EFFICACY OF TRICHODERMA AND BOTANICALS IN CONTROLLING ALTERNARIA LEAF BLIGHT OF MUSTARD AND STUDY ON MORPHOLOGY OF THE PATHOGEN

A THESIS

BY

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This is to certify that the thesis entitled *"EFFICACY OF TRICHODERMA AND BOTANICALS IN CONTROLLING ALTERNARIA LEAF BLIGHT OF MUSTARD AND STUDY ON MORPHOLOGY OF THE PATHOGEN"* 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 *Abu Bakar Siddique*, Registration no. *04-01440*, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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ABSTRACT

Experiments were conducted at the Farm of Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur and in the laboratory of Plant Pathology division, during rabi season from the month of November'2009 - February'2010 to evaluate the efficacy of some selected *Trichoderma harzianum* strains and plant extracts against *Alternaria brassicae* and *Alternaria brassicicola* causing gray blight of mustard (*Brassica campestris* var. BARI Sarisha-15). Three *Trichoderma harzianum* strains and three plant extracts viz. Bashok (*Adhatoda vasica*) leaf extract, Bishkatali leaf extract and Marigold (*Tagetes patula*) were explored in the experiment. Among the *Trichoderma harzianum* strains and plant extracts, *Trichoderma harzianum* S1 showed the best performance in reducing disease incidence and disease severity as well as increasing seed yield against leaf blight of mustard. Seed infection by *Alternaria spp.* was reduced by 64.79% and seed yield was increased by 48.19% over control by the application of *Trichoderma harzianum* S1. Among the botanicals, Marigold showed the better performance. No promising results were shown by the rest of the treatments against leaf blight of mustard.

INTRODUCTION

A good number of oilseed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower and soybean are being cultivated in Bangladesh. The first three are considered as the major oil crops. Among these three the highest area and production of oil seed is contributed by mustard. Presently the requirement of the country's edible oil is about 1.4 million metric tons of which a maximum of about 0.55 million metric ton is being supplied from the local oil seed production. This huge shortage is being met by importing, which amount to about Tk 76,729 million (Bakr and Ahmed 2009). Rapeseed-mustard (*Brassica spp*) is the major oilseed crop of Bangladesh. Out of total cropped area of 13.728 million hectare, oil crops occupy only 0.34 million hectare which is about 2.5% of the total cropped area. Rapeseed-mustard covers 0.19 million hectare of land (60%) of the total oil-seed cropped area and produces 0.246 million tons yield (52.2%) (BBS, 2006-07)

Nevertheless, we get 10-12 g/capita/day oil from local production and import. The developed country like USA and EU countries, the consumption rate is 60g/capita/day. Mustard seeds contain 40-45% oil and 20-25% protein. Using local grain average 33% oil may be extracted. In this sub continent three species of *Brassica* are cultivated for oil purposes, viz. *Brassica campestries, Brassica juncia, Brassica napus* (Anonymous 2001).

Many factors are associated with the poor yield of rape seed mustard in Bangladesh. Diseases have been identified as one of the major causes (Ahmed, 1992). Rapeseed mustard suffers from about 14 diseases (Fungi 9, Virus-2, Bacteria-1, Nematode-1 and parasitic plant-1) in Bangladesh (Bakr *et al.* 2009). Among these diseases leaf blight caused by *Alternaria brassicae* is widely distributed and the most serious and devastating disease of rape seed mustard. The characteristic symptom is the development of circular spots on leafs and pods with concentric ring. Later on spots coalesce and ultimately leaves become blighted. The disease may cause 25% yield reduction at severe condition of infection (Anonymous, 2001).

The disease has otherwise been called leaf blight (*Alternaria brassicae*) causing blight of leaf, pod and stem. The disease has been reported as endemic in Bangladesh and all the cultivated mustard variety is susceptible to the disease. This disease causes 30-60% yield loss in Bangladesh. It also adversely affects the seed quality reducing seed size, seed discoloration and reduction in oil contents (Howlader *et al*, 1991).

There is no information on the resistant sources. The only way of controlling the disease is use of chemicals. Few fungicides have been identified for controlling the disease (Meah *et al*, 1988 and Howlader *et al,* 1985). There are non chemical methods which should be tried for controlling the disease. These include use of biological agents, botanicals, adjustment in cultural practices etc. Researches with these ideas have yielded good results but not much better than the use of chemicals.

Therefore the present study was undertaken to achieve the following objectives:

1. To determine the effect of different *Trichoderma* strains and plant extracts on the incidence and severity of leaf blight of mustard.
2. To determine the morphological variability of *Alternaria spp.* causing leaf blight of mustard.

REVIEW OF LITERATURE

Meena *et al* (2004) reported that the efficacy of plant extracts (from leaves of Azadirachta indica, Ocimum sanctum [O. tenuiflorum], Datura stramonium, Eucalyptus longifolia, Calotropis gigantea, Acacia nilotica, Parthenium hysterophorus, Bougainvillea sp. and Lantana camara; and bulbs of Allium sativum), biological control agent (Trichoderma viride isolates H-1, H-2, SI-1, GR, SI-2, B. P and T) and fungicides (mancozeb and carbendazim), alone or in combination, in controlling Indian mustard alternaria blight (Alternaria brassicae). Fungicides mancozeb and carbendazim caused 100% reduction in mycelial growth of Alternaria brassicae over control in vitro, while the 1% (w/v) aqueous bulb extract of Allium sativum and leaf extract of Acacia nilotica caused significant reductions. In dual culture, GR isolate of T. viride performed best among the test isolates of Trichoderma, causing 81 and 82% reduction in mycelial growth of Alternaria brassicae over the control. Performance of isolates SI-2, P and SI-1 of T. viride were at par ($P<0.01$) with that of GR isolate. Spraying of Alternaria brassicae at different ages of the mustard host plant showed that 75 days after sowing (d.a.s.) was the most critical age of the mustard plant for the development of Alternaria blight, followed by 45 d.a.s.

Rai and Singh (1980) reported the antagonistic effect of some leaf surface fungi against the pathogenic fungi *Alternaria brassicae* and *Drechslera graminea* in the field and in the laboratory. Application of spores of the test fungi to leaves, either collectively or individually, inhibited lesion development by the pathogens. Inhibition increased with increasing spore concentration and was highest when a composite spore mixture was used. The most antagonistic fungi were *Epicoccum*

purpurascens, Aureobasidium pullulons and *Cladosporium cladosporioides* in the case of *A. brassicae* and *E. purpurascens, Alternaria alternata* and *Aureobasidium pullulans* in the case of *D. graminea.* The metabolites of *Acremonium roseogriseum, Aspergillus terreus* and *C. cladosporioides* inhibited *A. brassicae.* Those of *A. pullulans, E. purpurascens* and *Trichoderma viride* reduced the activity of *D. graminea.* The most pronounced inhibitory effects were caused by a mixture of metabolites of all the test fungi. The most significant effects were observed when the spores of the leaf surface fungi or their metabolites were sprayed on leaves prior to inoculation of the pathogens. Maximum inhibition of *A. brassicae* in vitro was caused by *A. alternata,* followed by *T. viride* and *E. purpurascens.*

Stefania *et al* (2008) stated that the evaluation of several *Trichoderma* isolates as possible biocontrol agent in preliminary in vitro and in vivo assays led to the selection of two *Trichoderma* isolates characterized by their ability to reduce pathogen sporulation and antagonism towards the pathogen or competence for sugar beet phyllosphere. Repeated foliar applications of the liquid culture homogenate preceded by a single treatment of difenoconazole in 2 year trials under natural inoculum in field reduced the disease incidence and pathogen sporulation from the necrotic spots.

Mora and Earle (2001) reported that progeny from transgenic broccoli (cv. Green Comet) expressing a *Trichoderma harzianum* endochitinase gene were used to assess the interaction between endochitinase and the fungicide Bayleton in the control of *Alternaria brassicicola.* In vitro assays have shown synergistic effects of endochitinase and fungicides on fungal pathogens. Two month old transgenic and non-transgenic plants were sprayed with ED50 levels of Bayleton and/or

inoculated with an *A. brassicicola* spore suspension. Disease levels in non-sprayed transgenic plants were not statistically different from sprayed transgenic plants or from sprayed non-transgenic controls. Thus endochitinase-transgenic plants alone provided a significant reduction of disease severity, comparable to the protection by fungicide on non-transgenic plants.

Studies on assessment of treatment efficacy with fungicides and *Trichoderma harzianum* against *Alternaria* leaf spot using cucumber leaf disk assay (Anonymous 2003) indicated the presence of significant differences between the treatments with 4 types of new, low-residual fungicides or 2 forms of *T. harzianum* and the control treatments with a blank formulation of invert emulsion or sterile distilled water. Application of formulated conidia of *T. harzianum* in invert emulsion at a concentration of $2.0X10^8$ conidia /ml significantly suppressed the disease-lesion diameter on treated cucumber leaf-discs.

Verma *et al* (2007) showed that *Trichoderma* spp. has been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which chiefly contribute to antagonism of these fungi. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defense system are typical biocontrol actions of these fungi. On the other hand, *Trichoderma* spp. has also been used in a wide range of commercial enzyme productions, namely, cellulases, hemicellulases, proteases, and _-1, 3-glucanase. Information on the classification of the genus, *Trichoderma*, mechanisms of antagonism and role in plant growth promotion has been well documented. However, fast paced current research in this field should be carefully updated for the full-proof commercialization of the fungi.

Maketon *et al* (2008) used two biological control agents, *Bacillus subtilis* AP-01 (Larminar) and *Trichoderma harzianum* AP-001 (Trisan) alone or in combination in controlling three tobacco diseases, including bacterial wilt (*Ralstonia solanacearum*), damping-off (*Pythium aphanidermatum*), and frogeye leaf spot (*Cercospora nicotiana*). Tests were performed in greenhouse by soil sterilization prior to inoculation of the pathogens. Bacterial-wilt and damping off pathogens were drenched first and followed with the biological control agents and for comparison purposes, two chemical fungicides. But for frogeye leaf spot, all treatments including a chemical fungicide was applied as foliar spray instead of drenching. Results showed that neither *B. subtilis* AP-01 nor *T. harzianum* AP-001 alone could control the bacterial wilt, but when combined, their controlling capabilities were as effective as a chemical treatment. These results were also similar for damping-off disease when used in combination. In addition, the combined *B. subtilis* AP-01 and *T. harzianum* AP-001 resulted in a good frogeye leaf spot control, which was statistically similar to significantly different from the chemical treatment.

Perello (2008) determined the effect of six isolates of *Trichoderma harzianum* and one isolate of *T. koningii* on the incidence and severity of tan spot, caused by *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*) under field conditions. In 2003, two of the isolates assayed (T5, T7) showed the best performance against the disease when applied as seed treatments or sprayed onto wheat leaves at different stages. The application of six treatments on wheat plants significantly reduced disease severity by 16 to 35% in comparison with the control. Disease control provided by isolate T7 was similar to that provided by the fungicide treatment (56% reduction).

Jegathambigai *et al* (2008) reported that *Trichoderma* is a potential bio agent, which has definite role in suppressing the inoculums of *Helminthosporium sp.* The result revealed that leaf spot incidence was lowered significantly in cane palms treated with *Trichoderma* species followed by treatment with combination of *Trichoderma* sp. and fungicides.

Perello *et al* (2008) studied the effect of six isolates of *Trichoderma harzianum* and one isolate of *T. koningii* on the incidence and severity of tan spot (*Pyrenophora tritici-repentis*) and leaf blotch of wheat (*Mycosphaerella graminicola*) under field conditions. Significant differences between wheat cultivars, inoculum types and growth stages were found. Three of the isolates tested (T2 for *M. graminicola* , T7 for *P. tritici-repentis* and T5 for both of them) showed the best performance in controlling leaf blotch and tan spot when coated onto seed or sprayed onto wheat leaves at different growth stages, with significant severity reduction up to 56%. At tillering, six isolates reduced the severity of *P. tritici-repentis* and *M. graminicola* compared to the control by up to 39% and 12-53%, respectively. In some experiments, the biocontrol preparation (T2 and T5) gave a level of disease control similar to that obtained with Tebuconazole (70 and 48%, respectively). The effect of *Trichoderma* against *P. tritici-repentis* was also observed at the heading stage, when six of the treatments reduced disease severity by 16-35%.

Sobowale *et al* (2005) reported that five strains of *Trichoderma pseudokoningii* (antagonists) suppressed radial growth of *Fusarium verticillioides* (Sacc.) Nirenberg (*Fusarium moniliforme* Sheldon) *invitro*. These were *T. pseudokoningii* strain1 (IMI 380933), strain2 (IMI 380937), strain3 (IMI 3809 39), strain4 (IMI 380940) and strain5 (IMI 380941). Growth suppression of *F. verticillioides* by all strains of *T. pseudokoningii* was significantly different (R2=0.98, p=0.05) from

control. It differed significantly ($p>0.0003$) among the strains in all pairing methods. Growth suppression also differed significantly among ($p>0.0001$) and within ($p>0.018$) pairing methods. Growth suppression was best when antagonists were inoculated before pathogen. Suppression mechanisms include mycoparasitism and competition for space and nutrients. *T. pseudokoningii* strains 3 and 4 had the best ($p=0.05$) growth suppression of *F. verticillioides*

Intana *et al* (2005) reported that a total of 13 isolates of *Trichoderma virens* were isolated from soil samples collected from 9 different sites. All isolates inhibited the mycelial growth of *Alternaria brassicicola* that causes Chinese kale leaf spot. There were two strains, T-ST-01 and T-NST-01 that gave high values of inhibition, 72.5 and 67.0%, respectively. Strains T-ST-01 and T-NST-01 were used to evaluate antifungal properties using dialysis membrane technique testing with *A. brassicicola*. The results showed that mycelial growth of *A. brassicicola* was completely inhibited. Antifungal metabolite was extracted and tested against germination of *A. brassicicola* spores. The results showed that spores of *A. brassicicola* germinated 9.4% compared with 98.5% for the control. Use of antifungal metabolite was effective both in laboratory and glasshouse conditions.

Karthikeyan *et al* (2008) tested that three antagonists: *Pseudomonas fluorescens* (Pf1), *Bacillus subtilis* and *Trichoderma viride*, either tested alone and in combination for suppression of onion leaf blight (*Alternaria palandui*) disease under glasshouse and field conditions. The average mean of disease reduction was 24.81% for single strains and 42.44% for mixtures. In addition to disease suppression, treatment with a mixture of antagonists promoted plant growth in terms of increased plant height and ultimately bulb yield. Though seed treatment of either single strain or strain mixtures alone could reduce the disease, subsequent

application to root, leaves or soil further reduced the disease and enhanced the plant growth. The mixture consisting of *Pseudomonas fluorescens* Pfl plus *Bacillus subtilis* plus *Trichoderma viride* was the most effective in reducing the disease and in promoting plant growth and bulb yield in greenhouse and field tests.

Patni and Kolte (2006) reported the activity of some plant extracts against the Alternaria blight pathogen, *Alternaria brassicae*. Among the extracts tested, the *Eucalyptus globulus* leaf extract showed significant reduction in the radial growth, sporulation and spore germination of *A. brassicae*. Under laboratory conditions, leaf extracts of *Eucalyptus globulus*, *Ocimum sanctum Ocimum tenuiflorum* and *Anagallis arvensis* showed maximum reduction (92.5, 91.6 and 91.4 decrease over the control, respectively) in radial growth. *Ocimum sanctum*, *Eucalyptus globulus* and *Urtica dioica* showed minimum sporulation intensity (0.26, 0.28, and 0.81×10^5, respectively). The lowest reduction of spore germination was observed with *U. dioica* followed by *Ocimum sanctum* and *E. globulus* (86.6, 79.4 and 78.9%, respectively). Studies conducted under glasshouse conditions revealed that *E. globulus* spray gave significantly lesser number of spots/leaf (2.05), minimum size of spot (1.28 mm), minimum sporulation intensity (1.22×10^5) and minimum disease index (13.96) followed by *Calotropis procera*, *Ocimum sanctum* and *Polyalthia longifolia* extracts spray.

Chand and Singh (2006) prove that the effects of extracts of oak (*Calotropis procera*), eucalyptus (*Eucalyptus globulens* [*E. globulus*]), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*) and bulbs of garlic (*Allium sativum*) on Alternaria blight (*Alternaria brassicae*) of Indian mustard cv. RH-30 studied under laboratory conditions. *Alternaria brassicae* was isolated from infected leaves and mass multiplied on potato dextrose agar (PDA) medium. Indian mustard leaves

were sprayed with spore suspension, and after 24 h, were sprayed with the various plant extracts (obtained from leaves and bulbs) at different concentrations (10, 20 and30%) except the (untreated) control. All the extracts effectively reduced the disease. Foliar spray with bulb extract of *Allium sativum* showed the lowest disease intensity (2.87%), followed by *E. globulens* (5.3%) and *Azadirachta indica* (7.4%) compared to 20% in the control. *J. multifida* and *C. procera* were comparatively less effective than the other plant extracts, but these also reduced the disease intensity from 20% to 7.5 and 11.9%, respectively. Generally, the disease intensity decreased non-significantly with increasing extract concentration. However, in *A. indica*, the disease intensity at 30% concentration (2.3%) was significantly less than that at 10% concentration (13.3%). Similar observations were recorded for *C. procera.*

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

Daya and Ram (1997) studied the *in vitro* fungitoxicity of leaf extracts of *Cassia tora, Azadirachta indica, Anisomeles ovata, Aegle marmelos, Adhatoda vasica, Mentha arvensis, Dalbergia sissoo, Tinospora cordifolia, Pongamia pinnata, Cyperus rotundus, Ocimum adscendens* and *Ocimum sanctum*, a resin extract of Ferulafioetida and bulb extracts *of Allium sativum* and *A. cepa.* The leaf extract of *O. sanctum* was found to be most effective and completely inhibited spore germination of *A. brassicae*, the causal agent of *Alternaria* blight of mustard at 10000 ppm. *A. sativa* and *A. cepa* were next in efficacy and inhibited spore germination by 40% at 10000 ppm.

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

Chand and Singh (2004) studied the effects of extracts of oak (*Calotropis procera*), eucalyptus (*Eucalyptus globulens* [*E. globulus*]), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*) and bulbs of garlic (*Allium sativum*) on *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. RH-30 under laboratory conditions. *Alternaria brassicae* was isolated from infected leaves and mass multiplied on potato dextrose agar (PDA) medium. Indian mustard leaves were sprayed with spore suspension, and after 24 h, were sprayed with the various plant extracts (obtained from leaves and bulbs) at different concentrations (10, 20 and 30%) except for the (untreated) control. All the extracts effectively reduced the disease. Foliar spray with bulb extract of *Allium sativum* showed the lowest disease intensity (2.87%), followed by *E. globulens* (5.3%) and *Azadirachta indica* (7.4%) compared to 20% in the control. *J. multifida* and *C. procera* were comparatively less effective than the other plant extracts, but these also reduced the disease

intensity from 20% to 7.5 and 11.9%, respectively. Generally, the disease intensity decreased non-significantly with increasing extract concentration. However, in *A. indica*, the disease intensity at 30% concentration (2.3%) was significantly less than that at 10% concentration (13.3%). Similar observations were recorded for *C. procera*.

Prasad (2006) conducted a field trial during rabi 2002/03 and 2003/04, in India to evaluate the efficacy of different spraying combinations of three fungicides (ridomil [metalaxyl], carbendazim and mancozeb) and five plant extracts (*Datura stramonium*, *Eucalyptus globosus*, *Azadirachta indica*, *Allium sativum* and *Allium cepa*) against *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. Varuna. Comparative analysis of various spraying schedules revealed that first spray of carbendazim (0.1%) + mancozeb (0.2%) followed by two sprays of mancozeb (0.2%) at early sowing (20 October) was the best combination in reducing the disease severity on leaves (18.7%) and pods (10.4%) and in increasing yield (1295.8 kg/ha), 1000-seed weight (5.12 g) and oil content (42.6%). Sowing on 20 October also gave higher seed yield and reduced disease intensity on leaves and pods in comparison to later sowing. Among the botanicals integrated with the standard fungicide (mancozeb), 5% aqueous extract of *D. stramonium*, *E. globosus* and *Allium sativum* reduced the disease intensity by 21.7, 23.3 and 25.5% on leaves, respectively. However, mancozeb provided the highest reduction (20.9%) of the disease on leaves and was statistically at par with these plant extracts. Apart from mancozeb, *D. stramonium* was found to be most effective in increasing seed yield.

MATERIALS AND METHODS

3.1 Experimental sites

The experiment was in Block No. 15 in central farm of Bangladesh Agricultural Research Institute (BARI) at Joydevpur in Gazipur (Photograph 1). Details are in Appendix.1.

Photograph 1. Field experiment in controlling leaf blight of mustard with botanicals and *Trichoderma harzianum*

3.2 Experimental period

The experiment was carried out during the Rabi season form October, 2009 to March, 2010

.3.3 Soil type

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

3.4 Climate

The climate of the experimental field area was of sub-tropical in nature characterized by high temperature associated with heavy fog and dew during Rabi season (October to March).

3.5 Weather

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

3.6 Variety

The mustard (*Brassica campestries*) variety BARI Sarisha-15 released from Bangladesh Agricultural Research Institute was used for the experiment. Seeds were collected from Oilseed Research Centre of Bangladesh Agricultural Research Institute, Gazipur.

3.7 Treatments of the experiment

Seven treatments were assessed in the experiment as follows:

T_1 = Foliar application of *Trichoderma harzianum S1*

T_2 = Foliar application of *Trichoderma harzianum S2*

T_3 = Foliar application of *Trichoderma harzianum S3*

T_4 = Foliar application of Bashok leaf extract

T_5 = Foliar application of Bishkatali extract

T_6 = Foliar application of Marigold extract

T_7 = Control

Photograph 3. Pure culture of *Trichoderma harzianum* S1

3.8 Design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The whole plot was divided into four blocks each containing seven (7) plots of 4m x 3m size, giving 28 units plots. The space was kept 1m between the blocks and 0.5 m between the plots (Appendix-III).

3.9 Land preparation

The land was firstly ploughed with a power tiller in the first week of November 2009 and left exposed to sunlight for 7 days. Then the land was ploughed and cross-ploughed by a power draw cultivator 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 (Kodal) 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 were used for carrying out the field study (Anonymous, 2001).

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

3.11 Collection of botanicals and preparation of extract

Botanicals such as leaf of Marigold, Bishkatali, Bashok were collected from Bangladesh Agricultural Research Institute area. For preparation of extract, collected plant materials were weighed in an electric balance and then were washed in water. After washing these were chopped into small pieces. For getting extract, chopped plant materials were blended in an electric blender and then distilled water was added to make the solution 1:10 (w/v) for foliar spray.

3.12 Preparation and application of spray solution

The suspensions of *Trichoderma* strains were prepared by mixing with required amount of water. Some suspensions of *Trichoderma* strain, some plant extract (concentration @ 1:10) and plain water was sprayed with compressed hand sprayer. Sprays were done at 45, 55, 65 days after sowing. Adequate precautions were taken to avoid drifting of spray materials from one plot to the neighboring ones.

3.10 Application of manure and fertilizers

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

Manures Fertilizers	Rate /ha
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Boric acid	10 kg

3.11 Collection of botanicals and preparation of extract

Botanicals such as leaf of Marigold, Birikati, Bashok were collected from Bangladesh Agricultural Research Institute area. For preparation of extract collected plant materials were weighed in an electric balance and then were washed in water. After washing those were chopped into small pieces. For getting extract chopped plant materials were blended in an electric blender and then distilled water was added to make the solution 1:10 (*w/v*) for foliar spray.

3.12 Preparation and application of spray solution

The suspensions of *Trichoderma* strain were prepared by mixing with required amount of water. Some suspensions of *Trichoderma* strain, some plant extract (concentration @ 1:10) and plain water was sprayed with compressed hand sprayer. Sprays were done at 45, 55, 65 days after sowing. Adequate precautions were taken to avoid drifting of spray materials from one plot to the neighboring ones.

3.13 Thinning

Thinning was done to keep the population in proper ratio and to maintaining the suitable density and number of plant in the plot. It was done at 15 days after sowing. It is also helpful for maintaining proper spacing of plants.

3.14 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 57 EC was applied three times at 10 days intervals to control aphid.

3.15 Germination and seed health test

Germination test of BARI Sarisha-15 seeds received from treated plot with different treatments was conducted in plastic Petri dishes. Four hundred seeds were randomly collected from each treatment. Seeds were placed on three layers of moist blotting paper (Whatman No.1) contained in plastic petri dishes. Twenty-five seeds were placed in each petri dish 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 to keep the blotting paper moist 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. The results were presented as percent seed infection for individual pathogen.

3.16 Collection of data

The following parameters were considered for data collection.

On diseases incidence

Percent leaf infection

Percent leaf area diseases (% LAD)

Percent pod infection

Number of spots/pod

On growth parameters

Number of leaf/plant

Number of branches/plant

Plant height (cm)

On yield and yield contributing characters

a. Number of pods/plant

b. 1000-seed weight

c. Yield (Kg/ha)

On harvest seed

Percent seed germination

Percent seed infection

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Percent pod infection

Number of spots pod

On growth parameters

Number of leaf/plant

Number of branches/plant

Plant height (cm)

On yield and yield contributing characters

a. Number of pods/plant

b. 1000-seed weight

c. Yield (Kg/ha)

On harvest seed

Percent seed germination

Percent seed infection

3.17 Procedure of data collection

Ten plants per plot were selected and tagged for collection of data. Data on percent leaf infection were recorded 45, 55 and 65 days after sowing by visual observation of symptoms. Percent leaf infection was calculated by the following formula.

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

Data on percent leaf area diseased were recorded 45, 55 and 65 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 65, 75 and 85 days after sowing by visual observation of symptoms. Percent pod infection was calculated by the following formula.

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

3.18. Collection of diseased sample for morphological study of *Alternaria brassicae*

For studying morphological variation of the pathogen *Alternaria brassicae* infected mustard leaves were collected from four different geographical locations namely, Jamalpur, Ishurdi, Shatkhira and Gazipur. Four samples were collected from each location elapsing a distance of 0.5km from sample to sample. Infected leaf having characteristic *Alternaria* infected spots with concentric zones was selected. The diseased leaf samples were placed two layer of news paper and were taken to the laboratory at Joydebpur. Pieces of leaves about 0.5mm sq size containing diseased as well as healthy portion were cut from the collected samples. The pieces were surface sterilized with 0.5% of Sodium Hypochlorite for one minute. The pieces were then placed on a sterilized blotting paper to remove the excess liquid. The pieces were then placed on previously prepared 9 cm diameter PDA petri dishes putting five pieces in each plate. The plates were then incubated at room temperature (26 ±2°C). After three days incubation when the pathogen started mycelial growth on the PDA medium a portion of the growing tip was transferred to PDA Slants as well as on PDA plates for obtaining pure culture of the pathogen. The microscopic study was done for observing morphological variation of the conidia of the pathogen collected from different geographical location. Data recorded from each of the four samples of each location included number of conidia per microscopic field at 10x magnification, number of muriform conidia per microscopic field, maximum number of septation in each conidia, number of transverse and longitudinal septation in each conidia.

3.18. Collection of diseased sample for morphological study of Alternaria brassicae

For studying morphological variation of the pathogen Alternaria brassicae infected mustard leaves were collected from four different geographical locations namely, Jamalpur, Jahurdi, Shatkhira and Gazipur. Four samples were collected from each location elapsing a distance of 0.5km from sample to sample. Infected leaf having characteristic Alternaria infected spots with concentric zones was selected. The diseased leaf samples were placed two layer of news paper and were taken to the laboratory at Joydebpur. Pieces of leaves about 0.5 cm sq size containing diseased as well as healthy portion were cut from the collected samples. The pieces were surface sterilized with 0.5% of Sodium Hypochlorite for one minute. The pieces were then placed on a sterilized blotting paper to remove the excess liquid. The pieces were then placed on previously prepared 9 cm diameter PDA petri dishes putting five pieces in each plate. The plates were then incubated at room temperature (26 ±2°C). After three days incubation when the pathogen started mycelial growth on the PDA medium a portion of the growing tip was transferred to PDA Slants as well as on PDA plates for obtaining pure culture of the pathogen. The microscopic study was done for observing morphological variation of the conidia of the pathogen collected from different geographical location. Data recorded from each of the four samples of each location included number of conidia per microscopic field at 10x magnification, number of muriform conidia per microscopic field, maximum number of septation in each conidia, number of transverse and longitudinal septation in each conidia.

3.19 Analysis of data

The data were statistically analyzed using computer package program. Treatment means were compared by DMRT (Duncan's Multiple Range Test). ANOVA table was shown in appendix-V. Correlation and Regression study was done to determine the relationship between percent leaf infection, percent leaf area diseased (% LAD) and percent pod infection with days after sowing for each of the treatments.

RESULTS AND DISCUSSION

4.1 Percent leaf infection

The effect of different treatments on percent leaf infection of mustard at different days after sowing (DAS) were summarized and presented in Table 1 and figure 1. Different *Trichoderma harzianum* strains and plant extracts had significant influence on percent leaf infection of mustard (BARI Sarisha-15) at different days after sowing (DAS). Percent leaf infection of mustard increased gradually with the advancement of crop growth. At 65 days after sowing (DAS), the highest percent leaf infection (35.08%) was found in control and the lowest percent leaf infection (14.44%) was recorded in T_1 (*Trichoderma harzianum S1*) treated plot followed by T_2 (*Trichoderma harzianum S2*), T_3 (*Trichoderma harzianum S3*), T_6 (Marigold) and T_4 (Bashok). Perello *et al* (2006) reported that *Trichoderma harzianum* was the most effective in controlling of *Aalternaria* blight of mustard.

Table 1: Effect of different *Trichoderma* strains and plant extract on percent leaf infection of mustard at different days after sowing (DAS)

Treatments	% Leaf infection		
	45 DAS	55 DAS	65 DAS
T_1 (*T. harzianum S1*)	10.03d	12.05e	14.44f
T_2 (*T. harzianum S2*)	11.34c	13.87d	17.38e
T_3 (*T. harzianum S3*)	12.42c	14.40d	18.20de
T_4 (Bashok)	15.29b	18.60c	23.07c
T_5 (Bishkatali)	16.32b	21.07b	25.92b
T_6 (Marigold)	11.51c	14.28d	18.70d
T_7 (Control)	17.97a	26.07a	35.08a
CV (%)	5.95	5.33	2.90

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

4. 2 Percent leaf area diseased (% LAD)

The effect of different treatments on percent leaf area diseased (% LAD) of mustard at different days after sowing (DAS) were recorded and presented in Table 2 and Fig.2. Percent leaf area diseased (% LAD) of mustard was found to be significantly different at different days after sowing (DAS) in response to the application of different treatments. Percent leaf area diseased (LAD) increased gradually with the advancement of crop growth. At 65 days after sowing the highest percent leaf area diseased (13.24%) was found at T_7 (control) treatment and the lowest percent leaf area diseased (2.59%) was recorded from the treatment T_1 (*Trichoderma harzianum S1*) followed by T_2 (*Trichoderma harzianum S2*), T_6 (Marigold), T_3 (*Trichoderma harzianum S3*), T_4 (Bashok), and T_5 (Bishkatali). Stefania *et al* (2008) reported that repeated foliar applications of the liquid culture homogenate preceded by a single treatment of *Trichoderma harzianum* in 2 year trials under natural inoculum in field reduced the disease incidence and pathogen sporulation from the necrotic spots. Similar findings are reported by Verma *et al* (2007), Maketon *et al* (2008) and Intana *et al* (2005). So, the present findings are in agreement with the earlier findings.

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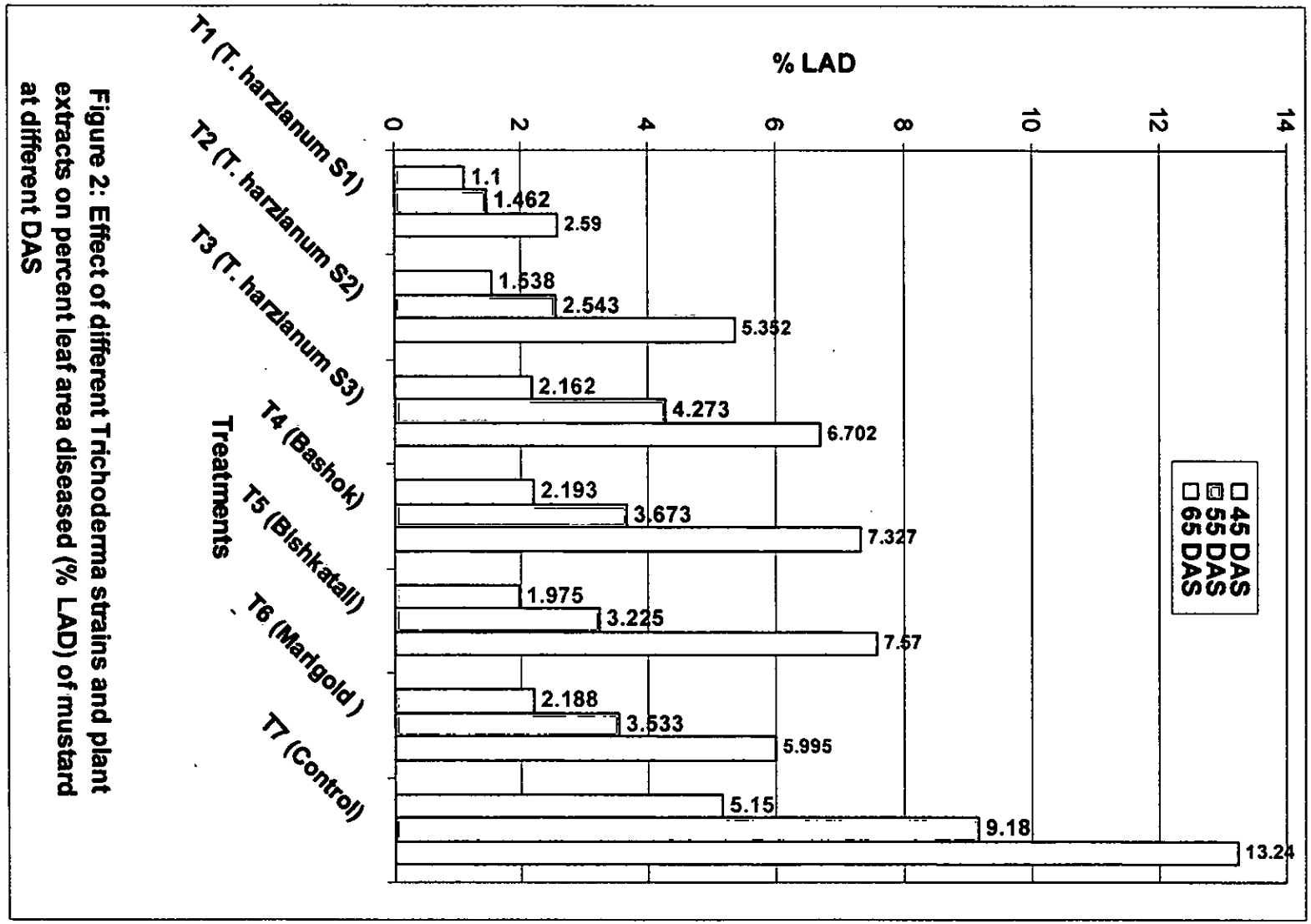


Figure 2: Effect of different Trichoderma strains and plant extracts on percent leaf area diseased (% LAD) of mustard at different DAS

4. 3 Percent pod infection

The data on effect of different treatments on percent pod infection of mustard at different days after sowing (DAS) was summarized and presented in table 3. It is seen from the data of the table that there is significant variation on the effect of different treatments on percent pod infection of mustard (BARI Sarisha-15) on different days after sowing (DAS). Percent pod infection of mustard increased gradually with the increase of crop age. Very little pod infection was recorded at 65 DAS while it was raised to the range from 19.39 – 37.47 at 85 DAS in response to applying different treatments. At 85 days after sowing (DAS) the highest percent pod infection (37.47%) was recorded from T_7 (control) treatment (Photograph-4) and the lowest percent pod infection (19.39%) was recorded from T_1 (*Trichoderma harzianum S1*) treatment (Photograph-5) followed by T_2 (*Trichoderma harzianum S2*), T_3 (*Trichoderma harzianum S3*), T_4 (Bashok), T_6 (Marigold) and T_5 (Bishkatali). These findings are agreed with the findings of Stefania *et al* (2008), Mora and Earle (2001), Verma *et al* (2007), Maketon *et al* (2008) and Intana *et al* (2005).

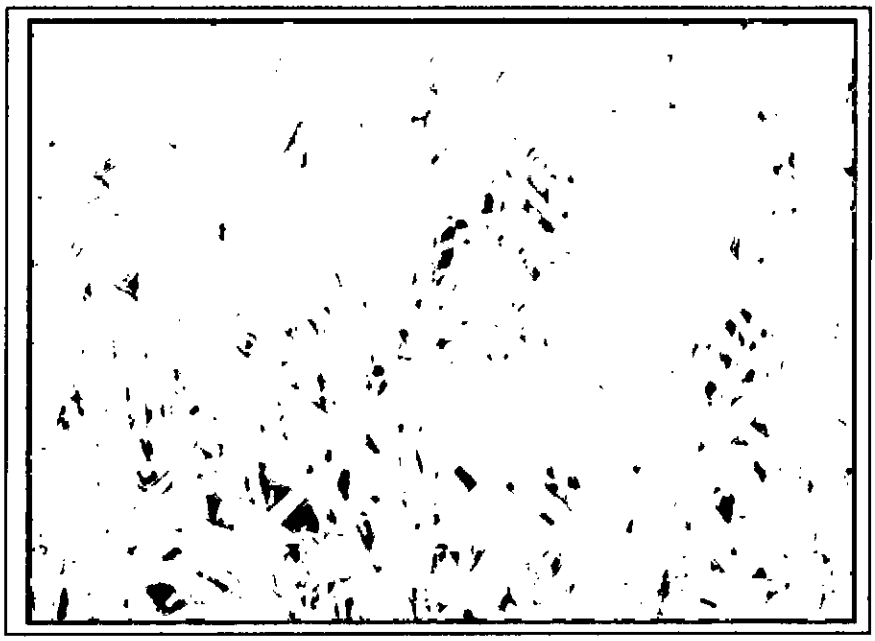
Table 3: Effect of different *Trichoderma* strains and plant extracts on percent pod infection of mustard at different days after sowing (DAS)

Treatments	% Pod infection		
	65 DAS	75 DAS	85 DAS
T_1 (*T. harzianum S1*)	1.519e	9.515c	19.39d
T_2 (*T. harzianum S2*)	1.895c	10.24bc	19.69cd
T_3 (*T. harzianum S3*)	1.683d	9.810bc	21.00c
T_4 (Bashok)	2.005b	11.02b	22.45b
T_5 (Bishkatali)	1.873c	9.960bc	23.45b
T_6 (Marigold)	2.040b	10.33bc	22.82b
T_7 (Control)	3.145a	17.94a	37.47a
CV (%)	2.65	7.67	3.72

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

Photograph-2. Showing infected pods of mustard

Photograph 4: Untreated (control) plot of mustard plant



Photograph 5: *T. harzianum* s1 treated plot of mustard

4. 4 Number of spots per pod

The effect of different treatments on number of spots per pod of mustard on different days after sowing (DAS) was recorded. The summarized data have been presented in Table 4 The treatment significantly influenced the number of spots per pod of mustard (BARI Sarisha-15) with the application of different *Trichoderma harzianum* strains and plant extracts at different days after sowing (DAS). Number of spots per pod increased gradually from 65 DAS to 75 DAS and finally after 85 DAS. Very little number of spots per pod was recorded at 65 DAS. At 85 DAS, the maximum number of spots per pod (1.867) was recorded from T_7 (control) treatment while minimum number of spots per pod (1.217) was counted from applying with T_1 (*Trichoderma harzianum S1*) treatment followed by T_3 (*Trichoderma harzianum S3*). On the other hand application of Bashok (T_4), Bishkatali (T_5), *Trichoderma harzianum S2* (T_2) and Marigold (T_6) produced similar result in which there was found statistically no significant difference. The

similar indication of results was reported by Stefania *et al* (2008), Mora and Earle (2001), Verma *et al* (2007), Maketon et *al* (2008) and Intana *et al* (2005). So these findings are agreement with the earlier reports.

Table 4: Effect of different *Trichoderma* strains and plant extracts on number of spots/pod of mustard at different days after sowing (DAS)

Treatments	Number of spots/pod		
	65 DAS	75 DAS	85 DAS
T_1 (*T. harzianum S1*)	0.001d	0.785d	1.217c
T_2 (*T. harzianum S2*)	0.014cd	0.888cd	1.352b
T_3 (*T. harzianum S3*)	0.039bcd	0.908cd	1.320b
T_4 (Bashok)	0.059bc	0.970c	1.335b
T_5 (Bishkatali)	0.085ab	1.160b	1.348b
T_6 (Marigold)	0.082ab	1.330a	1.368b
T_7 (Control)	0.111a	1.285ab	1.867a
CV (%)	12.78	9.56	2.50

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

4. 5. 1 Number of leaf/plant

The treatments produced significant effect on number of leaf per plant due to the application of different *Trichoderma harzianum* strains and plant extracts. The highest number of leaf per plant (8.547) was recorded in case of T_6 (Marigold) treatment and the lowest number of leaf per plant (7.45) was obtained from T_7 (Control) treatment (Fig:-5).

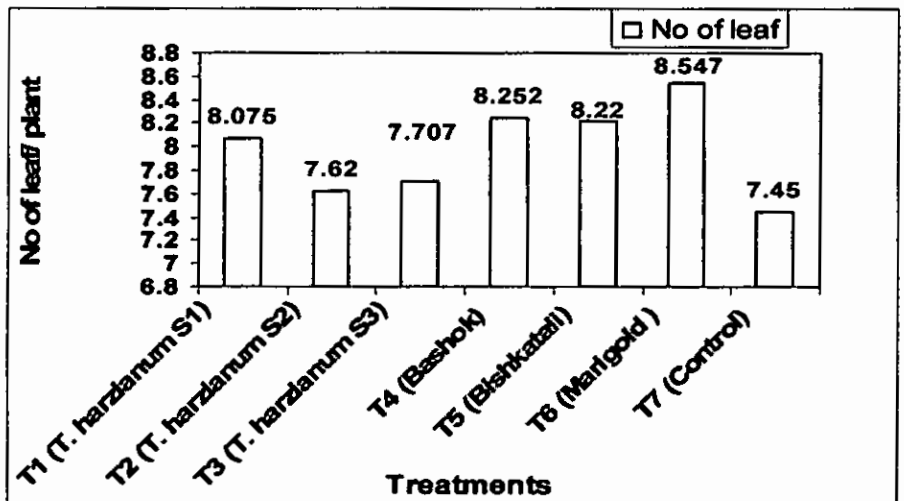


Fig.5: Effect of Trichoderma and plant extract on no. of leaf per plant

4. 5. 2 Number of branches/plant

Number of branches per plant differed significantly due to application of different *Trichoderma harzianum* strains and plant extracts. The maximum number of branches (7.585) was recorded in case of T_3 (*T. harzianum* S3) which was followed by T_6 (Marigold), T_1 (*T. harzianum* S1), T_5 (Bishkatali), T_2 (*T. harzianum* S2) and T_7 (Control). T_4 (Bashok) produced the lowest (6.68) number of branches per plant (Fig:6).

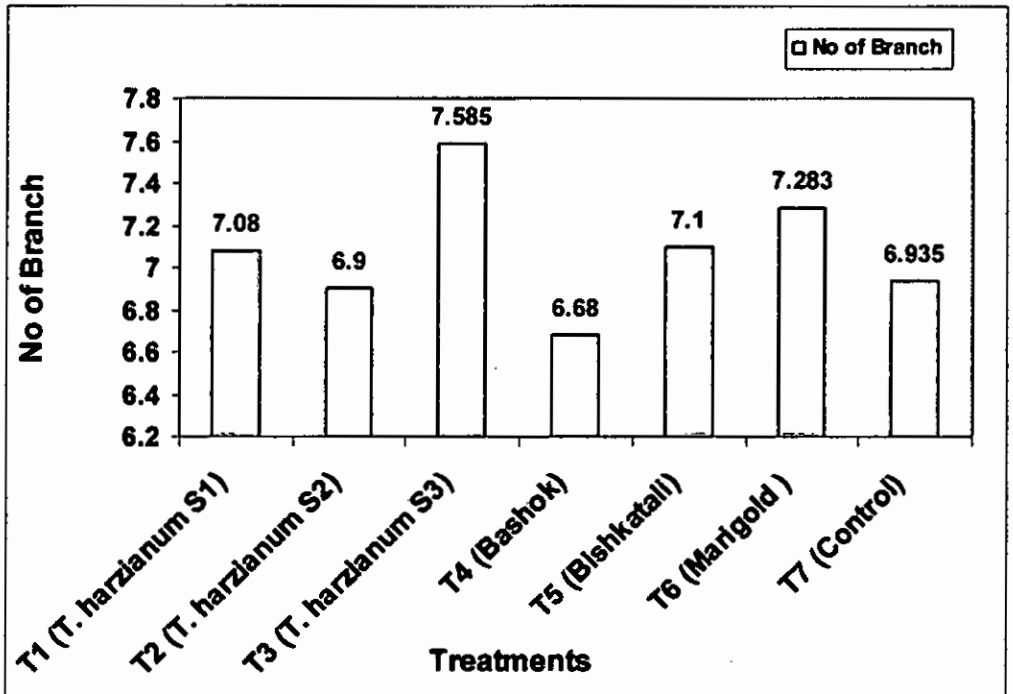


Fig. 6: Effect of Trichoderma and plant extract on no. of Branch per plant

4. 5. 3 Plant heights (cm)

Different *T. harzianum* strains and plant extracts had significant influence on plant height (cm) of mustard. The tallest plant was obtained from T_3 (*T. harzianum* S3) (96.93 cm) which was statistically identical with T_2 (*T. harzianum* S2) and T_1 (*T. harzianum* S1). The lowest plant height (88.07cm) was recorded in case of T_7 (Control) (Fig: 7).

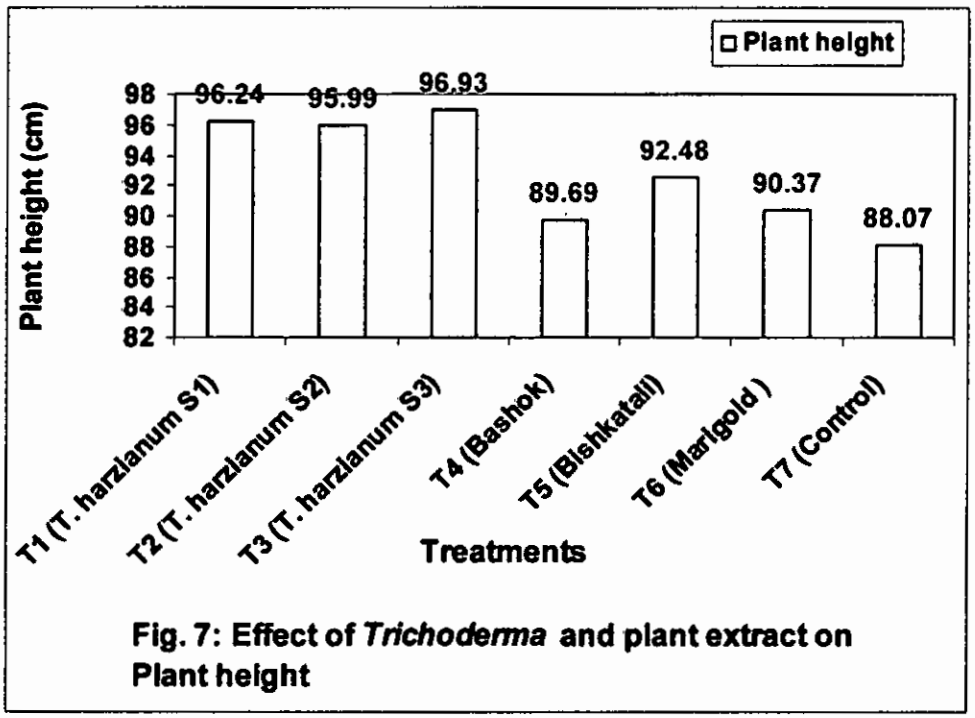


Fig. 7: Effect of *Trichoderma* and plant extract on Plant height

4. 6.1 Number of pods/plant

Different *T. harzianum* strains and plant extracts had significant influence on plant height (cm) of mustard. The tallest plant was obtained from T_6 (Marigold) (76.86) which was statistically identical with T_2 (*T. harzianum* S2) and T_1 (*T. harzianum* S1). The lowest plant height (70.07) was recorded in case of T_7 (Control) (Table-6).

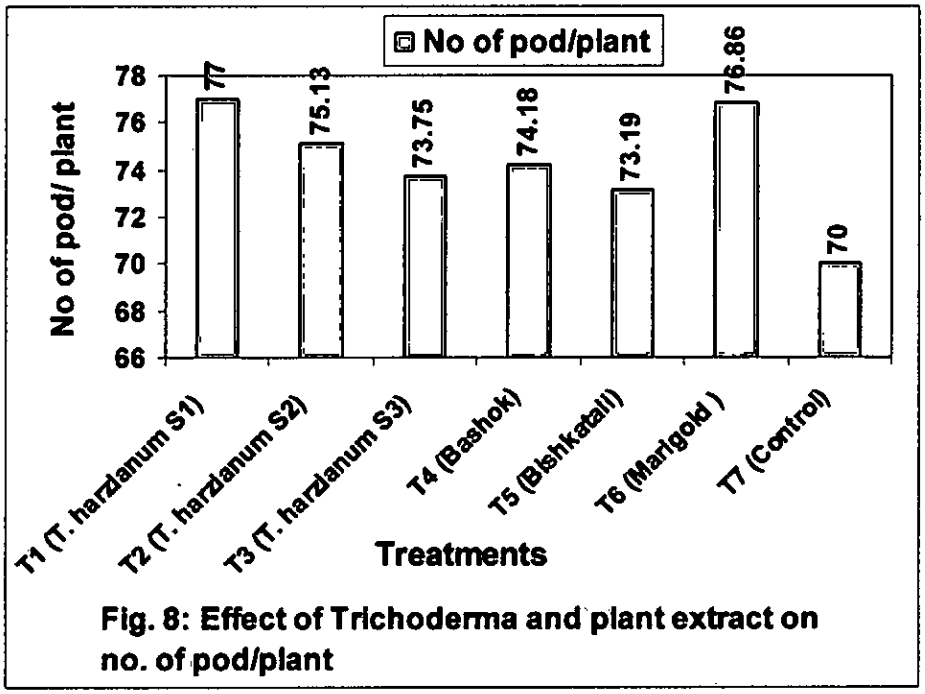


Fig. 8: Effect of Trichoderma and plant extract on no. of pod/plant

4. 6. 2 1000-Seed weight

1000-seed weight was found to be significant due to application of different *Trichoderma harzianum* strains and plant extracts. Spraying with *T. harzianum* S2 (T_2) produced the maximum 1000-seed weight (3.483g) (Table-6) while T_7 (Control) produced the minimum 1000-seed weight (3.065g).

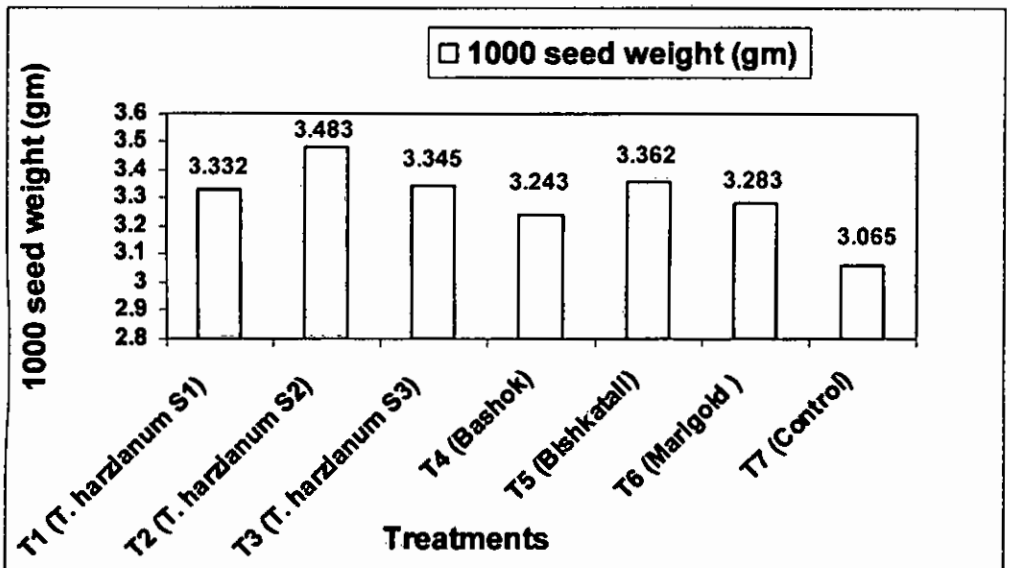


Fig. 9: Effect of Trichoderma and plant extract on 1000 seed weight (gm)

4. 6. 2 1000-Seed weight

1000-seed weight was found to be significant due to application of different Trichoderma harzianum strains and plant extracts. Spraying with T. harzianum (T2) produced the maximum 1000 seed weight (2.48g) (Table-6) while T4 (control) produced the minimum 1000-seed weight (2.06g).

Fig. 9: Effect of Trichoderma and plant extract on 1000 seed weight (gm)

4. 6. 3 Yield

Significant variation of different treatments was found on yield per plant (g) and yield Kg per hectare. Maximum yield per plant (4.01 g) and per hectare (1336 kg) was obtained from T_1 (*Trichoderma harzianum S1*) treated plot which was statistically similar with T_2 (*Trichoderma harzianum S2*), T_3 (*Trichoderma harzianum S3*) and followed by T_6 (Marigold), T_5 (Bishkatali) and T_4 (Bashok). The minimum yield per hectare (856.5 kg) was recorded from T_7 (Control) treatment which was not statistically identical with any treatment (Table-6). Similar findings have been reported Stefania *et al* (2008), Mora and Earle (2001), Verma *et al* (2007), Maketon *et al* (2008) and Intana *et al* (2005). So, the present findings are corroborating with the previous findings.

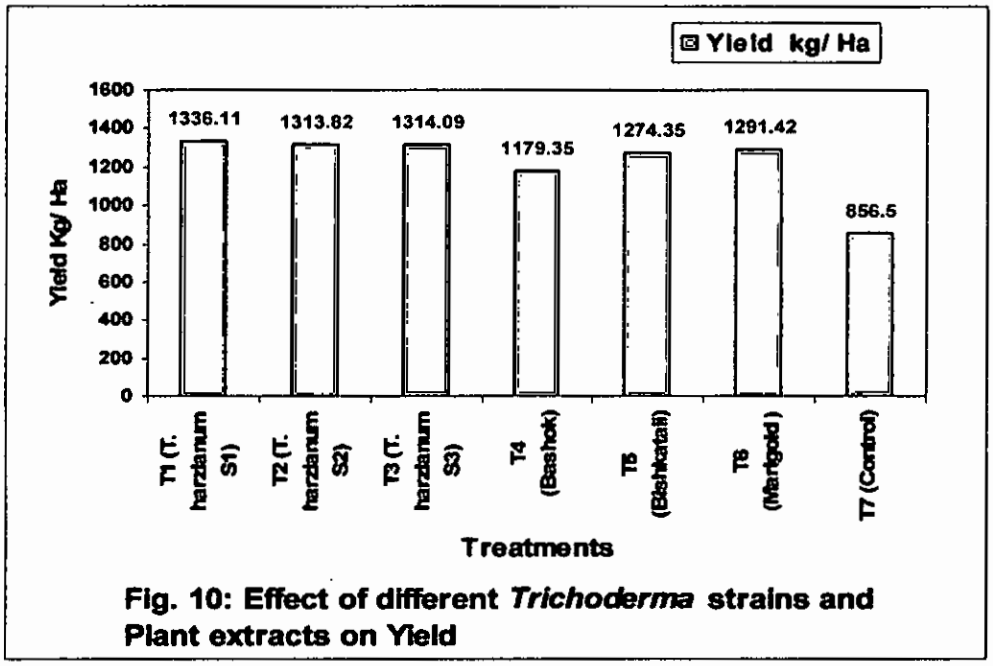


Fig. 10: Effect of different *Trichoderma* strains and Plant extracts on Yield

Table 6: Effect of different treatments on yield and yield contributing characters of mustard

Treatments	Yield and yield contributing characters		
	No. of pods/plant	1000-Seed weight (g)	Yield (kg/ha)
T_1 (*T. harzianum* S1)	77.00a	3.332b	1336.11a
T_2 (*T. harzianum* S2)	75.13b	3.483a	1313.82a
T_3 (*T. harzianum* S3)	73.75bc	3.345b	1314.09a
T_4 (Bashok)	74.18bc	3.243b	1112.49d
T_5 (Bishkatali)	73.19c	3.362b	1188.96c
T_6 (Marigold)	76.86a	3.283b	1245.03b
T_7 (Control)	70.00d	3.065c	856.50e
CV (%)	1.35	2.36	2.13

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

4. 7. 1 Percent seed germination

Percent seed germination was found to be significant due to the application of different fungicides and plant extracts in comparison to control. Seed obtained from *Trichoderma harzianum* S1 (T_1) treated plot showed the maximum percent germination (99.36%) (Plate 1) which was statistically similar (98%) to seed obtained from *Trichoderma harzianum* S1 (T_2) followed by T_3, T_6, T_4 and T_5 treated plot. Seed obtained from control (T_7) showed the minimum percent germination (91.40%). The percent seed germination in case of rest of the treatment revealed statistically identical (Fig. 11).

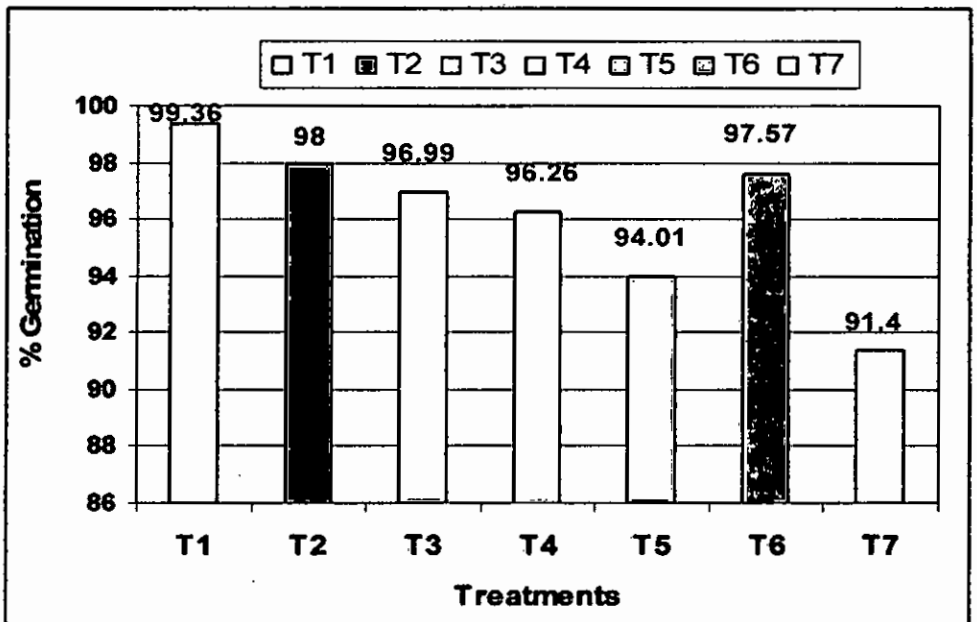


Fig. 11: Effect of *Trichoderma* and plant extract on percent seed Germination

Legend:

T_1 = *T. harzianum* S1, T_2 = *T. harzianum* S2, T_3 = *T. harzianum* S3,

T_4 = Bashok, T_5 = Bishkatali, T_6 = Marigold and T_7 = Control

4. 7. 2 Percent seed infection

After harvest percent seed infection was examined. Percent seed infection by *Alternaria brassicae* and *Alternaria brassicicola* of harvested seed received from treated plot with different fungicides and plant extracts was found to be significant. Seed obtained from control treatment showed the highest percent seed infection (19.02%) while seed obtained from Control (T_7) treated plot showed the lowest seed infection (6.47%) preceded by *Trichoderma harzianum S1* (T_1) and followed by *Trichoderma harzianum* S2 (T_2) (11.6%), *Trichoderma harzianum* S3 (T_3) (12.26%) and Marigold (12.66%), Bishkatali (14.43%) and Bashok (16.44%)(Fig12).

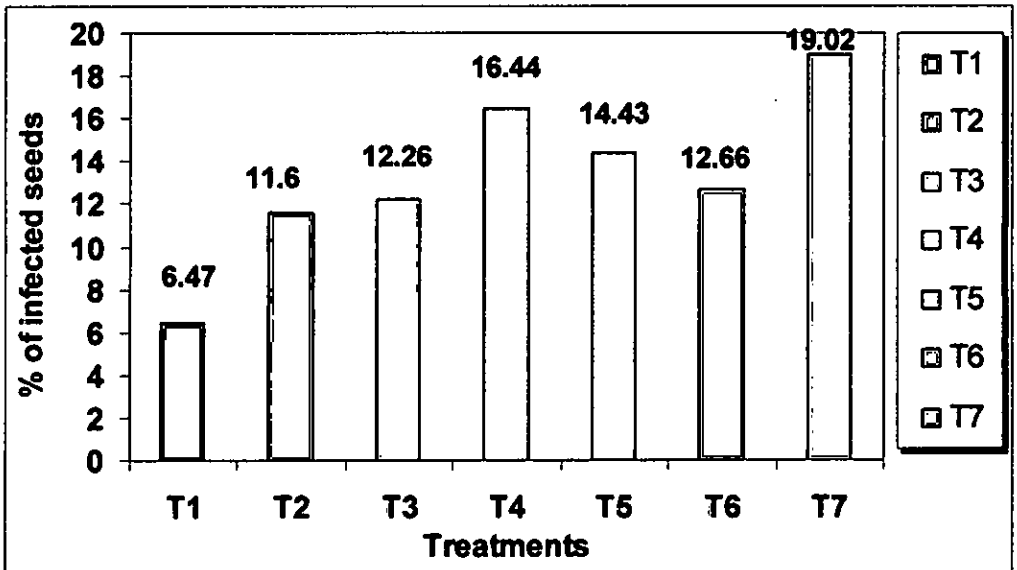


Fig. 12: Effect of *Trichoderma* and plant extract on Seed Infection

Legend:

T_1 = *T. harzianum* S1, T_2 = *T. harzianum* S2, T_3 = *T. harzianum* S3,

T_4 = Bashok, T_5 = Bishkatali, T_6 = Marigold and T_7 = Control

Plate 1: Showing maximum germination of seed obtained from *Trichoderma* treated plot

Plate 2: Showing poor germination of seed obtained from untreated plot

4.8. Study on morphological variation of the pathogen collected from different locations

For studying morphological variation of the pathogen isolated from the diseased leaf samples from four different locations, cultural characters of the isolates were studied which showed practically no difference. The mycelial growth of the culture was appressed and ashy white in color. The conidial characters studied under compound microscope have been summarized and presented in the Table 7.

It was observed from the table that the number of muriform conidia per microscopic field varied from eight to 23 in isolates of Jamalpur, 10 to 19 in isolates of Shatkhira, 17 to 26 in isolates of Ishurdi, and 15 to 24 conidia per microscopic field in isolates of Gazipur. Similarly the number of muriform conidia per microscopic field varied from 8 to 23 in Jamalpur isolates, 8 to 15 in Shatkhira isolates, 10 to 20 in Ishurdi isolates and 9 to 18 in Gazipur isolates. In recording total number of septations including transverse as well as longitudinal septations, the highest was in Jamalpur isolates which ranged from 4.2 to 4.8, while in isolates of three other locations the total number of septations in conidia are more or less

same ranging 3.4 to 3.8 in Shatkhira, 3.2 to 3.4 in Ishurdi and 3.6 to 3.8 in Gazipur isolates.

The transverse septation was slightly higher in isolates of Jamalpur while it seems comparatively less in Ishurdi isolates. The Gazipur isolates also have higher number of transverse septation. Longitudinal septations were also slightly higher in Jamalpur isolates followed by Gazipur isolates.

Table 7: Morphological variation of *Alternaria spp.* collected from different location of Bangladesh

Location	Sample no	Number of conidia/ microscopic field	No. of Muriform conidia	*Septation	*Transverse section	*Longitudinal section
Jamalpur	1	14	8	4.2	3.2	2.0
	2	21	11	4.6	2.8	1.8
	3	30	23	4.6	2.6	1.8
	4	25	19	4.4	2.8	2.0
Shatkhira	1	10	8	3.8	3.4	1.2
	2	15	11	3.4	2.6	1.8
	3	19	15	3.8	2.2	1.4
	4	18	11	3.4	2.4	1.6
Ishurdhi	1	17	11	3.2	2.4	1.4
	2	26	20	3.4	2.2	1.4
	3	17	12	3.4	2.6	1.2
	4	18	10	3.3	2.8	1.2
Gazipur	1	23	13	3.8	2.9	1.8
	2	15	9	3.8	2.4	1.8
	3	24	18	3.6	2.6	1.8
	4	20	14	3.6	2.8	2.0

*Figures are mean of five observations

SUMMARY AND CONCLUSION

The experiment was conducted at central farm of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, during the period from October, 2009 to March, 2010. The objectives of this experiment were to control leaf blight of mustard through some selected strains of *Trichoderma harzianum* and plant extracts.

The experiment was laid out in a RCBD with four replications. There were seven treatments, Viz.T_1 (*T. harzianum* S1), T_2 (*T. harzianum* S2), T_3 (*T. harzianum* S3), T_4 (Leaf extract of Bashok), T_5 (Leaf extract of Bishkatali), T_6 (Leaf extract of Marigold) and T_7 (Control). The unit plot size was 4m x 3m with row and plant spacing of 25 cm × 15 cm. The spaces between blocks and unit plots were 1 m and 0.5 m, respectively. Data were collected on disease incidence and severity of the disease, yield and yield contributing characters. Data were analyzed and the mean values were adjudged with Duncan Multiple Range Test (DMRT).

The study revealed that application of *Trichoderma harzianum* S1 and plant extract significantly influenced all most all of the parameters. The lowest percent leaf infection (10.03%), percent leaf area diseases (1.10%), percent pod infection (1.52%) and number of spots per pod (0.62) were recorded from spraying with *Trichoderma harzianum S1*. The highest percent leaf infection (35.08%), percent leaf area diseased (13.24%), percent pod infection (37.47%) and number of spots per pod (1.867) were recorded from control.

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The highest yield (1336.11 kg/ha) was obtained from the plot spraying with *Trichoderma harzianum S1*. The highest germination percentage and the lowest seed infection obtained from the plot spraying with *Trichoderma harzianum S1*. The lowest germination percentage and the highest seed infection of harvested seeds were obtained from the control treatment. The lowest yield (856.50 kg/ha) was obtained from untreated plot.

Among the botanicals the lowest percent leaf infection (11.51%), percent leaf area diseases (2.18%), percent pod infection (2.04%) and number of spots per pod (0.82) were recorded from spraying with Marigold. Among the botanicals the highest yield (1245.03 kg/ha) was obtained from the plot spraying with Marigold. So Marigold had good effect in controlling *Alternaria* blight which was found significantly higher among all other botanicals.

From the present findings it may be concluded that seed treatment as well as spraying with *Trichoderma harzianum S1* was found to be best and Marigold was also better in case of botanicals for lowering leaf blight incidence and severity and the highest yield of good quality seed of mustard (BARI sarisha-15).

For studying morphological variation of the pathogen isolated from the diseased leaf samples from four different locations, cultural characters of the isolates were studied which showed practically no difference. The number of muriform conidia per microscopic field varied from 8 to 24 in isolates collected from four different locations. In recording total number of septations including transverse as well as longitudinal septations, the highest was in Jamalpur isolates which ranged from 4.2 to 4.8, while in isolates of three other locations the total number of septations in conidia are more or less same ranging 3.4 to 3.8 in Shatkhira, 3.2 to 3.4 in Ishurdi

The highest yield (1330.11 kg/ha) was obtained from the plot spraying with *Trichoderma harzianum* SJ. The highest germination percentage and the lowest seed infection obtained from the plot spraying with *Trichoderma harzianum* SJ. The lowest germination percentage and the highest seed infection of harvested seeds were obtained from the control treatment. The lowest yield (850.50 kg/ha) was obtained from untreated plot

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and 3.6 to 3.8 in Gazipur isolates. The transverse septation was slightly higher in isolates of Jamalpur while it seems comparatively less in Ishurdi isolates. The Gazipur isolates also have higher number of transverse septation. Longitudinal septations were also slightly higher in Jamalpur isolates followed by Gazipur isolates.

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APPENDICES

Appendix-I: Particulars of the Agro-ecological Zone of the Experimental site

Agro-ecological region : Madhupur Tract (AEZ-28).
Land type : High land.
General soil type : Shallow and brown terrace soil
Soil series : Tejgaon
Topography : Fairly leveled
Location : Central farm of BARI
Field level : Above flood level.
Drainage : Well drained.
Firmness (consistency): Compact to friable when dry.

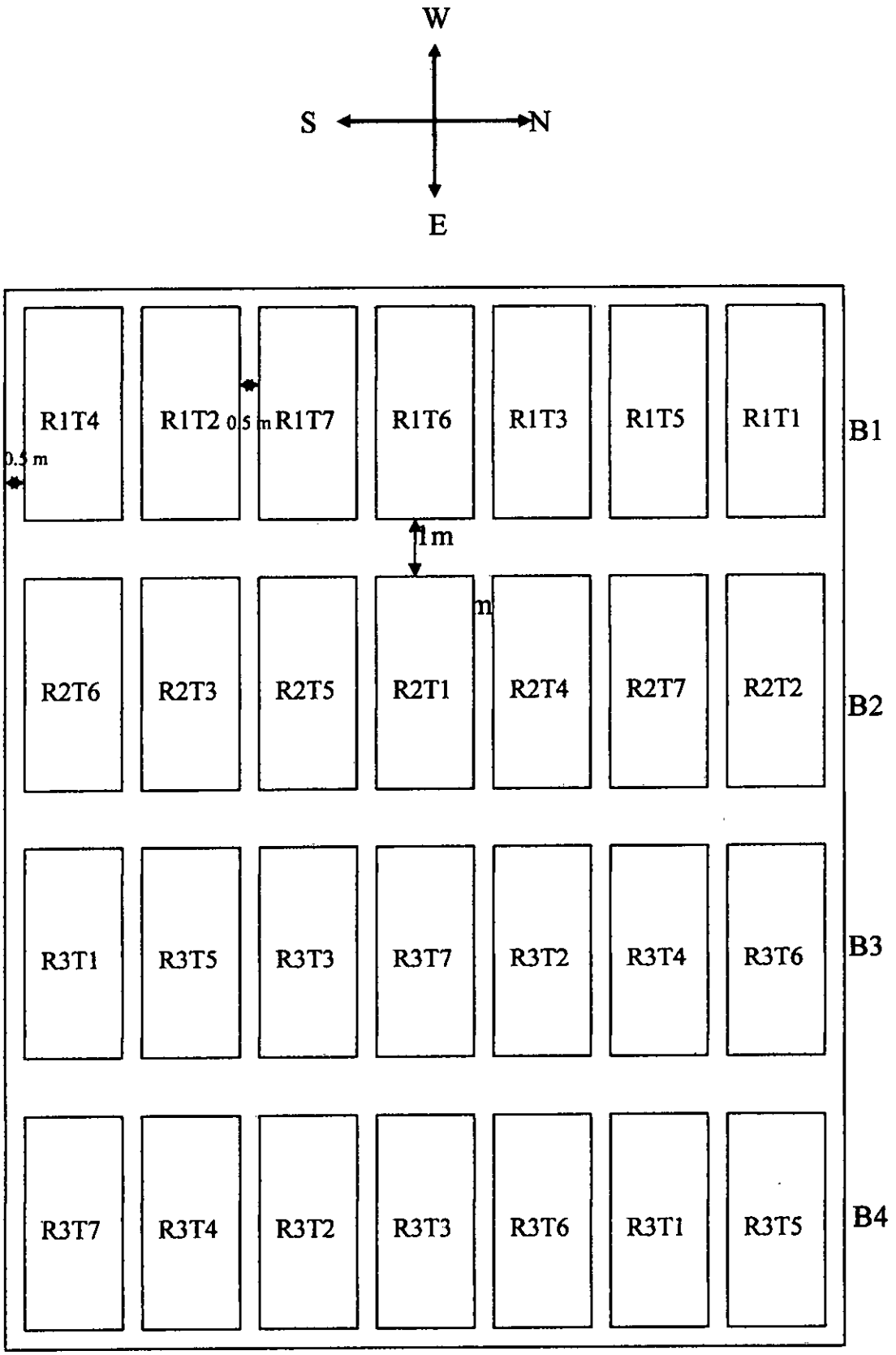
Appendix-II: Monthly mean weather

Monthly mean of daily maximum, minimum and average temperature, relative humidity and total rainfall during November/2009 to February/2010.

Year	Month	Air temperature (^{0}c)			Relative humidity (%)		Rain fall (mm)
		Maximum	Minimum	Mean	Max	Min	
2009	October	32.15	23.15	27.65	97	62	1.57
2009	November	30.38	18.19	24.29	97	59	1.5
2009	December	25.69	13.13	19.41	98	53	0.0
2010	January	24.10	11.22	17.66	99	31	0.0
2010	February	28.66	13.81	21.24	99	31	5.0
2010	March	34.14	21.5	27.82	98	23	12.0

Source: Bangladesh Meteorological Department (Climate division) of Bangladesh Agricultural Research Institute (BARI) at Joydevpur in Gazipur.

Appendix-III: Layout of the field experiment



Appendix-IV: Inspection for morphological variation

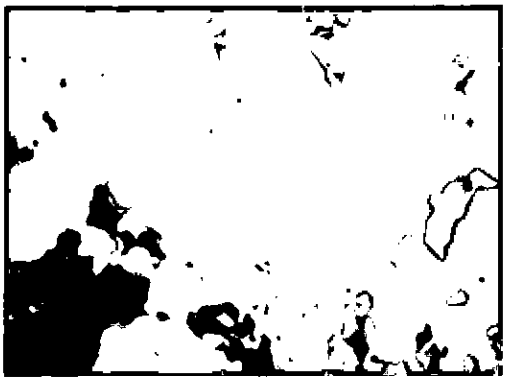
Photograph 6: Inspection of morphological variation of *Alternaria brassicae* under microscope

Photograph 7: leaves of Bashok

Photograph 8: leaves of Marigold

Photograph 9: leaves of Bishkatali

Photograph 10: Pure culture of *Trichoderma harzianum* S3



Photograph 11: Symptom of infected leaves of Mustard

Photograph 12: Conidia of *Alternaria*

Appendix-V: ANOVA table of the experiment

01: Percent leaf infection at 45 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	29.548	9.849	2.5241	0.0902**
Factor A	6	123.555	20.593	5.2772	0.0027**
Error	18	70.239	3.902		
Total	27	223.343			
Coefficient of variation: 14.78%					

**Significance at 5% level

02: Percent leaf infection at 55 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	32.171	10.724	2.8088	0.0690
Factor A	6	291.365	48.561	12.7194	0.000**
Error	18	68.722	3.818		
Total	27	392.258			
Coefficient of variation: 13.73%					

**Significance at 5% level

03: Percent leaf infection at 65 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	83.359	27.786	10.4448	0.000**
Factor A	6	930.702	155.117	58.3075	0.000**
Error	18	47.886	2.660		
Total	27	1061.947			
Coefficient of variation: 8.59%					

**Significance at 5% level

04: Percent LAD at 45 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.031	0.010	0.3947	
Factor A	6	41.147	6.858	259.1794	0.000**
Error	18	0.476	0.026		
Total	27	41.654			
Coefficient of variation: 6.98%					

**Significance at 5% level

05: Percent LAD at 55 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.024	0.008	0.7688	
Factor A	6	145.578	24.263	2305.7930	0.000**
Error	18	0.189	0.011		
Total	27	145.792			
Coefficient of variation: 2.57%					

**Significance at 5% level

06: Percent LAD at 65 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	1.988	0.663	0.8739	
Factor A	6	250.494	41.749	55.0593	0.000**
Error	18	13.649	0.758		
Total	27	266.131			
Coefficient of variation: 12.50%					

**Significance at 5% level

07: Percent Pod infection at 65 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.004	0.001	0.5103	
Factor A	6	6.690	1.115	389.2297	0.000**
Error	18	0.052	0.003		
Total	27	6.746			
Coefficient of variation: 2.65%					

**Significance at 5% level

08: Percent Pod infection at 75 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	1.519	0.506	0.6793	
Factor A	6	213.949	35.658	47.8366	0.000**
Error	18	13.417	0.745		
Total	27	228.886			
Coefficient of variation: 7.67%					

**Significance at 5% level

09: Percent pod infection at 85 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.372	0.124	0.1585	
Factor A	6	935.396	155.899	199.2097	0.000**
Error	18	14.087	0.783		
Total	27	949.854			
Coefficient of variation: 3.72%					

**Significance at 5% level

10: No. of spots/pod at 65 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.000	0.000	0.0916	
Factor A	6	0.039	0.007	125.9771	0.000**
Error	18	0.001	0.000		
Total	27	0.040			
Coefficient of variation: 12.78%					

**Significance at 5% level

11: No. of spots/pod at 75 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.030	0.010	0.9931	
Factor A	6	1.076	0.179	17.9010	0.000**
Error	18	0.180	0.010		
Total	27	1.286			
Coefficient of variation: 9.56%					

**Significance at 5% level

12: No. of spots/pod at 85 DAS

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.005	0.002	1.4106	0.2722
Factor A	6	1.074	0.179	143.8193	0.000**
Error	18	0.022	0.001		
Total	27	1.102			
Coefficient of variation: 2.52%					

**Significance at 5% level

13: No. of branches/plant

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.177	0.059	2.8121	0.0688
Factor A	6	3.791	0.632	30.1737	0.000
Error	18	0.377	0.021		
Total	27	4.344			
Coefficient of variation: 1.81%					

14: No. of leaf/plant

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.158	0.053	1.7436	0.1940
Factor A	6	2.039	0.340	11.2278	0.000**
Error	18	0.545	0.030		
Total	27	2.743			
Coefficient of variation: 2.46%					

**Significance at 5% level

15: Plant height (cm)

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	9.658	3.219	3.3887	0.0408
Factor A	6	307.683	51.281	53.9775	0.0000
Error	18	17.101	0.950		
Total	20	334.442			
Coefficient of variation: 1.05%					

16: No. of pods/plant

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	5.621	1.874	1.8589	0.1728
Factor A	6	138.363	23.061	22.8772	0.000**
Error	18	18.144	1.008		
Total	27	162.129			
Coefficient of variation: 1.35%					

**Significance at 5% level

17:1000-Seed weight

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	0.010	0.003	0.5499	
Factor A	6	0.396	0.066	10.8577	0.0000
Error	18	0.110	0.060		
Total	27	0.516			
Coefficient of variation: 2.36%					

18: Yield (Kg/ha)

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	4646.601	1548.867	2.3840	0.1031
Factor A	6	688569.343	114761.557	176.6394	0.0000
Error	18	11694.490	649.694		
Total	27	704910.434			
Coefficient of variation: 2.13%					

**Significance at 5% level

20: Percent Germination

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	28.800	9.600	0.8405	
Factor A	6	174.269	29.045	2.5430	0.0582
Error	18	205.589	11.422		
Total	27	408.658			
Coefficient of variation: 3.51%					

**Significance at 5% level

19: Percent seed infection

Source of variance	Degrees of freedom	Sum of squares	Mean square	F value	Prob
Replication	3	2.417	0.806	0.0966	
Factor A	6	378.994	63.166	7.5759	0.0004
Error	18	150.078	8.338		
Total	27	531.489			
Coefficient of variation: 21.76%					