EVALUATION OF SELECTED BOTANICALS EXTRACT AND BAU- BIOFUNGICIDE AGAINST *CERCOSPORA* LEAF SPOT OF LETTUCE

MD. YUNUS ALI

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

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BY

MD. YUNUS ALI

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Approved By:

Dr. Khadija Akhter Professor **Supervisor** Department of Plant Pathology Sher-e-Bangla Agricultural University	**Dr. F. M. Aminuzzaman** Professor **Co-Supervisor** Department of Plant Pathology Sher-e-Bangla Agricultural University

Professor Dr. Fatema Begum
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

Sher-e-Bangla Agricultural University

CERTIFICATE

*This is to certify that thesis entitled, "**EVALUATION OF SELECTED BOTANICALS EXTRACT AND BAU- BIOFUNGICIDE AGAINST CERCOSPORA LEAF SPOT OF LETTUCE**" 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 (M. S.) IN PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by **Registration No. 18-09032** 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 source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 20/12/2020

Dhaka, Bangladesh

Dr. Khadija Akhter

Professor

Supervisor

Department of Plant Pathology

Sher-e-Bangla Agricultural University

Dhaka-1207

DEDICATED

TO

BELOVED PARENTS

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ABSTRACT

The study was conducted to evaluate selected botanicals and BAU- biofungicide against *Cercospora* leaf spot of lettuce in the Central Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during October, 2019 to February, 2020. Seven botanicals namely Allamanda leaf extract (1:3 w/v), Garlic clove extract (1:3 w/v), Neem leaf extract (1:3 w/v), Onion bulb extract (1:3 w/v), Lantana leaf extract (1:3 w/v), Turmeric rhizome extract (1:3 w/v), Mint leaf extract (1:3 w/v) and BAU- biofungicide (1ml/L) were applied including a control. At different days after transplanting (DAT), BAU- biofungicide and all botanicals significantly showed the minimum disease severity (1.17%), minimum plant disease incidence (15%) with increased yield (47.82%) of lettuce. However, the highest disease reduction with maximum yield and yield components were recorded in BAU-biofungicide applied plot followed by Neem leaf extract and Allamanda leaf extract. The BAU- biofungicide most effective against *Cercospora* leaf spot of lettuce compare to other treatment used in this experiment.

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ACRONYMS AND ABBREVIATIONS

ACRONYMS	ABBREVIATIONS
AEZ	Agro-Ecological Zone
ANOVA	Analysis of Variance
Agricultural	Agril.
Agriculture	Agric.
BARI	Bangladesh Agricultural Research Institute
Biology	Biol.
Biotechnology	Biotech.
Cm^2	Centimeter squares
CV	Coefficient of Variation
CDA	Carrot Dextrose Agar
DAT	Days After Transplanting
Entomology	Entomol.
et al.	And others
e. g.	exempli gratia (L), for example
etc.	Etcetera
FAO	Food and Agricultural Organization
G	Gram (s)
Horticulture	Hort.
i. e.	id est (L), that is
International	Int.
Journal	J.
Kg	Kilogram (s)
LSD	Least Significant Difference
M	Meter
mL	Milliliter
M. S.	Master of Science
No.	Number
SAU	Sher-e-Bangla Agricultural University
Science	Sci.
Naocl	Sodium Hypochloride
Species	Spp.
Var.	Variety
^oC	Degree Celsius
%	Percentage

CHAPTER I

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an annual plant of the family, Asteraceae. It is the world's most popular leafy salad vegetable (Raid, 2004). It is a leafy herb. It produces a short term stem early in the season, a cluster of leaves varying considerably in shape, character and color in different varieties. It is mainly a cold loving crop. The day temperature range for lettuce cultivation is 18°C to 25°C and the night temperature is 10°C to 15°C (Ryder, 1998). China, U.S.A, Spain, Italy, India and Japan are among the world's largest producers of lettuce (Lebeda *et al*. 2007; Mou 2008). China produced around 11,005,000 metric tonnes of lettuce on 500,250 hectares of land. FAO (2012) reported that some 12,574,500 tonnes of lettuce were produced during that year. In Bangladesh, huge quantity of lettuce is used in fast food shop and in various star hotels as fresh vegetable like salad. The cost of lettuce cultivation was found to be Tk. 145538 per hectare whereas the yield of lettuce was found 10887 kg per hectare. The gross margin was found to be Tk. 205284 per hectare (Afroj *et al*., 2013).

Lettuce is attacked by many diseases caused by fungi, bacteria, viruses. Among them nine fungal diseases, e. g., anthracnose, bottom rot, *Cercospora* leaf spot, damping-off, downy mildew, drop, gray mold, Septoria leaf spot and southern blight are identified (Raid and Nagata, 2003). Among the fungal diseases *Cercospora* leaf spot caused by *Cercospora longissima* is fairly common in china. In Thailand, it has been reported *Cercospora* leaf spot cause by *Cercospora lactucae-sativae* (To-Anun *et al*., 2011). The causal agent is *Cercospora longissima* is mainly a seed born pathogen. However; the pathogen is also able to survive for at least one year in plant debris and soil. These diseases cause reduction of yield of lettuce. Leaves are the prime edible parts of lettuce vegetable. Since, the yield or production data of this vegetable is not available in Bangladesh; it is assumed that, a vast amount of yield is lost in terms of quantity and quality due to various constraints. Among the constraints, diseases especially C*ercospora* leaf spot plays a vital role for the qualitative loss of this vegetable. The typical

symptoms of *cercospora* leaf spot appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded by a purple to brown border. The initial symptoms of the disease appear as water-soaked spot on leaves**.** As spots become older may coalesce together causing enlarged dead area on the infected leaves. Primarily their spores are dispersed by wind and is favored by prolong rainfall, high relative humidity and 25^0C to 35^0C temperature (Recardo *et al.*, 2015). Due to *Cercospora* leaf spot disease, photosynthetic process is disturbed and leaves becomes deformed resulting weakens plant, premature defoliation which ultimately lowers the yield and market value. The fungus *Cercospora* also causes disease on numerous economically plant and other leafy vegetable like Palong (Poornima, 2011), egg- plant (Srivastava and Nelson, 2012), legume crop like seasame, groundnut (Enikuomehin, 2005; Debele and Ayalew, 2015) and also in mungbean (Uddin *et al*., 2013) where the yield loses were recorded up to 58% (Lal *et al*., 2001).

Use of botanicals extract against plant diseases control is however a recent approach to plant diseases management and it has drawn the special attention of the plant pathologist all over the world. Many researchers reported plant extracts having antifungal properties and thus having potential to be used against many plant diseases (Singh and Dwivedi, 1987; Tariq and Magee, 1990; Lakshmanan *et al*., 1990). Most of the plant extracts are cost effective and do not have harmful effects on beneficial soil microorganisms. As chemicals have undescribed contribution on agriculture to control diseases and yield improvement but indiscriminate and frequent use of this chemical ultimately creates serious health hazards by entering in food web. So, now days, plant pathologist gives an attention on botanicals and BAU-biofungicide for the management of plant diseases.

In Bangladesh, only a few attempts have been made to evaluate plant extracts against plant diseases (Ashrafuzzaman and Hossain, 1992; Hossain *et al*., 1993; Ashrafuzzaman and Khan, 1992; Suratuzzaman *et al*., 1994, Hasan *et al*., 2005; Bdliya and Aikali, 2008).

Trichoderma have the ability to increase plant growth and induce plant resistance. Along with mycoparasitism, antibiotics and competition, induced resistance is one of the most important mechanisms of *Trichoderma* action against fungal plant pathogens (Cumagun, 2012). It would help to avoid environmental pollution caused by chemicals and thus become most rewarding one to our existing socio-economic conditions and environmental threat.

Considering the above scenario, the present piece of research was undertaen to find out the efficacy of foliar spray with seven botanicals extracts and BAU-biofungicide to control *Cercospora* leaf spot of lettuce. The specific objectives were considered:

1. To identify the causal organism of *Cercospora* leaf spot of lettuce;
2. To find out the effects of foliar spray with botanicals extract and BAU-biofungicide to control *Cercospora* leaf spot of lettuce; and
3. To determine the effect of foliar spray with botanicals extracts and BAU-biofungicide on yield and yield contributing characters of lettuce plant.

CHAPTER II
REVIEW OF LITERATURE

Use of botanicals instead of chemicals fungicides is one of the recent approaches for plant disease control but it is not commonly practiced. The research work so far done in Bangladesh and elsewhere is not adequate and conclusive. Nevertheless, some of the important and informative works and research findings related to the control through plant extract at home and abroad have been reviewed in this chapter.

Mandal and Mandal (2015) observed that all the extracts were used at 5% concentration had antifungal effects on *Cercospora longissima*. This potential of inhibiting growth could be due to the presence of biologically active secondary compounds, such as cinnamaldehyde in cinnamon, eugenol in both clove and cinnamon, and linalool in coriander.

Meghvansi *et al.* (2013) reported that the conidiophores of *Cercospora* spp. damage the host tissue by rupturing the epidermal layer of the leaves or emerging out from the host tissue through stomatal openings.

Nahunnaro and Tunwari (2012) evaluated five plant extracts against *C. sesame* and found that all the extracts reduced the disease by 7.1% to 8.64% compared to unsprayed plot.

Venturoso *et al*. (2011) reported that Several authors have studied the toxic effect of plant extracts on the pathogens. An antifungal effect of the extracts of garlic, cinnamon, and clove, on the in vitro development of pathogens, was observed at 20% concentration on six phytopathogens but only the aqueous extract of clove completely inhibited all fungi that were tested.

Das *et al*. (2010) reported that natural plant-based chemicals show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms.

According to Crous *et al*. (2007) *Cercospora* is classified as under the Kingdom – Fungi, Phylum – Ascomycota, Class – Dothideomycetes, Order – Capnodiales, Family – Mycosphaerellaceae, Genus *Cercospora*.

Hemachandra (2007) reported that among plant extracts tested, *Allium sp.* showed 100 percent inhibition of mycelial growth against Cercospora leaf spot of sugarbeet caused by *Cercospora beticola* Sacc, followed by *prosopis julifera.*

Islam *et al*. (2006) evaluated eight plant extracts including Vitavax-200 against leaf spot of wheat. Among eight plant extracts onion, garlic, kalijira, ginger, bishkatali and neem extract showed statistically similar grain yield as of seed treatment with vitavax-200. Seed treatment with bishkatali extract increased 29.74% grain yield over untreated control.

Hossain *et al*. (2005) reported that extract of different plant viz. bishkatali, vatpata, garlic, gagra, bitter gourd and neem were effective against fungi associated with wheat seed. Out of six plant species, neem extract was turned up as superior among the selected extracts followed by garlic, bishkatali and vatapta.

Chowdhury (2005) observed that highly infected/contaminated seed samples with seed borne fungi of rice, wheat, cosmos, zinnia, sunflower and radish were subjected to seed treatment with 1:0, 1:1, 1:5, 1:10 and 1:20 dilution of crude/nascent extract of garlic, datura and turmeric; 1:5, 1:10 and 1:20 dilution of commercially available oil extracts of neem, mahogany and koromcha. Botanicals at all concentrations reduced the occurrence of mycoflora on the seed significantly and thereby increased seed germination. Some fungi were totally removed at 1:10 dilution of commercially available plant oil extract.

In an experiment conducted by Shyam *et al*. (2004) during kharif season of 2000/01 and 2001/02, at Lucknow, Uttar Pradesh, India, the destructive yellow mosaic disease of mungbean (*Vigna radiata*), caused by mungbean yellow mosaic virus, was successfully controlled by the application of the aqueous root extract of Boerhaavia diffusa [*Boerhavia diffusa*]. Treatments were administered weekly, as foliar sprays, at a concentration of 10%, commencing from the seedling stage. Six sprays of *B. diffusa* root extract was found most effective, as it considerably delayed symptom appearance, suppressed symptom severity and decreased disease incidence by 80-90%. This treatment also increased root nodulation, plant height, primary and secondary branches, pod formation and grain yield.

Kaur *et al*. (2004) reported minimum disease intensity (16.72) and maximum disease control (64.58%) with two sprays of Topsin-m followed by Bavistin against *Cercospora* leaf spot of mungbean.

Crous and Braun (2003) stated that the genus *Cercospora* is currently considered as one of the largest and most heterogeneous genera of hyphomycetes.

Vincelli (2002) observed the risk of resistance can also be reduced by using IPM strategies that include an appropriate crop rotation schedule, planting resistant cultivars, and using good sanitation practices to reduce overall disease pressure.

Uttam *et al*. (2001) reported that neem products are being exploited for their pesticidal value but sometimes these products have some adverse effect on the germination of seeds. Keeping this in view, the experiment was planned to find out the non-toxic dose of neem products. Seeds of mungbean were coated with neem seed powder, Neemgold, Neemark, Fieldmarshal, Achook and Nimbecidine @ 5% and 10% w/w (v/w for liquid formulations). Coated seeds were tested for germination in moist sand and in moist filter paper. Germination percentage was calculated after one week of sowing and also after 14 days of sowing to observe the delayed germination, if any. It was found that germination was suppressed and delayed more in sand as compared with filter paper. All the neem based commercial formulations delayed germination and it was more pronounced with 10% dose of the products than in case of 5% dose. It was revealed that for pesticidal purposes only the dose of 5% w/w would be preferred.

Kapadiya and Dhruj (2001) reported that garlic extract was good inhibitor of growth as well as which plant spore germination with 79.86 and 92.34 per cent mean inhibition respectively.

Rahman *et al*. (1999) observed that bishkatali (*Polygonum hydropiper*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and neem (*Azadirachta indica*) extracts were effective against seed borne infections by *Alternaria tenuis*, *Bipolaris sorokiniana*, *Curvularia lunata*, *Fusarium* spp. of wheat. However, garlic was found superior to ginger and neem.

Singh *et al*. (1999) observed that plant quaternary alkaloid 3-alstovenine inhibited the spore germination of most of the *Cercospora* spp.

Khan (1999) studied the effect of plant extacts (allamanda, bel and neem) for the managemnt of *phomopsis* blight/fruit rot of eggplant in field condition by sparaying and observed that among the 3 plant extracts, allamonda was most effective than bel and neem extract.

Hossain *et al*. (1997) demonstrated from their experiment that the extract of *Allium sativum* and *Lawsonia alba* showed marked effect in inhibiting the spore germination and mycelial growth of *Bipolaris sorokiniana* and pathogenicity to wheat leaves and *Nigella sativa* showed positive antifungal activity in reducing the pathogenicity of *Bipolaris sorokiniana* of wheat leaves.

Kurucheve and Padmavathi (1997) evaluated five selected plant products against *Pythium aphanidermatum*, the causal organism of damping off of chilli. Among them *Allium sativum* (garlic) bulb recorded the minimum mycelium growth (176 mg) followed by *Lawsonia inermis* leaf extract. Maximum percentage of seed germination, growth and vigour of chilli seedlings were observed with garlic bulbs.

Mahfuzul (1997) evaluated some plant extract viz. garlic (*Allium sativum*), ginger (*Zingiber officinale*), nisinda (*Vitex negundo*), Dolkalmi (*Ipomoea fistulosa*) and marigold (*Tagetes erecta*) against major seed borne fungal pathogens of chilli. Among the plant extracts garlic was found to be most effective followed by neem leaf. The garlic and neem leaf extracts at the dilution ratio of 1:1were almost equally effective.

Benagi (1995) reported that leaf and kernel extract of *Azadirachta indica* at 2.5 % concentration was found inhibitory to conidial germination of *Phaeoisariopsis personata*.

Mohanty (1995) demonstrated that leaf extract of neem was significantly effective causing 52.23% growth inhibition of *Phomopsis vexans*, the causal agent of phomopsis blight and fruit rot of brinjal.

Suratuzzaman (1995) performed an experiment with plant extracts to control seed borne *Colletotricum dematium* var. *truncatum*, *Macrophomina phaseolina* and

Cercospora kikuchi of soyabean seed. Seed treatment with garlic and ginger extracts gave excellent control of pathogens.

Mohanty *et al*. (1995) observed that garlic bulb extract (1:1) and allamonda leaf extract sprays in the field reduced phomopsis blight and fruit rot by 66% and 75%, respectively.

Miller *et al*. (1994) reported that harmful chemical substances enter into the food chain that ultimately causes serious health hazards. Commercial varieties generally have only moderate levels of resistance and require fungicide applications to obtain acceptable levels of protection against *Cercospora* leaf spot.

Hossain and Schlosser (1993) found neem seed extracts/cake effective against *Bipolaris sorokiniana*. The extract inhibited the growth of the fungus and also reduced its pathogenecity on wheat leaves. Germination rate of wheat seeds increased after treatment with extracts of neem seed and cake.

Cammue *et al*. (1993) reported that the seed extracts of *Mirabilis jalapa, Amaranthus caundatus, Raphanus sativus* were having antifungal activity against *C. beticola.*

Khan and Hossain (1993) observed that extracts of *Allium alba*, *Ricinus communis*, *Leomurussi biricus* and *Metha viridus* completely inhibited spore germination of *B. sorokiniana* at 1:3 (w/v) dilution ratio

Achimu and Schlosser (1992) carried out an experiment to find out the effect of neem seed extracts against downy mildew (*Plasmopara viticola*) of grapevine. They found that raw neem seed extract and commercial neem products had high (80-90%) antifungal properties against *Plasmopara viticola*.

Ashrafuzzaman and Hossain (1992) evaluated pudina (Mentha viridis) extract against *Bipolaris sorokiniana* and observed that the extract inhibited mycelial growth and spore germination. In the same work they found that extract of caster (Ricinus communis) and Dantha kalash (Leucas aspera) were inhibitory against mycelial growth and spore germination of *Bipolaris sorokiniana*.

Ashrafuzzaman and Khan (1992) evaluated thankuni (*Hydrocotyl asiatica*), mehedi (*Lawsonia alba*) and duranta (*Duranta plumeiri*) against *Rhizoctonia solani* and

found all the extracts effective in reducing mycelial growth and sclerotia formation effectively.

Fakir and Khan (1992) reported that garlic bulb extract was effective in controlling seed borne fungal pathogen of jute such as *Macrophomina phaseolina* and *Fusarium* spp. by seed treatment.

Dubey and Dwivedi (1991) reported that fungistatic properties of extracts of leaves, bulb of onion and garlic and fruit, bark of *Allium cepa* against vegetative growth, *Sclerotia* variability of *Macrophomina phaseolina.* They observed that all the extracts inhibited growth but garlic bulb extract was more effective than other extracts employed in the tests.

Thakhur *et al*. (1991) studied on extracts of medicinal plants against cotton pathogens *Myrothecium roridum*, *Alternaria tenuis* and *Xanthomonus campestris* pv. *malvacearum* showed that among the nine extracts tested, *Punica granatum* and *Dutra metel* had the best antifungal and antibacterial activity against cotton pathogens.

Tewari and Mandakini (1991) reported that extract of *Piper betle*, *Ocimum sanctum*, *Nyctanthes arbortristis* and *Citrus lemon* were effective in reducing the radial growth of *Pyricularia oryzae*, *C. miyabeans* and *Rhizoctonia solani* in vitro, with extracts of *P. betle,* follwed by *O. sanctum* the most effective.

Lakshmanan *et al*. (1990) found that extract of pan (*Piper betel*) found to be effective against collar rot pathogen, *Thanatephorus cucumeris*. They also observed that garlic clove extract was most effective in inhibiting mycelial growth and spore germination of *Corynespora cassiicola*.

Miah *et al*. (1990) examined the efficacy of extract of eight different plant species against seed borne fungi of rice through eight hours seed soaking. Out of the plant species tested, extracts of *Allium sativum* and *Curcuma longa* reported to be promising.

Shetty *et al*. (1989) found that rice seeds soaked in 10%, 20% and 30% extracts (w/v) of garlic bulb and rhizome of ginger significantly reduced seed-borne infection of *Alternaria padwickii*.

Mishra *et al*. (1989) investigated the fungitoxic effect of lemon (*Citrus medica*) extract against *Aspergillus flavus* and found that the extract inhibited the fungus considerably.

Pons and Sutton (1988) mentioned it was used for naming any cercosporoid fungus, i. e. a dematiaceous hyphomycete with filiform conidia.

Assadi and Behroozin (1987) conducted an experiment to evaluate the efficacy of bulb extracts of onion and clove extracts of garlic against mycelial growth of *Fusarium* spp. and *Sclerotium cepivorum*. Garlic extract was found more active than that of onion in inhibiting growth of *Fusarium solani*, *Fusarium oxysporum* and *Fusarium acuminatum*.

Alice and Rao (1987) evaluated 31 plant extracts *in vitro* against *Drechslera oryzae* in rice using paper disc technique (inhibition zone technique) and found that maximum inhibition of *D. oryzae* was obtained with *Mentha piperita* followed by *Piper nigrum* seed extract and *Allium sativum* extract.

Singh and Dwivedi (1987) estimated that hyphal dry weight and sclerotia production of *Sclerotium rolfsii* Sacc. were significantly reduced by bark extracts of *Acacia arabica*. They evaluated bulb and leaf extracts of garlic and onion, leaf extracts of *Rauwolfia sarpentina*, *Lowsonia alba*, *Dutara stramonium*, *Solanum xarhocarpum*, *Calotropis procera*, *Eucalyptus globus*, *Emblica officinalis*, fruit extract of *Azadirachta indica* and rhizome extracts of turmeric and ginger against *Sclerotium rolfsii* and found that those extracts more or less effective in inhibiting the growth of the fungus.

Chalfo and Carvalho (1987) compared the garlic extract and chemical fungicide Captafol in controlling mycelial growth of *Gibberella zeae* where most effective concentration being 8000 ppm for garlic extract and 10000 ppm for Captafol.

Siddaramaiah (1986) reported that leaf extract of *Allium sativum, Ocimum sanctum* flower extracts of *Allium cepa, Mangifera indica,* and stem extracts of *A. cepa, A. sativum* and *Zingiber officinalis* were found inhibitory to conidial germination of *Cercospora moricola.*

El-Shami *et al*. (1986) observed that the antifungal property of garlic juice was also demonstrated against *Fusarium* wilt of watermelon caused 6 by *Fusarium oxysporum* f. sp. *niveum*. They also observed that garlic extract successfully inhibited spore germination and mycelial growth of fungus. But *in vitro* experiment, soaking water melon seeds in the extract gave better control of seedling infection than that of seed treatment with Benlate, Vitavax, Carboxin (captan + carboxin) or Thiram.

Dharam and Sharma (1985) observed that neem oil inhibited the growth of *Alternaria alternata* at different concentration in watermelon cultivation.

Ahmed and Sultana (1984) observed that bulb extract of garlic was most effective against the major seed borne pathogen of Jute viz. *Macrophomina phaseolina*, *Botryodiplodia theobromae* and *Colletotrichum corchori.* They reported that jute seeds treated with garlic paste increased seed germination and decreased the rate of post emergence seedling mortality over untreated control. The growth of jute plant was higher in treated plot than the control condition.

Siddaramaiah *et al.* (1980) reported that spore germination of *Cercospora* spp.and *Puccinia arachidicola* was completely inhibited in garlic extract at concentration of 1:8.

Szeto and Bau (1975) described the symptoms of *Cercospora* leaf spots of lettuce. it first appears as minute, water-soaked specks that gradually enlarge into circular to irregular spots that turn various shades of tan to brown. Sometime, spot centers are dingy gray. Occasionally, dead areas sufficiently numerous to kill an entire leaf. The disease progresses from older outer leaves to newer leaves.

Solheim (1929) described that the *Cercospora* mycelium appears to be very fine to coarse, fairly regular to very irregular, septate hyphae which for the most part ramify the host tissue, and the conidiophores may be continuous or septate, straight or flexuous, geniculate, denticulate, hyaline or of various shades and tints of brown up to blackish brown.

Atkinson (1891) reported teleomorphs of *Cercospora* has a few species viz. *Sphaerella gossypina*, as perfect stage of *Cercospora gossypina* and in majority of the cases, *Mycospherella* species have been observed as the perfect stage of *Cercospora*.

Saccardo (1876) at first reported the *cercospora* leaf spot disease, also known as frogeye leaf spot disease. This disease is caused by a fungus referred to as *Cercospora*. The asexual spores (conidia) of *Cercospora* infect the leaves by penetrating directly or through stomata. Fungal mycelium develops in leaves and, damages the tissues, which become visible in the form of circular or irregularly shaped brownish spots with light whitish centre resembling ‘frogeye’. A photosensitizer toxin, cercosporin, isproduced by the pathogen which incites the production of reactive oxygen species (ROS) that disrupts the cell membrane. By this mechanism, *Cercospora* infects the plant and consumes the nutrients available in them to flourish self.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted at Central Farm and Plant Pathology Laboratory of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka- 1207. The location of the experimental site was at 23^0 46' N latitude and 90^0 22'E longitudes with an elevation of 8.24 meter from sea level details are given in Appendix I.

3.2 Experimental Period

The experiments were conducted during the period from October, 2019 to February, 2020 (winter months).

3.3 Soil Characteristics

The experimental plote was a medium high land belonging to the Modhupur Tract under the Agro Ecological Zone (AEZ) 28. The soil texture was silty loam, non-calcarious, dark grey of Tejgaon soil series with a pH 6.7. The characteristics of soil are shown in Appendix II.

3.4 Weather Condition

During the experiments, the average temperature, precipitation and relative humidity of those months are given below:

Table 1. Average weather conditions in Dhaka, Bangladesh in 2019-20

Season and year	Average Temperature (°C)	Average Precipitation (mm)	Average Humidity (%)
October 2019 to February 2020	20.5	16.67	72

Source: Metrological Department of Bangladesh

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3.5. Treatment

The following treatments were used for the treatment:

$T_{0=}$ Control,

$T_{1 =}$ Allamanda (*Allamanda cathertica*) leaf extract (1:3 w/v)

$T_{2=}$ Garlic (*Allium sativum*) clove extract (1:3 w/v)

$T_{3=}$ Neem (*Azadirachta indica*) leaf extract (1:3 w/v)

$T_{4=}$ Onion (*Allium cepa*) bulb extract (1:3 w/v)

$T_{5=}$ Lantana (*Lantana camara*) leaf extract (1:3 w/v)

$T_{6=}$ Turmeric (*Curcuma longa*) rhizome extract (1:3 w/v)

$T_{7=}$ BAU-biofungicide (1ml/L)

$T_{8=}$ Mint (*Mentha lamiales*) leaf extract (1:3 w/v)

3.6 Variety and Collection of seeds

Grand Rapid variety was used in the experiment. The seeds were collected from siddique Bazar, Dhaka.

3.7 Collection of botanicals

Botanicals were collected from Sher-e-Bangla Agricultural University Campus, Dhaka

3.8 Preparation of botanical extracts

The extracts were prepared following the method of Ashrafuzzaman and Hossain, (1992). For preparation of extracts, collected plant parts were weighted on an electric balance and washed with running tapwater. After washing the plant parts were were cut into small pieces. For getting extract, weighted plant parts were blended and distilled water was added into the blender. The pulverized mass was squeezed through 3-folds of chese cloth. For getting 1:3 (w/v) ratio 300 ml of distilled water was added to 100 g plant parts.

3.9 Procedure of application of plant extract

Plant extracts were applied in the field as foliar spray. Spraying was done 4 times at 10 days interval start from 25 days after transplanting.

3.10 Collection of BAU-biofungicide

BAU-biofungicide which was a formulated product of *Trichoderma harzianum*, collected from Integrated Pest Management Laboratory, Department of Plant Pathology, Bangladesh Agricultural University campus, Mymensingh. Distributor, Biotech Care.

3.11 Preparation and application of BAU-biofungicide

For getting 1ml/L, 1ml of BAU-biofungicide was added with 1L distilled water. The BAU-biofungicides were applied as foliar spray. Spraying was done 4 times at 10 days interval start from 25 days after transplanting.

3.12 Design and layout of the Experiment

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The layout of the experiment was prepared for distributing the treatment combinations in each plot in each block. There were 27 units plot in the experiment. The size of the plot was 3.0 m × 1.0 m. The distance between two blocks and two plots were 0.75 m (Appendix III).

3.13 Raising of seedlings

The seedlings were raised at the Farm of SAU, Dhaka with special care in a 1m x 0.5m size seed bed. The soil of the seed bed was well ploughed with spade and prepared into loose friable dried masses to obtain good tilth to provide a favorable condition for vigorous growth of seedlings. Weeds, stubbles and dead roots of the previous crop were removed. The seedbed was dried in the sun to destroy the soil insect and to protect seedlings from the attack of damping off disease. To control damping off disease cupravit fungicide were applied. Cocodust was applied to prepare seedbed. Lettuce seeds were soaked in water for 48 hours and then seeds

were mixed with soil and sown in seed bed. Twenty (20) grams of seeds were sown in each seedbed on October 30, 2019.

3.14 Preparation of the main field

The selected experimental plot was opened in the last week of November 2019 with a power tiller and was exposed to the sun for a week for soil solarization. After one week the land was harrowed, ploughed and cross-ploughed several times followed by laddering to obtain a good tilth. Weeds and stubbles were removed and finally obtained a desirable tilth of soil for sowing seeds of lettuce. The experimental plot was partitioned into the unit plots in accordance with the experimental design.

3.15 Transplanting of seedlings in the main Field

Apparently healthy and uniform sized seedlings were transplanted in the main field according to the treatments on 26 November, 2019. The seedlings were uprooted carefully from the seedbed to avoid any damage to the root system. To minimize the roots damage of the seedlings the seedbed was watered before uprooting the seedlings. Transplanting was done in the afternoon. During transplanting a spacing of 25 cm × 40 cm row to row and plant to plant were maintained. A number of seedlings were also planted in the border of the experimental plots for gap filling necessary.

3.16 Application of manure and fertilizers

Three impotant fertilizers N as urea, TSP and MP were applied. The entire amounts of TSP and MP were applied at final land preparation. Urea was applied in three equal installments at 10, 20 and 30 days after seedling transplanting. Well-decomposed cowdung at 10 t/ha was applied during final land preparation. Urea, TSP and MP was applied at 200, 75 and 75 kg/ha (Rashid *et al.* 1993).

3.17 Intercultural operation

After emergence of seedlings, various intercultural operations were accomplished for better growth and development of plants.

3.17.1 Irrigation

Light over-head irrigation was provided with a watering can to the plots at 4 and 7 weeks after the seed sowing respectively. Irrigation also applied two times considering the moisture status.

3.17.2 Weeding

Weeding was done two times in the experimental plot. First weeding was done one month after sowing followed by another with 20 days interval.

3.18 Crop sampling and data collection

During the growing period the plots of lettuce were inspected regularly to record the leaf spot disease and to measure different agronomic parameter. Dead plants were removed from the field after counting and infected leaves were collected to identify leaf spot pathogens. Number of infected leaves was obtained from randomly selected five plants and marked with sample sticks and following data were recorded.

3.18.1 Plant height

Plant height was measured from five randomly selected plants using meter scale in centimeter from the ground level to the tip of the longest leaf at 10 days interval starting from 25 days after transplanting (DAT) and continued upto 55 DAT and their mean value was calculated.

3.18.2 Number of leaves per plant

Number of leaves per plant was counted from five randomly selected plants at 10 days interval starting from 25 days after transplanting (DAT) and continued upto 55 DAT and their average was recorded.

3.18.3 Leaf area

Fresh leaves of plant were recorded from five randomly selected plant and leaf area was measured by centimeter scale at 25, 35, 45 and 55 days after transplanting (DAT).

3.18.4 Number of leaf spot per leaf

Number of leaf spot per leaf was recorded from infected leaves at 25, 35, 45 and 55 DAT.

3.18.5 Assessment of disease incidence

The disease incidence was recorded 10 Days interval from the first appearance of disease. The incidence of disease was recorded four times at 25, 35, 45 and 55 DAT at 10 days interval, Disease incidence data were calculated following standard formulae (Nutter *et al*., 2006; Agrios, 2005; Kranz, 1988):

$$\text{Plant incidence (\%)} = \frac{\text{Numbers of infected plants}}{\text{Numbers of inspected plants}} \times 100$$

$$\text{Leaf incidence (\%)} = \frac{\text{Numbers of infected leaves}}{\text{Numbers of inspected leaves}} \times 100$$

Reduction of disease incidence over control was calculated by using the following formula (Abbott, 1925):

$$\text{Disease Incidence Reduction over Control (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = % disease incidence in control plot

T = % disease incidence in treated

3.18.6 Assessment of disease severity

Five infected plants were selected randomly from each plot for scoring. Four sprays were applied at an interval of 10 days. The first spraying was done at the first appearance of disease symptom. Disease data were recorded before every spray. Infected plants were scored at 25, 35, 45, 55 DAS using (0-5) rating scale 3which was developed as follows (Mehta and Mandal, 1978).0 = No infection

1 = 10% leaf area infection

2 = 11-30% leaf area infection

3 = 31-50% leaf area infection

4 = 51-70% leaf area infection

5 = 71%and above leaf area infection

Percent disease index (PDI) was calculated using the recorded data according to (Prasad *et al*., 1979)

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of total ratting} \times 100}{\text{Total number of observations} \times \text{Highest grade in the scale}}$$

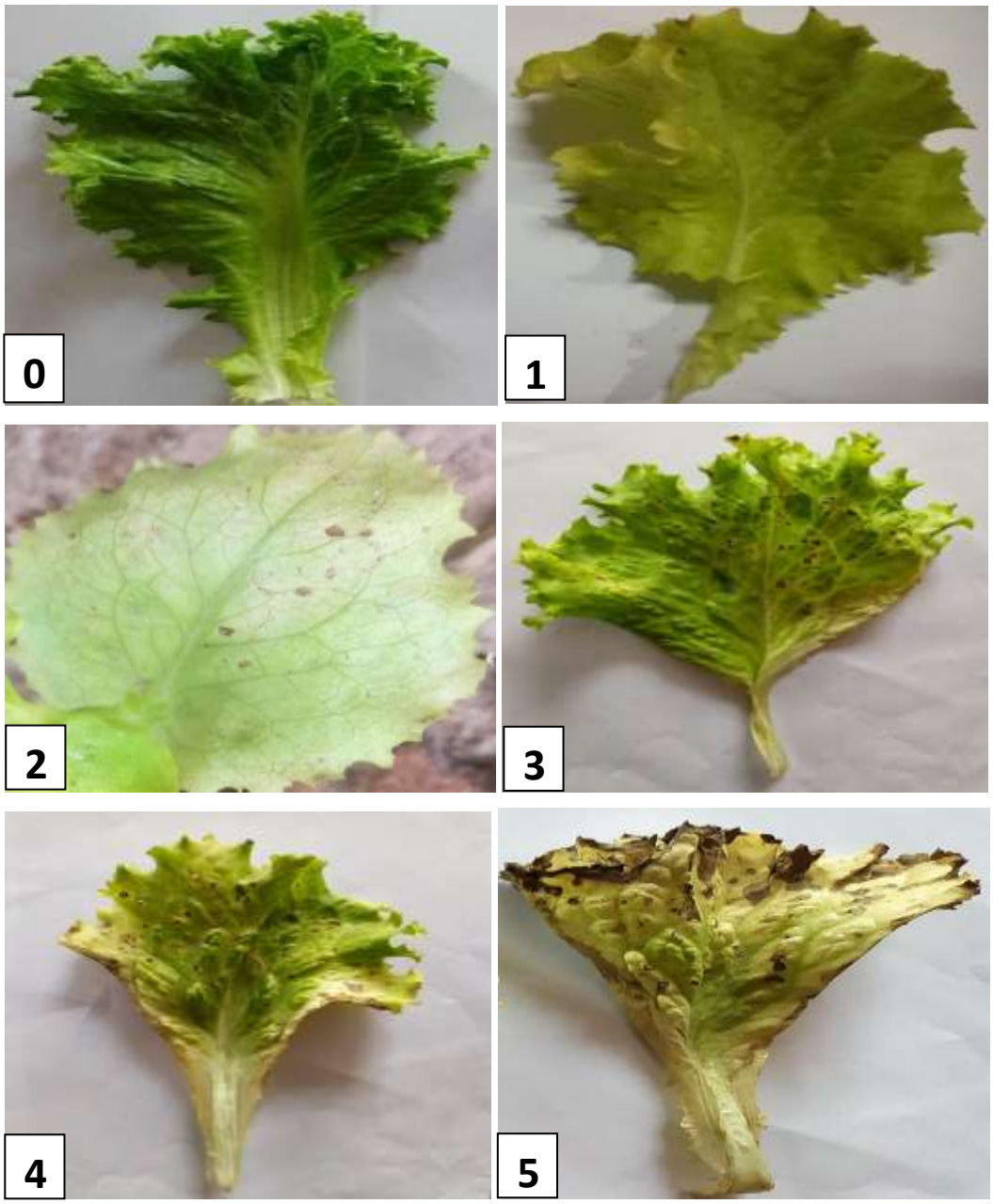

Plate 1. Disease severity grade of *Cercospora* leaf spot of lettuce. Grade 0 = 0%, Grade 1= 10%, Grade 2=11-30%, Grade 3 = 31-50%, Grade 4 = 51-70%, Grade 5 = above 70%.

3.18.7 Total yield per plot

Total yield of lettuce per plot was recorded by adding the yield of different harvesting time and it was included weight of leaves at different harvesting time and was expressed in kilogram.

3.18.8 Yield per hectare

Yield/hectare was computed by converted total yield per plot into yield per hectare and was expressed in ton. It included weight of leaves at different harvesting time from 25 days after transplanting (DAT) and continued upto 55 DAT at 10 days interval.

3.19 Laboratory experiment

3.19.1 Collections of diseased specimens

Diseased leaves of lettuce were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka- 1207. Collected leaf samples were put in polyethylene bags immediately after collection. Then the samples were preserved in refrigerator at 4 °C for future investigation.

3.19.2 Preparation of carrot dextrose agar (CDA) media

The present studies a standard carrot dextrose agar medium was used 200 g cleaned, washed and peeled carrot tubers were chopped into small pieces. Later pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose (20 g) and agar (20 g) were dissolved in the carrot extract and the volume was made upto 1000 ml by adding distilled water. Known quantity of medium was dispensed into number of conical flasks. The flask was plugged with non absorbent cotton and finally wrapped with alluminium foil. The flasks containing dispensed medium was sterilized at 121 °C temperature with 15 PSI pressure for about 30 minutes. Then the medium was allowed to cool and acidified by adding of lactic acid (1:10) in medium. The medium was dispensed aseptically into sterile glass Petri-dishes inside the inoculating chamber and allowed to cool down to solidify.

3.19.3.1 Moist blotter method

The causal organism was isolated by moist blotter method. Margins of infected leaves (2- 5 mm diameter) were cut to contain both diseased lesions and healthy uninfected tissues using flame-sterilized scissors and forceps. Cut pieces of leaves were surface-sterilized (0.1 % NaOCl for 5 minutes then rinsed in five times with sterile distilled water) and blottered dry with tissue paper in the laminar flow (Plate 2).

3.19.3.2 Agar plate method

Then placed the hyphal tip of fungi on carrot dextrose agar (CDA) containing petridishes and incubated at 28 ± 2 °C for 7 to 10 days. Fungi grew from the plant parts (Plate 3) were subcultured until pure cultures were obtained. (Holliday, 1980; Domsch *et al*., 1981; Barnett and Hunter, 1999).

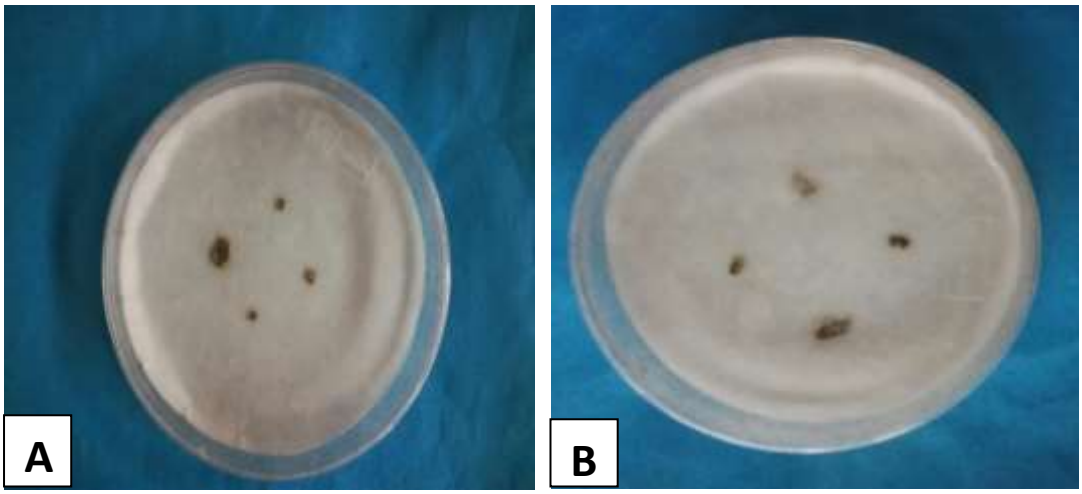

Plate 2 (A-B): Leaf sample with leaf spot on moist blottered paper

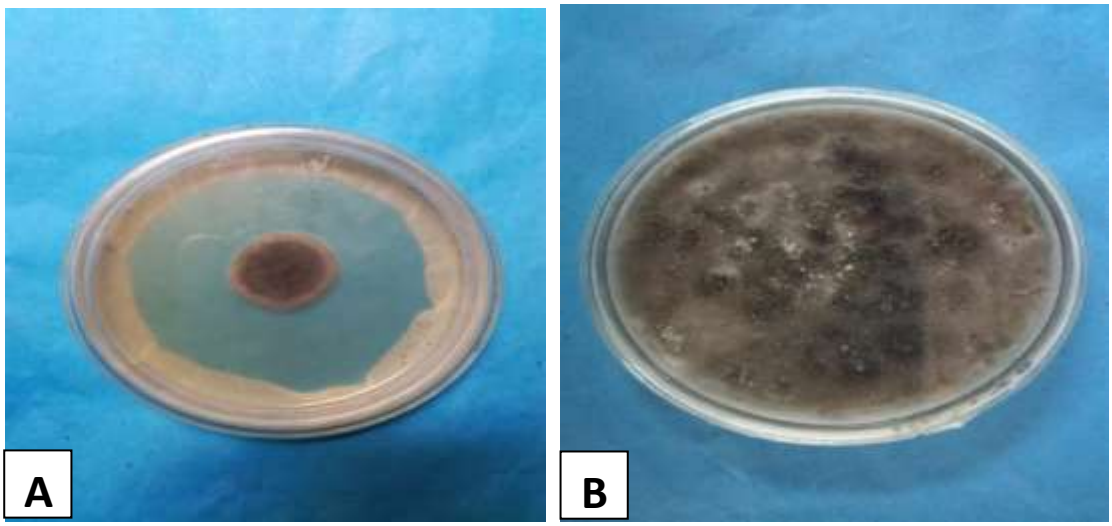

Plate 3 (A-B): Pure culture of *Cercospora longissima* on CDA media

3.19.4 Identification of *Cercospora longissima*

Tuff of conidiophore was observed. The Conidiophores of *Cercospora longissima* were septate, straight or flexuous, geniculate, hyaline, blackish to brown colour. The growth of the conidiophores was determinate or sympodial (Plate 6-A, B, C) They damage the host tissue by rupturing the epidermal layer of the leaves or emerging out from the host tissue through stomatal openings. The shape of conidia cylindrical, solitary, hyaline, filiform, straight to slightly curved with obtuse to subacute at the apex and subtruncate bases, multiseptate (Plate 6- C, D)

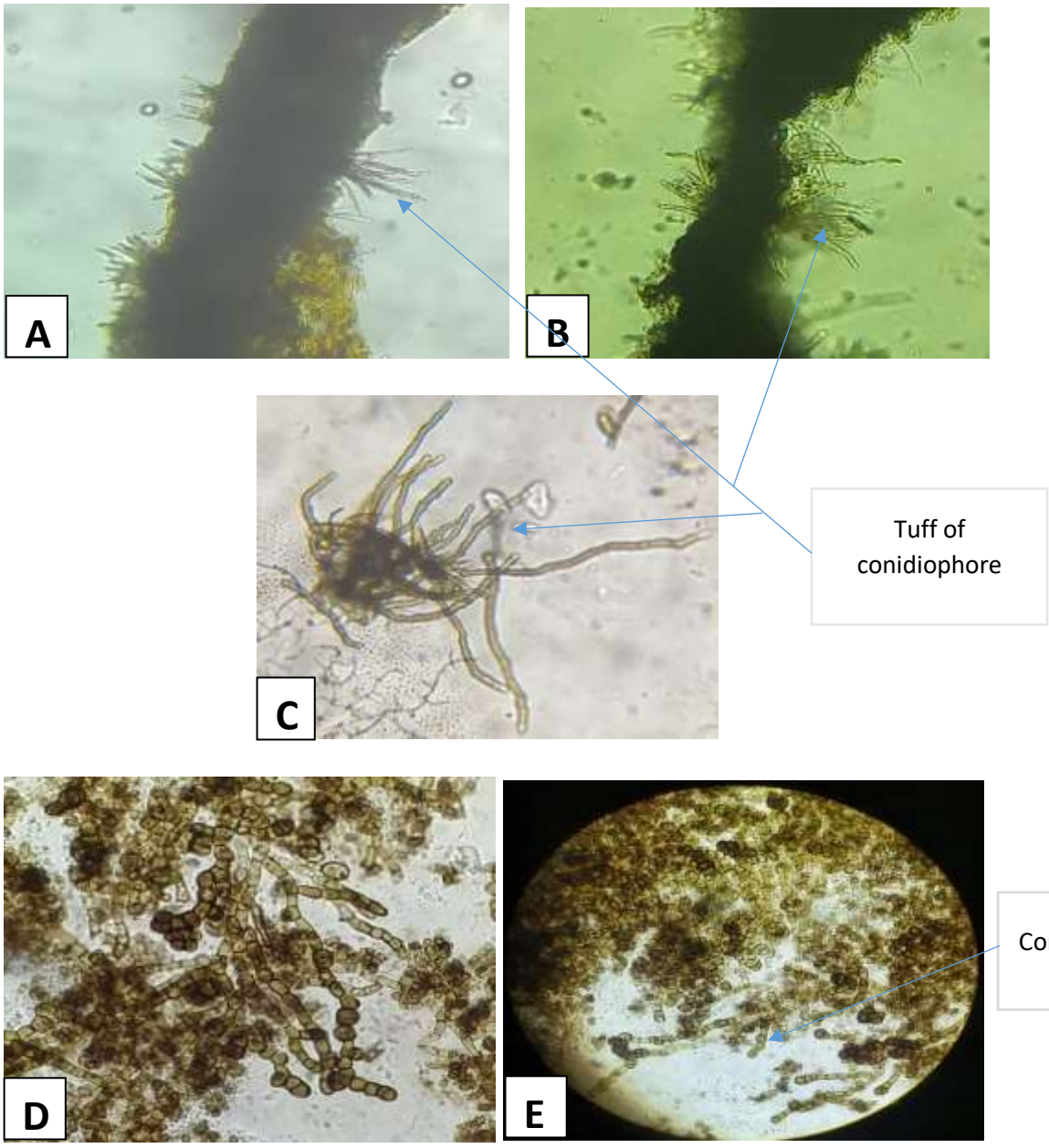

Plate 4. (A-C) Pathogenic structure of *Cercospora longissima* by cross sectioning of infected leaf tissue (Compound Microscopic view, 10X)), **(D-E)** from pure culture (Sterio Microscopic view, 10X).

3.20 Nethouse experiment

3.20.1 Soil collection

Soil was collected from the experimental fields of Sher-e-Bangla Agricultural University, Dhaka-1207.

3.20.2 Soil Sterilization

Soil was sterilized with 40% formalin solution @ 200 ml/cft soil and kept covered with polyethylene sheets for 2-3 days. Then the soil was uncovered and pulverized enough and kept for 2-3 days to release the gas of formalin.

3.20.3 Preparations of pots

Sterilized soil was applied at the rate of desired amount per pot. Then the pots were arranged.

3.20.4 Seeds sowing in pot

Disinfected viable seeds of lettuce *var*. grand rapid were sown in pots with 4-6 seeds per pot in the net house. Watering was continued till the seedlings were established.

3.20.5 Pathogenicity test of *Cercospora longissima*

Conidia of fungus was removed by flooding each petridish with 10 ml of sterile distilled water and scrubbing the surface with a glass slide. Conidial suspension was prepared and filtered through three layers of muslin cloth to remove mycelial fragments. The suspension was made to 100 ml. The conidial concentration was determined using the Hemocytometer. The adaxial surfaces of the leaves were sprayed with hand sprayer and at a concentration of 0.5×10^3 spore/ml. Each plant was covered with a transparent polythene bag for 48 hours to 72 hours for maintaining with high humidity. Control plants were sprayed with sterile distilled water. Disease assessment was done by visual observation for presence of symptoms from 10 to 30 days after inoculation (Plate 5).

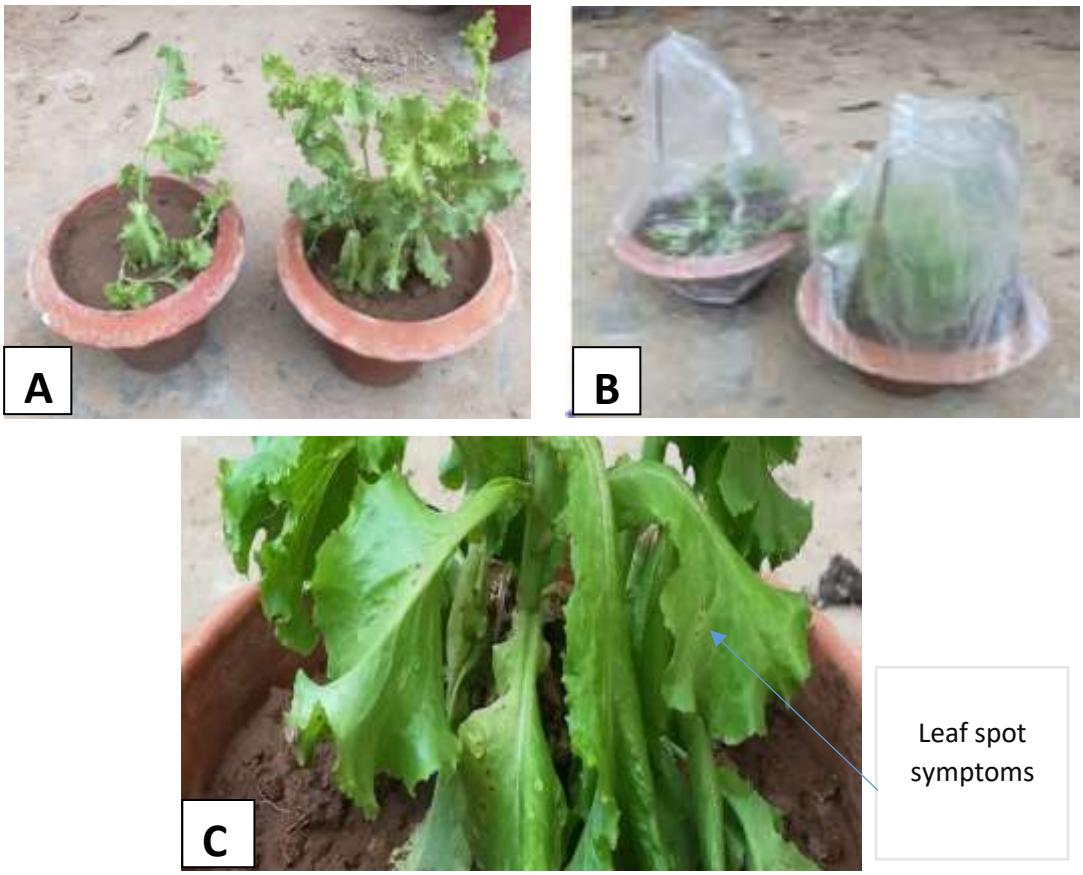

Plate 5 (A-C). Pathogenicity test for C*ercospora longissimi*

3.21 Statistical analysis

Field experimental data were analysed by using Randomized Completely Block Design (RCBD). Data was recorded on disease incidence, severity, yield contributing characters and yield of lettuce. The analysis of variance was performed by using MSTAT-C program. The significance of the difference among the treatment means was estimated by LSD at 5% level of probability.

Plate 6. (A-B) Seeds of lettuce Grand Rapid

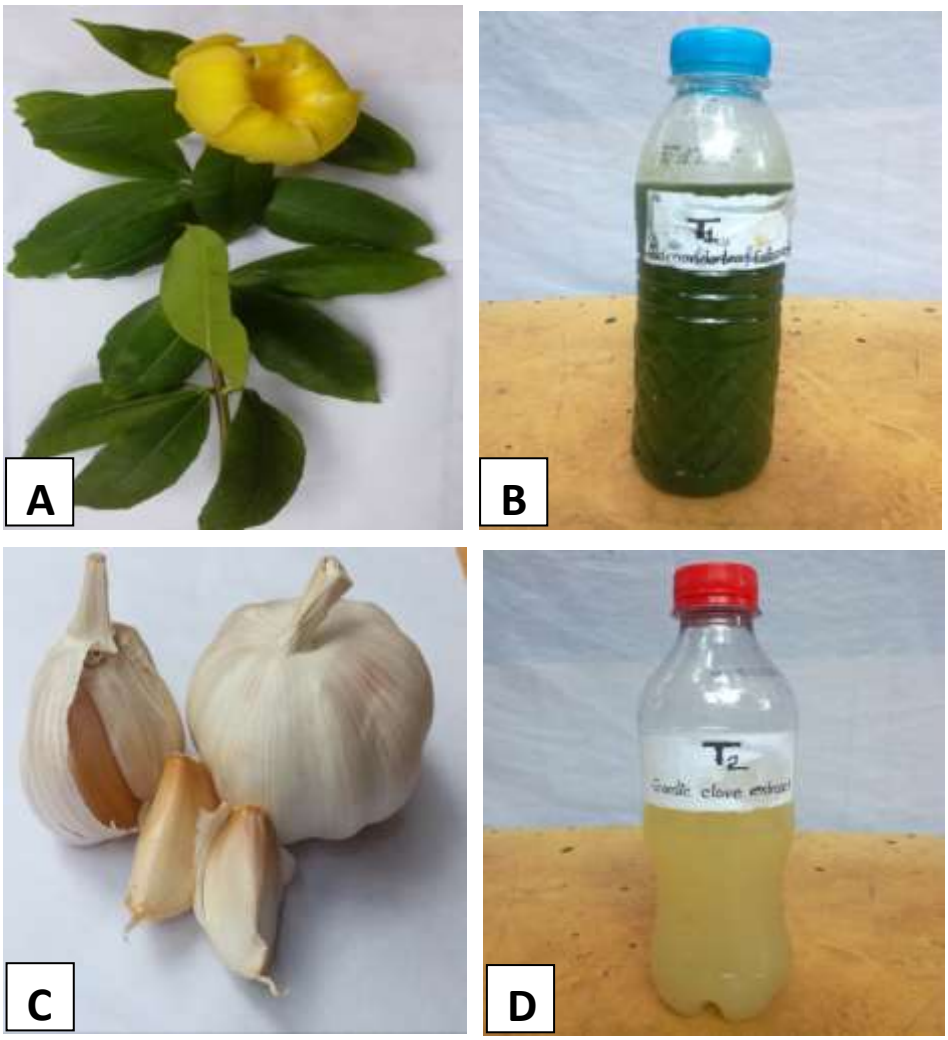

Plate 7. (A-D**)** Used treatments in the experiment

A. Allamanda (*Allamanda cathertica*) leaf, **B**. Allamanda leaf extract, **C**. Garlic (*Allium sativum*) clove, **D**. Garlic clove extract

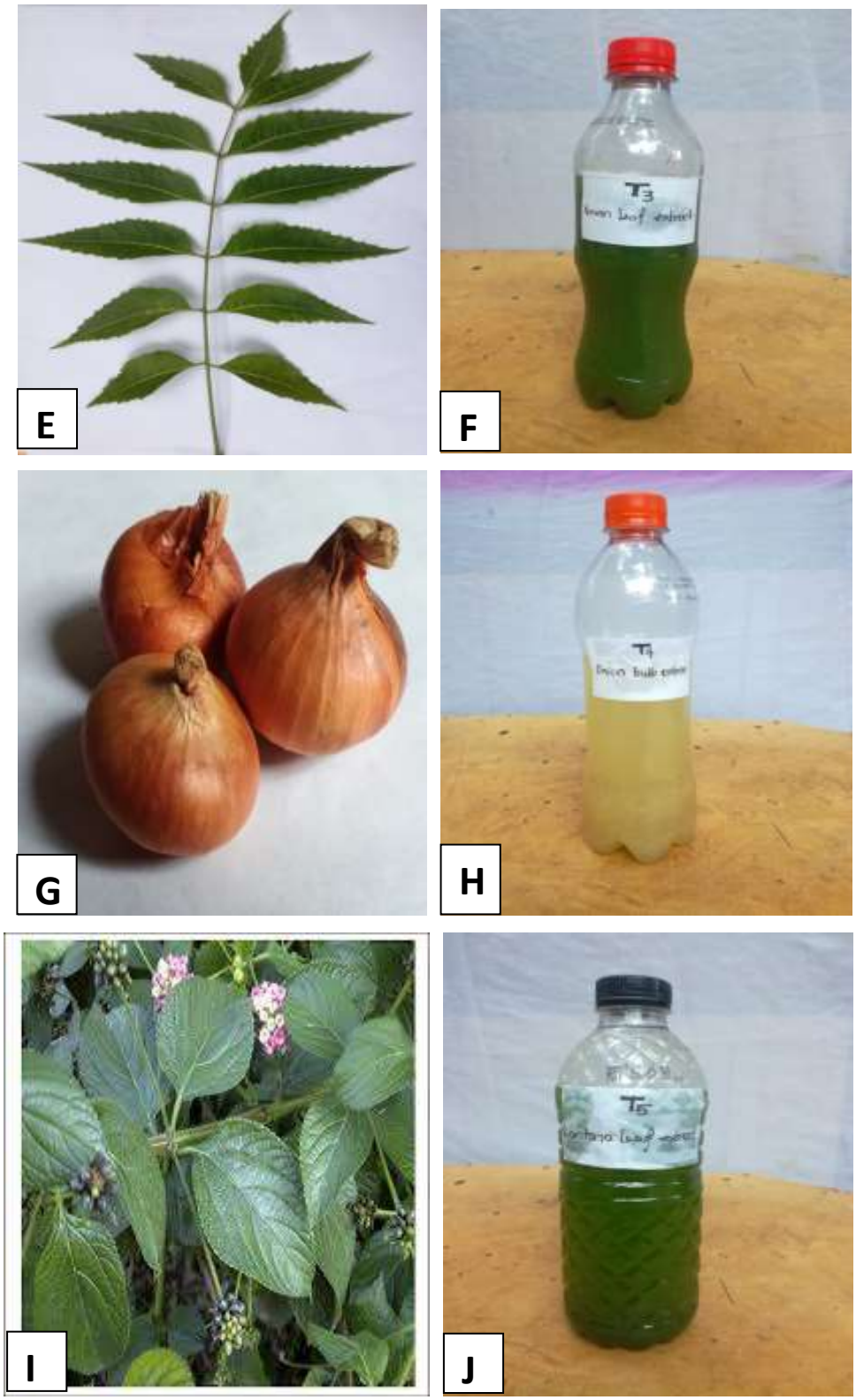

Plate 8. (E-J) Used treatments in the experiment

E. Neem (*Azadirachta indica*) leaf, **F**. Neem leaf extract,

G. Onion (*Allium cepa*) bulb, **H**. Onion bulb extract,

I. Lantana (*Lantana camara*) leaf, **J**. Lantana leaf extract

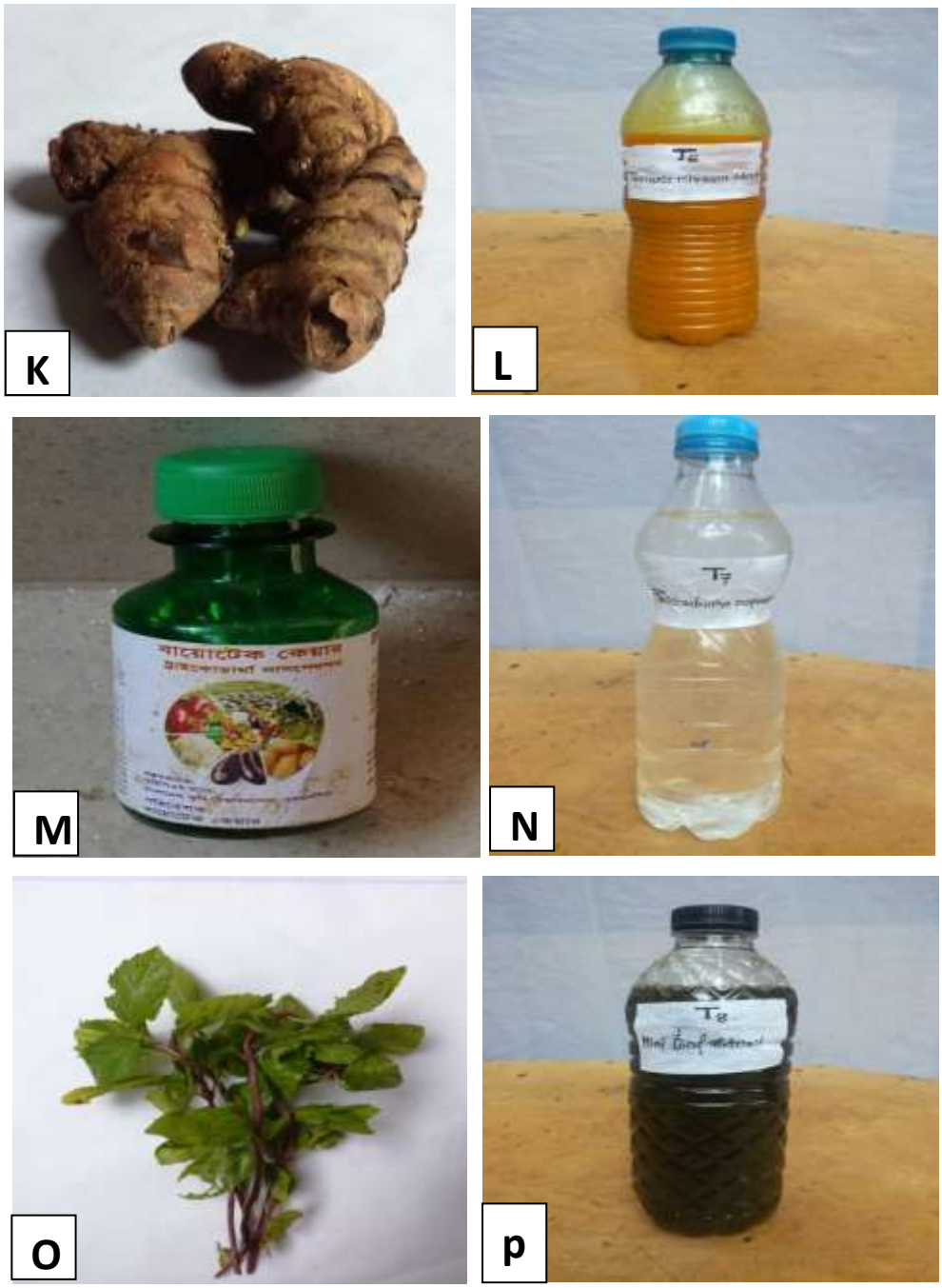

Plate 9. (K-P) Used treatments in the experiment

K. Turmeric (*Curcuma longa*) rhizome, **L**. Turmeric rhizome extract,

M. Trichoderma suspension, **N**. Trichoderma solution,

O. Mint (*Mentha lamiales*) leaf, **P**. Mint leaf extract.

Plate 10. Field view of experimental plot

CHAPTER IV
RESULTS

4.1 Symptoms of *Cercospora* leaf spot disease of lettuce

The characteristics leaf spot symptoms were observed in the experimental field of SAU under natural conditions. Disease symptoms include at initial stage, slightly pale areas were on the upper surface of the leaves (Plate 12). After few days, small brownish spots were turn to become visible which later on increase in their size. The shape and size of these spots is quite variable. Shape of the leaf spots may be circular to irregular size less than 1 mm to 10 mm in diameter. Margins of infected leaves (2 - 5 mm diameter). In severe cases, the spots coalesce and affect a significant proportion of leaf area there by reducing photosynthetic capability. The spots, in some cases are surrounded by a yellow halo (Plate 11). Spots may be light brown, reddish brown or dark brown in colour with whitish centre. sometimes with yellow halos, and joining together to cover large areas of the leaf. The older leaves are infected first.

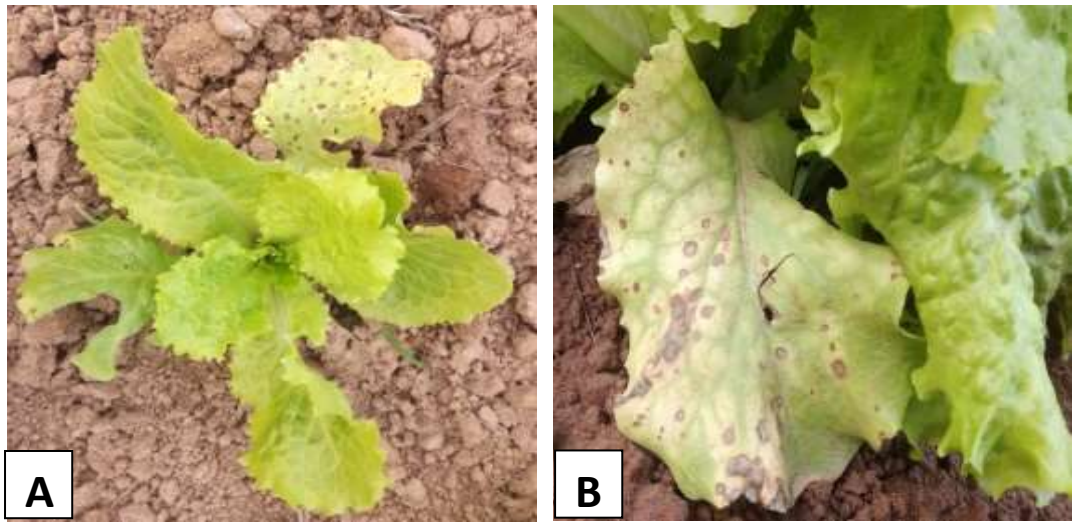

Plate 11. (A-B) Symptoms showing in standing plants

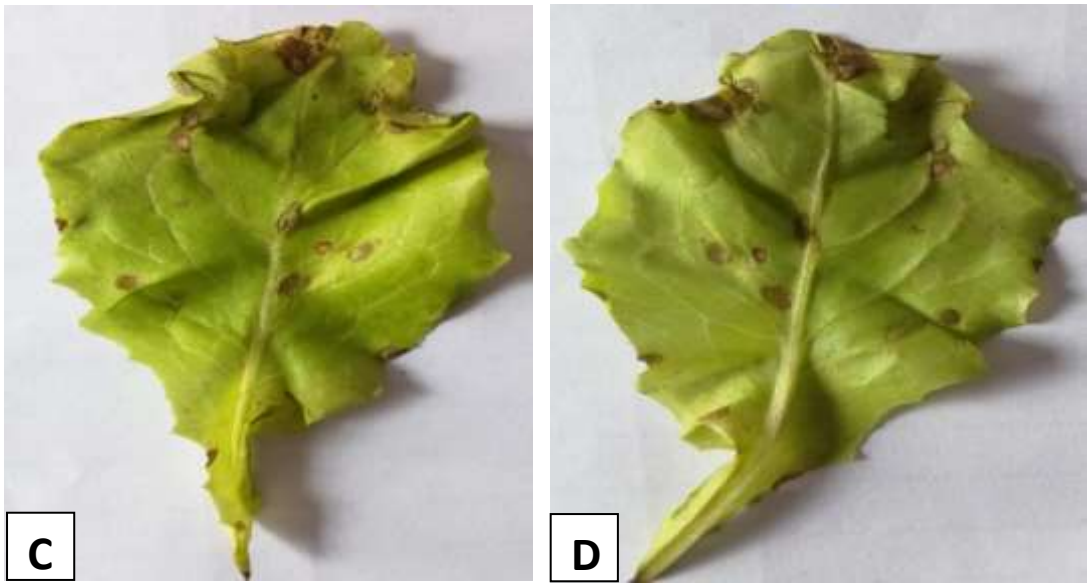

Plate 12. (C-D) Symptoms of leaf spot disease on both side of the leaves of lettuce A (upper side), B (lower side)

4.2 Number of leaf spot per leaf influenced by botanicals extract and BAU-biofungicide

At 25 days after transplanting (DAT), the number of leaf spot ranged from 5.82 to 7.87 per leaf. The highest number of leaf spot was recorded from control, which was statistically similar to leaf spot from treatments with Onion bulb extract, Turmeric rhizome extract and Mint leaf extract. On the other hand, BAU-biofungicide, extracts of Neem leaf, Lantana leaf, Allamanda leaf, Garlic clove (in order of efficacy) significantly reduced spot number over control. The highest number of 24.82, 27.83 and 34.79 *Cercospora* spot per leaf was recorded from Control at 35, 45 and 55 DAT. Foliar spray with extracts of all botanicals and BAU-biofungicide significantly reduced the parameter over control at those three days after transplanting of lettuce seedlings. At 35 DAT, the number of leaf spot under treated plots ranged 9.51 – 18.92 per leaf. The highest reduction in leaf spot was obtained from BAU-biofungicide followed by Neem and Allamanda leaf extract. At 45 DAT, all treatments with BAU-biofungicide and botanicals extract significantly reduced the number of leaf spot within the ranged of 14.60 – 20.23 spots/leaf. The mximum reduction was obtained with BAU-biofungicide (1ml/L), which was statistically similar, neem leaf extract and Allamanda leaf extract. At 55 DAT, the effective treatments to reduce spot number within the range of 15.04 - 21.25 spots/leaf. The lowest number of leaf spot was recorded from BAU-biofungicide, which was statistically similar to Neem leaf extract and Allamanda leaf extract (Table 2).

Table 2. Influenced of botanicals extract and BAU-biofungicide on number of *Cercospora* leaf spot per leaf of lettuce

Treatment with botanicals extract and BAU-biofungicide	Number of leaf spot/ leaf			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	7.87 a	24.82a	27.83a	34.79a
Allamanda leaf extract (1:3 w/v)	6.19 cd	14.34bcd	16.63cd	17.21cd
Garlic clove extract (1:3 w/v)	6.73 bcd	16.37b	18.50bc	19.28bc
Neem leaf extract (1:3 w/v)	5.92 d	11.30cd	16.01cd	16.57cd
Onion bulb extract (1:3 w/v)	7.09 abc	17.29b	18.57bc	19.40bc
Lantana leaf extract (1:3 w/v)	6.30 cd	14.61bc	17.53b-d	18.23b-d
Turmeric rhizome extract (1:3 w/v)	7.79 a	18.92b	20.23b	21.25b
BAU-biofungicide (1ml/L)	5.82 d	9.51d	14.60d	15.04d
Mint leaf extract (1:3 w/v)	7.55 ab	18.66b	18.83bc	19.78bc
CV (%)	7.77	17.26	9.20	9.24
LSD $_{(0.05)}$	0.91	4.84	2.98	3.22

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.3 Disease incidence (%)

4.3.1 Effect of botanicals extract and BAU-biofungicide on disease incidence of lettuce (leaf)

At 25 DAT, incidence of *Cercospora* leaf spot on leaf of lettuce ranged 1.63 - 2.53% under different treatments including an untreated control. Differences in this parameter under eight different treatments (including control) was not significantly different. Under other three days after transplanting of lettuce seedlngs, the disease incidence was 4.27, 7.55 and 10.75%, which were reduced to 1.90 – 3.11, 3.28 – 5.10, and 3.51 – 6.10%, respectively due to foliar spray with BAU-biofungicide and extract of seven botanicals. At 35 DAT, the lowest disease incidence of 1.90% was achieved with BAU-biofungicide followed by Neem (2.23%) and Allamanda (2.36%). Their differences were significant. The least effective material was Turmeric followed by Onion and Mint. At 45 DAT, the most effective treatment was BAU-biofungicide, followed by Neem, Allamanda and Lantana leaf extracts, the four treatments were statistically similar. At 55 DAT, the lowest disease incidence of 3.51% was recorded from BAU-biofungicide, which was statistically similar to Neem, and Allamanda extracts (Table 3).

Table 3. Efficacy of botanicals extract and BAU- biofungicide on disease incidence of lettuce (leaf)

Treatments with botanicals extract and BAU- biofungicide	(%) Disease incidence (Leaf)			
	25 DAT*	35 DAT	45 DAT	55 DAT
Control	2.53a	4.27a	7.55a	10.75a
Allamanda leaf extract (1:3 w/v)	1.80a	2.36e	3.86cde	4.40de
Garlic clove extract (1:3 w/v)	1.98a	2.55d	4.20cd	5.24bcd
Neem leaf extract (1:3 w/v)	1.70a	2.23f	3.64de	4.00e
Onion bulb extract (1:3 w/v)	2.13a	2.72c	4.49bcd	5.54bc
Lantana leaf extract (1:3 w/v)	1.98a	2.42e	4.08cde	4.91cd
Turmeric rhizome extract (1:3 w/v)	2.40a	3.11b	5.10b	6.10b
BAU- biofungicide (1ml/L)	1.63a	1.90g	3.28e	3.51e
Mint leaf extract (1:3 w/v)	2.17a	2.83c	4.63bc	5.69bc
CV (%)	6.12	2.83	11.07	9.40
LSD $_{(0.05)}$	0.92	1.32	0.86	0.91

*Non significant

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.3.2 Efficacy of seven botanicals extract and BAU-biofungicide on disease incidence of lettuce (plant)

At 25 DAT, the plant disease incidence ranged 7.33 – 11.67% under all treatments including control. The highest plant disease incidence was recorded from control which was statistically similar to only Turmeric rhizome extract, Except Tumeric rhizome extract, all other treatments significantly reduced plant disease incidence over control. The most effective treatment to reduce disease incidence was foliar spray with BAU-biofungicide followed by Neem leaf, Allamanda leaf and Onion bulb. At 35, 45 and 55 DAT, plant disease incidence was 20.00, 30.67 and 35.00%, respectively under control. All treatments significantly reduced the parameter over control within the range of 11.33 – 14.00, 14.33 – 20.67 and 15.00 – 22.33%, respectively. The lowest incidence was observed under BAU-biofungicide, which was statistically similar to extract of Onion bulb, Lantana leaf, Turmeric rhizome, Neem leaf, Garlic clove and Allamanda leaf at 35 DAT. The most effective treatment to reduce plant disease incidence was BAU-biofungicide, which was followed by Neem leaf, Allamanda leaf, and Lantana at 45 DAT. At 55 DAT, maximum reduction in plant disease incidence was recorded from spray with BAU-biofungicide, which was statistically similar to extract of Allamanda leaf, Neem leaf and Lantana leaf (Table 4).

Table 4. Efficacy of seven botanicals extract and BAU-biofungicide on disease incidence of lettuce (plant)

Treatments with botanicals extract and BAU-biofungicide	(%) Disease incidence (Plant)			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	11.67a	20.00a	30.67a	35.00a
Allamanda leaf extract (1:3 w/v)	8.33bcd	12.33bc	16.33def	17.67d
Garlic clove extract (1:3 w/v)	9.00bc	12.67bc	18.33b-e	19.33cd
Neem leaf extract (1:3 w/v)	8.00cd	12.00bc	16.00ef	17.67d
Onion bulb extract (1:3 w/v)	9.33bc	13.00bc	18.67bcd	20.33bc
Lantana leaf extract (1:3 w/v)	8.67bcd	12.67bc	17.33cde	19.00cd
Turmeric rhizome extract (1:3 w/v)	11.33a	14.00b	20.67b	22.33b
BAU-biofungicide (1ml/L)	7.33d	11.33c	14.33f	15.00e
Mint leaf extract (1:3 w/v)	9.67b	13.67b	19.00bc	20.67bc
CV (%)	9.32	8.62	7.52	7.15
LSD $_{(0.05)}$	1.49	2.01	2.47	2.57

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.4 Disease severity (%) due to *Cercospora* leaf spot of lettuce

Under control, the highwst plant disease severity of 0.89, 3.13, 4.08, 5.10 was observed at 25, 35, 45 and 55 DAT. Foliar spray of lettuce plant with botanicals extract and BAU-biofungicide reduced disease severity within the range of 0.55 – 0.86, 0.96 – 1.82, 1.13 – 2.02 and 1.17 – 2.13% at at 25, 35, 45 and 55 DAT, respectively. At 25 DAT, the reduction in disease severity was significant under all treatments except Turmeric rhizome extract. The lowest disease severity of 0.55% was recorded from BAU-biofungicide, which was statistically similar to Lantana leaf, Neem leaf and Allamanda. At 35, 45 and 55 DAT, foliar sprays with BAU-biofungicide and all botanicals extract significantly reduced disease severity compared to control. The lowest disease severity was obtained with BAU-biofungicide, Lantana, Neem and Allamanda leaf extract. The least effective treatment to reduce disese severity was Turmeric followed by Onion bulb (Table 5).

Table 5. Efficacy of seven botanical extracts and BAU-biofungicide on disease severity of lettuce

Treatments with botanicals extract and BAU-biofungicide	(%) Disease severity			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	0.89a	3.13a	4.08a	5.10a
Allamanda leaf extract (1:3 w/v)	0.62bcd	1.43cde	1.48cde	1.53cde
Garlic clove extract (1:3 w/v)	0.67bc	1.63b-d	1.69bcd	1.76bcd
Neem leaf extract (1:3 w/v)	0.57cd	1.13de	1.23de	1.27de
Onion bulb extract (1:3 w/v)	0.68b	1.63b-d	1.77bc	1.85bc
Lantana leaf extract (1:3 w/v)	0.62b-d	1.46b-e	1.58b-e	1.65b-e
Turmeric rhizome extract (1:3 w/v)	0.86a	1.96b	2.02b	2.13b
BAU-biofungicide (1ml/L)	0.55d	0.96e	1.13e	1.17e
Mint leaf extract (1:3 w/v)	0.68b	1.82bc	1.85cde	1.94cde
CV (%)	9.00	17.55	15.31	13.08
LSD $_{(0.05)}$	0.10	0.52	0.48	0.46

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.5 Plant height of lettuce as influenced by BAU-biofungicide and extract of botanicals

The lowest plant height of 12.32, 15.49, 15.70 and 16.00 cm was recorded from control at 25, 35, 45 and 55 DAT, respectively, Except Turmeric extrract at 25 DAT and Turmeric and Mint extrracts at 35 DAT, foliar spray with BAU-biofungicide and other botanicals significantly increased plant height compared to control. At 25, 35, 45 and 55 DAT, the maximum plant height was achieved with BAU-biofungicide follwed by Neem leaf and Lantana leaf. On the other hand, the minimum increase in plant height was recorded from Tumeric rhizome followed by Mint leaf and Onion bulb, respectively (Table 6).

Table 6. Efficacy of seven botanicals extract and BAU-biofungicide on plant height of lettuce

Treatments with botanicals extract and BAU-biofungicide	Plant height (cm)			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	12.32f	15.49d	15.70e	16.00e
Allamanda leaf extract (1:3 w/v)	15.98bc	20.31b	20.59bc	20.89bc
Garlic clove extract (1:3 w/v)	15.12cd	19.03bc	19.22cd	19.52cd
Neem leaf extract (1:3 w/v)	16.60b	20.70b	21.32b	21.62b
Onion bulb extract (1:3 w/v)	14.07de	17.84c	18.39d	18.69d
Onion bulb extract (1:3 w/v)	15.53bc	20.06b	20.53bc	20.83bc
Turmeric rhizome extract (1:3 w/v)	13.67ef	17.34cd	17.85d	18.15d
BAU-biofungicide (1ml/L)	18.16a	23.43a	24.13a	24.43a
Mint leaf extract (1:3 w/v)	13.88de	17.48cd	17.95d	18.25d
CV (%)	5.22	6.54	6.08	5.99
LSD $_{(0.05)}$	1.36	2.16	2.05	2.04

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD

4.6 Effect of botanicals extract and BAU-biofungicide on number of leaves per plant of lettuce

At 25, 35, 45 and 55 DAT, the lowest leaf number of 8.77, 13.07, 23.08 and 24.20 was recorded from control. Foliar spray with botanical extracts and BAU-biofungicide against *Cecopora* leaf spots significantly increased the parameter within range of 9.38- 14.00, 15.20 – 23.00, 26.13 – 32.60 and 24.52 – 33.30, respectively. The highest increase was achieved with BAU-biofungicide followed by Neem leaf and Allamanda (Table 7).

Table 7. Number of leaves per plant of lettuce as influenced by the application of botanicals extract and BAU-biofungicide recorded at different dates

Treatments with botanicals extract and BAU-biofungicide	Number of leaves per plant			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	8.77d	13.07f	23.80e	24.20f
Allamanda leaf extract (1:3 w/v)	11.27bc	19.07bc	28.93bc	29.65bc
Garlic clove extract (1:3 w/v)	11.03bc	17.90cd	27.73cd	28.43cd
Neem leaf extract (1:3 w/v)	12.52ab	20.68ab	30.27ab	30.97 ab
Onion bulb extract (1:3 w/v)	10.08cd	17.40c-e	27.07cd	27.77cd
Lanatana leaf extract (1:3 w/v)	11.21bc	18.47b-d	28.27b-d	28.96 b-d
Turmeric rhizome extract (1:3 w/v)	9.38cd	15.20ef	23.80e	24.52de
BAU-biofungicide (1ml/L)	14.00a	23.00a	32.60a	33.30a
Mint leaf extract (1:3 w/v)	9.93cd	16.53de	26.13de	26.72ef
CV (%)	10.94	7.65	4.92	4.79
$LSD_{(0.05)}$	2.06	2.37	2.35	2.34

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.7 Effect of botanicals extract and BAU-biofungicide on leaf area of lettuce

At 25, 35, 45 and 55 DAT, the lowest leaf area of 124.40, 173.70, 1911.33 and 194.16 cm^2 was found in control plots. Spray of BAU-biofungicide gave the maximm and significant increase in the parameter to 209.80, 278.99, 293.00 and 298.86 cm^2, which was followed by Neem leaf (196.42, 271.30 279.00 and 283.91 cm^2) and Allamanda leaf (189.26, 268.45, 260.73 and 265.95 cm^2). Treatments with Garlic, Onion, Lantana, Turmeric and Mint at 25 DAT and Turmeric at 35 DAT failed to increase leaf area (Table 8).

Table 8. Effect of foliar spray with botanicals extract and BAU-biofungicide on leaf area of lettuce at different dates of data collection

Treatments with botanicals extract and BAU-biofungicide	Leaf area (cm^2)			
	25 DAT	35 DAT	45 DAT	55 DAT
Control	124.40c	173.70d	191.33f	194.16f
Allamanda leaf extract (1:3 w/v)	189.26ab	268.45ab	260.73bc	265.95bc
Garlic clove extract (1:3 w/v)	162.78ac-	242.35a-c	229.27de	233.85de
Neem leaf extract (1:3 w/v)	196.42ab	271.30a	279.00ab	283.91ab
Onion bulb extract (1:3 w/v)	159.61a-c	236.93a-c	226.01de	230.53de
Onion bulb extract (1:3 w/v)	172.95a-c	266.93ab	242.40cd	247.58cd
Turmeric rhizome extract (1:3 w/v)	146.65bc	200.90cd	213.53e	215.47e
BAU-biofungicide (1ml/L)	209.80a	278.99a	293.00a	298.86a
Mint leaf extract (1:3 w/v)	157.82a-c	222.35bc	218.60e	222.97e
CV (%)	10.82	11.11	5.35	5.05
$LSD_{(0.05)}$	60.85	46.19	22.17	21.87

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

4.8 Effect of botanicals extract and BAU-biofungicide on leaf weight and yield of lettuce

The lowest leaf weight of 1.90 kg/plot and leaf yield of 9.55 t/ha was harvested from untreated control plots. Due to foliar spray of lettuce plants with botanicals extract and BAU-biofungicide against *Cercospora* leaf spot disease caused significant increase in both the parameters. The range of increase in leaf weight and leaf yield was 2.20 – 2.82 kg/plot and 11.00 – 14.12 t/ha. The maximum (47.82%) increase in yield per plot over control was recorded from BAU-biofungicide and the minimum (15.18%) was recorded from Turmeric rhizome extract. The highest increase was obtained with BAU-biofungicide followed by Neem leaf and Allamanda leaf. Least effective treatments were Turmeric rhizome followed by Mint leaf (Table 9).

Table 9. Effect of foliar spray with botanicals extract and BAU-biofungicide on leaf weight and leaf yield of lettuce recorded at different dates

Treatments with botanicals and BAU-biofungicide	Leaf weight (kg/plot)	Leaf yield (t/ha)	Increased yield over control (%)
Control	1.91e	9.55f	-
Allamanda leaf extract (1:3 w/v)	2.53b	12.63bc	32.29
Garlic clove extract (1:3 w/v)	2.36cd	11.82cd	23.73
Neem leaf extract (1:3 w/v)	2.66ab	13.30ab	39.27
Onion bulb extract (1:3 w/v)	2.28cd	11.40de	19.37
Onion bulb extract (1:3 w/v)	2.48bc	12.40c	29.84
Turmeric rhizome extract (1:3 w/v)	2.20d	11.00e	15.18
BAU-biofungicide (1ml/L)	2.82a	14.12a	47.82
Mint leaf extract (1:3 w/v)	2.30cd	11.48de	20.24
CV (%)	4.57	11.93	
$LSD_{(0.05)}$	0.16	0.83	

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

CHAPTER V

DISCUSSION

*Cercospora l*eaf spot is a serious disease of lettuce in Bangladesh. It may be considered as one of the major limiting factors to grow lettuce which is widely distributed all over the country wherever the crop is cultivated. In the present study, application of botanical extracts and BAU-biofungicide against *Cercospora* leaf spot was studied under field conditions. It is evident that some of the treatments showed significant effect leaf spot and plant disease incidence (% infected plant) at 25, 35, 45 and 55 DAT. It has been observed that neem leaves extract resulted significant reduction of *Cercospora* leaf spot of lettuce which give the simillar result of mungbean and other crops over untreated (control) (Anon., 1984; BARI, 2007). Simillar findings were recoded by Islam (2005) the antifungal action of garlic extracts, Allamanda extracts, neem extracts have been reported by many researchers. Garlic bulb extract and Allamanda leaf extract were found promising inhibiting mycelial growth of fungus in *in vitro* condition and also reduced the disease incidence and severity of vegetables in field condition Similar results were found from Stefania *et al.*, (2008) 0bserved that foliar applications of trichoderma biofungicide in field condition reduced the disease incidence and pathogen sporulation from the necrotic spots. Fakir (2000); Afzal *et al*., (1999); Bakr (1994); Razzaque and Hossain (1991) observed antifungal potentiality of different botanical extracts. Miah *et al.,* (1990) and Ahmed (1985) also reported that neem extract was effective for controlling *Cercospora* leaf spot in mungbean. But present study reported that BAU-biofungicide is better than neem leaf extract to control the *Cercospora* leaf spot of lettuce. It is evident that the treatments showed significant effect in respect of disease severity (0-5 scale) at 25, 35, 45 and 55 DAT. Simillar results were found by Ngegba *et al.,* (2017) revealed that plant extracts can effective control *cercospora* leaf spot disease of groundnut ant its causative organisms. However, *T. diversifolia*, *C. odorata*, and *T. procumbens*, should br used as a potential biocide in plant disease management, as they showed fungicidal and

fungitoxic ability. Uddin *et al*., (2013) suggested that the use of neem leaves extracts is effective for minimizing *Cercospora* leaf spot incidence, severity and increasing yield of mungbean. Dharam and Sharma (1985), Mondal, 1978, Alpana and Singh (1994) and Ahmed and Sultana (1984) used neem oil and found that 100% effective against the growth of disease of anthracnose and stem rot rot jute. Islam *et al*., (2006) found a significant reduction of the disease severity of leaf blight of wheat by seed treatment with garlic and bishkatali. The works of Achimu and Schloesser (1992) and others confirmed that neem leaf have high antifungal properties. In the present experiment, it has been observed that lettuce plant treated with different botanical extracts specially with BAU-biofungicide showed a better effect on number of plant height, leaf per plant and leaf area. The highest plant height, number of leaves per plant and and leaf area was recorded in BAU-biofungicide where the lowest value was recorded in untreated (control). Similar result was found by Hossain *et al.*, (2009) the BAU biofungicide resulted an increase of shoot length, root length, shoot weight, root weight and vigour index of the vegetables seedlings. Roy and Malathi, (2001) reported the similar effect on number of inflorescences per plant and plant height of mungbean. Further works of Singh Dwvedi (1987); Tariq and Magee (1990); Lakshmanan *et al.*, (1990) supported the earlier findings and recommended the use of garlic as an antifungal agent. Dubey and Dwivedi (1991) reported that fungistatic properties of extracts of leaves, bulb of onion and garlic and fruit, bark of *Allium cepa* against vegetative growth, *Sclerotia* variability of *Macrophomina phaseolina*. They observed that all the extracts inhibited growth but garlic bulb extract was more effective than other extracts employed in the tests. Mohanty *et al*., (1995) observed that garlic bulb extract (1:1) and allamanda leaf extract sprays in the field reduced phomopsis blight and fruit rot by 66% and 75%, respectively.

CHAPTER VI
SUMMARY AND CONCLUSION

The present study was carried out to evaluate different botanicals extract and BAU-biofungicide on *Cercospora* leaf spot of lettuce in the farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during October, 2019 to February, 2020. The experiment consists of 9 treatments such as T_0 = Control, T_1 = Allamanda leaf extract (1:3 w/v), T_2 = Garlic clove extract (1:3 w/v), T_3 = Neem leaf extract (1:3 w/v), T_4 = Onion bulb extract (1:3 w/v), T_5 = Lantana leaf extract (1:3 w/v), T_6 = Turmeric rhizome extract (1:3 w/v), T_7 = BAU-biofungicide (1ml/L) and T_8 = Mint leaf extract (1:3 w/v). Data was recorded on disease incidence, severity, growth and yield contributing characters and yield of lettuce for different treatment. The analysis of variance was performed by using MSTAT-C program. The significance of the difference among the treatment means was estimated by LSD at 5% level of probability

At 25, 35, 45 and 55 DAT, the maximum number of leaf spot 7.87, 24.82, 27.83, 34.79 was recorded from control, respectively, while the minimum number of leaf spot 5.82, 9.51, 14.60, 34.79 from application of BAU-biofungicide.

At 25, 35, 45 and 55 DAT, the disease incidence (leaf) was ranged from 1.63% to 2.53%, 1.90% to 4.27%, 3.28% to 7.55% and 3.52% to 10.75% at different treatments, respectively. At all cases, the highest leaf incidence was recorded from control (T_0) and the lowest was found from T_7 followed by T_3 (Spraying with Neem leaf extract).

Simillarly at 25, 35, 45 and 55 DAT, the disease incidence (plant) was noted from control which was 11.67%, 20%, 30.67% and 35.00%, respectively at different application. On the other hand, the lowest plant disease incidence was noted from 7.33%, 11.33%, 14.33% and 15% at 25, 35, 45 and 55 DAT.

At 25, 35, 45 and 55 DAT, the maximum disease severity (0-5 scale) 0.89%, 3.13%, 4.08% and 5.10% was recorded from control, respectively, while the minimum disease severity 0.55%, 0.96%, 1.13% and 1.17% from application of BAU-biofungicide.

At 25, 35, 45 and 55 DAT, significant difference was found at plant height of lettuce as influenced by different botanicals extract and BAU-biofungicide. At 25 DAT, the highest (18.16 cm) plant height was recorded from T_7 (Spraying with BAU-biofungicide (1ml/L)) and the lowest (12.13 cm) plant height was recorded from T_0 (Control). At 35 DAT, the highest plant height (23.43 cm) was recorded from T_7 (Spraying with BAU-biofungicide (1ml/L)) and the lowest (15.49 cm) plant height was recorded from T_0 (Control). At 45 DAT, the highest (24.13 cm) plant height was recorded from T_7 (Spraying with BAU-biofungicide (1ml/L)) and the lowest (15.70 cm) plant height was recorded from T_0 (Control). At 55 DAT, the highest (24.43) plant height was recorded from T_7 (Spraying with BAU-biofungicide (1ml/L)) and the lowest (16.00 cm) plant height was recorded from T_0 (Control).
At 25, 35, 45 and 55 DAT, significant difference was found in case of number of leaves per plant of lettuce due to application of different botanicals extract and BAU-biofungicide. The highest numbers of leaf per plant was recorded from 14, 23, 32.60 and 33.30 from T_7 (Spraying with BAU-biofungicide (1ml/L)). At 25, 35, 45 and 55 DAT, respectively followed by T_3 (Spraying with Neem leaf extracts) and T_1 (Spraying with Allamanda leaf extracts). On the other hands the lowest number of leaves per plant was counted 8.77,13.07, 23.80 and 24.20 from control (Without applying any treatment) At 25, 35, 45 and 55 DAT.

Statistical deviation was found at leaf area of lettuce by application of different botanicals extract and BAU-biofungicide at 25, 35, 45 and 55 DAT. the lowest leaf area 124.40 cm^2, 173.70 cm^2, 191.33 cm^2 and 194.16 cm2 was recorded from control, respectively. While the maximum leaf area 209.80 cm^2, 278.99 cm^2, 293.00 cm^2 and 298.86 cm2 from application of T_7 BAU-biofungicide.

Leaf weight of lettuce per plot also showed a significant variation among for controlling *Cercospora* leaf spot of lettuce. The maximum (2.82 kg) leaf weight per plot was recorded from T_7 (Spraying with BAU-biofungicide (1ml/L)) and the minimum (1.91 kg) leaf weight per plot was recorded from the T_0 (Control) without application of botanicals extract and BAU-biofungicide. no extract was applied. The maximum (14.12 tonnes) yield per hectare was recorded for T_7 (Spraying with BAU-biofungicide (1ml/L)) and the minimum (9.55 tonnes) yield per hectare was recorded from Control. The maximum 47.82% yield was increased over control was recorded from BAU-biofungicide (1ml/L)) while the minimum 15.18% was increased from T_6 (Spraying with Turmeric rhizome extracts).

From the present study it may be concluded that,

- *Cercospora longissimi* was identified as a causal organism of leaf spot of lettuce.
- BAU-biofungicide was the most effective for management of the disease incidence and severity of *Cercospora* leaf spot of lettuce than different botanicals extract.
- Among the botanicals extract, neem leaf extract and allamanda leaf extract performed better in case of growth and yield contributing characters including yield.

Considering the situation of the present experiment, further studies in the following areas may be suggested:

- BAU-biofungicide (1ml/L) may be used for management of *Cercospora* leaf spot disease of lettuce.

CHAPTER VI

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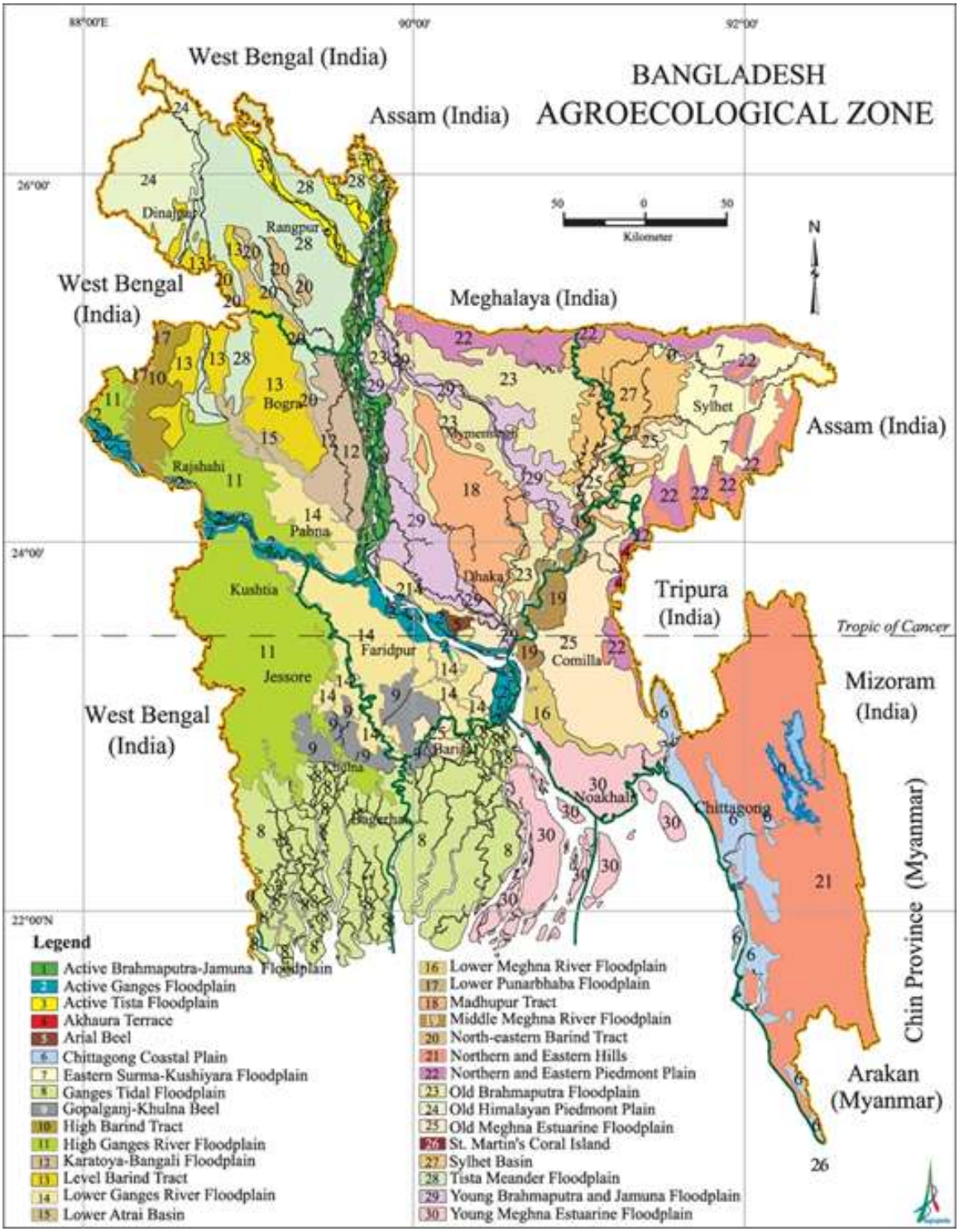
Appendix I: Map showing the experimental site



Appendix II: Soil characteristics of experimental farm of Sher-e-Bangla Agricultural University are analyzed by soil Resources Development Institute (SRDI) Farmgate, Dhaka.

A. Characteristics of the experimental field

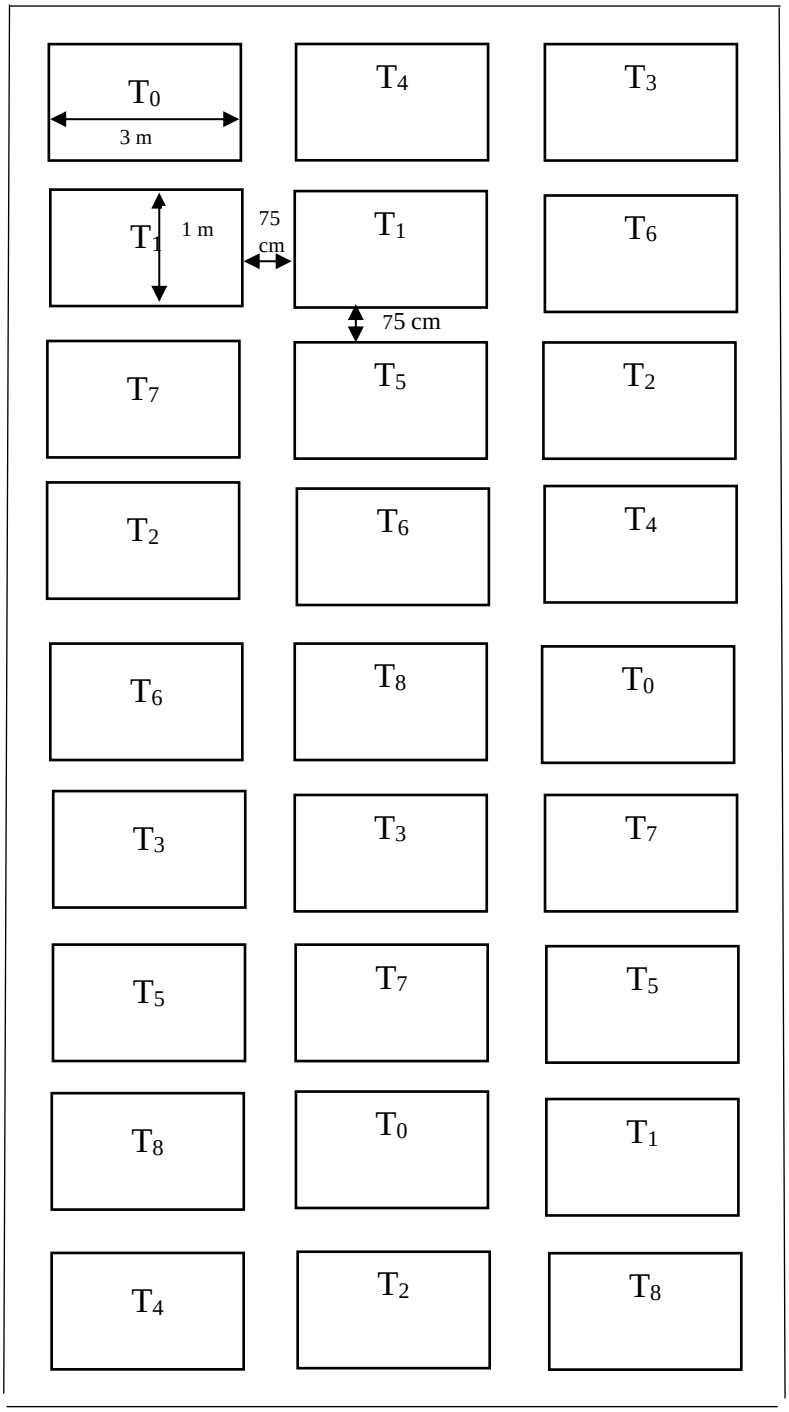
Morphological features	**Characteristics**
Location	Farm, SAU, Dhaka
AEZ	Modhupur tract (28)
General soil type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled
Flood level	Above flood level
Drainage	Well drained
Cropping pattern	**N/A**

B. Physical and chemical properties of the initial soil

B. Physical and chemical properties of the initial soil Characteristics	**Value**
Practical size analysis	
Sand (%)	16
Silt (%)	56
Clay (%)	28
Silt + Clay (%)	84
Textural class	Silty clay loam
pH	5.56
Organic matter (%)	1.00
Total N (%)	0.06
Available P (µgm/gm soil)	42.64
Available K (me/100g soil)	0.13

Source: SRDI

Appendix III: Experimental Field Layout



Legend:

1. Width of the plot = 1 m	
2. length of the plot = 3.0 m	
3. Space around the land = 75 cm	
4. Space between the block =75cm	
5. Space between the plot =50cm cm	