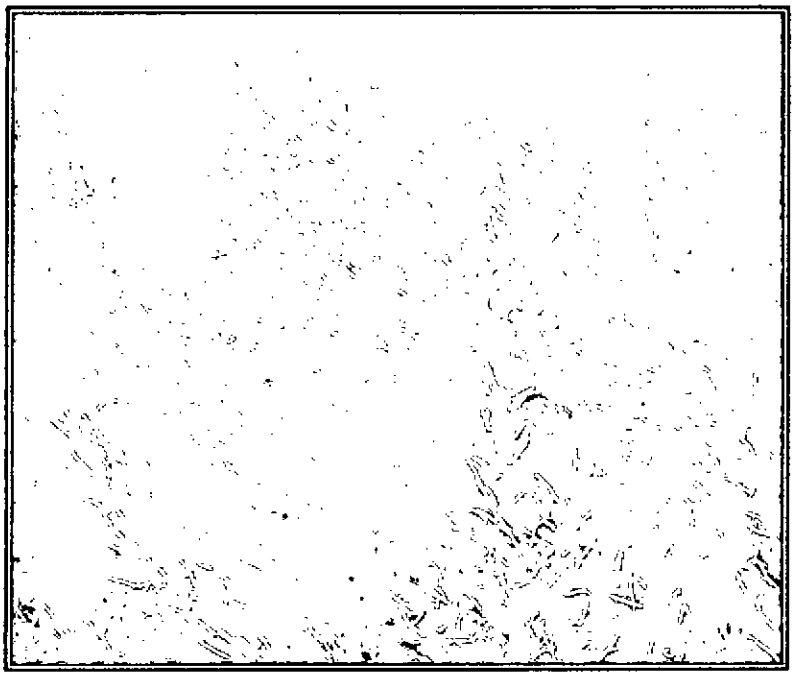


Fig.5 Maturing stage of Tori-7

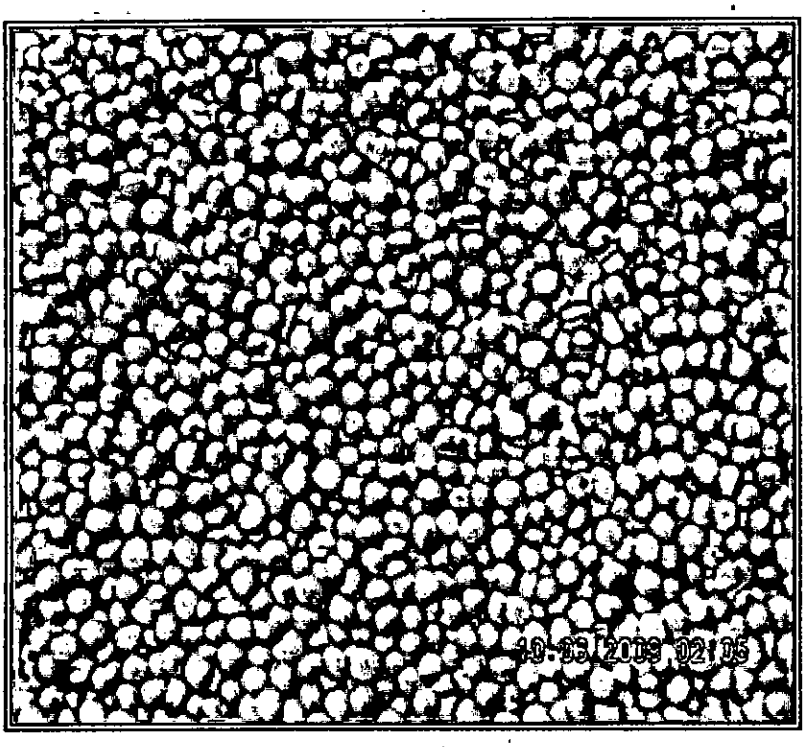


Fig.6 Healthy seed of mustard (BARI-6)

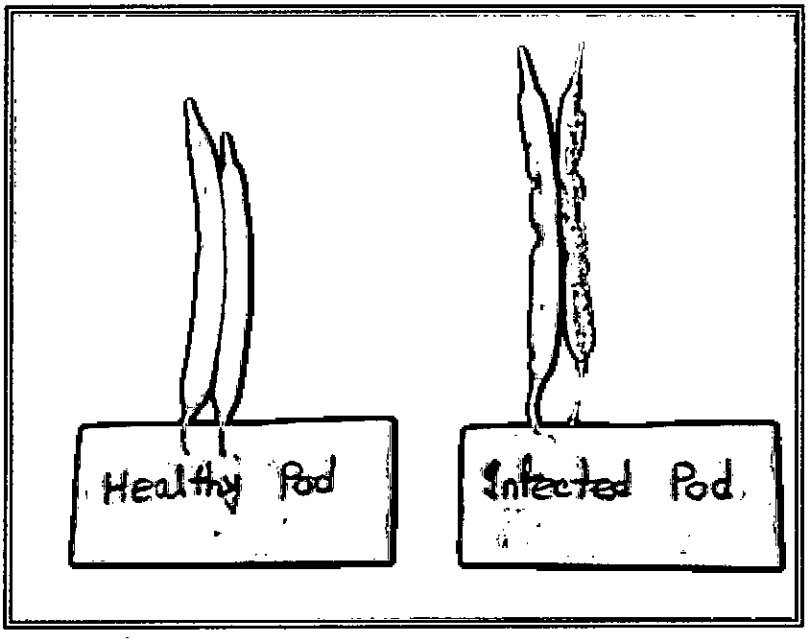


Fig 7. Healthy and infected pod of SAU-Sharisha

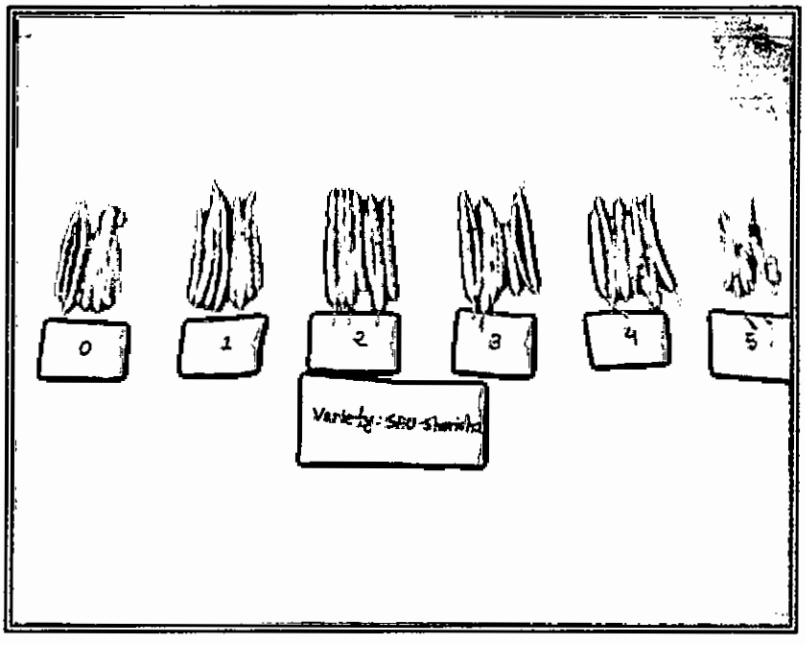


Fig.8 Grading of pod using 0-5 scale (SAU- Sharisha)

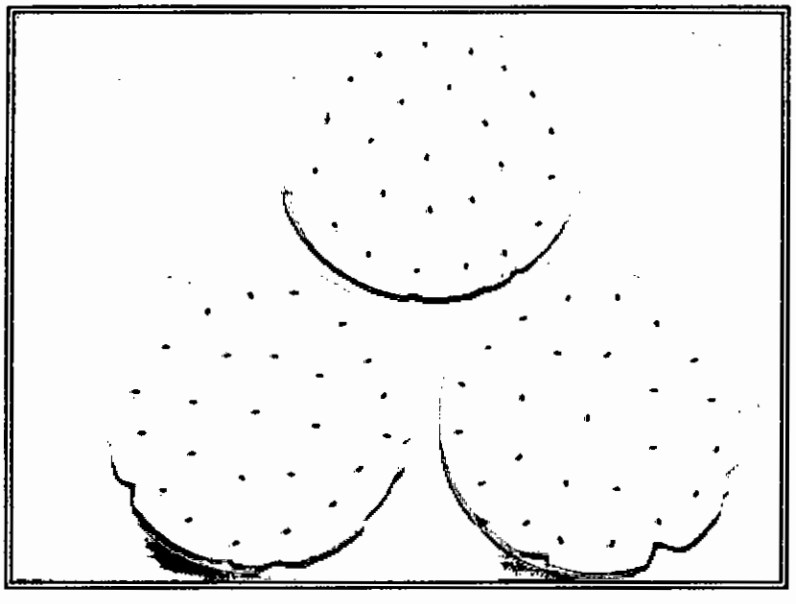


Fig.9 Seed is plated on filter paper

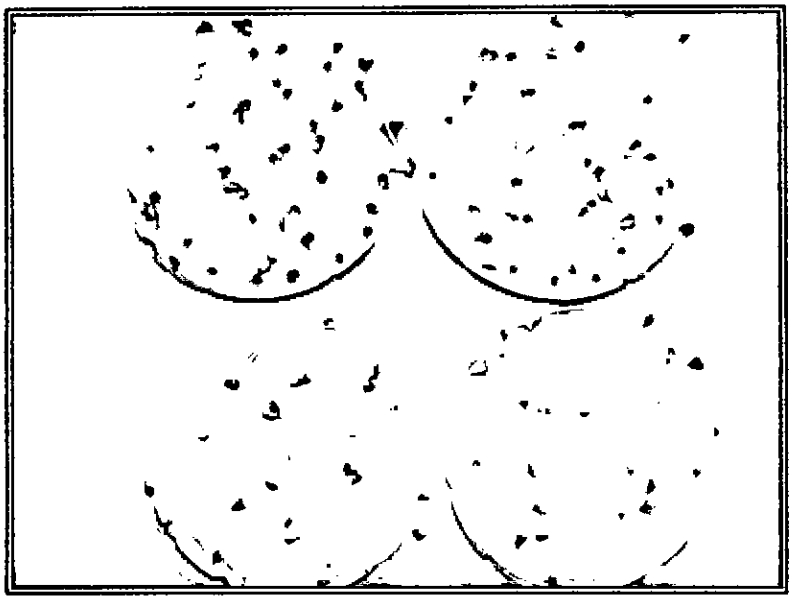


Fig.10 Seed is germinated on filter paper

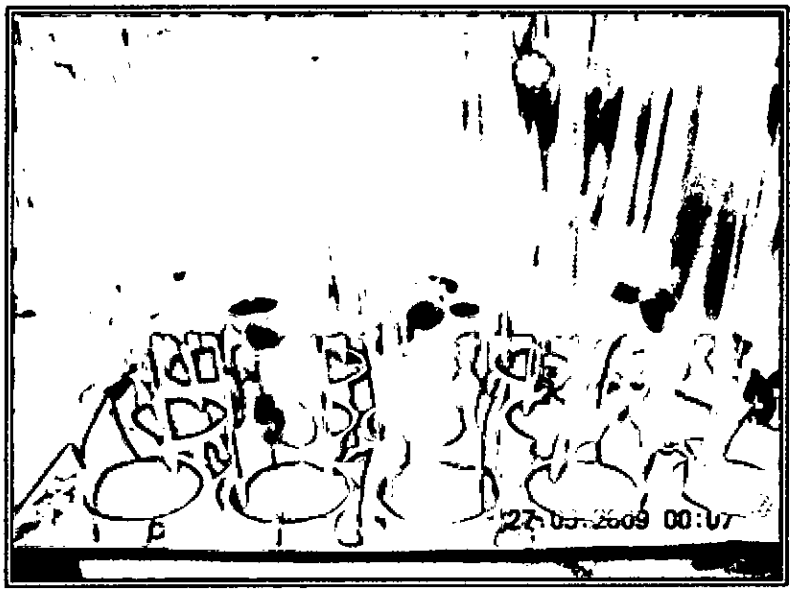


Fig. 11 Seedling is growing on test tube.

Fig.12 Growth of *Alternaria* spp. on incubated mustard seed under stereomicroscope (X 40)

Fig. 13 Conidia of *Alternaria* spp. (x 100)

Fig. 14 Growth of *Aspergillus flavus* on incubated mustard seed seen under stereomicroscope (X 40)

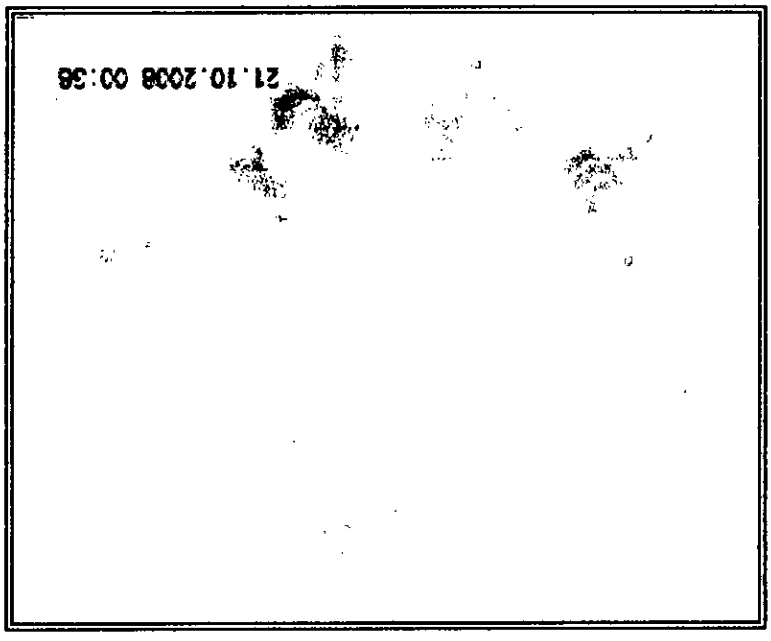


Fig. 15 Growth of *Aspergillus niger* on incubated mustard seed seen under stereomicroscope (X 40)

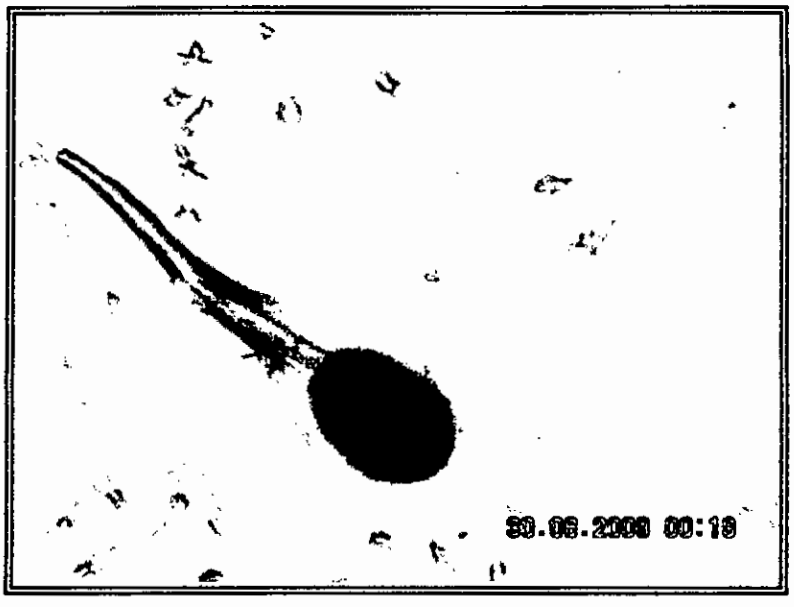


Fig. 16 Conidia and conidiophore of *Aspergillus niger* (x 100)

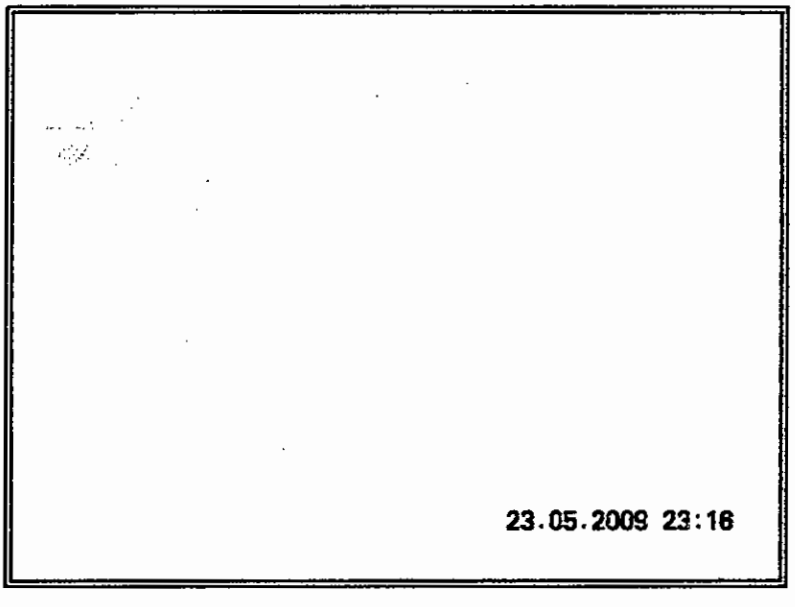


Fig.17 Growth of *Fusarium* spp. on incubated mustard seed seen under stereomicroscope (X 40)

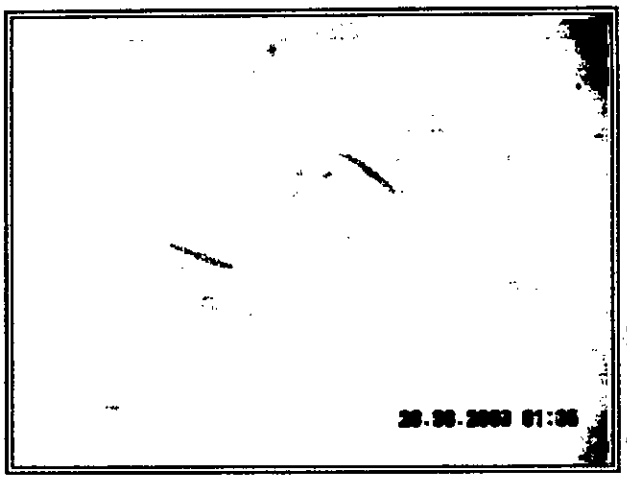


Fig.18 Conidia of *Fusarium* spp. (x 100)

Fig.19 Pure culture of. *Fusarium spp.*

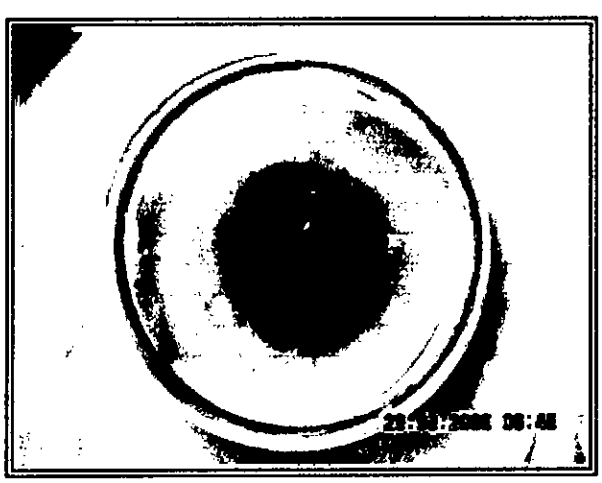


Fig.20 Pure culture of *Alternaria spp.*

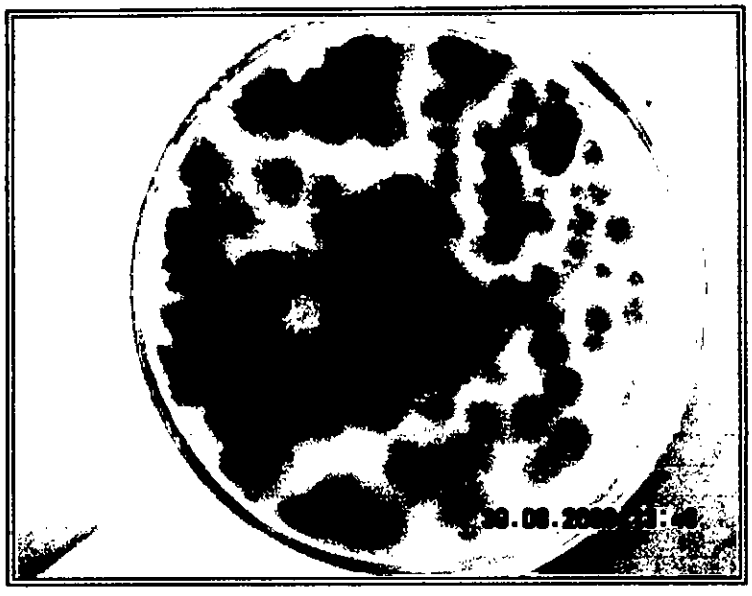


Fig.21 Pure culture of *Aspergillus flavus*

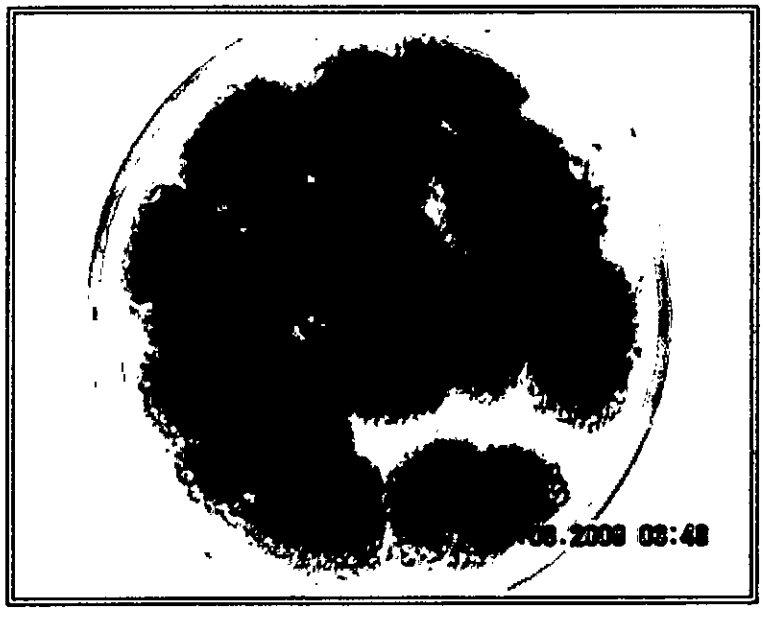


Fig.22 Pure culture of *Aspergillus niger*

3.2. Effect of variety and treatment on number of spot on leaf and siliqua during the management of *Alternaria* blight of Mustard

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against spot on leaf and spot on siliqua of mustard caused by *Alternaria* blight and their significant effects were observed (Table 4 to Table 6).

Varietal effect

There is no significant effect of different mustard varieties on the number of spot per leaf for different date after sowing (DAS) of mustard (Table 4). But considering the numerical values, spot per leaf was ranged from 0.70-1.06 for 60 DAS and 1.19-1.33 for 80 DAS where the lowest spot per leaf was recoded in BARI Sarisha-6 and the highest spot per leaf was recoded in SAU Sarisha-1. In case of spot per siliqua there is no significant effect for different date after sowing (DAS) of mustard seeds (Table 4). But considering the numerical values, spot per siliqua was ranged from 4.35-7.14 for 60 DAS and 1.19-1.33 for 80 DAS where the lowest number of spot per leaf was recoded in BARI Sarisha-6 and the highest percent LAD was recoded in SAU Sarisha-1.

Table 4. Varietal effect on the spot of siliqua during the management of *Alternaria* blight of Mustard

Mustard Variety		Spot per leaf (No.)		Spot per siliqua (No.)	
		60 DAS	80DAS	65 DAS	85DAS
V_1	BARI Sarisha-6	1.06	1.19	4.35	6.40
V_2	Tori-7	0.78	1.31	6.02	8.64
V_3	SAU Sarisha-1	0.70	1.33	7.14	9.29
$LSD_{(0.01)}$		NS	NS	NS	NS
CV (%)		50.60	22.30	29.75	21.78

NS= Non Significant

3.2. Effect of variety and treatment on number of spot on leaf and siliqua during the management of *Alternaria* blight of Mustard

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V_2	Tori-7	0.78	1.31	6.02	8.64
V_3	SAU Sarisha-1	0.70	1.33	7.14	9.29
$LSD_{(0.01)}$		NS	NS	NS	NS
CV(%)		50.60	22.30	29.75	21.78

NS= Non Significant

Treatment effect

The significant effects of different seed treating chemicals and BAU-biofungicide were observed on the spot per leaf and spot per siliqua for different data recording times (Table 5). In case of 60 DAS, the spot per leaf were ranged from 0.68-1.35, where the highest spot per leaf was recorded for untreated control, which was statistically different from all other treatements followed by Brine solution (0.95) and Provax -200 (1.00). On the other hand the lowest spot per leaf was recorded for Rovral 50 WP (0.26) followed by BAU-biofungicide (0.68).

In case of 80 DAS, numbers of spots per leaf were ranged from 1.79-0.67 where the highest spot per leaf was recorded for untreated control, which was statistically different from all other treatements followed by Brine solution (0.95) and Provax-200 (1.00). On the other hand the lowest number of spot per leaf was recorded for BAU-biofungicide (0.67) followed by Rovral 50 WP (1.07).

Similarly at 65 DAS , number of spot per siliqua were ranged from 2.79-9.06 where the highest spot per siliqua was recorded for untreated control, which was statistically different with all other treatments followed by Brine solution (6.75) and Provax -200 (6.18). On the other hand the lowest spot per leaf was recorded for (0.67) Rovral 50 WP (2.79) followed by BAU-biofungicide (4.41)

Similarly at 85 DAS , the spot per siliqua were ranged from 4.53-13.80 where the highest spot per siliqua was recorded for untreated control, which was statistically different with all other treatments followed by Brine solution (8.41) and Provax -200 (7.51). On the other hand the lowest number of spots per leaf (4.53) was recorded for Rovral 50 WP followed by BAU-biofungicide (6.30).

Treatment effect

The significant effects of different seed treating chemicals and BAU-biofungicide were observed on the spot per leaf and spot per siliqua for different data recording times (Table 5). In case of 60 DAS, the spot per leaf were ranged from 0.68-1.35, where the highest spot per leaf was recorded for untreated control, which was statistically different from all other treatements followed by Brine solution (0.95) and Provax -200 (1.00). On the other hand the lowest spot per leaf was recorded for Rovral 50 WP (0.26) followed by BAU-biofungicide (0.68).

In case of 80 DAS, numbers of spots per leaf were ranged from 1.79-0.67 where the highest spot per leaf was recorded for untreated control which was statistically different from all other treatements followed by Brine solution (0.95) and Provax-200 (1.00). On the other hand the lowest number of spot per leaf was recorded for BAU-biofungicide (0.67) followed by Rovral 50 WP (1.07).

Similarly at 65 DAS, number of spot per siliqua were ranged from 2.79-9.06 where the highest spot per siliqua was recorded for untreated control, which was statistically different with all other treatments followed by Brine solution (6.75) and Provax -200 (6.18). On the other hand the lowest spot per leaf was recorded for (0.67) Rovral 50 WP (2.79) followed by BAU-biofungicide (4.41).

Similarly at 85 DAS, the spot per siliqua were ranged from 4.53-13.80 where the highest spot per siliqua was recorded for untreated control, which was statistically different with all other treatments followed by Brine solution (6.41) and Provax -200 (7.61). On the other hand the lowest number of spots per leaf (4.53) was recorded for Rovral 50 WP followed by BAU-biofungicide (6.30).

Table 5. Effect of different seed treating chemicals including BAU-biofungicide on the number of spot per leaf and siliqua during the management of *Alternaria* blight of Mustard

Treatments		Number of spot per leaf (No.)		Number of spot per siliqua	
		60 DAS	80 DAS	65 DAS	85 DAS
T_1	Provax	1.00 ab	1.30 abc	6.18 ab	7.51 b
T_2	Brine solution	0.95 ab	1.54 ab	6.75 ab	8.41 b
T_3	BAU-Biofungicide	0.68 ab	0.67 c	4.41 b	6.30 b
T_4	Rovral 50 wp	0.26 b	1.07 bc	2.79 b	4.53 b
T_5	Control	1.356 a	1.79 a	9.06 a	13.80 a
$LSD_{(0.01)}$		0.9730	0.6421	3.922	3.987
CV (%)		50.60	22.30	29.75	21.78

Interaction effect

Significant variations among the effects of four management practices and one untreated control treatments were observed on the number of spots per leaf and sliqua by number of three mustard varieties at different data recording times (Table 6). The number of spot per leaf was ranged from 0.33 to 1.4 at 60 DAS, and 0.63 to 1.86 at 80 DAS, where the minimum number of spot per leaf was recorded for Rovral-50 WP at 60 DAS, and for BAU-Biofungicide at 80 DAS. On the other hand, the maximum number of spot per leaf was recorded for untreated control treatment at all data recording times.

In case of number spot per siliqua, more or less similar results were also observed for all treatments at all data recording times among three mustard varieties. That is the minimum number (1.13) of spot per leaf was recorded for Rovral-50 WP at 65 DAS, (18.27 to 11.23) at 85 DAS. On the other hand, the maximum number of spot per leaf was recorded for untreated control treatment at all data recording times.Considering the mustard varieties, no significant effects were observed on the number of spot per leaf and number of spot per siliqua of three varieties.

Table 6. Effect of different treatments on number of spot per leaf and siliqua of three mustard varieties at different days after sowing (DAS)

Variety	Treatments	Number of spots per leaf		Number of spots per siliqua	
		60 DAS	80DAS	65 DAS	85DAS
BARI Sarisha-6	Provax-200	1.40 a	1.13 abcd	6.00 d	15.57 bc
	Brine solution	0.93 ab	1.36 abcd	6.19 d	13.77 cde
	BAU-Biofungicide	1.26 ab	0.73 cd	2.93 ef	12.73 de
	Rovral-50WP	0.33 ab	1.03 bcd	1.63 gh	11.23 e
	Control	1.40 a	1.68 ab	7.39 c	17.43 ab
Tori-7	Provax-200	1.06 ab	1.36 abcd	6.16 d	12.57 de
	Brine solution	1.00 ab	1.60 ab	5.94 d	14.40cd
	BAU-Biofungicide	0.33 ab	0.66 cd	3.27 e	11.53 e
	Rovral-50WP	0.26 ab	1.10 bcd	1.13 h	13.47 cde
	Control	1.26 ab	1.86 a	9.26 a	17.63 ab
SAU Sarisha-1	Provax-200	0.53 ab	1.40 abc	5.48 d	13.47cde
	Brine solution	0.93 ab	1.66 ab	7.16 c	13.80 cde
	BAU-Biofungicide	0.46 ab	0.63 d	2.36 fg	11.27e
	Rovral-50WP	0.20 b	1.10 bcd	2.22 fg	12.23 de
	Control	1.40 a	1.85 a	8.28 b	18.27 a
$LSD_{(0.01)}$		0.9730	0.6421	0.7584	2.270
CV(%)		50.60	22.30	6.67	7.21

3.3. Effect of variety and treatment on siliqua infection of mustard caused by *Alternaria brassicae*

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against siliqua infection by number and percent disease index of mustard caused by *Alternaria* blight and their significant effects were observed (Table 7 to Table 9).

Varietal effect

There is significant effect of different mustard varieties on the percent siliqua infection for different date after sowing (DAS) of mustard seeds (Table 7). The percent siliqua infection was ranged from 36.29-62.93% where the lowest percent of siliqua infection was recoded in Tori-7 and the highest percent of siliqua infection was recoded in BARI Sarisha-6.

There is no significant effect of different mustard varieties on the percent Disease Index (PDI) for different treatment of mustard seeds (Table 7). But considering the numerical values, percent Disease Index (PDI) was ranged from 38.21-47.35, where the lowest percent PDI was recoded in BARI Sarisha-6 and the highest percent PDI was recorded in Tori-7.

Table 7. Varietal effect on the disease incidence on siliqua during the management of *Alternaria* blight of Mustard

Mustard Variety		Disease incidence on Siliqua	
		% Siliqua infection by number	% Disease Index (PDI)
V_1	BARI Sarisha-6	62.93 a	38.21 a
V_2	Tori-7	36.29 b	47.35 a
V_3	SAU Sarisha-1	42.19 b	44.15 a
$LSD_{(0.01)}$		15.06	11.17
CV (%)		14.16	11.45

Treatment effect

The significant effects of different seed treating chemicals and BAU-biofungicide were observed on the percent siliqua infection for different treatment (Table 8). The percent siliqua infection were ranged from 35.38-62.11, where the highest percent siliqua infection was recorded for untreated

control, which was statistically different from all other treatements followed by Brine solution (43.76%) and Provax-200 (53.82%). On the other hand the lowest percent siliqua infection was recorded for Rovral 50 WP (35.38%) followed by BAU-biofungicide (40.61%).

Similarly the percent disease index (PDI) was ranged from 27.12-67.76% and where the highest percent disease index (PDI) was recorded for untreated control, which was statistically different with all other treatments. The lowest percent disease index (PDI) was recorded for Rovral 50 WP (27.12%) followed by BAU-biofungicide (31.64%).

Table 8. Effect of different seed treating chemicals including BAU-biofungicide on the disease incidence on siliqua during the management of *Alternaria* blight of Mustard

Treatments		Disease incidence on Siliqua	
		% Siliqua infection by number	% Disease Index (PDI)
T_1	Provax	53.82 ab	41.79 bc
T_2	Brine solution	43.76 bc	47.88 b
T_3	BAU-Biofungicide	40.61 bc	31.64 cd
T_4	Rovral 50 wp	35.38 c	27.12 d
T_5	Control	62.11 a	67.76 a
$LSD_{(0.01)}$		15.06	11.17
CV (%)		14.16	11.45

Interaction effect

Significant variations among the effects of different treatments were observed on the percent siliqua infection and percent disease index (PDI) by number among three mustard varieties (Table 9). The percent siliqua infection was ranged from 36.30 to 93.50%, where the lowest siliqua infection was recorded in Provax-200 for all varieties and the highest percent was recorded in untreated control. In case of percent disease index, the PDI was ranged from

control, which was statistically different from all other treatements followed by Brine solution (43.76%) and Provax-200 (53.82%). On the other hand the lowest percent siliqua infection was recorded for Rovral 50 WP (35.38%) followed by BAU-biofungicide (40.61%).

Similarly the percent disease index (PDI) was ranged from 27.12-67.76% and where the highest percent disease index (PDI) was recorded for untreated control, which was statistically different with all other treatments. The lowest percent disease index (PDI) was recorded for Rovral 50 WP (27.12%) followed by BAU-biofungicide (31.64%).

Table 8. Effect of different seed treating chemicals including BAU-biofungicide on the disease incidence on siliqua during the management of *Alternaria* blight of Mustard

Treatments		Disease incidence on Siliqua	
		% Siliqua infection by number	% Disease Index (PDI)
T_1	Provax	53.82 ab	41.79 bc
T_2	Brine solution	43.76 bc	47.88 b
T_3	BAU-Biofungicide	40.61 bc	31.64 cd
T_4	Rovral 50 wp	35.38 c	27.12 d
T_5	Control	62.11 a	67.76 a
$LSD_{(0.01)}$		15.06	11.17
CV (%)		14.16	11.45

Interaction effect

Significant variations among the effects of different treatments were observed on the percent siliqua infection and percent disease index (PDI) by number among three mustard varieties (Table 9). The percent siliqua infection was ranged from 36.30 to 93.50%, where the lowest siliqua infection was recorded in Provax-200 for all varieties and the highest percent was recorded in untreated control. In case of percent disease index, the PDI was ranged from

to 71.27%, where the lowest PDI was recorded in Rovral-50 WP for all varieties and the highest PDI was recorded in untreated control.

Considering the mustard varieties, lower siliqua infection and PDI were observed for SAU Sarisha-1 followed by BARI Sarisha-6 and Tori-7 varieties.

Table 9. Effect of different seed treating chemicals on number of spot/ leaf and percent diseases index (PDI) of 3 mustard varieties

Variety	Treatments	Disease incidence on Siliqua	
		% Siliqua infection by number	% Disease Index (PDI)
BARI Sarisha-6	Provax-200	36.30 d-f	32.83 e-h
	Brine solution	51.10 cd	39.80 d-f
	BAU-Biofungicide	57.33 c	25.83 gh
	Rovral-50WP	76.40 b	21.30 h
	Control	93.50 a	71.27 a
Tori-7	Provax-200	33.30 ef	51.43 b-d
	Brine solution	26.97 f	51.73 b-d
	BAU-Biofungicide	36.80 d-f	32.03 e-h
	Rovral-50WP	41.40 c-f	27.60 f-h
	Control	43.00 c-f	73.97 a
SAU Sarisha-1	Provax-200	36.53 d-f	41.10 c-e
	Brine solution	43.77 c-f	52.10 b-c
	BAU-Biofungicide	37.13 d-f	37.07 e-g
	Rovral-50WP	43.67 c-f	32.47 e-h
	Control	49.83 c-e	58.03 b
$LSD_{(0.01)}$		15.06	11.17
CV (%)		14.16	11.45

3.4. Effect of variety and treatment on yield contributing of mustard caused by *Alternaria* blight

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against leaf infection of mustard caused by *Alternaria* blight and their significant effects were observed (Table 10 to Table 12).

Varietal effect

Significant variations among the effects of different treatments were observed on the different yield contributing characters viz. plant height (cm), number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weight (gm) and 1000 seed weight (gm) of three mustard varieties (Table 10). The plant height was ranged from 74.71-106.5cm where the lowest plant height was recoded in Tori-7 and the highest plant height was recoded in BARI Sarisha-6. Considering the number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weights (gm) and 1000 seed weight (gm), more or less similar trends of results were also observed for varietal effect (Table 10).

Table 10. Varietal effect on the yield contributing characters during the management of *Alternaria* blight of Mustard

Mustard Variety		Yield contributing characters					
		Plant height (cm)	Pod per plant (No.)	Pod length (cm)	Branch per plant (No.)	100 siliqua wt (g)	1000 seed wt (g)
V_1	BARI Sarisha-6	106.5 a	108.3 b	5.802 a	8.23 a	10.84 a	3.29 a
V_2	Tori-7	74.71 c	166.1 a	4.779 b	9.17 a	5.81 b	2.54 b
V_3	SAU Sarisha-1	92.17 b	104.6 b	5.583 ab	8.33 a	8.95 ab	2.47 b
$LSD_{(0.01)}$		1.262	5.161	0.9652	3.962	3.371	0.2670
CV (%)		0.61	1.81	7.94	20.47	17.50	4.20

4. Effect of variety and treatment on yield contributing of mustard -used by *Alternaria* blight

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against leaf infection of mustard caused by *Alternaria* blight and their significant effects were observed (Table 10 to Table 12).

Varietal effect

Significant variations among the effects of different treatments were observed on the different yield contributing characters viz. plant height (cm), number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weight (gm) and 1000 seed weight (gm) of three mustard varieties (Table 10). The plant height was ranged from 74.71-106.5cm where the lowest plant height was recoded in Tori-7 and the highest plant height was recoded in BARI Sarisha-6. Considering the number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weights (gm) and 1000 seed weight (gm), more or less similar trends of results were also observed for varietal effect (Table 10).

Table 10. Varietal effect on the yield contributing characters during the management of *Alternaria* blight of Mustard

Mustard Variety		Yield contributing characters					
		Plant height (cm)	Pod per plant (No.)	Pod length (cm)	Branch per plant (No.)	100 siliqua wt (g)	1000 seed wt (g)
V_1	BARI Sarisha-6	106.5 a	108.3 b	5.802 a	8.23 a	10.84 a	3.29 a
V_2	Tori-7	74.71 c	166.1 a	4.779 b	9.17 a	5.81 b	2.54 b
V_3	SAU Sarisha-1	92.17 b	104.6 b	5.583 ab	8.33 a	8.95 ab	2.47 b
$LSD_{(0.05)}$		1.262	5.161	0.9652	3.962	3.371	0.2670
CV (%)		0.61	1.81	7.94	20.47	17.50	4.20

Treatment effect

The significant effects of different seed treating chemicals and BAU-biofungicide were observed on the plant height (Table 11). The tallest plant (104.5) was obtained from Rovral 50 WP which was statistically different with other seed treating elements. The lowest plant height (78.00) was recorded in case of control plot.

Similarly the significant effects of different seed treating chemicals and BAU-biofungicide were observed on pod per plant (Table 11). The highest number of pod (159.8) was obtained from Rovral 50 wp treated plot which was statistically different with other seed treating elements. The lowest number of pod (110.1) was recorded in case of control plot.

Similarly the pod lengths were ranged from 4.56-6.12cm and where the highest pod length was recorded for Rovral 50 wp which was statistically identical with Brine solution. The lowest pod length was recorded for untreated plot.

Considering the number of branches per plant and100 siliqua weight (gm) more or less similar trends of results were also observed for different treatments (Table 11).

1000-seed weight differed significantly due to application of different seed treating chemicals. The maximum seed weight (2.97) was recorded in case of Rovral 50 wp which was statistically different with Brine solution, BAU-Biofungicide and Provax. The minimum seed weight (2.63) was recorded in case of control plant.

Table 11. Effect of different seed treating chemicals including BAU-biofungicide on the yield contributing characters during the management of *Alternaria* blight of Mustard

Treatments		Yield contributing characters					
		Plant height (cm)	Pod per plant (No.)	Pod length (cm)	Branch per plant (No.)	100 siliqua wt (gm)	1000 seed wt (gm)
T_1	Provax-200	91.40 c	117.1 c	5.31 ab	8.37 a	8.47 a	2.76 ab
T_2	Brine solution	85.37 d	117.2 c	5.17 ab	7.88 a	8.43 a	2.68 b
T_3	BAU-Biofungicide	96.41 b	127.4 b	5.76 a	9.31 a	8.82 a	2.80 ab
T_4	Rovral 50 wp	104.5 a	159.8 a	6.12 a	10.83a	9.50 a	2.97 a
T_5	Control	78.00 e	110.1 d	4.56 b	6.50 a	7.44 a	2.63 b
$LSD_{(0.01)}$		1.262	5.161	0.9652	3.962	3.371	0.2670
CV (%)		0.61	1.81	7.94	20.47	17.50	4.20

Interaction effect

Significant variations among different treatments were observed on the different yield contributing characters viz. plant height (cm), number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weight (g) and 1000 seed weight (g) of three mustard varieties (Table 12). The plant height was dependent on the variety as well as effect of different treatments, where BARI Sarisha-6 showed maximum height (85.93 to 123.7 cm) and minimum height showed by Tori-7 (63.10 to 80.00 cm). In case of treatment effects, the highest plants were produced for irrespective of all mustard varieties. Considering the number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weights (g) and 1000 seed weight (g), more or less similar trends of results were also observed for both varietals and treatments (Table 12).

Table 12. Effect of different seed treating chemicals on the yield Contributing characters of mustard

Variety	Treatments	Yield contributing characters					
		Plant height (cm)	Pod per plant (No.)	Pod length (cm)	Branch per plant (No.)	100 siliqua wt (g)	1000 seed wt (g)
BARI Sarisha-6	Provax-200	106.3 c	102.0 h	5.81 bc	8.73 b	10.55 ab	3.26 a
	Brine solution	105.0 d	112.7 g	5.37 b-d	7.22 b	10.88 ab	3.25 a
	BAU-Biofungicide	111.7 b	113.7 g	6.24 ab	9.20 b	11.12 ab	3.30 a
	Rovral-50WP	123.7 a	132.0 e	7.14 a	9.47 b	12.40 a	3.44 a
	Control	85.93 g	81.2 j	4.43 de	6.53 b	9.24 a-d	3.23 a
Tori-7	Provax-200	77.93 ij	146.7 cd	5.00 c-e	8.06 b	5.94 de	2.60 bc
	Brine solution	73.37 k	142.7 d	4.42 de	8.03 b	5.65 de	2.43 c
	BAU-Biofungicide	80.00 h	151.7c	5.15 b-d	10.13 ab	6.02 de	2.61 bc
	Rovral-50WP	79.17 hi	222.0 a	5.27 b-d	14.07 a	6.18 c-e	2.73 b
	Control	63.10 l	167.3b	4.04 e	5.58 b	5.28 e	2.33 c
SAU Sarisha-1	Provax-200	89.93 f	102.7h	5.12 cd	8.33 b	8.93 a-e	2.42 c
	Brine solution	77.73 j	96.23i	5.73 bc	8.40 b	8.787a-d	2.36 c
	BAU-Biofungicide	97.57e	117.0g	5.90 bc	8.60 b	9.32 a-d	2.50 bc
	Rovral-50WP	110.7b	125.3f	5.94 bc	8.95 b	9.93 a-c	2.75 b
	Control	84.97 g	81.73 J	5.217 b-d	7.40 b	7.81 b-e	2.35 c
$LSD_{(0.01)}$		1.262	5.161	0.9652	3.962	3.371	0.2670
CV (%)		0.61	1.81	7.94	20.47	17.50	4.20

3.5. Effect of variety and treatment on the yield of mustard

The variety and different management practices comprising seed treating chemicals and bio-control agent BAU-biofungicide were tested against on the yield of mustard and their significant effects were observed (Table 13 to Table 15).

Varietals effect

There is significant effect of different mustard varieties on the yield of mustard seeds (Table 13). The yield of mustard was ranged from 105.0-228.9 g where the lowest yield was recoded in Tori-7 and the highest yield was recoded in SAU Sarisha-1.

There is significant effect of different mustard varieties on the yield of straw (Table 13). The yield of straw was ranged from 637.3-2006 g where the lowest straw was recoded in Tori-7 and the highest straw was recoded in BARI Sarisha-6.

Table 13. Varietal effect on the yield of mustard during the management of *Alternaria* blight

Mustard Variety		Seed yield per plot (g)	Straw yield per plot (g)
V_1	BARI Sarisha-6	199.5 b	2006. a
V_2	Tori-7	105.0 c	637.3 c
V_3	SAU Sarisha-1	228.9 a	1545 b
$LSD_{(0.01)}$		7.694	43.60
CV(%)		1.92	1.38

Treatment effect

Significant variation of different treatments was found on yield per plot (g). Maximum yield per plot (214.1g) was obtained from Rovral treated plot followed by BAU-Biofungicide, Provax and in Brine solution. The minimum

yield per plot (149.1g) was recorded from control treated plot which was statistically identical with Brine solution (Table-14).

Significant variation of different treatments was found on straw yield per plot (g). Maximum straw yield per plot (1679g) was obtained from Rovral-50WP treated plot followed by BAU-Biofungicide, Provax and in Brine solution. The minimum straw yield per plot (1134g) was recorded from control treated plot which was statistically identical with Brine solution (Table-14).

Table 14. Effect of different seed treating chemicals including BAU-biofungicide on the yield of mustard during the management of *Alternaria* blight

Treatments		Seed yield per plot (g)	Straw yield per plot (g)
T_1	Provax-200	176.0 c	1412. c
T_2	Brine solution	156.7 d	1244. d
T_3	BAU-Biofungicide	193.1 b	1511. b
T_4	Rovral-50 WP	214.1 a	1679. a
T_5	Control	149.1 d	1134. e
$LSD_{(0.01)}$		7.694	43.60
CV (%)		1.92	1.38

Interaction effect

Significant variations among the effects of different treatments were observed on the different straw and seed yield per plot of three mustard varieties (Table 15). The both straw and seed yield of mustard were dependent on the variety as well as effect of different treatments, where BARI Sarisha-6 produced maximum seed (172.3 to 237.7 g) and straw (1622 to 2373 g) yield per plot. On the other hand, the minimum seed (95.13 to 131.30 g) and straw (512.70 to 762.7 g) yield per plot in Tori-7. In case of treatment effects, the maximum seed and straw yield per plot were produced for Rovral-50 WP and minimum seed and straw yield were produced for untreated control irrespective of all mustard varieties (Table 15).

-ele per plot (149.1g) was recorded from control treated plot which was statistically identical with Brine solution (Table-14).

Significant variation of different treatments was found on straw yield per plot (g). Maximum straw yield per plot (1679g) was obtained from Rovral-50WP treated plot followed by BAU-Biofungicide, Provax and in Brine solution. The minimum straw yield per plot (1134g) was recorded from control treated plot which was statistically identical with Brine solution (Table-14).

Table 14. Effect of different seed treating chemicals including BAU-biofungicide on the yield of mustard during the management of *Alternaria* blight

Treatments		Seed yield per plot (g)	Straw yield per plot (g)
T_1	Provax-200	176.0 c	1412. c
T_2	Brine solution	156.7 d	1244. d
T_3	BAU-Biofungicide	193.1 b	1511. b
T_4	Rovral-50 WP	214.1 a	1679. a
T_5	Control	149.1 d	1134. e
$LSD_{(0.01)}$		7.694	43.60
CV (%)		1.92	1.38

Interaction effect

Significant variations among the effects of different treatments were observed on the different straw and seed yield per plot of three mustard varieties (Table 15). The both straw and seed yield of mustard were dependent on the variety as well as effect of different treatments, where BARI Sarisha-6 produced maximum seed (172.3 to 237.7 g) and straw (1622 to 2373 g) yield per plot. On the other hand, the minimum seed (95.13 to 131.30 g) and straw (512.70 to 762.7 g) yield per plot in Tori-7. In case of treatment effects, the maximum seed and straw yield per plot were produced for Rovral-50 WP and minimum seed and straw yield were produced for untreated control irrespective of all mustard varieties (Table 15).

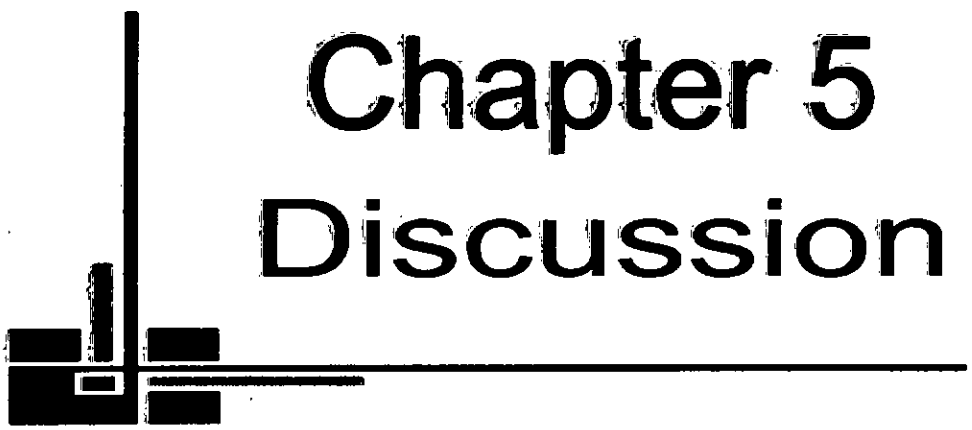
Table 15. Effect of different seed treating chemicals on yield and yield contributing characters of 3 mustard varieties

Variety	Treatments	Yield	
		Seed yield per plot (g)	Straw yield per plot (g)
BARI Sarisha-6	Provax-200	192.0 e	2050 c
	Brine solution	180.3 f	1771 e
	BAU-Biofungicide	215.0 d	2213 b
	Rovral-50WP	237.7 c	2373 a
	Control	172.3 f	1622 f
Tori-7	Provax-200	101.7 h	665.3 k
	Brine solution	95.03 h	561.7 l
	BAU-Biofungicide	102.0 h	684.3 k
	Rovral-50WP	131.3 g	762.7 j
	Control	95.10 h	512.7 m
SAU Sarisha-1	Provax-200	234.3 c	1522 g
	Brine solution	194.7 e	1400 h
	BAU-Biofungicide	262.3 b	1634 f
	Rovral-50WP	273.3 a	1900 d
	Control	180.0 f	1267 i
$LSD_{(0.01)}$		7.694	43.60
CV (%)		1.92	1.38

The incidence of *Alternaria brassicae* on seeds ranged from 3.5 to 42.5% in BARI-6, 9 to 29.5% in Tori-7 and 6 to 36% in SAU-Sharisha. The lowest seed infection was found in BARI-6 when the plot was treated by Rovral-50WP. The highest seed infection was found in BARI-6 untreated plot.

Table 16. Effect of different treatments on incidence of *Alternaria brassicae* on three mustard varieties.

Incidence of *Alternaria brassicae*						
Treatments	BARI-6	Percent reduction over control	Tori-7	Percent reduction over control	SAU-Sharisha	Percent reduction over control
Provax-200	20.5	51.76	12.5	59.67	23.5	34.72
Brine solution	31	27.05	29	6.45	24	33.33
BAU-Biofungicide	22	48.23	29.5	4.83	10	72.22
Rovral-50WP	3.5	91.76	9	70.96	6	83.33
Control	42.5		31		36	



Chapter 5
Discussion

CHAPTER V

DISCUSSION

The present study was carried with seed treating chemicals and BAU-Biofungicides to evaluate their efficacy in controlling *Alternaria* blight disease caused by *Alternaria brassicae.*

Rovral (Iprodione) 50WP when applied as fungicide tremendously decreased *Alternaria* infection of mustard. Rovral has been reported to be the most effective fungicide against *Alternaria* leaf blight of mustard (Kumara, 1996; Meah, 1992; Meah and Hossain 1989 and Shivpuri *et al.* 1988. The effect of different treatments on percent leaf area diseased (% LAD) of mustard at different days after sowing (DAS) was summarized. 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 diseases (LAD) of mustard increased gradually with the advancement of crop growth. At 85 Days after sowing the maximum percent of LAD (18.30%) was found at T_5 (control) treatment (SAU- sharisha) and the minimum % of LAD (7.40%) was found at Rovral for (BARI-6) variety. The findings of the present study are in agreement with different researchers. Rahman (2000) reported that, Rovral at 1000ppm sprayed for 3 times was the best treatment for reducing the disease intensity and increasing yield. Percent leaf area diseased, % siliqua infection and number of spots per siliqua were reduced by 64.9%, 57.1% and 70.5% with 3 sprays.

The effect of different treatments on number of spots per pod of mustard at different days after sowing (DAS) was also summarized. Number of spots per pod influenced significantly with the application of different treatment and different days after sowing (DAS). Very little number of spots per pod was

recorded at 65 Days after sowing at 85 DAS, the maximum number of spots per pod (18.27) was recorded from T_5 (in case of SAU- sharisha) and minimum number of spots per pod (11.23) was obtained from applying with T_4 (Rovral) treatment in BARI-6 followed by T_3 (BAU- Biofungicide), T_1 (Provax) and T_2 (Brine solution). Meah and Hossain (1989) found that Rovral 50 WP (0.1%) sprayed three times from fruiting stage of mustard resulted disease free pods and significantly increased seed yield. Sultana *et al.* (2009) was demonstrated that single spray of Rovral @ 2 g LG1 of water at 30, 40, 50 and 60 days after sowing (DAS) control the disease severity of leaf blight compare to 70 DAS and control. These results indicate that disease severity, seed yield and yield contributing characters were significantly influenced by variety and single time of spray. Three time application of Rovral produced the lowest disease severity and produced highest seed yield. The highest seed yield (1747.33 kg haG1), lowest disease severity (1.7) and PDI (8.89) were recorded from the treatment combination V T (BARI Sarisa-9 with 1 6 3 spray). The second highest seed yield (1588.10 kg haG1) and lowest severity (2.0) were obtained from treatment.

Different treatments had significant influence on plant height (cm) of mustard. The tallest plant (123.7) was obtained from in the treatment combination from Rovral × BARI-6. The lowest plant height (63.10) was obtained from in the treatment combination from Tori-7 × control (T_5). Number of pod was highest in Tori- 7 in treatment (T_4) and it gives 222.16 pods. The lowest number of pod was obtaining in BARI-6 for (T_5) and it gives only 81.27 pods. The highest pod length (7.14) cm was obtained from in treatment combination from var. BARI-6 × T_4 (Rovral) and lowest pod length (4.04) cm was obtained from in treatment combination from var. Tori-7 × T_5 (control) plot. The maximum number of branches (14.06) was recorded in case of Tori-7 in T_4 (Rovral). In case of Tori-7, T_5 (control) produced the lowest (5.58) number of branches per

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plant. The maximum straw produced 2373g in BARI-6 × T_4 (Rovral) treatment and lowest straw produced 512.99g in Tori-7.

Weight of 100 siliqua (12.40g) was highest in treatment combination of BARI-6 × T_4 (Rovral) and lowest in (5.28g) in treatment combination of Tori-7 × T_5 (control). Another experiment was conducted in BARI, (1987) and total yield, 1000 seed weight and 100 pod weight were recorded. All the chemicals reduced the disease incidence in all the spray schedules. Among the tested fungicides, Rovral showed the best performance when spraying was started at 40 days. In case of 100 pods wt. and 1000 seed weight all are increased over control. 1000 seed weight was found to be significant due to application of different treatment. Seed treatment with Rovral (T_4) in BARI-6 produced the maximum 1000 seed weight (3.44g) while minimum 1000 seed weight (2.33) was recorded when no treatment applied in Tori-7 plant (T_5). The 1000-seed weight was increased due to Rovral spray (Anonymous, 1992) which is supporting the present findings. The present findings also similar with the result of Ayub *et al.* (1996).

The highest number of seed was produced (237.7) in treatment combination of SAU- Shorisha × T_4 (Rovral) and lowest number of seed was produced (95.10) in treatment combination of Tori-7×T_5 (control). 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 500g ai/ha during the early pod stage. Iprodione was more effective in compare to untreated plot in reducing Alternaria blight incidence. The yield of mustard in Iprodione treated plot was higher by 24-59% than that in the control plots. Singh *et al.* (2006) reported that spraying of Rovral (iprodione) was more effective than other fungicides and the highest yield were recorded with Rovral. These findings are agreed

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with the findings of Chattopadhyay and Bhuian (2003), Mukherjee *et al.* (2003), Pandya *et al.* (2000) and Ayub *et al.* (1996). This view is also strengthened from economic point of view and also from the findings of Meah *et al.* (1992) who obtained disease reduction and yield increase by 115 and 147% over control with 2 sprays of Rovral starting at 50 days age and also ORC (1995) who obtained lowest disease and highest yield with Rovral spray starting at siliqua filling stage.

Number of spots/leaf of mustard increased gradually with the advancement of crop growth of spot was observed in treatment combination of BARI-6 × T_5 (control) (93.50) and lowest number of spot was observed in treatment combination of SAU- shorisha × T_4 (Rovral) followed by T_3 (BAU-Biofungicide), T_1 (Provax) and (Brine- solution).

Percent disease index was highest (73.97) in treatment combination of Tori-7 × T_5 (control) plot and the lowest PDI was found in treatment combination BARI-6 × T_4 (Rovral) followed by T_3 (BAU- Biofungicide) T_1 (Provax) and T_2 (Brine - solution). Hossain (2003) stated that the maximum of 66.7 PDI was recorded under control plots. The PDI was reduced to a range of 21.1-39.4 and the lowest PDI was recorded in the plots treated with Rovral-50 WP.

Seed health regarding incidence of *Alternaria brassicae* were differ due to application of different treatments. The lowest seed infection (3.5%) by *Alternaria brassicae* was found in the seed lot obtained from treated plot with Rovral-50 WP in BARI-6 plot compared to control. 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-50 WP. Anonymous (1992) reported that foliar spray of Rovral significantly reduced the seed borne infection of *Alternaria* spp.

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Chapter 6

Summary and conclusion

CHAPTER VI

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The experiment was conducted at the experimental field of Sher-e-Bangla Agricultural University, Dhaka and in the seed Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The experiment was held during the period from November 2008 to March 2009.

The experiment was laid out in a RCBD with three replications. There were five treatments, Viz, T1 (Provax-200), T2 (Brine solution), T3 (BAU-Biofungicide), T4 (Rovral), T5 (control). The unit plot size was 1m × 1m. Data were collected on disease incidence and severity of the disease, yield and yield contributing characters. Three varieties namely BARI-6, Tori-7 and SAU-Sharisha-1 were used in this study.

The study revealed that application of seed treating chemicals and BAU-Biofungicides significantly influenced all most all of the parameters. Seed treatment with Rovral as well as spraying resulting better performance. The lowest percent leaf area diseased (7.40%), lowest number of spots/siliqua (11.23%), highest plant height 123.90cm, pod length increases 7.14 cm, weight of 100 siliqua 12.40g, weight of 1000 seed weight 3.44g and lowest PDI (21.30%) were recorded, where, BARI-6 was treated with Rovral-50 WP. The highest number of pod 222.16 was recorded where; Tori-7 was treated with Rovral-50 WP. The lowest number of spot per leaf (33.33%) were recorded where, Tori-7 was treated with Provax-200. The highest numbers of LAD (18.30%), Number of spot (14.97) were recorded from control plot.

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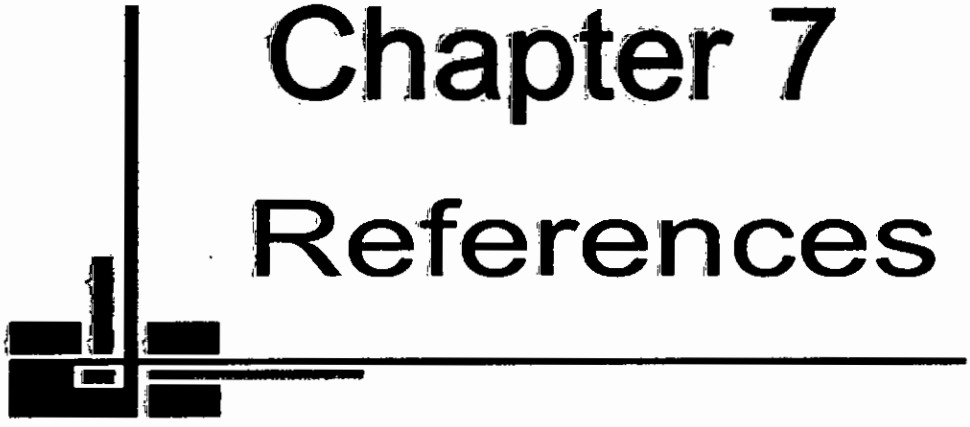
The highest seed yield was (273.47g) was obtained form SAU-shorisha plot spraying with Rovral-50 WP. The lowest yield (95g) was obtained from untreated plot.

The lowest seed infection (3.5%) obtained from BARI-6 plot spraying with Rovral-50 WP. The highest seed infection of harvested seeds (42.5%) were obtained from the control treatment.

From the present findings it may be concluded that seed treatment as well as spraying with Rovral 50 WP was found to be best in reducing *Alternaria* blight incidence and severity and increasing quality seed of mustard. BAU-Biofungicide also has some promising effect against the disease. Therefore, use of BAU- Biofungicide as seed treatment would be an immense and potential means for controlling *Alternaria* blight of mustard with an additional benefit of avoiding environmental pollution. Moreover, it is ecofriendly comparing with the chemical fungicides that are being used in the country.



Chapter 7
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CHAPTER VII

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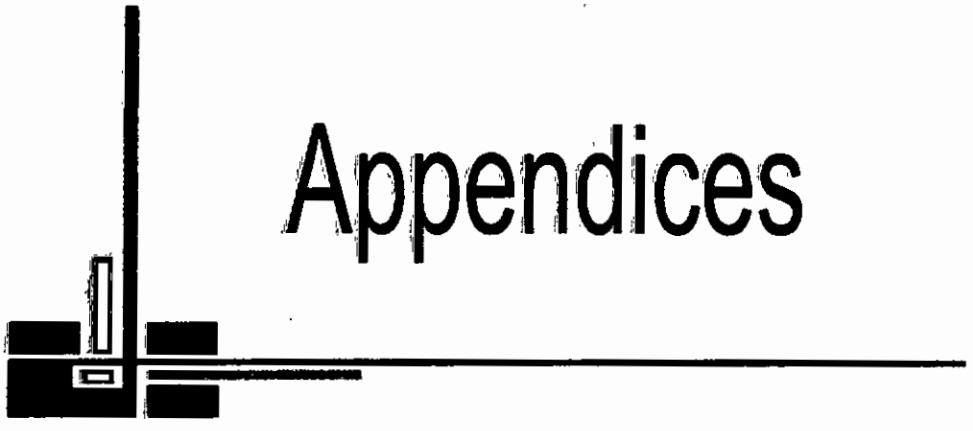

Appendices

8. APPENDICES

Appendix I. Particulars of the Agro-ecological Zone of the Experimental Site

Agro-ecological region: Madhupur Tract (AEZ-28)

Land Type	: Medium high land
General soil type	: Non-Calcareous Darkgray floodplain soil
Soil series	: Tejgaon
Topography	: Up land
Elevation	: 8.45
Location	: SAU Farm, Dhaka.
Field level	: Above flood level
Drainage	: Fairly good

Firmness (Consistency): Compact to friable when dry.

Appendix II. Records of meteorological information (monthly) of the experimental site during the period from October 2008-February 2009.

Month (2008-09)	Average Temperature (° C)		Rainfall (mm)	Relative humidity (%)
	Maximum	Minimum		
October, 2008	34.8	18.0	227	77
November, 2008	32.3	16.3	0	69
December, 2008	29.0	13.0	0	79
January, 2009	28.1	11.1	1	72
February, 2009	33.9	12.2	1	55

Source: Bangladesh Meteorological Department (Climate & Weather Division) Agargoan, Dhaka - 1212

Appendix III

Prevalence of Seed borne pathogen on mustard

Seed Health Report: Seedling Symptom Test

Accession Number: Date of Receipt: Host variety: Mustard(SAU Sharisha -1)	Date of Sowing: 15.11.08
	Type of substrate/ media: Water Agar Media
	Date of examination: 02.06.09
	No. of seed tested: 50
	No. of test tube: 50
	1. number of healthy seedlings: 40
	2.Number of Seedling showing symptoms: 2
	3.Short account of symptoms: 3
	4.Name of the fungus/ fungi causing symptoms: *Alternaria brassicae, Aspergillus niger*
	5. No. of ungerminated seeds with fungi: 3
	6. No. of ungerminated seeds without fungi: 2
	7. Final infection counts: 10
Remarks : Here total number of seed was tested 50. Number of healthy seedling was 40. A fungal infection was 10. ..... Signing Authority	

Appendix IV

SEED HEALTH REPORT: Blotter method & Modification

Accession Number:

Date of Receipt:

Host variety: Mustard (BARI-6)

Method: Blotter method
Number of seed tested: 400

Date of planting: 12.05.09

Date of recording: 19.05.09

Fungi	No. of dish/ plates																Infection (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Alternaria brassicae	6	2	0	5	3	2	4	3	2	1	4	3	2	0	6	1	10
Fusariu oxysporum	3	2	0	0	0	2	1	1	0	0	0	2	2	2	2	0	4.25
Aspergillus niger	2	1	2	0	0	0	0	1	2	0	2	1	2	3	0	1	4.25
Rhizopus sp	2	0	1	1	1	0	0	2	2	0	1	2	4	1	1	3	5.25
Aspergillus flavus	2	3	1	1	0	2	0	2	0	1	0	2	0	2	1	1	4.5

Remarks: Highest infection caused by *Alternaria brassicae* and lowest infection caused by *Fusarium oxysporum & Aspergillus niger*

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Appendix V

SEED HEALTH REPORT: Agar plate Method

Accession Number:	**Method: Agar** Number of seed tested: 200								
Date of Receipt:	Date of planting: 12.05.09								
	Date of recording: 19.05.09								
Host variety: Mustard (Tori-7)	Pathogen	No of dish /plate							Infection(%)
		1	2	3	4	5	6	7	8	
	Alternaria brassicae	1	3	2	0	0	0	1	2	4.5
	Fusarium sp	7	5	4	5	6	5	4	5	21.5
	Aspergillus niger	7	8	7	0	0	0	4	6	16
	Aspergillus flavus	3	0	1	7	2	0	2	0	7.5
	Trichoderma	5	7	0	0	4	2	4	0	16

Remarks: Here *Alternaria brassicae* was found 4.5% & the highest infection percentage of *Fusarium sp* is 21.5%

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Appendix VI

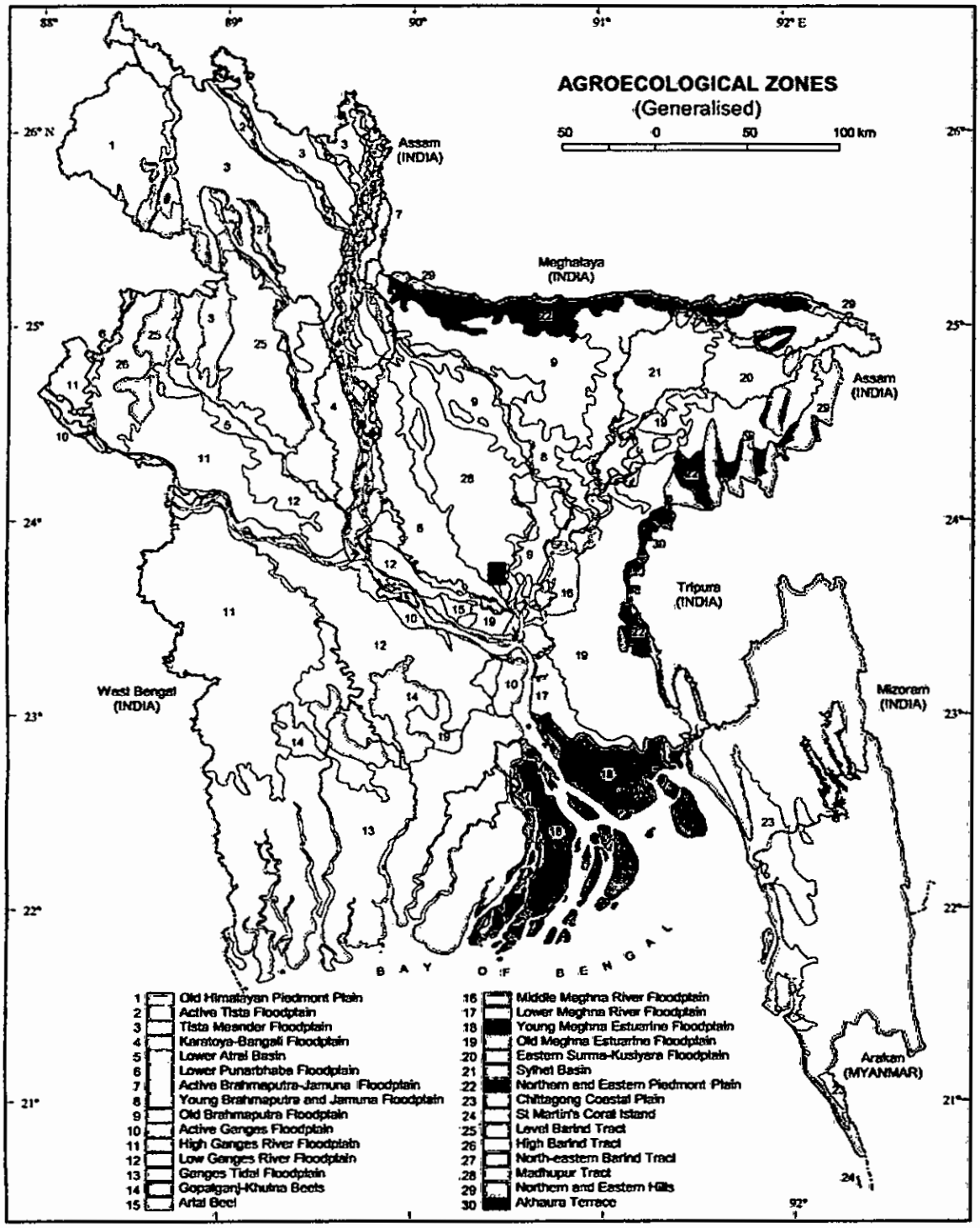

Fig. Location of experimental site under study