YIELD LOSS ASSESSMENT FOR GREY BLIGHT OF MUSTARD CAUSED BY *Alternaria* spp.

REGISTRATION NO. 15-06476



DEPARTMENT OF PLANT PATHOLOGY SHER-E-BANGLA AGRICULTURAL UNIVERSITY SHER-E-BANGLA NAGAR, DHAKA-1207, BANGLADESH

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YIELD LOSS ASSESSMENT FOR GREY BLIGHT OF MUSTARD CAUSED BY Alternaria spp.

BY

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A Thesis

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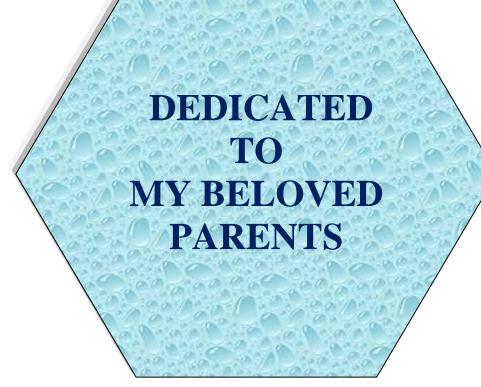
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CERTIFICATE

This is to certify that thesis entitled "YIELD LOSS ASSESSMENT FOR GREY BLIGHT OF MUSTARD CAUSED BY Alternaria spp." submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) in Plant Pathology, embodies the result of a piece of bona fide research work carried out by BASAD AL MAHAMUD, Registration No. 15-06476 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged and style of this thesis have been approved and recommended for submission.

Dated: June, 2022 Place: Dhaka, Bangladesh **Prof. Dr. Md. Rafiqul Islam** Department of Plant Pathology Sher-e-Bangla Agricultural University **Supervisor**



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Date: June, 2022 SAU, Dhaka The Author

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ABSTRACT

A pot experiment was carried out during the period from December 2021 to March 2022 to assess the yield loss of mustard due to grey blight disease. The experiment consists of twelve treatments viz, $T_0 = Control$ (Field spraying with plain water); $T_1 =$ One field spraying with Rovral 50 WP @ 0.2%; T₂ = Two field spraying with Rovral 50 WP @ 0.2%; T_3 = Three field spraying with Rovral 50 WP @ 0.2%; T_4 = Four field spraying with Rovral 50 WP @ 0.2%; T_5 = Five field spraying with Rovral 50 WP @ 0.2%; T_6 = Six field spraying with Rovral 50 WP @ 0.2%; T_7 = Seven field spraying with Rovral 50 WP @ 0.2%; $T_8 = Eight$ field spraying with Rovral 50 WP @ 0.2%; T_9 = Nine field spraying with Rovral 50 WP @ 0.2%; T_{10} = Ten field spraying with Rovral 50 WP @ 0.2% and T_{11} = Eleven field spraying with Rovral 50 WP @ 0.2%.. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Different treatments comprising different number of sprayings had remarkable effect on the disease incidence and severity of grey blight, yield and yield contributing characters of mustard. The lowest (0.0%) percent disease index (PDI) and the highest yield (934.3 kg/ha) was recorded in case of treatment T_{11} where eleven foliar spraying were applied with Rovral 50 WP @ 0.2% at 7 days interval. The highest PDI (84%) and the lowest yield (357.6 kg/ha) was counted in case of treatment T₀ (control). The disease severity (PDI) and yield were varied in case of other treatments on the basis of number of fungicide spraying. Using the varied disease severity (PDI) and corresponding yield, the mathematical yield loss assessment model was constructed as Y = 0.50 + 2.32Xi using the regression equation.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Full meaning
%	Percent
@	At the rate
⁰ C	Degree Centigrade
Agril.	Agricultural
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
cm	Centi-meter
CV	Coefficient of variation
d.f.	Degrees of freedom
DAS	Days After Stimulation
DMRT	Duncan's Multiple Range Test
e.g.	For example
et al.	And others
FAO	Food and Agriculture Organization
G	Gram
J.	Journal
Kg	Kilogram
LSD	Least Significant Difference
Mg	Milligram
m ²	Meter Squares
RCBD	Randomized Complete Block Design
SAU	Sher-e-Bangla Agricultural University

CHAPTER I

INTRODUCTION

Mustard (Brassica spp.) belongs to the family Brassicaceae (or Cruciferae) is an important oil crop next to soybean. It is the second-most significant edible oil in the world (Singh et al., 2017). In Bangladesh, it is widely cultivated during the winter season from the month of October to February and its contribution in total oil seed production is approximately 70% (Kabir, 2021). Mustard seed and oil has multiple uses in health care system. The leaves of young plants are used in the human diet as green vegetable. Mustard seed and oil has multiple uses in health care system. It improves the body complexion because of its antifungal property. It is used as a very good massage oil, which brings vitality and strength to the body and improves the circulatory system and cures bodyache. It also kills various microbes and thus, keeps skin infections away. Oral doses of oil help in strengthening the teeth and cure various mouth related diseases. It helps in healing wounds by stopping the pus formation and in curing various skin disorders by removing unwanted fluids from the body (Kumar and Chauhan, 2005). Erucic acid and glucosinolate are the two major deterrents of oil and seed meal in oilseed brassica, respectively (Singh et al., 2013). The oilseed brassicas usually contain 38-57% of erucic acid, 4.7-13% linolenic acid and 27% of oleic and linoleic acid, which are of high nutritive value required for human health (Singh et al., 2012). Rapeseed and mustard are rich source of oil and contains 44% to 46% good quality oil (Rashid, 2013). The different varieties of mustard seed contain 40-44% oil and mustard oil cake contains 40% protein (Chowdhury and Hassan, 2013). It is also an important row material for industrial use such as; soaps, paints, varnish, hair oil, lubricants, etc. Mustard oil cake used as animal feeds also as manure (Haque, 2012).

It is the major oil seed crop of Bangladesh yielding 36.83% of total oilseed production from 64.6% of the total area coverage (BBS, 2020). During 2020-21 growing year, about 0.81 million hectares of land are covered to mustard cultivation in Bangladesh with yield of mustard seed in order of 0.39 million tons per year (BBS, 2022). The average production of the world is 0.87 ton/ha (FAO, 2020), whereas in Bangladesh is 0.5 ton/ha. Yield of mustard is very low in Bangladesh in comparison to other countries. Biotic and abiotic factors are responsible with the poor yield of mustard in Bangladesh. Among the biotic factors, diseases have been identified as one of the major factors. Mustard suffers from about 14 diseases (fungus 9, virus 2, bacteria 1, nematode 1 and parasitic plant 1) in Bangladesh (Bakr *et al.*, 2009). Of these disease, Alternaria leaf blight caused by Alternaria species is one of the major diseases of mustard (Meena *et al.*, 2016, Selvamani *et al.*, 2014). Alternaria blight caused by *Alternaria brassicae* has been reported to cause heavy yield losses to 35-60% in mustard crop (Karthikeyan *et al.*, 2021). The disease reduces mustard yield up to 47% in India (Sharma, 2009) and 30-40% in Bangladesh (Kabir *et al.*, 2021).

The most severe and devasting disease of rapeseed-mustard is grey leaf blight, which is caused by Alternaria sp. (Fakir, 2008). The majority of Alternaria species are saprophytes and common in nature (Simmons, 2007). The pathogens are greatly influenced by weather with the highest disease incidence reported in wet seasons and areas with relatively heavy rainfall (Meena et al., 2010). The Spore is produced in chains or in branching fashions which are multicellular pigmented. The spores are broadest near the base and elongate beak taper gradually. Alternaria morphologically creates a series of concentric rings around the initial site of the host leaf (Anju et al., 2013). The pathogen infects all aerial plant parts, reducing photosynthetic area and accelerating senescence and defoliation. Alternaria species can affect plant species in all growth stages, including seed. At seedling stages the disease is characterized by dark stem lesions just after germination that leads damping off, or stunted seedlings. The symptoms may vary with host and environment. The disease may cause 25% yield reduction at severe condition of infection (Anonymous, 2001). Grey blight causing yield loss which is approximately 30-40% in Bangladesh. The yield losses of mustard due to grey blight disease affect the market price of edible oil in the country. The market price closely depends on the local oilseed production. But for such as important disease, the crop loss assessment model is not yet been constructed. Thus, the present study was undertaken to estimate the yield losses of grey blight of mustard caused by Alternaria spp.

Considering the above facts, the experiment has been undertaken with the following specific objectives:

- To calculate the disease incidence and severity at the critical disease stage.
- To calculate yield loss of mustard for grey blight disease caused by *Alternaria* spp.
- To develop a mathematical point model for yield loss assessment of mustard due to grey blight disease.

CHAPTER II

REVIEW OF LITERATURE

Gray blight disease of mustard caused by *Alternaria* spp. is a common and most important disease in our country. This disease causes serious yield loss of the crop. Researchers all over the world have carried out intensive investigation on the gray blight of mustard. Literature in relation to management, severity and yield loss assessment of gray blight of mustard is reviewed and presented below:

Kumar *et al.* (2019) reported that foliar sprays of ridomil MZ 72 WP @ 2g/l significantly reduced alternarial blight, increased yield, followed by foliar sprays with Trichoderma harzianum @ 10g/kg, foliar sprays with bulb extract of garlic 1% (w/v), foliar sprays with bulb extract of onion 1% (w/v), foliar sprays with carbendazim 50% WP @ 2g/l, foliar sprays with mancozeb 75% WP @ 2.5g/l, and foliar sprays with ridomil MZ 72 WP @ 2g/l. However foliar sprays of mancozeb, carbendazim and bulb extract of garlic have shown results at par with the foliar sprays of ridomil in increasing the yield.

Ahmed *et al.* (2018) found 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.

Mahapatra and Das (2017) carried out an experiment to assess the yield losses of mustard due to Alternaria leaf blight in Gangetic plains of West Bengal and found that three sprays of iprodion @ 0.2% at 10 days interval beginning from 45 days after sowing resulted in lowest *Alternaria* blight under moderate disease pressure and four sprays for high disease pressure resulted in highest seed yield and cost benefit ratio of 5.19 and 4.61, respectively for commercial seed and 8.43 and 7.48 for certified seed. Highest avoidable losses of seed yield and 1000 seed weight due to the *Alternaria* blight infection were 27.24% and 5.98% for three sprays and 30.71% and 0.74% for four sprays respectively. The results indicated that three sprays of Iprodion (0.2%) resulted in minimum disease severity and maximum profit whereas under high disease pressure four sprays were essential for minimum disease severity and maximum profit in West Bengal.

Das (2015) reported that a number of fungicides have been reported to be effective against the spread of against *Alternaria* under different field conditions e.g. Dithane M-45 (0.2%), Dithane Z-78 (0.2%), Iprodione (Rovral) (0.2%), Blitox 50 (0.3%), Baycor (0.2%) and Mancozeb (64%)

Akhter *et al.* (2012) reported that, eight mustard varieties (SAU-1, BINA-6, TORI-7, BARI-9, BARI-6, SOFOL, AGRANI and SS-75) were evaluated for their reaction against *Alternaria* blight (*Alternaria brassicae*) under natural condition at the experimental field of Sher-e-Bangla Agricultural University, Dhaka during winter season from November 2007 to February 2008. At 60 days after sowing (DAS) disease severity did not exceed 5% and no symptoms were observed in the siliqua. Results revealed that, among the varieties the lowest disease severity was observed in Agrani in all stages of plant growth. Maximum disease severity (97.17%) was found in SAU Sarishsa 1 giving lowest yield (1266.55kg/ha).

Kumar (2008) conducted field resistance/partial resistance to *Alternaria* blight (*Alternaria brassicae*) was assessed in nine genotypes of Indian mustard under field conditions. Three genotypes viz. PR 8988, PR 9024 and Kranti exhibited partial resistance and had lowest severity. The yield potential of the genotypes was negatively correlated with the disease severity.

Alam (2007) evaluated the efficacy of some selected fungicides and plant extracts against *Alternaria brassicae* and *Alternaria brassicicola* causing grey blight of mustard (var. SAU Sarisha-1, Brassica campestris). Experiments were conducted at the Farm of Sher-e-Bangla Agricultural University, Dhaka and in the laboratory of Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Rahmatpur, Barishal during rabi season during the month of November, 2006 to February, 2007. Four fungicides viz. Rovral 50 WP (0.2%), Dithane M-45 (0.3%), Ridomil 68 WP (0.2%), Bavistin DF (00.15 and two plant extracts viz. Garlic clove extract, Allamanda leaf extract were employed in the experiment. Among the fungicides and plant extracts tested, Rovral WP (0.2%) showed the best performance in reducing disease incidence and disease severity as well as increasing seed yield against gray blight of mustard. Seed infection by Alternaria spp. was reduced by 64.90% and seed yield was increased by 48.19% over control by the application of Rovral 50 WP.

Prasad and Lallu (2006) found that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early date of sowing was the best combination in reducing the grey blight of mustard disease severity on leaves (18.7%) and pods (10.4%) higher realization yield (1295.8 kg/ha), 1000 seed weight (5.12 g) and oil content (42.6%).

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

Shrestha *et al.* (2005) reported that mancozeb and iprodione had effectively reduced grey blight disease in the sprayed plots and increased seed yield by 48% and 130%, respectively. The correlation between disease severity and yield, and yield components was negative and highly significant. Average yield loss was estimated to be in the range of 32 to 57%. Seed infection was also significantly higher in non-sprayed treatment than sprayed one. The disease showed a negative effect on oil content causing losses on oil between 4.2 to 4.5%.

Singh and Singh (2005) were carried out an experiment in India for controlling *Alternaria* blight (AB) caused by *Alternaria brassicae* and *A. brassicicola* and observed that seed treatment combined with three foliar sprays of Mancozeb 75% WP (0.2%) at 15-day intervals, beginning at 45 days after sowing, resulted in the lowest AB incidence and the highest seed yield and cost-benefit ratio of 1:5.2. It was followed by foliar sprays of Mancozeb 75% WP alone in all cases. Highest avoidable losses due to the combined effect of these diseases in seed yield, seed test weight and oil content were 34.7, 13.1 and 4.2%, respectively.

Mukherjee *et al.* (2003) studied the efficiency 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.

Prasad et al. (2003) investigated an experiment in Kanpur, Uttar Pradesh, India, during the 1999/2000 and 2000/01 rabi seasons on Indian mustard genotypes PAB 9534, PAB 9511, JMM 915, RN 490 and Varuna to determine the losses due to Alternaria blight (Alternaria brassicae) under protected and unprotected conditions. Varuna and PAB 9511 were used as the susceptible and resistant controls, respectively. The protected plots were sprayed with 0.25% mancozeb starting from 40 days after sowing and 3 subsequent sprays at 15-day intervals. The disease appeared 45 days after sowing. The highest disease intensity was recorded at flowering and pod formation. Treatment with mancozeb reduced disease incidence in all the genotypes. There was a 72.6 and 59.0% reduction in disease severity for RN 490 and the lowest disease intensity (17.8 and 16.1%) was recorded in the protected plots compared to the unprotected plots (39.6 and 32.5%) in both the years. The highest seed yield loss (20.8 and 21.9%) was observed in Varuna under unprotected conditions; however, it also gave the highest seed yield (20.3 and 19.5 q/ha) followed by RN 490 (18.5 and 18.3 q/ha) in the protected plots. Pooled analysis of data revealed that Varuna had the highest disease intensity (22.0 and 44.0%)and yield performance (19.9 and 15.7 q/ha) in protected and unprotected plots, respectively. The 1000-seed weight of RN 490 in protected (5.2 g) and unprotected (4.8 g) plots was similar with Varuna.

Chattopadhyay and Bhunia (2003) studied with seven fungicides viz; mancozeb 0.2%, captan 0.2% metalaxyl M.Z 0.25%. iprodione 0.2%, bayletan 0.05% (triadimefon), copper oxychloride 0.3% and antracol 0.2% (propineb) against Alternaria leaf blight of rapseed-mustard (*Brassica campestris* cv. Yellow Sarson) caused by *Alternaria brassicae*. Best control of the disease was observed by iprodione followed by mancozeb. Higher seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione followed by mancozeb. Highest seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione followed by mancozeb. Highest seed yield and significant increase of 1000-seed weight were also recorded from single spray of iprodione followed by mancozeb.

iprodione at post flowering stage. But maximum economic return was obtained from two spraying of mancozeb at 45 DAS and 60 DAS.

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

Hossain (2003) conducted an experiment where Seed health regarding incidence of *Alternaria brassicae* were different 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.

Ferdous *et al.* (2002) conducted an experiment to investigate the effect of three plant extracts and one fungicide on the incidence of Alternaria blight (caused by *Alternaria brassicae*) of mustard (*Brassica* sp.) cv. Sonali Sarisha under neutral field conditions in Gopalgonj, 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 also used. All the 4 treatments were used at 1 litre/10m² areas. 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.

Singh and Singh (2002) investigated on timely sown (15-20 October) of mustard crops during 1995/96-2001-02 revealed Alternaria blight (AB-*Alternaria brassicae*), white rust (WR-*Alugo candida*), downy mildew (DM-*Peronospora parasitica*) were the major mustard diseases in mid-eastern India and together caused 44.06% avoidable yield loss. In trails conducted in the same field during 2001-02 and 2002-03 crop seasons, 3 spray of Iprodione 50 WP (Rovral @ 0.20%). Followed by mancozeb 75 WP (Indofil M 45 @ 0.2%) and propineb 70 WP (Antracol @ 0.2%) gave the most effective AB control and yield gain. Significantly superior WR control was obtained by 2 sprays of metalaxyl+ mancozeb 72 WP (Ridomil MZ @ 0.25%) followed by 3 sprays of captan 50 WP (Captaf @ 0.20%).

Godika *et al.* (2001) conducted a fiefd experiment from 1994/95 to 1996/97 in Rajasthan, India to evaluate the efficacy of different fungicides, named Mancozeb, Ridomil MZ, (mancozeb+metalaxyl), Captan, Rovral (iprodione), Bayletan (tridimefon), and copper oxycloride, against Alternaria blight (*Alternaria brassicae*) and white rust (*Albugo candida*) of Indian mustard. All the fungicides significantly controlled both diseases, but their efficacy varied. Rovral was the most effective in controlling of Alternaria blight; mean disease intensity in 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 stag head were 8.5 and o.5 %, respectively. Yield was highest with Rovral (2.1 t/ha), followed by Mancozeb and Ridomil MZ, each recording a yield of 1.9 t/ha.

Rahman (2000) observed that Rovral at 1000 ppm 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.

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

CHAPTER III MATERIALS AND METHODS

The experiment was conducted during the period from December 2021 to March 2022 to assess the yield loss of mustard for grey blight disease caused by *Alternaria* spp. The chapter includes a brief description of the location of experiment, materials used for the experiment, design of the experiment, data collection on disease incidence and severity, growth parameters, yield and yield contributing characters and data analysis procedure which are presented below under the following headings-

3.1. Experimental site

The experiment was conducted at the farm of Sher-e-Bangla Agricultural University, Dhaka. The location of the study site was situated in 23°74'N latitude and 90°35'E longitudes. The altitude of the location was 8m from the sea level as per the Bangladesh Metrological Department, Agargaon, Dhaka-1207, which have been shown in the Appendix I.

3.2 Duration of the experiment

The experiment was carried out during the Rabi season from December 2021 to March 2022. Seeds of mustard was sown on 16th December 2021 and was harvested on 8th March 2022.

3.3 Characteristics of soil

The soil of the experimental area belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with adequate irrigation facilities and remains fallow during previous growing season. The nutrient status of the farm soil under the experimental pot was collected and analyze in the Soil Resource Development Institute (SRDI), Dhaka and result has been presented in Appendix II-III.

3.4 Climate condition of the experimental site

The experimental site is situated in the subtropical monsoon climatic zone, which is characterized by heavy rainfall during the months from April to September (Kharif season) and scanty of rainfall during rest of the year (Rabi season). Plenty of sunshine and moderately low temperature prevail during October to March (Rabi season), which are suitable for growing of mustard in Bangladesh. The weather information regarding temperature, rainfall, relative humidity and sunshine hours prevailed at the experimental site during the cropping season December 2021 to March 2022 have been presented in Appendix IV-VI.

3.5 Planting materials

The mustard (*Brassica campestries*) variety SAU Sharisha 3 released from Sher-e-Bangla Agricultural University was used for the experiment. Seed was collected from Department of Genetics and Plant Breeding, Sher-e- Bangla Agricultural University, Dhaka.

3.6 Treatments of the experiment

Twelve different treatments were codified with three replications to achieve the desired objectives. The treatments were as follows:

 T_0 = Foliar spraying with distill water only

- T_1 = One foliar spraying with Rovral 50 WP @ 0.2%
- T_2 = Two foliar spraying with Rovral 50 WP @ 0.2%
- T_3 = Three foliar spraying with Rovral 50 WP @ 0.2%
- T_4 = Four foliar spraying with Rovral 50 WP @ 0.2%
- T_5 = Five foliar spraying with Rovral 50 WP @ 0.2%
- T_6 = Six foliar spraying with Rovral 50 WP @ 0.2%
- T_7 = Seven foliar spraying with Rovral 50 WP @ 0.2%
- T_8 = Eight foliar spraying with Rovral 50 WP @ 0.2%
- T_9 = Nine foliar spraying with Rovral 50 WP @ 0.2%
- T_{10} = Ten foliar spraying with Rovral 50 WP @ 0.2%
- T_{11} = Eleven foliar spraying with Rovral 50 WP @ 0.2%

3.7 Experimental design

The experiment was set up in a single factor Randomized Complete Block Design (RCBD) with three replications. Thus 36 experimental pots were placed in ambient air

at the research Farm premises of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.8 Preparation of soil and filling of pots

There were 36 earthen pots. The size of the pot was 30 cm top diameter with a height of 25 cm. Plant parts, inert materials, visible insects and pests were removed from soil by sieving. Collected soil was dried under the sun. The dry soil was thoroughly mixed with well decomposed cow dung and fertilizers before filling the pots. Each pot was prepared with 10 kg air dried soil.

3.9 Pot preparation

A ratio of 1:3 well decomposed cow dung and soil were mixed and pots were filled 10 days before seed sowing. Silty loam soils were used for pot preparation. All 36 pots were filled on December 2021. Weeds and stubbles were completely removed from the soil.

3.10 Application of manure and fertilizers in the pot

The required amount of fertilizers (N, P, K, Gypsum, Zinc oxide and Boric acid kg ha⁻¹) and manure (cow dung) was calculated for each pot considering the dose of 1 hectare soil at the depth of 20 cm as per recommendation of Fertilizer Recommendation Guide, 2012 (FRG 2012). As per such recommendation, 15.0 g of urea, 7.0 g of triple super phosphate (TSP), 4.0 g of muriate of potash (MoP), 5.0 g of gypsum, 0.25 g of Zinc oxide, 0.5 g of Boric acid and 100.0 g of cow dung pot⁻¹ was applied. Half of urea and entire amount of cow dung, TSP, MoP, Gypsum, Zinc oxide, Boric acid were mixed with the soil in each pot before seed sowing. Rest of the urea was applied as side dressing at the time of flower initiation.

3.11 Intercultural operation

Intercultural operations, such as weeding, thinning, irrigation, pest management, etc. were done uniformly in the pots. One post sowing irrigation was given by sprinkler after sowing of seeds to bring proper moisture condition of the soil to ensure uniform germination of the seeds. The first weeding was done at 15 days after sowing. During

the same time, thinning was done for maintaining a proper distance. A total of ten plant were kept in each pot. Second weeding was done after 35 days after sowing. The crop was protected from the attack of aphids by spraying Ektara @ 2 ml/litre of water. The insecticide Ektara @ 2 ml/litre was applied for the first time 15 days after sowing and it was applied with a regular interval. The insecticides were applied in the evening and not spray in same days of fungicide spray.

3.12 Preparation and application of spray solution

The fungicidal suspension was prepared by mixing with required amount of fungicide (Rovral 50 WP @ 0.2%) with tap water. 20 g Rovral 50 WP was mixed in 10 L water for preparing 0.2% spray solution. The first spray was done at 7 days after sowing and others were sprayed with 7 days interval. The last spray was done at 77 days after sowing. Every time the fungicide was freshly prepared prior to application and the spray tank was thoroughly cleaned before filling with new materials. The insecticides were applied in the evening and not spray in same days of insecticide spray. Special attention was given to complete coverage of the growing plants with the fungicides. Adequate precaution was taken to avoid drifting of spray materials from one plot to neighboring ones. The control pot was sprayed with plain water only.

Common	Chemical name	Active	Doses used
name		ingredients	
Rovral 50 WP	3-(3,5 dichlorophenyl)-N-	Iprodione	0.2% of the
	(methylethyl)-2,4	(50%)	commercial
	dioxoimidazolidene carboxamide		formulation
	$(C_{3}H_{13})_{3}N_{3}C_{12}$		

Table 1: Details of fungicio	le
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3.13 Isolation and identification of pathogens from leaf

From experimental plot, diseased leaves were collected and cut into pieces (4 diameter) and surface sterilized with $HgCl_2$ (1:1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and then blot dry and placed into acidified PDA media in petridish. The plates containing leaf pieces were placed at room temperature for seven

days for incubation. When the fungus grew well and sporulated, then the slide was prepared from the pure culture and was identified under microscope with the help of relevant literature (Haque 2012).

3.14. Harvesting of crop

When 80% of the plants showed symptoms of maturity i.e. straw colored leave, stem, siliquae was noticed the crop was harvested as seed yield taken. At maturity, plants were harvested by uprooting and then they were tagged properly. Data were recorded on different parameters from these harvested plants.

3.15 Collection of data

The following parameters were considered for data collection.

Disease incidence and severity

- a. Percent leaf infection
- b. Percent leaf area diseased (% LAD)
- c. Percent pod infection
- d. Percent pod area diseased (% PAD)
- e. Percent Disease Index (PDI) was calculated using %LAD rate

Growth parameters

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

Yield and yield contributing characters

- a. Number of pods/plant
- b. 1000-seed weight (g)
- c. Yield (kg/ha)

Harvested seed

- a. Percent seed germination
- b. Percent seed infection

3.16 Procedure of data collection

As there were ten plants in each pot, so all plant were selected for collection of data.

3.16.1 Percent leaf infection

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

% leaf infection = Total number of inspected leaf × 100

3.16.2 Percent leaf area diseased

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

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

3.16.3 Percent pod infection

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

% Pod infection = Total number of inspected pod × 100

3.16.4 Percent pod area diseased

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

% Pod area diseased = Total pod area inspected Total pod area inspected

3.16.5 Number of leaves per plant

Number of leaves per plant data was also recorded before and after flowering from all the plants of each pot.

3.16.6 Number of branches per plant

Number of branches per plant data was also recorded before and after flowering from all the plants of each pot.

3.16.7 Plant height

Plant height was measured in centimeter by a meter scale at vegetative and reproductive stage and their average data was recorded per replication. For plant height the ground surface to the top of the main shoot and the mean height were expressed in cm.

3.16.8 Number of pods per plant

Number of pods per plant data was recorded by counting all the pods from all of the plants in a pot.

3.16.9 1000 seed weight (g)

One thousand grains were randomly counted and selected from the stock seed and weighed in gram by digital electric balance. It was expressed as 1000-seed weight in gram (g).

3.16.10 Yield (Kg/ha)

Seed yield were recorded from each pot. After harvesting the plant was sun-dried and threshed. Seed were properly sun-dried and their weights recorded. Seed yield was then converted to kg/ha.

3.16.11 Estimation of percent disease index (PDI)

Percent disease index is the measurement of the amount of a disease in a population. It is also named as percent disease index (PDI) and measured by the following formula-

Sum of total disease ratting

Percent Disease Index =

Total no. of observation \times Maximum grade in the scale

 $- \times 100$

Disease scoring scale:

Disease scoring was calculated by using "0-5" scale (Harsfall and Barnet, 1945), is given bellow-

% Leaf Area	Grade	No. of	Disease rating (No. of
Diseased (LAD)		observation	observation x Grade)
0	0		
0.1-5.0	1		
5.1-12.0	2		
12.1-25.0	3		
25.1-50.0	4		
>50%	5		
Total		Total No. of	Total sum of disease
		observation=	ratting=

Regression equation

For simulation of mathematical point model for estimation of yield loss regression equation was used as bellow:

 $\widehat{\mathbf{Y}} = \overline{\mathbf{Y}} + \mathbf{b} \ (\mathbf{Xi} - \overline{\mathbf{X}})$

Here, $\widehat{\mathbf{Y}}$ = Predicted yield loss (%)

Xi = Disease severity (i = 1,2,3,....n)

b = Regression value/Regression Co-efficient

$$\overline{X} = \frac{\Sigma X}{N}$$
 (N = No. of observation)

$$\overline{Y} = \frac{\Sigma Y}{N}$$
 (N = No. of observation)

Regression value (b) = $\Sigma XY - \frac{\Sigma X \cdot \Sigma Y}{N} = \frac{\Sigma XY - \frac{\Sigma X \cdot \Sigma Y}{N}}{\Sigma (Xi - \overline{X})^2}$

3.17 Germination and seed health test

For germination and seed health testing 400 seeds randomly drawn from each sample were tested in the standard technique (ISTA, 2000). Seeds were placed on three layers of moist blotting paper (Whatman no. 1) contained in petridishes. In each petridish, 25 seeds were placed in equidistance. All the plates with seeds were incubated at room temperature $(25\pm 20 \text{ C})$ under 12 hours cycle of alternate Near Ultra Violet (NUV) light and darkness. Watering was done as and when required. Germination of seedling and seed infection by Alternaria spp. were recorded. Results were expressed as percent seed germination. After 7-10 days of incubation, each seed was observed under sterio-binocular microscope to detect the presence of *Alternaria* spp.

3.18 Statistical analysis

All the data collected on different parameters were statistically analyzed by following the analysis of variance (ANOVA) technique and mean differences were adjusted by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) using the MSTAT-c computer package program. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.



Figure 1. Application of treatment according to the pot



Figure 2: Light irrigation was applied in the plot



Figure 3: Growing crops in the pot

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect on percent leaf infection

The effect of selected treatments on percent leaf infection for grey blight of mustard at different days after sowing (DAS) was recorded and presented in Table 2. Different treatments had significant influence on percent leaf infection for grey blight of mustard (SAU Sharisha 3) at different days after sowing (DAS) and the percent leaf infection ranged from 0-31.06%, 0-59.42% and 0-82.33% respectively at 65, 75 and 85 DAS (Table 2 and appendix VI). Percent leaf infection of mustard increased gradually with the advancement of crop growth. In all growth stage, treatment T₁₁ performed the best in respect of percent leaf infection, whereas T₀ performed the worst. At 85 days after sowing (DAS), the highest percent leaf infection (82.33%) was found in T₀ (control) and no leaf infection (0.00%) was recorded in treatment T₁₁ where eleven spraying were done with Rovral 50 WP @ 0.2% at every 7 days interval. The inhibition of leaf infection gradually decreased with the decrease of number of sprays. As a result, the order of efficacy of management practices in terms of leaf infection (percentage) is T₁₁>T₁₀>T₉>T₈>T₇>T₆>T₅>T₄>T₃>T₂>T₁>T₀.

4.2 Effect on percent leaf area diseased

The effect of selected treatments on leaf area diseased (LAD) for grey blight of mustard at different days after sowing (DAS) was presented in Table 3. Different treatments (number of spray) had significant effect on percent leaf area diseased for grey blight of mustard at different days after sowing (DAS) and the percent leaf area diseased ranged from 0 - 11.45%, 0 - 19.52% and 0 - 30.59% at 65, 75 and 85 DAS, respectively (Table 3 and appendix VI). Percent leaf area diseased for grey blight of mustard increased gradually with the advancement of crop growth. In all growth stages, treatment T₁₁ performed the best in respect of percent leaf area diseased, whereas T₀ performed the worst. At 85 days after sowing (DAS), the highest percent LAD (30.59%) was found in T₀ (control) and LAD (0.00%) was recorded in treatment T₁₁ where eleven spraying were done with Rovral 50 WP @ 0.2% at every 7 days interval. The inhibition of % LAD for grey blight of mustard was 100% in case of T_{11} where eleven sprays were applied. The inhibition of leaf area diseased gradually decreased with the decrease of number of sprays. As a result, the order of efficacy of management practices in terms of leaf area diseased (percentage) is $T_{11}>T_{10}>T_9>T_8>T_7>T_6>T_5>T_4>T_3>T_2>T_1>T_0$. (Table 3).

Treatments	%	Leaf infection	% Inhibition of percent	
	65 DAS	75 DAS	85 DAS	leaf infection over control
				at 85 DAS
To	31.06 a	59.42 a	82.33 a	0.00
T_1	20.00 b	50.72 b	73.67 b	10.52
T_2	12.84 c	39.11 c	63.33 c	23.08
T 3	10.58 d	33.64 d	52.33 d	36.44
T_4	9.83 d	29.14 e	41.67 e	49.39
T 5	8.61 e	27.11 f	30.00 f	63.56
T 6	4.70 f	19.43 g	23.00 g	72.06
T_7	3.52 g	10.87 h	14.00 h	83.00
T_8	1.30 h	6.40 i	10.33 i	87.45
Т9	1.12 hi	3.20 ј	5.67 j	93.11
T 10	0.57 hi	1.07 k	2.67 jk	96.76
T ₁₁	0.00 i	0.00 k	0.00 k	100.00
CV (%)	8.04	4.65	6.35	
LSD(0.05)	1.16	1.80	3.50	

 Table 2. Effect of different treatments on percent leaf infection for grey blight of

 mustard at different days after sowing (DAS)

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[Here, T_0 = Control (Foliar spraying with plain water only); T_1 = One foliar spraying with Rovral 50 WP @ 0.2%; T_2 = Two foliar spraying with Rovral 50 WP @ 0.2%; T_3 = Three foliar spraying with Rovral 50 WP @ 0.2%; T_4 = Four foliar spraying with Rovral 50 WP @ 0.2%; T_5 = Five foliar spraying with Rovral 50 WP @ 0.2%; T_6 = Six foliar spraying with Rovral 50 WP @ 0.2%; T_7 = Seven foliar spraying with Rovral 50 WP @ 0.2%; T_8 = Eight foliar spraying with Rovral 50 WP @ 0.2%; T_9 = Nine foliar spraying with Rovral 50 WP @ 0.2%; T_{10} = Ten foliar spraying with Rovral 50 WP @ 0.2% and T_{11} = Eleven foliar spraying with Rovral 50 WP @ 0.2%]

8 1	8		·	8	
Treatments	% Leaf A	Area Diseaseo	d (LAD)	% Inhibition of LAD ove	
-	65 DAS	75 DAS	85 DAS	control at 85 DAS	
To	11.45 a	19.52 a	30.59 a	0.00	
T ₁	10.00 b	17.34 b	28.55 b	6.67	
T_2	7.69 c	14.96 c	23.52 c	23.11	
T 3	6.76 d	12.68 d	20.83 d	31.91	
T_4	5.84 e	11.02 e	18.39 e	39.88	
T 5	4.85 f	9.07 f	15.97 f	47.79	
T 6	3.66 g	6.76 g	13.23 g	56.75	
T 7	2.67 h	5.83 h	10.97 h	64.14	
T 8	1.97 i	3.32 i	9.54 i	68.81	
Т9	1.42 j	2.29 j	7.18 ј	76.53	
T 10	0.64 k	0.93 k	3.41 k	88.85	
T 11	0.001	0.001	0.001	100.00	
CV (%)	5.40	6.26	3.97		
LSD(0.05)	0.43	0.90	1.00		

 Table 3. Effect of different treatments on percent leaf area diseased (%LAD) for

 grey blight of mustard at different days after sowing (DAS)

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[Here, T_0 = Control (Foliar spraying with plain water only); T_1 = One foliar spraying with Rovral 50 WP @ 0.2%; T_2 = Two foliar spraying with Rovral 50 WP @ 0.2%; T_3 = Three foliar spraying with Rovral 50 WP @ 0.2%; T_4 = Four foliar spraying with Rovral 50 WP @ 0.2%; T_5 = Five foliar spraying with Rovral 50 WP @ 0.2%; T_6 = Six foliar spraying with Rovral 50 WP @ 0.2%; T_7 = Seven foliar spraying with Rovral 50 WP @ 0.2%; T_8 = Eight foliar spraying with Rovral 50 WP @ 0.2%; T_9 = Nine foliar spraying with Rovral 50 WP @ 0.2%; T_{10} = Ten foliar spraying with Rovral 50 WP @ 0.2% and T_{11} = Eleven foliar spraying with Rovral 50 WP @ 0.2%]

4.3 Effect on percent pod infection

The effect of selected treatments on percent pod infection for grey blight of mustard at different days after sowing (DAS) was recorded and presented in Table 4. Different treatments had significant influence on percent pod infection for grey blight of mustard (SAU Sharisha 3) at different days after sowing (DAS) and the percent pod infection ranged from 0 - 20.68%, 1 - 41.25% and 0 - 53.33% at 70, 80 and 90 DAS, respectively (Table 4 and appendix VII). Percent pod infection of mustard increased gradually with the advancement of crop growth. In all growth stage, treatment T₁₁ performed the best in respect of percent pod infection, whereas T₀ performed the worst. At 90 days after sowing (DAS), the highest percent pod infection (53.33%) was found in T₀ (control) and lowest pod infection (1.00%) was recorded in treatment T₁₁ where eleven spraying were done with Rovral 50 WP @ 0.2% at every 7 days interval. The inhibition of pod infection gradually decreased with the decrease of number of sprays.

4.4 Effect on percent pod area diseased

The effect of selected treatments on pod area diseased (PAD) for grey blight of mustard at different days after sowing (DAS) was presented in Table 5. Different treatments (no. of spray) had significant effect on percent pod area diseased for grey blight of mustard at different days after sowing (DAS) and the percent pod area diseased ranged from 0 -8.22%, 0 - 13.83% and 0 - 22.00% at 70, 80 and 90 DAS, respectively (Table 5 and appendix VIII). Percent pod area diseased for grey blight of mustard increased gradually with the advancement of crop growth. In all growth stage, treatment T₁₁ performed the best in respect of percent pod area diseased, whereas T₀ performed the worst. At 90 days after sowing (DAS), the highest percent pod area diseased (22.00%) was found in T₀ (control) and no pod area diseased (0.00%) was recorded in treatment T₁₁ where eleven spraying were done with Rovral 50 WP @ 0.2% at every 7 days interval. The inhibition of pod area diseased for grey blight of mustard was 100% in case of T₁₁ where eleven sprays were applied. The inhibition of pod area diseased gradually decreased with the decrease of number of sprays.

Treatments	% Pod infection			% Inhibition of percent
	70 DAS	80 DAS	90 DAS	pod infection over control
				at 90 DAS
To	20.68 a	41.45 a	53.33 a	0.00
T_1	16.40 b	35.49 b	41.67 b	21.86
T_2	10.40 c	31.04 b	37.00 b	30.62
T 3	9.98 c	31.00 b	31.67 c	40.62
T 4	7.08 d	21.46 c	25.00 d	53.12
T 5	5.65 de	21.00 c	24.67 d	53.74
T 6	4.03 ef	13.89 d	18.33 e	65.63
T 7	3.42 fg	7.22 e	10.00 f	81.25
T ₈	1.97 gh	3.94 ef	6.67 fg	87.49
Т9	0.98 hi	2.06 f	4.00 gh	92.50
T 10	0.83 hi	1.66 f	2.67 gh	94.99
T ₁₁	0.00 i	0.00 f	1.00 h	98.12
CV (%)	14.62	15.67	14.04	
LSD(0.05)	1.64	4.55	4.97	

 Table 4. Effect of different treatments on percent pod infection for grey blight of

 mustard at different days after sowing (DAS)

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

[Here, T_0 = Control (Foliar spraying with plain water only); T_1 = One foliar spraying with Rovral 50 WP @ 0.2%; T_2 = Two foliar spraying with Rovral 50 WP @ 0.2%; T_3 = Three foliar spraying with Rovral 50 WP @ 0.2%; T_4 = Four foliar spraying with Rovral 50 WP @ 0.2%; T_5 = Five foliar spraying with Rovral 50 WP @ 0.2%; T_6 = Six foliar spraying with Rovral 50 WP @ 0.2%; T_7 = Seven foliar spraying with Rovral 50 WP @ 0.2%; T_8 = Eight foliar spraying with Rovral 50 WP @ 0.2%; T_9 = Nine foliar spraying with Rovral 50 WP @ 0.2%; T_{10} = Ten foliar spraying with Rovral 50 WP @ 0.2% and T_{11} = Eleven foliar spraying with Rovral 50 WP @ 0.2%]

e		·	0
% Pod	Area Diseased	l (PAD)	% Inhibition of PAD over
70 DAS	80 DAS	90 DAS	control at 85 DAS
8.22 a	13.83 a	22.00 a	0.00
7.68 a	13.36 a	21.00 a	4.55
6.21 b	12.10 ab	17.00 b	22.73
5.88 b	13.26 d	14.67 c	33.32
4.51 c	10.49 bc	13.00 c	40.91
3.98 c	9.90 c	10.33 d	53.05
2.29 d	7.69 d	9.00 d	59.09
1.64 de	5.66 e	6.00 e	72.73
1.28 ef	4.48 e	5.00 ef	77.27
0.75 efg	1.84 f	2.33 fg	89.41
0.47 fg	1.05 f	1.00 gh	95.45
0.00 g	0.00 f	0.00 h	100.00
14.61	15.20	12.92	
0.86	0.69	2.15	
	70 DAS 8.22 a 7.68 a 6.21 b 5.88 b 4.51 c 3.98 c 2.29 d 1.64 de 1.28 ef 0.75 efg 0.47 fg 0.00 g 14.61	70 DAS80 DAS8.22 a13.83 a7.68 a13.36 a6.21 b12.10 ab5.88 b13.26 d4.51 c10.49 bc3.98 c9.90 c2.29 d7.69 d1.64 de5.66 e1.28 ef4.48 e0.75 efg1.84 f0.47 fg1.05 f0.00 g0.00 f14.6115.20	8.22 a $13.83 a$ $22.00 a$ $7.68 a$ $13.36 a$ $21.00 a$ $6.21 b$ $12.10 ab$ $17.00 b$ $5.88 b$ $13.26 d$ $14.67 c$ $4.51 c$ $10.49 bc$ $13.00 c$ $3.98 c$ $9.90 c$ $10.33 d$ $2.29 d$ $7.69 d$ $9.00 d$ $1.64 de$ $5.66 e$ $6.00 e$ $1.28 ef$ $4.48 e$ $5.00 ef$ $0.75 efg$ $1.84 f$ $2.33 fg$ $0.47 fg$ $1.05 f$ $1.00 gh$ $0.00 g$ $0.00 f$ $0.00 h$

 Table 5. Effect of different treatments on percent pod area diseased (%PAD) for

 grey blight of mustard at different days after sowing (DAS)

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

4.5 Effect on growth parameters

4.5.1 Number of leaves per plant

Number of leaves per plant was differ significantly due to the application of different treatments (Table 6 and appendix VIII). The highest number of leaves per plant (63.67) was recorded in T_{11} (eleven foliar spraying with Rovral 50 WP @ 0.2%) treatment and the lowest number of leaves per plant (58.00) was obtained from T_0 (control) treatment which was statistically identical with T_3 (58.33) treatment.

Treatments		Growth parameters	
	No. of leaf/plant	No. of	Plant height
		branches/plant	(cm)
To	58.00 d	5.97 k	76.60 d
T_1	59.00 cd	6.32 j	80.86 c
T_2	60.00 cd	6.68 i	81.48 bc
T 3	58.33 d	7.04 h	82.42 bc
T 4	60.67 abcd	7.43 g	83.20 abc
T 5	61.00 abcd	7.56 f	84.53 ab
T 6	61.00 abcd	7.67 e	84.50 ab
Τ7	60.67 abcd	8.11 d	84.33 abc
T ₈	62.00 abc	8.42 c	85.00 ab
Τ9	60.33 bcd	8.91 b	85.00 ab
T 10	63.33 ab	8.94 b	86.27 a
T 11	63.67 a	9.09 a	86.33 a
CV (%)	2.66	0.81	2.30
LSD(0.05)	2.68	1.10	3.18

Table 6. Effect of different treatments on growth parameters of mustard

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

4.5.2 Number of branches per plant

The differences in number of branches per plant with number of applications of fungicide was statistically significant. The highest number of branches per plant (9.09) was recorded in case of T_{11} (eleven foliar spraying with Rovral 50 WP @ 0.2% at 7 days interval) treatment followed by T_{10} (8.94) and T_9 (8.91). On the other hand, the lowest number of branches per plant (5.97) was obtained from T_0 (control) treatment, followed by T_1 (6.32) and T_2 (6.68) (Table 6).

4.5.3 Plant height

The number of sprays of fungicide had a significant impact on the height of mustard plant (Table 6 and appendix VIII). The tallest plant (86.33 cm) was recorded from T_{11} (eleven times spraying of Rovral 50 WP @ 0.2% at 7 days interval) which was statistically at par with T_{10} treatment (86.27 cm). On the other hand, when no fungicide was applied (T_0) then the shortest plant was recorded (76.60 cm) which was statistically identical with all other treatments.

4.6 Effect on yield and yield contributing characters of mustard

4.6.1 Number of pods per plant

Number of pod per plant was found to differ significantly due to the application of different number of spray. The highest number of pod per plant (178.8) was recorded in case of T_{11} (eleven foliar spraying with Rovral 50 WP @ 0.2% at 7 days interval) treatment and the lowest number of pod per plant (121.4) was obtained from T_0 (control) treatment (Table 7).

4.6.2 1000 seed weight (g)

1000 seed weight was significantly affected by grey blight disease of mustard under different treatments. Among the treatments, T_{11} was gave the best results (3.75 gm) for 1000-seed weight. The lowest 1000-seed weight (3.42 gm) was found in control treatment (T_0).

4.6.3 Yield (kg/ha)

Significant variation of different treatments was found on yield (kg/ha). Maximum yield (934.3 kg/ha) was obtained from T_{11} (eleven foliar spraying with Rovral 50 WP) treated plot which was statistically similar with T_{10} (899.7 kg/ha) and followed by T_9 (853.5 kg/ha), T_8 (807.4 kg/ha). The lowest yield (357.6) was recorded from T_0 (control) treatment.

Treatments	Yield and y	ield contributing	characters	% Yield increased
	No. of	1000 seed Yie		over control
	pod/plant	weight (g)	(kg/ha)	
To	121.4 e	3.42 d	357.6 i	0.00
T ₁	126.1 de	3.48 cd	392.2 hi	9.68
T 2	127.1 cde	3.50 cd	438.3 gh	22.57
T 3	131.3 cde	3.52 c	472.9 g	32.24
T 4	138.2 bcde	3.56 c	507.5 fg	41.92
T 5	152.9 abcd	3.56 c	553.6 ef	54.81
T 6	153.6 abcd	3.65 b	599.8 e	67.73
T 7	154.1 abcd	3.68 ab	726.6 d	103.19
T 8	156.5 abc	3.70 ab	807.4 c	125.78
Т9	162.2 ab	3.72 ab	853.5 bc	138.67
T 10	169.8 a	3.73 ab	899.7 ab	151.59
T ₁₁	178.8 a	3.75 a	934.3 a	161.27
CV (%)	10.65	1.34	7.11	
LSD(0.05)	26.07	0.07	7.64	

 Table 7. Effect of different treatments on yield and yield contributing characters of mustard

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

4.7 Percent seed germination

Percent seed germination was significant influenced by the application of different treatments (Fig.4). Seed obtained from T_{11} (Eleven foliar spraying with Rovral 50 WP @ 0.2% at 7 days interval) treated plot showed the maximum percent seed germination (100%). Seed obtained from control plots showed the minimum germination percentage (82.23%).

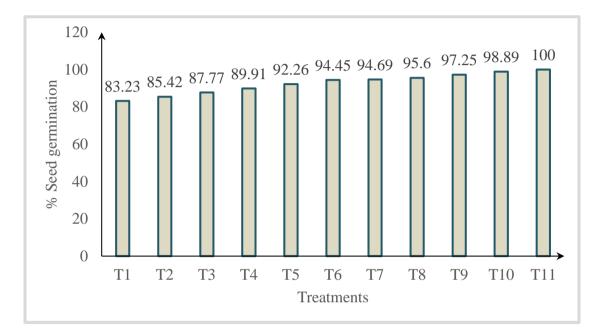


Figure 4. Effect of different treatments on percent seed germination of mustard.

4.7.2 Percent seed infection

Percent seed infection by *Alternaria* spp. of harvested seeds was varied due to the application of different number of treatments. Comparatively lower seed infection was found in the seed lot obtained from treated plot with higher number of sprays. Seeds obtained from control treatment showed the highest percent seed infection (21.69%) while seeds obtained from T_{11} (eleven foliar spraying with Rovral 50 WP) treated plots showed the lowest seed infection (0.0%).

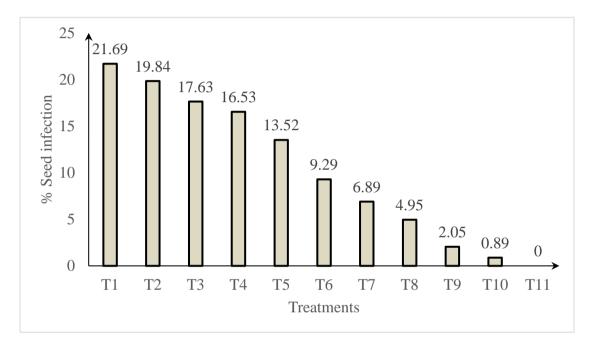


Figure 5. Effect of different treatments on percent seed infection of mustard.

4.8 Estimation of mathematical model for yield loss assessment

Using the variation of percent disease index (PDI) and corresponding yield loss from multiple treatment experiment, the predicted yield loss (Y) was calculated using the working formula of regression equation and presented in Table 8. Further, using the predicted yield loss and corresponding disease severity the yield loss assessment model was constructed as Y = 0.50 + 2.32Xi. By setting any Xi's value (PDI) in the formula, the yield loss of mustard due to grey blight disease could be estimated.

	1 81	-	-	
Treatment	Percent disease	Yield	Yield loss	% Yield loss
	index (PDI)	(kg/ha)	(kg/ha)	(Y)
T_0	Xi= 28.58	357.6 i	576.7	Yi=61.72
T_1	Xii=25.32	392.2 hi	542.1	Yii=58.02
T_2	Xiii=23.52	438.3 gh	496	Yiii=53.08
T ₃	Xiv=20.12	472.9 g	461.4	Yiv=49.38
T_4	Xv=15.42	507.5 fg	426.8	Yv=45.68
T ₅	Xvi=13.53	553.6 ef	380.7	Yvi=40.74
T_6	Xvii=12.34	599.8 e	334.5	Yvii=35.08
T_7	Xviii=10.34	726.6 d	207.7	Yviii=22.23
T_8	Xix=8.78	807.4 c	126.9	Yix=13.18
T 9	Xx=6.52	853.5 bc	80.8	Yx=8.64
T_{10}	Xxi=1.25	899.7 ab	34.6	Yxi=3.70
T ₁₁	Xxii=0.0	934.3 a	0	Yxii=0.00
Total	560			

 Table 8. Predicted yield loss calculated by percent disease index (PDI) and corresponding yield loss from multiple treatment experiment

[Here, T_0 = Control (Foliar spraying with plain water only); T_1 = One foliar spraying with Rovral 50 WP @ 0.2%; T_2 = Two foliar spraying with Rovral 50 WP @ 0.2%; T_3 = Three foliar spraying with Rovral 50 WP @ 0.2%; T_4 = Four foliar spraying with Rovral 50 WP @ 0.2%; T_5 = Five foliar spraying with Rovral 50 WP @ 0.2%; T_6 = Six foliar spraying with Rovral 50 WP @ 0.2%; T_7 = Seven foliar spraying with Rovral 50 WP @ 0.2%; T_8 = Eight foliar spraying with Rovral 50 WP @ 0.2%; T_9 = Nine foliar spraying with Rovral 50 WP @ 0.2%; T_{10} = Ten foliar spraying with Rovral 50 WP @ 0.2% and T_{11} = Eleven foliar spraying with Rovral 50 WP @ 0.2%]

Now making a correlation graph using the corresponding Xi's and Y values, the following graph was obtained.

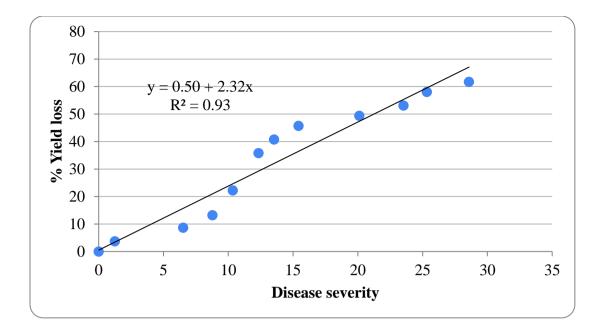


Figure 6. Mathematical model point for estimation of yield loss of mustard due to grey blight disease caused by *Alternaria* spp.



Figure 7. Growing seedling in the pot



Figure 8. Infected Grey light dieases leveas of mustar



Figure 9. Conidia of *Alternaria* spp. Under stereo microscore at (10×4x)

DISCUSSIONS

In the pot experiments the application of fungicides with different spray schedule had significant effect in reducing the disease incidence, severity and increasing the seed yield. Among the treatments, eleven foliar spraying with Rovral 50 WP @ 0.2% completely controlled leaf infection which was statistically identical with the application of ten foliar spraying. In case of percent leaf area diseased (LAD), no disease was observed in response to the application of eleven foliar spraying with Rovral 50 WP @ 0.2%. It was observed that percent leaf infection and leaf area diseased increased gradually with the advancement of crop growth but inhibited disease incidence and severity with the increasing number of sprayings of Royral 50 WP @ of 0.2%. The inhibition of pod infection as well as pod area diseased were for highest where 11 sprays were applied and the inhibition of pod infection and pod area diseased gradually decreased with the decrease of number of sprays. Results are also agreed with reported results regarding the disease severity index (%)/ leaf area diseased (% LAD) of mustard. Alam (2007) reported that the effect of different treatments on leaf area diseased (% LAD) was found to be significant at different days after sowing (DAS) in response to the application of different chemicals fungicides and botanicals. Percent leaf area diseases (LAD) of mustard (SAU Sarisha-1) increased gradually with the advancement of crop growth. Kolte and Awasthi (1980) observed that to get effective control of Alternaria blight, the fungicide should be sprayed at appropriate time and at appropriate intervals. Kolte et al. (1989) suggested the iprodione (Rovral) is superior to mancozeb for control of pod infection of Alternaria. Four sprays of Rovaral at 10 days interval reduced the Alternaria blight incidence and increase the seed yield of mustard (Hossain, 2003). Prasad and Lallu (2006) revealed that spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early date of sowing was the best combination in reducing the first disease severity on leaves (18.7%) and pods (10.4%) higher realization yield (1295.8 kg/ha), 1000 seed weight (5.12 g) and oil content (42.6%). Ayub et al. (1996) reported that iprodione (Rovral) reduced disease severity and increased seed yield when applied on 40 days old plants. So fungicidal sprays can reduce the disease was recorded earlier in ALB on mustard (Ayub et al., 1996; Mahapatra and Das, 2013). The results of the experiment clearly

indicate that eleven fungicidal sprays on the susceptible variety is sufficient to reduce the disease levels.

The effect of treatments on yield contributing characters like number of pod per plant, 1000 seed weight was remarkably influenced in seed yield. The highest seed yield (27.33 g/plot) was obtained from the plot where eleven foliar spraying was applied with Rovral 50 WP @ 0.2% against the disease that increased seed yield by 90.72% compared to control. It was also observed that seed yield increased gradually with the increase of number of spraying of Rovral 50 WP @ of 0.2%. The findings of the field experiments are well supported by the previous researchers. Alam (2007) while working with fungicides and plant extracts against the Alternaria blight of mustard caused by Alternaria brassicae and Alternaria brassicicola, reported that Rovral 50 WP (0.2%) was the potential fungicide in controlling disease incidence and severity and increasing seed yield by 48.19 % over control. Hossain and Miah (2006) reported that, in field trial, Rovral 50 WP (iprodione) significantly reduced the disease incidence and severity and increased seed yield when applied alone or in combination with other fungicides. Ferdous et al., (2002) reported that foliar spray of Rovral 0.1% concentration given at 7 days interval remarkably reduced Alternaria blight intensity increasing seed yield. These findings are in agreement with the reports of Singh and Singh (2006) that 3 sprayings of mancozeb 75% WP (0.25%) were most economical in managing the ALB on linseed on susceptible variety like Chambal and mustard (Mahapatra and Das, 2016a). These findings are also similar to those by Amaresh and Nargund (2004) that AUDPC values of alternaria leaf blight and rust of sunflower were less in higher number of spray of chlorothalonil, but low for ALB by Iprodion treatment. The present study also emphasizes that the treatment cost involved in the use of fungicides must be taken into consideration while selecting the fungicides for the effective and economical control.

From the regression equation Y = 0.50 + 2.32Xi, it is revealed that for increase of 1% disease severity value the mustard yield decreased by 2.32%. Thus, setting any disease severity value, the corresponding crop loss could be calculated.

Seed health regarding seed infection and seed germination were found to differ significantly due to the application of different treatments. No seed infection by *Alternaria* spp. and 100% seed germination was obtained from the plot treated with eleven fungicides spraying with Rovral 50 WP @ 0.2%. Seeds obtained from control plots showed the lowest seed germination and maximum seed infection. The present findings corroborate with the findings of previous research report. Haque (2012) also found 100% seed germination and no seed infection when nine sprays with Rovral @0.2% were done in the field. Anonymous (1992) reported that foliar spray of Rovral significantly reduced the seed borne infection of Alternaria spp. and increased germination percentage of mustard seed. It was reported that, seed born infection of Alternaria spp. was reduced above 90% and seed germination was increased above 9% over control while seed infection was reduced up to 18.8% with 3 times foliar spray of Rovral.

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in farm of Sher-e-Bangla Agricultural University, Dhaka during the period from December, 2021 to March, 2022 to determine the yield loss assessment of mustard due to grey blight disease. The experiment comprised 12 different treatments of fungicide viz., $T_0 = \text{Control}$ (Foliar spraying with plain water only); $T_1 = \text{One}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_2 = \text{Two}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_2 = \text{Two}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_3 = \text{Three}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_4 = \text{Four}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_5 = \text{Five}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_7 = \text{Seven}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_7 = \text{Seven}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_{10} = \text{Ten}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%; $T_9 = \text{Nine}$ foliar spraying with Rovral 50 WP @ 0.2%;

Data were collected on disease incidence and severity, yield and yield contributing characters. Data were analyzed and the mean value was adjudged with Duncan Multiple Ranges Test (DMRT).

SUMMARY

The study revealed that application of fungicide (Rovral 50 WP @ 0.2%) at different frequency significantly influenced almost all of the parameters. The lowest percent of leaf infection (0.0%), percent leaf area diseased (0.0%), pod infection (0.0%), pod area diseased (0.0%), were recorded from eleven foliar spraying with Rovral 50 WP. The highest percent of leaf infection (82.33%), percent leaf area diseased (30.59%), pod infection (53.33%), pod area diseased (22.00%) were recorded from control (T₀). Leaf infection, leaf area diseased, pod infection, pod area diseased decreased with increasing number of sprayings with Rovral 50 WP @ 0.2%. The order of efficacy of management practices in terms of leaf infection, LAD, pod infection and PAD (%) is $T_{11}>T_{10}>T_9>T_8>T_7>T_6>T_5>T_4>T_3>T_2>T_1>T_0$.

The number of sprays of fungicide had a significant impact of plant growth parameter. The no. of leaf/plant; no. of branches/plant and plant height also significantly influenced by the application of fungicide. The maximum no. of leaf/plant (63.67), no. of branches/plant (9.09) and plant height (86.33 cm) were recorded when Rovral 50 WP @0.2% at 7 days interval was applied in the pot and minimum number of leaf/plant (58.00), number of branches/plant (5.97) and plant height (76.60 cm) was found from control.

Yield contributing characteristics also influenced by the number of fungicide applied in the pot. Yield per pot was increased with the increase of number of spray of fungicide. The maximum yield (934.3 kg/ha) was recorded when eleven field spray was done with Rovral 50 WP @ 0.2% at 7 days interval.

The highest seed germination percentage and the lowest seed infection obtained from the pot of eleven foliar spraying with Rovral 50 WP @ 0.2%. The lowest seed germination percentage and highest seed infection obtained from untreated pot (control).

The result of the present study generated some information which may help to manage grey blight incidence in mustard. Hence, the present study may be concluded as follows:

Application of Rovral 50 WP @ 0.2% at 7 days interval for eleven times will be suitable for completely control of grey blight incidence and severity and giving the highest good quality seed of mustard under Agro-ecological zone of Modhupur Tract, AEZ-28 of Bangladesh. The regression equation Y = 0.50 + 2.32Xi could be used to calculate the yield loss of mustard due to grey blight. Further study might be conducted at the same Agro Ecological Condition for at least 3 consecutive years to justify to constructed yield loss assessment model.

CHAPTER VI

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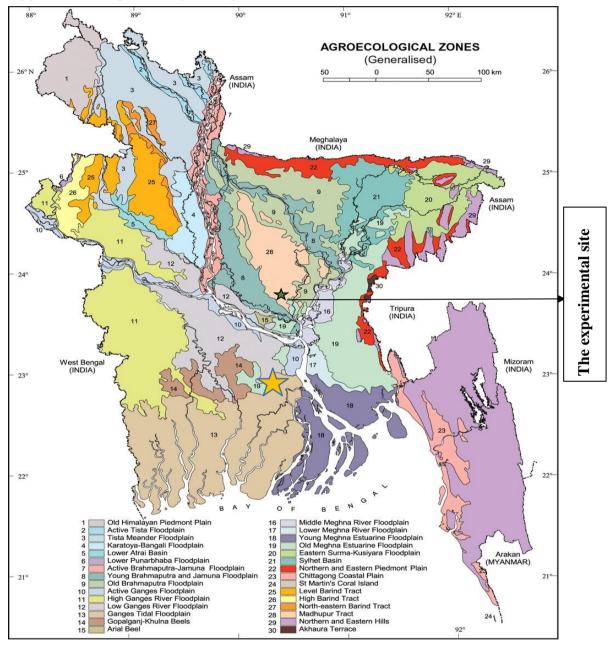
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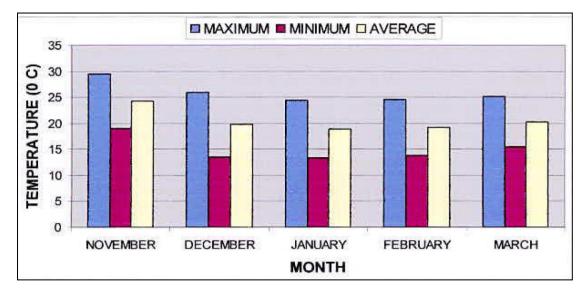
CHAPTER VII

APPENDICES

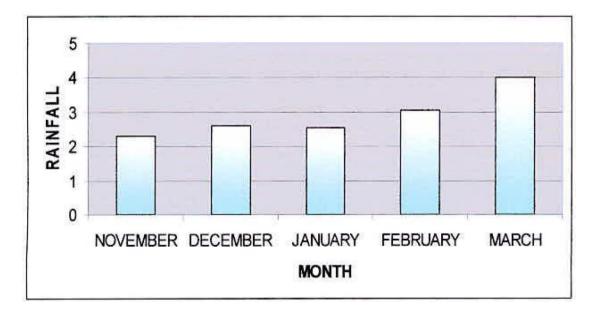
Appendix I: Map showing the experimental site



Appendix II: Monthly average, maximum and minimum air temperature (⁰C) of the experimental site, Dhaka during the growing time (November, 2021 to March 2022)



Appendix III: Monthly total rainfall (mm) of the experimental site, Dhaka during



the growing period (November, 2021 to March 2022)

Morphology	Characteristics
Location	SAU farm, Dhaka
Agro-ecological zone	Madhupur Tract (AEZ-28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur Clay
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level

Appendix IV: Morphological Characteristics of the Experimental Field

(FAO and UNDP, 1988)

Appendix V: Initial Physical and Chemical Characteristics of the Soil

Char	acteristics	Value
Mechanical fraction:	% Sand (2.0-0.02 mm)	22.26
	% Silt (0.02-0.002 mm)	56.72
	% Clay (<0.002 mm)	20.75
Textural Class		Silt Loam
pH (1:2.5 Soil-water)		5.9
Organi	c Matter (%)	1.09
Tot	al N (%)	0.06
Available K (ppm)		15.63
Available P (ppm)		10.99
Availa	ble S (ppm)	6.07

Appendix VI: ANOVA table of the experiment

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	10.21	2.104	10.49	0.0006
Treatment	11	2839.07	258.098	530.58	0.0000
Error	22	10.70	0.486		
Total	35	2859.98			

01: Percent leaf infection at 65 DAS

02: Percent leaf infection at 75 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	38.95	19.474	16.52	0.0000
Treatment	11	13075.52	1188.683	1008.09	0.0000
Error	22	25.94	1.179		
Total	35	13140.41			

03: Percent leaf infection at 85 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	147.17	73.583	16.49	0.0000
Treatment	11	27587.42	2507.947	562.05	0.0000
Error	22	98.17	4.462		
Total	35	27832.75			

04: Percent leaf area disease at 65 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	2.58	1.290	19.62	0.0000
Treatment	11	450.18	40.925	622.75	0.0000
Error	22	1.45	0.066		
Total	35	454.20			

05: Percent leaf area disease at 75 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	12.27	6.134	20.98	0.0000
Treatment	11	1410.61	128.237	438.59	0.0000
Error	22	6.43	0292		
Total	35	1429.31			

06: Percent leaf area disease at 85 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	21.73	10.867	29.96	0.0000
Treatment	11	3045.10	276.828	763.08	0.0000
Error	22	7.98	0.363		
Total	35	3074.82			

07: Percent pod infection at 70 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	25.13	12.564	12.76	0.0002
Treatment	11	1402.25	127.477	129.52	0.0000
Error	22	21.65	0.984		
Total	35	1449.03			

08: Percent pod infection at 80 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	184.37	92.186	12.23	0.0003
Treatment	11	7167.09	651.553	86.47	0.0000
Error	22	165.76	7.535		
Total	35	7517.22			

09: Percent pod infection at 90 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	27.17	13.583	1.51	0.2423
Treatment	11	9687.33	880.667	98.10	0.0000
Error	22	197.50	8.977		
Total	35	9912.00			

10: Percent pod area diseased at 70 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	7.21	3.603	13.19	0.0002
Treatment	11	278.06	25.278	92.54	0.0000
Error	22	6.01	0.273		
Total	35	291.28			

11: Percent pod area diseased at 80 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	37.58	18.789	13.34	0.0002
Treatment	11	854.05	77.641	55.12	0.0000
Error	22	30.09	1.408		
Total	35	922.61			

12: Percent pod area diseased at 90 DAS

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	7.06	3.528	2.10	0.1462
Treatment	11	1912.97	173.907	103.56	0.0000
Error	22	36.94	1.679		
Total	35	1956.97			

13: No. of leaf/plant

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	6.50	3.250	1.24	0.3079
Treatment	11	102.00	9.273	3.55	0.0055
Error	22	57.50	2.614		
Total	35	166.00			

14: No. of branches/plant

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	5.02	2.512	644.23	0.0000
Treatment	11	36.24	3.295	845.02	0.0000
Error	22	0.09	0.004		
Total	35	41.35			

15: Plant height (cm)

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	13.00	6.498	1.76	0.1952
Treatment	11	248.01	22.546	6.11	0.0002
Error	22	81.18	3.690		
Total	35	342.18			

16: No. of pods/plant

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	0.39	0.194	0.18	0.8355
Treatment	11	2128.97	193.543	180.34	0.0000
Error	22	23.61	1.073		
Total	35	2152.97			

17: 1000 seed yield (g)

Source of	Degree of	Sum of	Mean	F value	Probability
variance	freedom	squares	square		
Replication	2	0.13	0.064	27.46	0.0000
Treatment	11	0.42	0.038	16.33	0.0000
Error	22	0.05	0.002		
Total	35	0.60			