`EVALUATION OF SELECTED VARIETIES OF INDIAN SPINACH (*BASELLA ALBA* L.) AGAINST CERCOSPORA LEAF SPOT AND ITS MANAGEMENT

SADIA KADER OSHIN

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

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BY

SADIA KADER OSHIN

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Approved by

Prof. Dr. Fatema Begum	**Prof. Dr. Nazneen Sultana**
Department of Plant Pathology	Department of Plant Pathology
Supervisor	**Co-supervisor**

Prof. Dr. Fatema Begum
Chairman
Department of Plant Pathology
Sher-e-Bangla Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

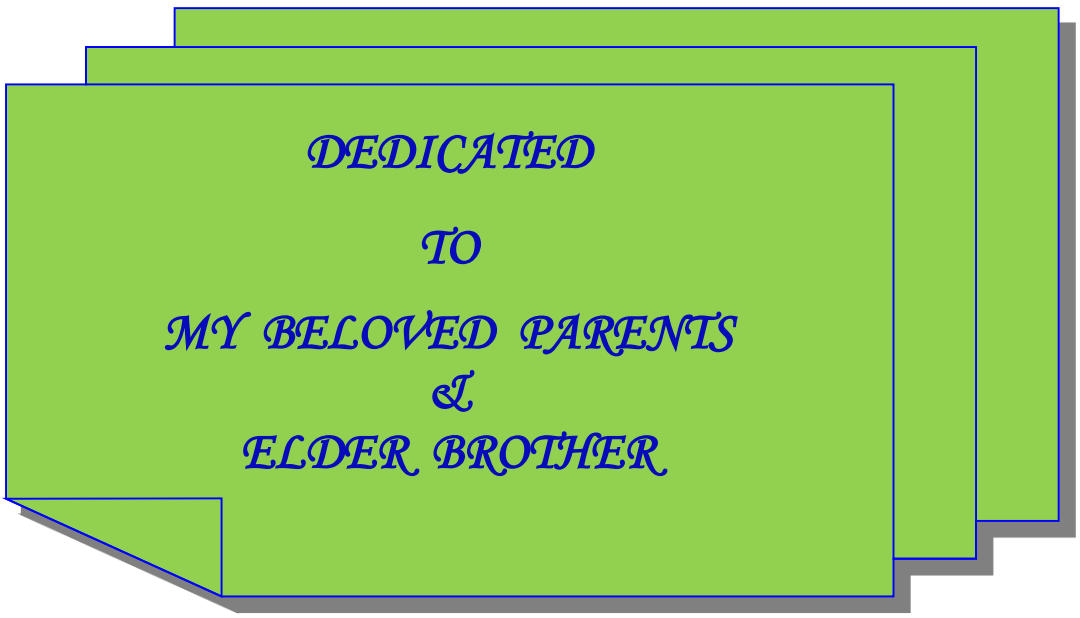
*This is to certify that the thesis entitled '**EVALUATION OF SELECTED VARIETIES OF INDIAN SPINACH (BASELLA ALBA L.) AGAINST CERCOSPORA LEAF SPOT AND ITS MANAGEMENT**' submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **PLANT PATHOLOGY**, embodies the results of a piece of bona fide research work carried out by **SADIA KADER OSHIN,** Registration No. **13-05597** under my direct supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: June, 2020
Dhaka, Bangladesh

Prof. Dr. Fatema Begum
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207

Supervisor


DEDICATED
TO
MY BELOVED PARENTS
&
ELDER BROTHER

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EVALUATION OF SELECTED VARIETIES OF INDIAN SPINACH (*BASELLA ALBA* L.) AGAINST *CERCOSPORA* LEAF SPOT AND ITS MANAGEMENT

By

SADIA KADER OSHIN

ABSTRACT

Consequently, two experiments were conducted in the experimental field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka to evaluate selected varieties of Indian spinach (*Basella Alba* L.) against *Cercospora* leaf spot and to manage the disease using bio-pesticides and selected botanicals in field condition. The 1st experiment was conducted during the period of April to June, 2018. Three varieties of Indian spinach namely BARI-1, BARI-2 and one local were selected to conduct the experiment and the experiment was designed at Randomized Complete Block Design (RCBD) with twelve replications. During evaluation, at early, mid and late growth stage, the lowest disease incidence (35.25%, 36.92% and 41.95%, respectively) was observed in BARI-2 variety, whereas the highest (59.36%, 64.49% and (71.37%) in local variety of Indian spinach. Similarly, the lowest disease severity (21.60%, 25.85% and 34.85%, respectively) was observed in BARI-2 variety, whereas the highest (45.25%, 52.45% and 62.50%, respectively) in local variety. The highest yield (23.79 t/ha) was recorded in BARI-2 variety, whereas the lowest yield (19.48 t/ha) in local variety. Based on morphological characteristics, the causal organism was identified as *Cercospora beticola* in CDA media in laboratory. The 2nd experiment was conducted during the period of February to April, 2019 and one local variety was used as planting material. The experiment comprised to manage the *Cercospora* leaf spot disease using one bio-fungicide and five botanicals. The treatment combinations were- T_0 = Untreated control (without any treatment); T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval; T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval; T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval; T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval; T_5 = Seed treated with BAU biofungicide followed by Biskatali leaf extract and spray at 7 days interval and T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval. The single factor experiment was designed in RCBD with three replications. At early, mid and late growth stage, the lowest disease incidence (3.38%, 4.74% and 5.24%, respectively) was observed in T_2, while the highest (57.31%, 64.10% and 71.31%, respectively) in T_0 treatment. The lowest disease severity (3.18%, 3.95% and 4.65%, respectively) was observed in T_2, while the highest (44.86%, 53.11% and 61.56%, respectively) in T_0 treatment at same growth stage. The highest yield (23.09 t/ha) was observed in T_2 and the lowest (19.66 t/ha) in T_0 treatment. Findings of the experiments revealed that BARI 2 showed best performance among all parameters whereas local variety was more susceptible to *Cercospora* leaf spot diseases and BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval gave better results compare to the other control measures of the study.

TABLE OF CONTENTS

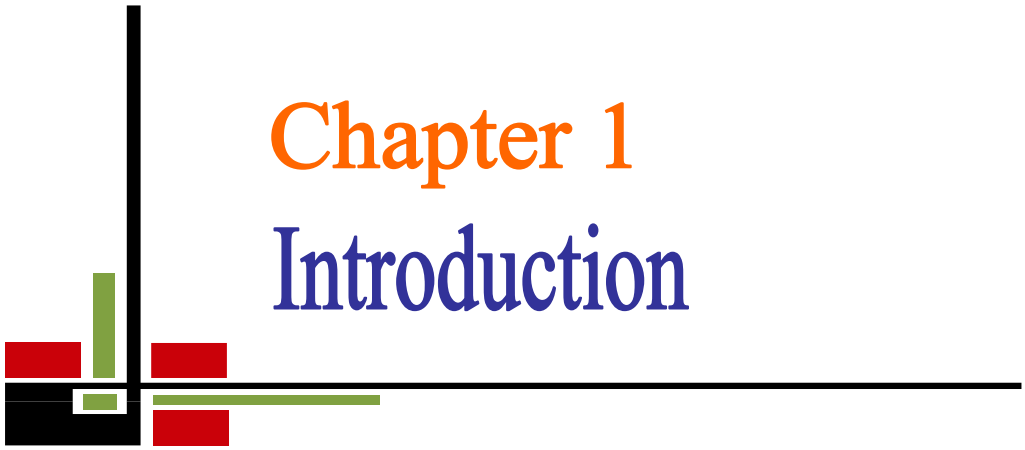
LIST OF TABLES

LIST OF FIGURE

LIST OF PLATES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES



Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Indian spinach (*Basella alba* L.) commonly known as Puishak, belongs to the family Basellaceae, is a popular tropical green vegetable crop widely cultivated for its edible leaves (Reddy *et al*., 2014; Kamruzzaman *et al*., 2015). It is a popular summer leafy vegetable in Bangladesh, India, and Africa. It is a very popular vegetable in Bangladesh and its demand is increasing day by day for its succulent, nutritious green tender stems and wholesome phyto-nutrients profile (Basunia *et al*., 2020). It is a good source of vitamins and minerals (Adhikari *et al*., 2012).

Indian spinach has been reported as having potential antiulcer, cytotoxic, antibacterial, antioxidant, anti-inflammatory, nephron-protective and wound healing properties and functioning as a central nervous system depressant (Kumar *et al*., 2013). Almost all plant parts are used as traditional medicine, which include laxative, rubefacient, demulcent, an astringent; febrifuge, diuretic, and to treat conjunctivitis, dysentery, diarrhoea, indigestion, constipation, boils and sores and as an antidote to poison (Encyclopedia of Life, 2017; National Parks Board, 2020). Red dye liquid that obtained from the fruits of Indian spinach is used as a rouge, ink, food coloring, in cosmetics and as a dye for official seals (Mitra and Das, 2015; Useful Tropical Plants, 2017).

In Bangladesh, Indian spinach is cultivated in around 10358.7 ha of land and the average yield of 7.3 t ha^{-1} with the production of 79,093 Metric ton (BBS, 2018).

Among the constraints, diseases especially *Cercospora* leaf spot plays a vital role for the qualitative loss of Indian spinach (Hasan *et al*., 2016). It is a succulent crop and that is why it is highly vulnerable to microbial infection, resulting leading to spoilage and loss of quality (Oladele, 2011). Sixteen different diseases of Indian spinach have so far been reported from different parts of the world (Hossain, 2007; Shova *et al*., 2020). In Bangladesh, only four diseases viz.

leaf spot caused by *Alternaria* sp., *Gloesporium* sp., and *Cercospora* sp., foot rot caused by *Sclerotium rolfsii* Sacc.; anthracnose caused by *Colletotrichum* sp., Macrophomina leaf spot and stem rot caused by *Macrophomina phaseolina* have been reported from different countries of the world (Sarker *et al*., 2017).

Cercospora leaf spot is a major disease of Indian spinach. The causal agent *Cercospora* sp. mainly is seed born, however; the pathogen is also able to survive for at least one year in plant debris and soil also. 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). The typical symptoms appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded by a purple to brown border (Recardo *et al*., 2015). Lesions. *Cercospora* leaf spot disease affect photosynthetic process in host plants, leaf becomes deformed resulting weakens and premature defoliation (Hasan *et al*., 2016).

In controlling *Cercospora* leaf spot disease of Indian spinach the farmers usually use different chemical control measures for the management of the disease (Hossain, 2007; Gomathinayagam *et al*., 2011). Some researchers also used different chemical fungicides to control *Cercospora* leaf spot disease and have achieved various degree of success (Khan and Smith, 2005). Extensive and non-judicious use of chemicals may cause serious environmental pollution. Since leaves of Indian spinach are consume fresh, so application of fungicides might be detrimental to human health.

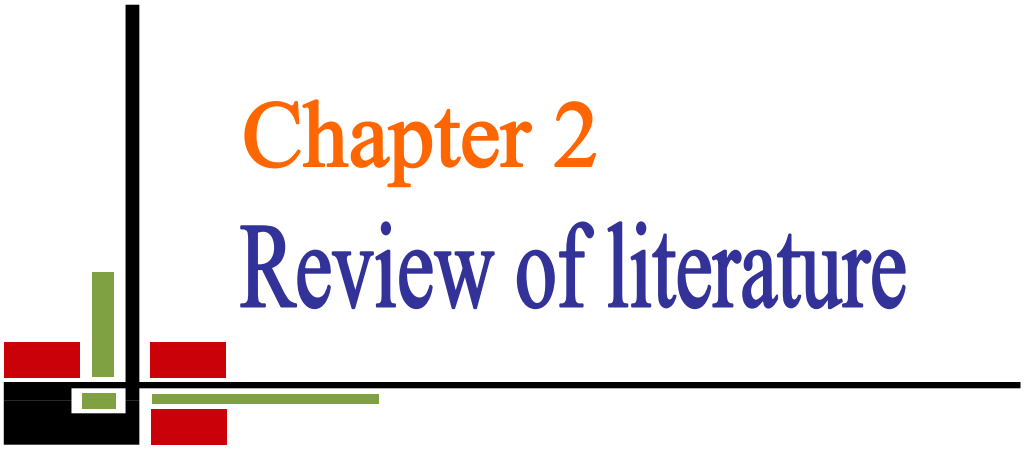
Considering present situations fungicide use it is necessary to look for economically better and environmentally sound means of control measures. The alternate approaches like using bio-fungicides and botanicals were found to be environmentally sound and effective against fungal pathogen (Uddin *et al*., 2013 and Maketon *et al*., 2008).

Recently, many plant pathologists give an attention on sustainable and eco-friendly agricultural practices for plant diseases management.

In Bangladesh few approaches have been taken to evaluate efficacy of bio-fungicide and botanicals against *Cercospora* leaf spot of Indian spinach (Hasan *et al*., 2016).

Considering above mentioned facts, the present study was undertaken to develop resistant varities of Indian spinach variety against *Cercospora* leaf spot and to determine the effect of selected bio-fungicides and botanicals, in reducing the disease incidence and severity of the disease. The following specific objectives were considered for the present study.

1. To evaluate three Indian spinach varieties for resistance against *Cercospora* leaf spot.
2. To evaluate bio-fungicide and botanicals for controlling *Cercospora* leaf spot of Indian spinach.



Chapter 2
Review of literature

CHAPTER 2

REVIEW OF LITERATURE

Cercospora leaf spot is one of the major disease of this crop that greatly influences the yield. But research works related to this disease and management practices using bio-fungicide and botanicals and also others is limited in Bangladesh context as well as the World. The research work so far done in Bangladesh and elsewhere also not adequate and conclusive. Nevertheless, some of the important and informative works and research findings related so far been done at home and abroad have been reviewed in this chapter under the following headings-

2.1 Origin and distribution of Indian spinach

There are two common species: *Basella rubra* and *Basella alba*. *B. rubra* is commonly found as a backyard/ornamental plant, whereas *B. alba* is usually cultivated in India. Both species are well adapted to diverse kind of soils. *B. alba* is having green stems and leaves, whereas *B. rubra* is having spectacular red stems and leaves (Kumar *et al*., 2015). Both are twining herbaceous vines, perennial, succulent, branched, smooth and annual growing to about 6-9 m in length. *B. alba* have thick, fleshy, broad, oval to heart-shaped leaves, whereas *B. rubra* have pink or red-violet stems and pink color veins running in the leaves.

They are considered to be native of India and are commonly found in tropical Asia (Bangladesh, Bhutan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Thailand and Vietnam), tropical Africa (Algeria, Egypt, Libya, Morocco, Mauritania and Tunisia), Brazil, Belize, Colombia, West Indies (Cuba, Haiti, Jamaica and Dominican Republic) and Fiji (Khare, 2004; Ernst, 2017). The crop is an ideal species for home garden and can be cultivated anywhere (in field or in pot culture). Importantly, *Basella* species are hardy and can grow in almost all kind of tropical soils and need relatively minimal attention and agronomic practices. Despite these attributes, the crop is a neglected and underutilized and not yet yearned a proper place in the

mainstream market of leafy vegetables. While spinach and other leafy vegetables are seasonal, the cultivation of couple of vines of Indian spinach in any home garden can supplement the dietary requirements of a family throughout the year (Singh *et al*., 2018).

2.2 Nutritional value of Indian spinach

The fresh leaves and stems of this climber are edible and reported to have protein, vitamins, essential minerals, amino acids and flavones (Singh *et al*., 2018). It is a highly nutritious and having all essential minerals such as calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), zinc (Zn), copper (Cu), manganese (Mn) and selenium (Se); important vitamins including vitamins C, A, Bi6, folate, niacin, panthotheinc acid; antioxidants like apigenin and all essential amino acids (Kumar *et al*., 2015; Das *et al*., 2017).

The plant is reported to contain moisture 93%, protein 1.2%, iron 1.4%, calcium 0.15%, vitamin A and vitamin C. Moreover, it is anadyne, sedative, diuretic and expectorant. Indian spinach is an excellent source of iron, an important trace element, required by the human body for red blood cell (RBC's) production (Basunia *et al*., 2020).

2.3 Evaluation of *Cercospora* leaf spot

Sixteen different diseases of Indian spinach have so far been reported from different parts of the world (Sarker *et al*., 2017). Shova *et al*. (2020) isolated ten fungi from the leaf spot of *Basella* and they were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Colletotrichum dematium*, *C. lindemuthianum*, *Curvularia lunata*, *Drechslera sacchari*, *Fusarium semitectum* and *Penicillium* sp. were found to be associated with the selected leaves of *Basella* spp. Among the isolated fungi *C. lindemuthianum, D. sacchari* and *F. semitectum* were found to be pathogenic to *Basella* spp.

Akter and Shamsi (2013) reported a total of 12 species of fungi with *Basella alba* and *B. rubra* those were *Alternaria alternata* (Fr.) Keissler, *Ascochyta* sp., *Aspergillus flavus* Link., *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A.*

terreus hom., *Colletotrichum dematium* Grove, *Curvularia pallescens* Boedijn, *Fusarium oxysporum* Schlecht, *Macrophomina phaseolina* (Tassi) Goid and two species of *Penicillium.*

2.4 Symptomology of *Cercospora* leaf spot

Cercospora leaf spot is caused by *Cercospora beticola* which affect mainly on leaf of Indian spinach. The typical symptoms appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded by a purple to brown border (Recardo *et al*., 2015).

The causal agent *Cercospora* mainly is seed born, however; the pathogen is also able to survive for at least one year in plant debris and as well as soil also. 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).

2.5 Incidence of *Cercospora leaf spot*

Indian spinach is a succulent crop that contains 90.50% moisture, it is highly vulnerable to microbial infection, resulting leading to spoilage and loss of quality (Oladele, 2011). Sixteen different diseases of Indian spinach have so far been reported from different parts of the world. In Bangladesh, only four diseases viz. leaf spot caused by *Alternaria* sp., *Gloesporium* sp., and *Cercospora* sp., foot rot caused by *Sclerotium rolfsii* Sacc.; anthracnose caused by *Colletotrichum* sp., Macrophomina leaf spot and stem rot caused by *Macrophomina phaseolina* have been reported (Sarker *et al*., 2017). Among the diseases leaf spot caused by *Cercospora beticola* is a major disease of *Basella alba* causes red spots and holes in leaves.

Hasan *et al*. (2016) carried out an experiment with two variety of Indian spinach BARI spinach 1 and local variety and reported that highest disease incidence (58.90 to 53.19) and the greater disease severity (35.00 to 52.20) in local variety and maximum disease incidence was found in BARI spinach 1 in compare with local variety.

2.6 Identification of *Cercospora Beticola*

Recardo *et al*. (2015) showed that in Indian spinach *Cercospora* leaf spot is mainly occurred by *Cercospora beticola* which affect mainly on leaf. The typical symptoms of *Cercospora* leaf spot is generally appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded by a purple to brown colour border in leaves.

Kirarei *et al*. (2019) observed that in the infection areas whitish-gray lesions revealed the presence of small and black stromata within the leaf substomatal cavities. The stromata produced loose fascicles of conidiophores, up to 15 conidiophores in a fascicle. Conidia of *Cercospora beticola* were solitary, hyaline, filiform, straight to slightly curved, with obtuse to subacute at the apex and subtruncate bases, multiseptate.

2.7 Management of *Cercospora* leaf spot

A field experiment was conducted by Chotangui *et al*. (2020) to investigate the effect of aqueous and ethanolic extracts of neem (*Azadirachta indica* A. JUSS) and garlic (*Allium sativum* L.) against *Cercospora* leaf spot (CLS) disease of groundnut (*Arachis hypogaea* L.). Results showed that CLS disease incidence ranged between 66.67% and 88.89% at 65 DAP for 10% ethanolic extract of garlic and 20% aqueous extract of garlic, respectively. Disease severity was lowest for 20% ethanolic extracts of neem seeds (14.74%) and garlic bulb (14.99%) at 65 DAP. Yield obtained from treatments of ethanolic extracts of garlic (3.62 t/ha) was significantly higher than the control (2.40 t/ha) but comparable to the spavozeb treatment (3.09 t/ha). Exotic varieties were more productive (3.13 and 3.43 t/ha) than the more resistant local variety (2.67 t/ha).

Akpan and Andrew (2018) conducted a field trial at the University of Uyo Teaching and Research farm, Use-offot, at various seasons to assess the fungicidal potentials of some plants as foliar sprays in the control of fungal diseases associated with (*Vigna radiata* L.) mungbean which include: *Cercospora* leaf spot caused by *Cercospora canescens* and *C. cruenta*, powdery

mildew by *Erysiphe polygoni* and anthracnose by *Colletotrichum lindemuthianum*. Plants extracts that served as foliar spray in the control of these fungal diseases were: neem leaves (*A. indica*), tassel flower (*E. coccinea*), drum stick (*Moringa oleifera*), and candle stick (*S. alata*) with sterile distilled water was used as the control. The results obtained showed that *Moringa oleifera* performed the best in increasing growth (vine length), more than all other extracts. Emilia coccinea performed best in terms of pod increase per plant, while *Senna alata* enhanced yield (seed weight) and so the high biomass. The result also indicated that neem showed the most effective response in reducing disease incidence and severity of mungbean disease more than all other plant extracts used in this study.

Ramesh and Zacharia (2017) carried out a study with an objective to find out the efficacy of bio-agents and botanicals against Leaf spot (*Cercospora arachidicola* Hori) disease of groundnut and they test the effect of foliar spray of bio-agents and botanicals against Leaf spot disease of groundnut. The treatments included mancozeb 75%WP 2g/lit (treated check), Garlic extract 20%, *Trichoderma viride* 5%, *Pseudomonas fluorescens* 5%, Neem leaf extract 20% and Datura leaf extract 20%. All the treatments used as foliage application and control with water spray. Mancozeb 75% WP 2g/lit was used as treated check and found superior among all the treatments that were applied in managing the leaf spot followed by *Trichoderma viride* 5%.

An experiment was carried out by Sarker *et al*. (2017) at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Sylhet, Bangladesh to estimate the efficacy of botanicals and biological agents to control *Cercospora* leaf spot disease of Indian spinach. In the field experiment, four treatments like as T_2: Custard apple leaf extract (1:2 w/v), T_3: Neem leaf extract (1:2 w/v), T_4: Biskatali leaf extract (1:2 w/v), T_5: Mahagoni leaf extract (1:2 w/v) were used as seed treatment along with another two treatments like T_1 : Trichocompost (2 kg/m^2) and T_6: Decomposed cowdung (2 kg/m^2) which were used as soil treatment before sowing. The four treatments

of leaf extracts were also used with concentration 1:4 (w/v) as spray solution in field condition. The lowest disease incidence (28.9%) and disease severity (14.4%) were found in treatments T_1 (Trichocompost) and gave best result in term of yield (8.9 t/ha). Treatment T_4 (Biskatali leaf extract) and T_6 (decomposed cowdung) also gave satisfactory result in yield and found effective to control *Cercospora* leaf spot of Indian spinach compared to other treatments. The results of the present studies suggested that, use of biological agents (Trichocompost) and botanical treatments especially Biskatali leaf extract has ability to minimize *Cercospora* leaf spot disease and increase yield of Indian spinach.

An experiment was carried out by Dey *et al*. (2017) at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Bangladesh to estimate the control measure of *Cercospora* leaf spot disease of country bean. Before sowing, the seeds were treated with six different treatments like as T1: Autostin 50 WP, T_2: Aimcozim 50 WP, T_3: Ata leaf extract (1:2 w/v), T_4: Neem leaf extract (1:2 w/v), T_5: Biskatali leaf extract (1:2 w/v) and T_6: Mahogani leaf extract (1:2 w/v). In field condition, similar four leaf extracts and two chemicals were used as spray solution with 1:4 w/v concentrations. The treatment T1: Autostin 50 WP was found to be most effective in controlling seed borne fungi and yield was highest (5.9 t/h) followed by T2: Aimcozim 50 WP (5.5 t/ha). The lowest disease incidence (33.3%) and disease severity (21.8%) was found in treatment T1: Autostin 50 WP and gave better response in yield (5.9 t/ha) compared to other treatments. Among the botanicals extract T_5: Biskatali leaf extract performed bestin case of disease incidence (44.4%), disease severity (25.7%) and yield (5.2 t/ha) The results of the present studies suggested that the use of the chemical Autostin 50 WP and botanical Biskatali leaf extract effectively minimizing *Cercospora* leaf spot disease severity and increase its yield.

Kumar *et al*. (2017) conducted a field trail to efficacy the effectiveness of botanicals namely neem oil, garlic oil and onion oil and bio-control agents *Trichoderma harzianum* and *Pseudomonas fluorescens* against leaf spot disease

(tikka) of groundnut caused by *Cercospora* Spp. *Pseudomonas fluorescens* @ 5% foliar spray led to a significant reduction of disease incidence (20.1%) followed by *Trichoderma viride* @ 5% (FS) (22.19%), neem oil @ 5% (FS) (23.29), garlic oil @ 4% (FS) (23.70), onion oil @ 4% (FS) (24.4) compared to treated and untreated control (27.90 and 20.12). Maximum cost benefit ratio was recorded *Pseudomonas fluorescens* @ 5% (FS) 1:2.05.

Neindow (2017) carried out a study to determine the effectiveness of Desert date seed extract (DDSE), Neem seed extract (NSE), Jatropha seed extract (JSE) and Tobacco leaf extract (TLE) for the control of CLS disease of groundnut. The study comprised field survey, laboratory studies, green house and field experiments. Multi-stage sampling technique was used for the field survey. Farmers' responses during the field survey showed CLS as a major constraint to groundnut production in Northern Region of Ghana. Farmers described the disease incidence as well as the disease severity to be above 50 %. In vitro studies indicated that aqueous DDSE, NSE, JSE and TLE at 100 g/l significantly inhibited mycelial growth of both *Cercospora arachidicola* and *Cercosporidium personatum* by 90.3 %, 80.8 %, 75.6 %, 54.5 % and 84.9 %, 73.3 %, 67.3 %, 59.4 % respectively. Pod yield was significantly more enhanced in plants treated with JSE, NSE, DDSE and Topsin-M, than those treated with TLE and the negative control plants for 2014 and 2015 cropping seasons with values ranging from 729 to 1095 and 931 to 1322 kg/ha respectively. For most of the parameters, DDSE produced similar results as Topsin-M followed by NSE and JSE. The adoption of DDSE, NSE and JSE as alternatives and better remedies to CLS disease control is recommended.

Hasan *et al*. (2016) carried out an experiment with *Trichoderma* based BAU-biofungicide, chemical Carbendazim and a synthetic plant growth promoting (PGP) hormone to study their effect on *Cercospora* leaf spot of Indian spinach. Number of leaf, number of infected leaf, disease incidence, disease severity, area under disease progress curve (AUDPC), plant height and plant weight were measured and significant variations was found against different treatment

combinations. Among the treatment combinations, seed treating with Carbendazim followed by foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim and only spray of PGP hormone significantly reduced number of leaf spot/leaf, disease incidence and severity, with increasing of plant height and weight. Interestingly, foliar application of PGP hormone reduces disease incidence and severity by 58.38% and 63.8% in both the variety of Indian spinach, respectively over control, whereas, in both the variety disease incidence and severity is reduced by seed treatment with Carbendazim followed by foliar spray with Carbendazim 69.72% and 77.63%; seed treatment with BAU bio-fungicide followed by foliar spray with Carbendazim 63.73% and 69.49%; foliar spray with Carbendazim 55.68% and 58.19%, respectively. Local variety of Indian spinach is more susceptible to *Cercospora* leaf spot than BARI spinach 1.

Nousraat *et al*. (2016) conducted this experiment to know the effects of botanicals and chemicals on *Cercospora* leaf spot disease of mungbean and direct effects on mungbean yield. A high yielding, disease tolerant variety (BARI mung-4) was used as experimental sample and treatment plots were set in Sylhet. Seven treatments mainly chemicals and plant leaf extracts, namely T_1 (Bavistin-50WP), T_2 (Secure600WP), T_3 (Neem leaf extracts), T_4 (Biskatali), T_5 (Bavistin + Secure), T_6 (mixed spray: Neem leaf extracts + Biskatali) and T_7 (water spray) were applied during experiment. Minimum disease incidence (5.87%) and disease severity (2.30%) were found in combined treatment of Bavistin 50WP + Secure 600WP (T_5) and gave better response in yield (1482.67 kg/ha). The result of the present study suggest that the integrated use of these treatments effectively minimize the incidence and severity of *Cercospora* leaf spot, as well as increase its yield.

A study was conducted by Hossain and Hossain (2014) to evaluate the effectiveness of three selected botanicals namely leaf extract of Neem (*Azadirachta indica*), Debdaru (*Polyalthia longifolia*) and datura (black)

(*Datura metel*) along with Bavistin (Carbendazim) and BAU-biofungicide (*Trichoderma harzianum*) against leaf spot disease (tikka) of groundnut caused by *Cercospora arachidicola* and *Cercosporidium personatum* at the Field Research Farm of Bangladesh Agricultural University, Mymensingh. The most effective treatments were Bavistin, BAU-biofungicide (seed treatment + spray), BAU-biofungicide (spray) and leaf extract of Neem. Bavistin increased pod and dry haulm yield by 53.51 and 24-80 %, respectively. Maximum pod yield and dry haulm weight were recorded under Bavistin. BAU-biofungicide (seed treatment + spray) produced the second highest pod and dry haulm yield followed by leaf extract of Neem and BAU-biofungicide (spray). BAU-biofungicide (seed treatment + spray) and leaf extract of Neem may be recommended for controlling leaf spot disease of groundnut.

Singh and Singh (2014) carried out a field experiment to evaluate the effect of different management options viz., carbedazim, neem leaf extract, intercropping with seasmum and their integration in integrated disease management. Minimum disease intensity (11.26%), maximum disease control (77.63%) and grain yield (9.70 q/ha) were observed with spraying of carbendazim + spray of neem (leaf) extract + inter-cropping of seasmum (2:1). Maximum increases yield (34.74%) was recorded in this treatment as compared to check having maximum disease intensity (50.33%) with lowest yield (6.33 q/ha).

Yadav *et al*. (2015) carried out an experiment with chemicals, bio-agents and botanicals for their antifungal efficacy against the growth of *Alternaria* spp, incitant of alternaria leaf spot of Cabbage (*Brassica olaracea* var. capitata L.). The minimum disease severity on leaves (15.13%) was recorded in Neem oil with 3% concentration. The other treatments viz, Neem leaf extract(100%), *Trichoderma viride* (5 gm./kg seed), Pseudomonas fluorescens (5 gm/kg seed), carbendazim (2 gm/kg seed) and mancozeb (2.5gm/kg seed) also showed the disease severity with 16.27%, 17.35%, 16.71%, 16.31% and 16.59% respectively when compared to control. All the treatments give the yield of fresh cabbage (Head) ranged from 26.88 to 32.21 t/ha significantly, the highest yield

was recorded in neem oil (3%) with 32.21 t/ha, followed by neem leaf extract 30.77, carbendazim 27.32, mancozeb 26.88, *T. viride* 25.99 and *P. fluorescens* 25.88 t/ha.

The experiment was conducted by Uddin *et al*. (2013) at Bangladesh Agricultural Research Institute farm, Joydebpur, Gazipur during to evaluate the bioefficacy of some plant extracts in controlling *Cercospora* leaf spot of mungbean. Six indigenous plant species i.e. Neem leaves extract (1:4 w/v), Garlic cloves extract (1:5 w/v), Biskatali leaves extract (1:4 w/v), Alamanda leaves extract (1:6 w/v), Arjun leaves extract (1:4 w/v) and Debdaru leaves extract (1:5 w/v) were used in this experiment. The lowest disease incidence (7.33%) at 60 DAS was found in T_1. Lowest and similar disease severity (PDI= 4.55) was found in T_2 and T_3 at the same DAS. Neem extract treated plots gave better response in yield (1.26 t/ha) and all the yield contributing parameters like inflorescences/plant (13.45), tallest plant (51.44 cm), the maximum number of pods/plant (26.81), length of pod (8.56 cm), number of seeds/pod (12.64) and 1000 seeds weight (27.33 g) followed by T_2 and T_3. The highest disease incidence (26.50%) and disease index (13.65%) were recorded in treatment T_7 at 60 DAS. The results of the experiment suggested that the use of neem leaves extracts are effective for minimizing *Cercospora* leaf spot incidence, severity and increasing yield of mungbean.

A study was undertaken by Hossain and Hossain (2013) to evaluate effectiveness of foliar spray with 33 plant extracts against leaf spot (Tikka) of groundnut caused by *Cercospora arachidicola* and *Cercosporidium personatum*. Bavistin and BAU-Biofungicide were included in the experiment as checks and spray of plain water represented control. Almost all treatments gave considerable reduction in disease incidence and increase in growth parameters, pod and haulm yield compared to control. The most effective materials were Bavistin 50 WP, BAU-Biofungicide, leaf extract of neem, tomato, datura black, and datura white. The materials decreased spot number per leaf, defoliation per plant, incidence of leaf spot, and number of infected leaf per plant by 35.45 -60.07, 42.06-72.20,

51.97–63.58, and 38.33 to 46.89 % and increased pod yield and haulm yield by 64.37-111.41 and 32.35- 74.71 %, respectively.

The study was conducted by Mohammed (2012) in the School of Agriculture and Agricultural Technology (SAAT) research farm with extracts from roots and seeds of *Moringa oleifera* and *Jatropha curcas* were tested at 10% concentration and sprayed after 2,3 and 4 weeks in three different growing periods of groundnut; 2, 3 and 4 weeks. Some plant extrated were mixed with 3ml of an emulsifier made from castor oil known as RIMULGAN and then separately added to each of the 150ml of the four different plant extracts. Neem oil at 0.6ml, hexaconazole material (6% EC) at 0.3ml and mancozeb (80% WP) at 0.4g/plot were equally used. The test materials significantly reduced the disease severity at 2, 4 and 6 Weeks spray in two, three and four weeks After Sowing (WAS) plots when compared with untreated plots. In three weeks after sowing, the test materials showed no significant differences on severity of *Cercospora* disease of groundnut after 4 and 6 weeks spray when compared with untreated plot. In four weeks after sowing, xii untreated plots (control) recorded highest severity of the disease (2.333) followed by RIMULGAN (2.00). While plots treated with Neem oil and Mancozeb 80% WP recorded lowest (0.333). After 6 weeks spray, plot treated with RIMULGAN + *J. curcas* root extract recorded lowest incidence of the disease (0.00%) in two weeks after sowing, followed by RIMULGAN + J. curcas seed extract and Neem oil (0.333%), and while plot treated with RIMULGAN and untreated plot recorded highest (2.00%). In four weeks spray, plots treated with RIMULGAN and control recorded the highest severity of the disease (2.333), followed by *M. oleifera* seed extract (2.00) while groundnut in plots sprayed with Neem oil and Mancozeb 80% WP recorded the least (0.333). Treatment materials showed significant differences on *Cercospora* disease incidence at 2, 4 and 6WAS. The summary of this study shows that the plant extracts were able to control both the *Cercospora* and other folia diseases of groundnut. However, the RIMULGAN + seed extracts (Jatropha and Moringa) were more effective and comparable with Neem oil and synthetic fungicides

(Hexaconazole 6% E.C and Mancozob 80% WP) than the root extracts and spray test plant after 2 WAS gave a better result than 3 and 4 WAS.

Yashoda *et al.* (2011) carried out an experiment with five bioagents viz *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* under in vitro and three biorationals like *Trichoderma harzianum*, *Pseudomonas fluorescens* and Azadiractin at 0.1%, 0.2% were tested under in vivo conditions against *Cercospora beticola*. Under in vitro conditions maximum reduction in colony growth was observed in *Trichoderma harzianum* which was significantly superior over other bioagents tested. Under in vivo conditions Pseudomonas fluorescens at 0.2% has found effective in reducing the leaf spot disease of palak with least % disease index.

Asaduzzaman *et al*. (2008) conducted a field experiment at the Plant Disease Clinic (PDC) and in the field laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. The aim of the experiment was to test the efficacy of garlic tablet in controlling *Cercospora* leaf spot of chili. Three different concentration of garlic tablet solution viz. 1:3, 1:2, 1:1 along with 0:0 and three frequencies spraying keeping 15 days interval were taken as treatment. In vitro screening of plant by garlic tablet solution against *Cercospora capsici* was found promising in inhibiting radial mycelia growth of *Cercospora capsici*. Garlic tablet solution spray at 1:1 w/v concentration for three times was found most effective to reduce per cent leaf infection, per cent fruit infection, per cent leaf area diseased (LAD%) and number of spots per leaf and considerable increase of yield. For successful management of *Cercospora* leaf spot of chili cv. Bindu, spray of garlic tablet solution may be recommended at 1:1 w/v concentration at flowering (95 days age), initial fruiting (110 days age) and fruiting stage (125 days age).

Uddin (2007) conducted and experiment for the management of *Cercospora* leaf spot of mungbean through botanicals. The experiment comprised of 7 treatments such as T_1 = Neem leaves extract (1:4 w/v); T_2 = Garlic cloves extract (1:5 w/v);

T_3 = Biskatali leaves extract (1:4 w/v); T_4 = Alamanda leaves extract (1:6 w/v); T_5 = Arjun leaves extract (1:4 w/v); T_6 = Debdaru leaves extract (1:5 w/v) and T_7 = Untreated (control) and recorded the highest (13.42% infected plant) disease incidence was recorded in treatment T_7 and the lowest (3.67% infected plant) disease incidence was recorded in treatment T_1 at 60 DAS and the highest (13.65) disease severity was recorded in treatment T_7 and the lowest (4.55) disease severity was recorded in treatment T_2 and T_3. The highest (13.45) number of inflorescence per plant was recorded in treatment T_1. The tallest (51.44 cm) plant was recorded in treatment T_1. The maximum number of bunches (8.56), number of pod per plant (26.81), length of pod (8.56 cm), number of seed per pod (12.64) and 1000 seed weight (27.33 g) was recorded in treatment T_1. The highest yield (1.51 kg/plot, 1.26 t/ha) also recorded in treatment T_1.

Above cited reviews revealed that application of different bio-pesticides and botanicals significantly influences the *Cercospora* leaf spot disease incidence and severity and growth as well as the yield of Indian spinach. The literature revealed that the effects of different bio-pesticides and botanicals on Indian spinach have not been studied well and have no definite conclusion in this aspects for the production of this crop in the agro climatic condition of Bangladesh and as well as other producing countries.

2.8 Researchs done in Bangladesh

An experiment was carried out by Sarker *et al*. (2017) at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Sylhet, Bangladesh to estimate the efficacy of botanicals and biological agents to control *Cercospora* leaf spot disease of Indian spinach. In the field experiment, four treatments like as T_2: Custard apple leaf extract (1:2 w/v), T_3: Neem leaf extract (1:2 w/v), T_4: Biskatali leaf extract (1:2 w/v), T_5: Mahagoni leaf extract (1:2 w/v) were used as seed treatment along with another two treatments like T_1 : Trichocompost (2 kg/m^2) and T_6: Decomposed cowdung (2 kg/m^2) which were used as soil treatment before sowing. The four treatments of leaf extracts were also used with concentration 1:4 (w/v) as spray solution in

field condition. The lowest disease incidence (28.9%) and disease severity (14.4%) were found in treatments T_1 (Trichocompost) and gave best result in term of yield (8.9 t/ha). Treatment T_4 (Biskatali leaf extract) and T_6 (decomposed cowdung) also gave satisfactory result in yield and found effective to control *Cercospora* leaf spot of Indian spinach compared to other treatments. The results of the present studies suggested that, use of biological agents (Trichocompost) and botanical treatments especially Biskatali leaf extract has ability to minimize *Cercospora* leaf spot disease and increase yield of Indian spinach.

An experiment was carried out by Dey *et al*. (2017) at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Bangladesh to estimate the control measure of *Cercospora* leaf spot disease of country bean. Before sowing, the seeds were treated with six different treatments like as T1: Autostin 50 WP, T_2: Aimcozim 50 WP, T_3: Ata leaf extract (1:2 w/v), T_4: Neem leaf extract (1:2 w/v), T_5: Biskatali leaf extract (1:2 w/v) and T_6: Mahogani leaf extract (1:2 w/v). In field condition, similar four leaf extracts and two chemicals were used as spray solution with 1:4 w/v concentrations. The treatment T1: Autostin 50 WP was found to be most effective in controlling seed borne fungi and yield was highest (5.9 t/h) followed by T2: Aimcozim 50 WP (5.5 t/ha). The lowest disease incidence (33.3%) and disease severity (21.8%) was found in treatment T1: Autostin 50 WP and gave better response in yield (5.9 t/ha) compared to other treatments. Among the botanicals extract T_5: Biskatali leaf extract performed bestin case of disease incidence (44.4%), disease severity (25.7%) and yield (5.2 t/ha) The results of the present studies suggested that the use of the chemical Autostin 50 WP and botanical Biskatali leaf extract effectively minimizing *Cercospora* leaf spot disease severity and increase its yield.

Hasan *et al*. (2016) carried out an experiment with *Trichoderma* based BAU-biofungicide, chemical Carbendazim and a synthetic plant growth promoting (PGP) hormone to study their effect on *Cercospora* leaf spot of Indian spinach. Number of leaf, number of infected leaf, disease incidence, disease severity, area

under disease progress curve (AUDPC), plant height and plant weight were measured and significant variations was found against different treatment combinations. Among the treatment combinations, seed treating with Carbendazim followed by foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim and only spray of PGP hormone significantly reduced number of leaf spot/leaf, disease incidence and severity, with increasing of plant height and weight. Interestingly, foliar application of PGP hormone reduces disease incidence and severity by 58.38% and 63.8% in both the variety of Indian spinach, respectively over control, whereas, in both the variety disease incidence and severity is reduced by seed treatment with Carbendazim followed by foliar spray with Carbendazim 69.72% and 77.63%; seed treatment with BAU bio-fungicide followed by foliar spray with Carbendazim 63.73% and 69.49%; foliar spray with Carbendazim 55.68% and 58.19%, respectively. Local variety of Indian spinach is more susceptible to *Cercospora* leaf spot than BARI spinach 1.

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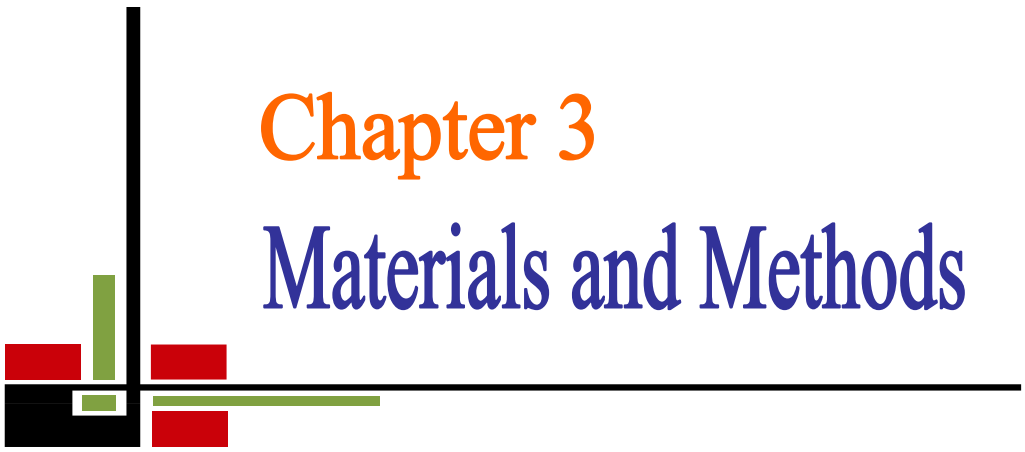


Chapter 3

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

3.1 Experiment conducted

Two field experiments were conducted to evaluate different varieties of indian spinach (*Basella Alba* L.) against *Cercospora* leaf spot and management of the disease using bio-pesticides and botanicals under field condition.

3.1.1 Experimental period

The first experiment was conducted during April to June, 2018 and the second experiments was conducted during February to April, 2019 (2^{nd} experiment).

3.1.2 Experimental location of experiment site

Both experiments were conducted in the experimental field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The site was $23^{0}74^{/}$N latitude and $90^{0}35^{/}$E longitude with an elevation of 8.2 meter from sea level. A map of the experimental location is presented in Appendix I.

3.1.3 Soil characteristics

The soil of the experimental field belongs to the Tejgaon series under the Agroecological Zone, Madhupur Tract (AEZ-28) and the General Soil Type was Deep Red Brown Terrace Soils (FAO, 1988). Top soil was Silty Clay in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. The experimental area having available irrigation and drainage system and situated above flood level. The soil having a texture of sandy loam with organic matter 1.15% and was composed of 26% sand, 43% silt and 31% clay particles. Details morphological, physical and chemical properties of the soil of experimental field are shown in Appendix II.

3.1.4 Climatic condition of experimental site

Experimental area was situated in the sub-tropical climate zone, which was characterized by heavy rainfall during the month of April to September and scanty rainfall during the rest of the year. The monthly average temperature,

humidity, rainfall and sunshine hour during crop growing period were collected from Weather Yard, Bangladesh Meteorological Department, Agargoan, Dhaka (Appendix III).

3.2 Details of Experiment-I: Evaluation of different varieties of Indian spinach (*Basella Alba* L.) against *Cercospora* leaf spot at natural condition

3.2.1 Planting material

For the experiment-I total 3 varieties of Indian spinach were used as the test crops in this experiment. Among the varieties BARI-1 and BARI-2 were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur and one local variety were collected from Siddique Bazar, Dhaka.Each variety represented as a treatment and designated as BARI-1=V_1, BARI-2 = V_2 and Local = V_3

A B C

Plate -1. Plant of Indian Spinach varieties (A) BARI-1, (B) BARI-2,(C) Local

3.2.2 Experimental design and layout

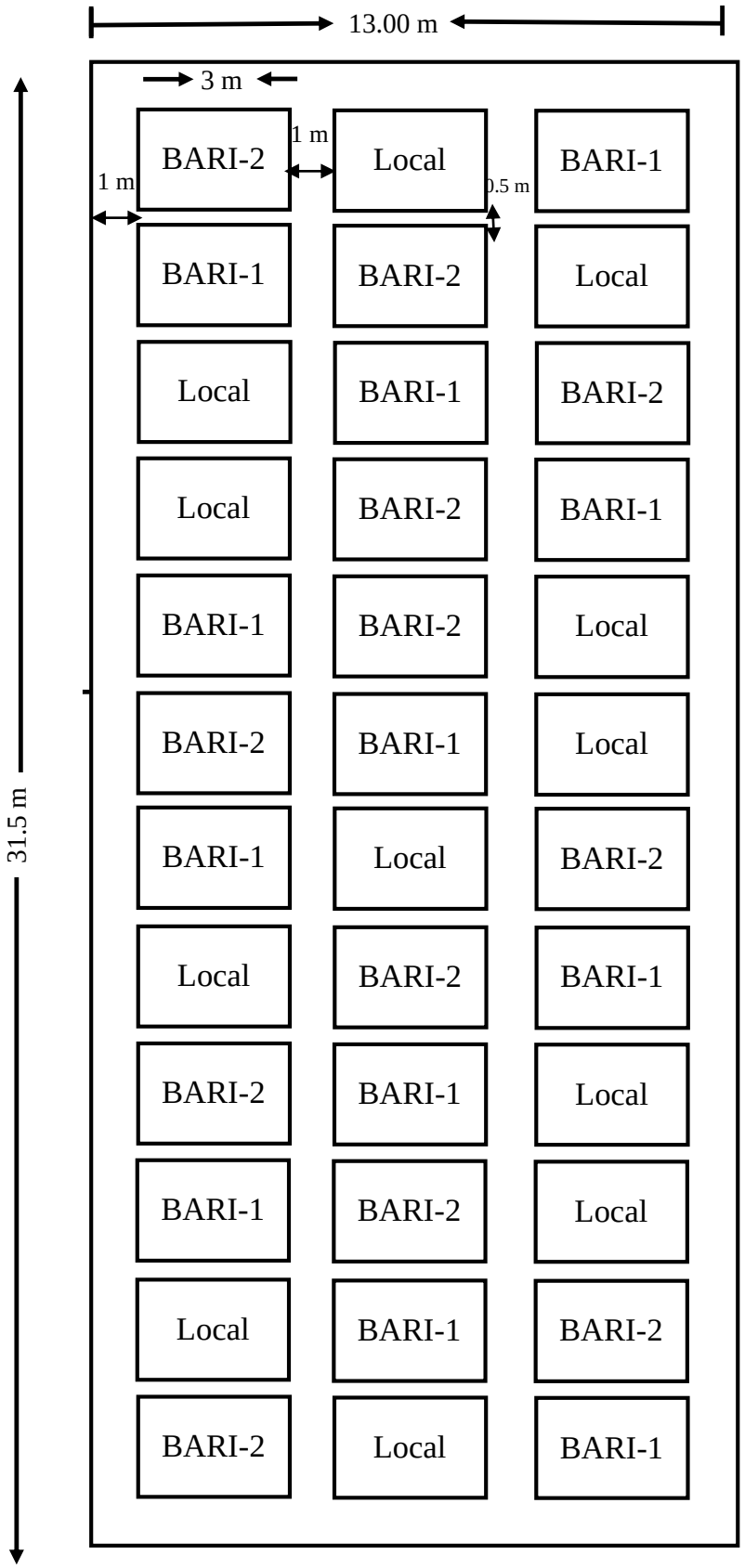
The single factor experiment was laid out in Randomized Complete Block Design (RCBD) with twelve replications. The total area of the experimental plot was 409.5 m^2 with length 31.5 m and width 13.0 m which were divided into twelve equal plots. Each block was divided into 3 plots where 3 varieties allotted at random. There were 36 unit plots and the size of each plot was 3.0 m × 2.0 m.

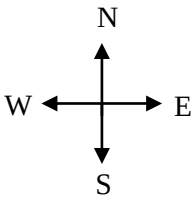
The distance between blocks was 1 m between two plots were 0.5 m, respectively. The layout of the experiment is shown in Figure 1.

3.2.3 Land preparation

The selected experimental plot was opened on the 16 April, 2018 with a power tiller and left exposed to the sun for a week. Subsequently cross ploughing was done three times followed by laddering to make the land suitable for seeds sowing. All weeds, stubbles and residues were removed from the field and finally, a good tilth was obtained. The soil treatment was done at the time of final land preparation to protect young plants from the attack of soil inhibiting insects. Final land preparation was done on 25 April, 2018.

Photograph- 1. Prepared field for evaluation of different varieties of Indian Spinach





Plot size: 3.0 m × 2.0 m

Plot spacing: 0.50 m

Between block: 1.0 m

Variety:

Indian spinach varieties:

BARI-1 = V_1

BARI-2 = V_2 and

Local = V_3

Figure 1. Layout of the evaluation experimental plot

3.2.4 Application of manure and fertilizer

Manures and fertilizers were applied to the experimental plot as per the recommended manures and fertilizer doses of Indian spinach and that were presented in Table 1.

Table 1. Doses and methods of application of manure and fertilizers in Indian spinach field

Manures and fertilizers	Dose ha^{-1}	Application (%)		
		Basal	14 DAS	28 DAS
Cowdung	3 ton	100	--	--
Urea	75 kg	33.33	33.33	33.33
TSP	20 kg	100	--	--
MoP	35 kg	33.33	33.33	33.33
Gypsum	30 kg	100	--	--

Source: Fertilizer Recommended Guide, 2018

3.2.5 Seeds sowing

Seeds of Indian spinach were sown on 26 April, 2018. Seeds were sown at a depth of 2 cm in the experimental plots. Light irrigation was provided in the evening after sowing seeds. There were four rows in a plot, each rows having six plant, in total there were twenty four plants in each plot.

3.2.6 Intercultural operation

After raising seedlings, various intercultural operations such as gap filling, weeding, earthing up, irrigation pest and disease control etc. were accomplished for better growth and development of the Indian spinach seedlings.

Gap filling

The seedlings in the experimental plot were kept under careful observation. Very few seedlings were damaged and such seedling were replaced by new seedlings from the same stock. Replacement was done with healthy seedling having a boll of earth which was also planted by the side of the unit plot. The transplants were given shading and watering for 5 days for their proper establishment.

Weeding

The hand weeding was done 14 and 28 days after sowing (DAS) of seeds to keep the plots free from weeds.

Irrigation

Light watering was given by a watering cane at every afternoon. It was continued for a week for rapid and well establishment of the seedlings. Irrigation was also provided at 20 and 40 days after transplanting followed by weeding.

3.2.7 Harvesting

Randomly selected 5 plants were harvested from each plot for data collection. Indian spinach was harvested six time at 30, 40, 50, 60, 70 and 80 days after sowing (DAS). Subsequently the 1st and 2nd harvest was treated as early growth stage and similary 3rd and 4th was treated as mid growth stage and 5th and 6th harvest treated as late growth stage.

3.2.8 Data collection

The growing plants of Indian spinach under different treatment were closely examined at regular intervals. Five plants were randomly selected from the middle rows of each unit plot for avoiding border effect and tagged with a sample card, which was done in plot wise. The following data were collected during the course of the experiment.

- Number of healthy and infected leaves/plant at early, mid and late growth stage
- Disease incidence (%) at early, mid and late growth stage
- Disease severity (%) at early, mid and late growth stage
- Number of *Cercospora* spot at early, mid and late growth stage in leaf
- Stem length (cm)
- Leaf area infection (%)
- Yield of Indian spinach (kg/ha and t/ha)

3.2.9 *Cercospora* leaf spot disease incidence

The experiment plots were examined 10 Days interval for the estimation of *Cercospora* leaf spot disease incidence. The disease incidence of *Cercospora* was recorded four times. The counting was made from 30 to 60 DAS at 10 days interval, which includes the per cent plant infection at different DAS to compare with, different other treatments. The infected plants were identified by comparing it visible symptoms critically with those already published by Bdliya and Gwio-Kura (2007). The disease incidence of *Cercospora* was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves/plant}}{\text{Total number of leaves/plant}} \times 100$$

3.2.10 Assessment of *Cercospora* disease severity

Disease severity was recorded by using a numeric severity score scale(0-5). In that 10 infected plants were selected randomly from each replicated plot. Five leaves were observed from each selected plant for scoring the disease severity data. At early, mid and late growth stage, the severity score was made in different plants due to rouge out of the infected leaves after scoring the severity data. The severity of *Cercospora* leaf spot disease was recorded following the grade as used by Bdliya and Gwio-Kura (2007).

The disease severity of *Cercospora* leaf spot was calculated using followings formula:

$$\text{Disease severity (\%)} = \frac{\text{Category No.} \times \text{Number of leaves/category}}{\text{Total number of leaves counted} \times \text{maximum disease category}} \times 100$$

Numeric score	Symptom severely on plants
0	No visible symptoms
1	1 to 10% leaf area affected
2	11 to 20% leaf area affected
3	21 to 50% leaf area affected
4	51 to 80% leaf area affected
5	above 80% leaf area affected

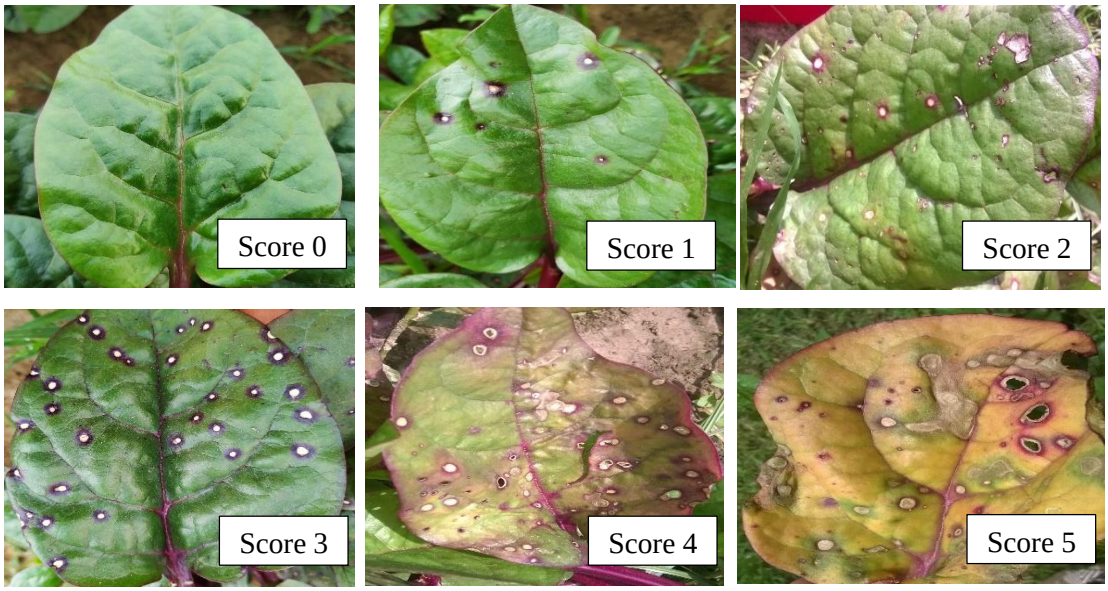


Plate 2. Numeric severity score scale for estimation of disease severity in Indian Spinach

3.2.11 Isolation and identification of *Cercospora beticola*

The pathogen that causes *Cercospora* leaf spot disease in Indian spinach was Isolated using tissue culture techniques. The surface of working clean bench was sterilized with ethanol (70%). That the infected leaf if Indian spinach were taken into clean bench and 0.5 -1.0 cm portion of the diseased section with early leaves of infection was cut from diseased leaves and washed in running tap water for 30 seconds and there after surface sterilizer in HgCl (1:100) for one and half minutes and then taken out with the help of sterile forceps and rinsed in three changes of sterile distilled water. After washing these *Cercospora* infected leaf cut pieces w**ere placed on sterilized blotter paper in petridishes and plated on Potato Dextrose Agar (PDA), Carrot Dextrose Agar (CDA) media and without

any media in 90 mm diameter petridishes under aseptic condition. The incubated petridishes were maintained at room temperature (25±2^{0}C) and observation made from 24 hours onwards. Seven days after incubation the fungal culture was studied under stereoscope and compound microscope for identification of the desired pathogen.

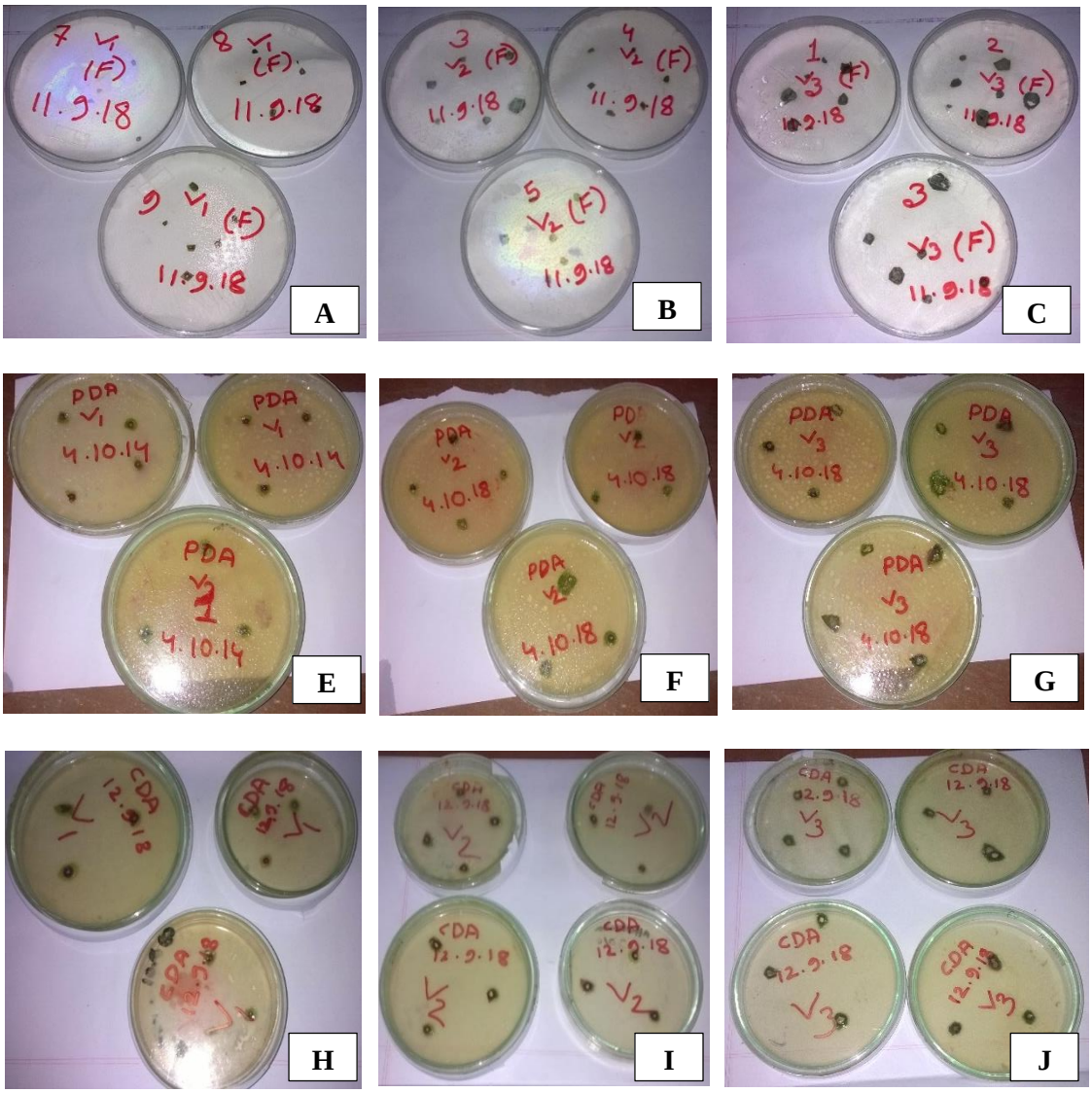


Plate 3. Cut pieces of disease leaves on blotter (A-C), PDA medium (E-G) and CDA medium (H-J) respectively

3.3 Details of Experiment--II: Test of Efficacy of Bio-pesticides for the Control

3.3.1 Test crop and treatment

Local variety of Indian spinach were used as the test crop in this experiment because it is more disease susceptible than other two varieties.

3.3.2 Treatment of the experiment

The experiment comprised of three bio-pesticides and three botanicals including an untreated control as treatment. The treatments were as follows:

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

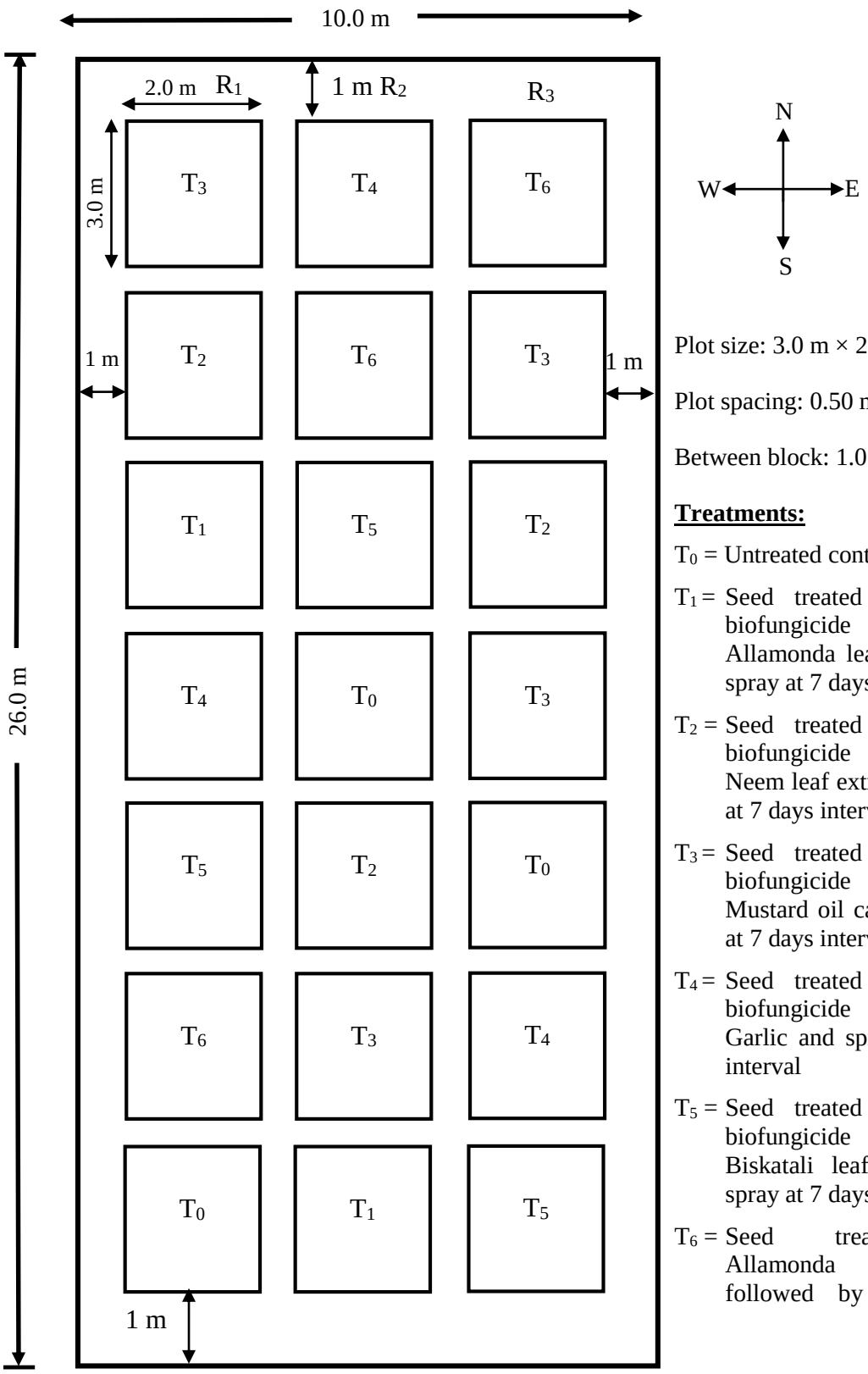
T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

3.3.3 Experimental design and layout

The single factor experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The total area of the experimental plot was 206 m^2 (length 26.0 m and width 10.0 m) which were divided into three equal blocks. Each block was divided into 7 plots where 7 treatments allotted in random.So there were 21 unit plots and the size of each plot was 3.0 m × 23.0 m. The distance between two blocks and two plots were 1.0 m and 0.5 m, respectively. The layout of this experiment shown in Figure 2.



Plot size: 3.0 m × 2.0 m

Plot spacing: 0.50 m

Between block: 1.0 m

Treatments:

T_0 = Untreated control

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf

Figure 2. Layout of the management experimental plot with three replications

3.3.4 Land preparation

The selected experimental plot for 2nd experiment was opened in the 1st February, 2019 with a power tiller and left exposed to the sun for a week. Subsequently cross ploughing was done three times followed by laddering to make the land suitable for seeds sowing. All weeds, stubbles and residues were eliminated from the field and finally, a good tilth was achieved. The soil treatment was done at the time of final land preparation to protect young plants from the attack of soil inhibiting insects. Final land preparation was done on 8th February, 2019.

Photograph-2. Prepared field for management study

3.3.5 Application of manure and fertilizer

For the Experiment-II, manures and fertilizers were applied as per Table 1 presneted in 3.2.4.

3.3.6 Preparation and application of different bio-pesticides and botanicals

3.3.6.1 Application of BAU Bio-Fungicide

A formulated product of *Trichoderma harzianum*, based BAU bio-fungicide developed by Prof. Dr. Ismail Hossain, Disease Resistance Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh was sprayed at 2% solution at 7 days interval by hand sprayer as per treatment.

Plate 4. *Trichoderma* based BAU Bio-Fungicide

3.3.6.2 Preparation and application of Allamonda, Neem and Biskatali leaf extract

- After collection of fresh leaves of Allamonda, Neem and Biskatali were washed with water and kept in shade for 15 days for air-drying.
- The dried leaves were grind separately with electrical grinder and sieved through 0.66 mm diameter sieve to obtain fine powder.
- The powder was suspended in clean water and preserved in plastic pot at low temperature and spray at 7 days interval as per treatment.

Plate 5. Green leaves of Allamonda, Neem and Biskatali

3.3.6.3. Preparation of Mustard oil cake and Garlic paste

- After collection of mustard oil cake and garlic from local marked they were cleaned with a soft cloth and the clove of garlic were separated and kept in the shade for 3 days for air-drying.
- They were growed grind separately with electrical grinder and sieved through 0.66 mm diameter sieve to obtain fine powder.

- The powder of mustard oil cake and garlic were suspended in clean water and preserved in plastic pot at low temperature and spray at 7 days interval as per treatment.

Plate 6. Paste of mustard oil cake and garlic

3.3.7 Seeds sowing

The seeds of Indian spinach were sown on 9th February, 2019. At a depth of 2 cm in the experimental plots. Light irrigation was provided in the evening after sowing of seeds. There were four rows in a plot, each rows having six plant, in total there are twenty four plants in each plot.

3.3.8 Intercultural operation

Intercultural operation were done as and when necssary

3.3.9 Harvesting

Harvesting of Indian spinach were done as per 3.2.7

Photograph-3. Field view of Experiment-II

3.3.10 Data collection

Data were also collected on

- Number of healthy and infected leaves/plant at early, mid and late growth stage
- Disease incidence (%) at early, mid and late growth stage
- Disease severity (%) at early, mid and late growth stage
- Number of *Cercospora* spot at early, mid and late growth stage in leaf
- Stem length (cm)
- Leaf area infection (%)
- Yield of Indian spinach (kg/ha and t/ha)

3.3.11 *Cercospora* leaf spot disease incidence

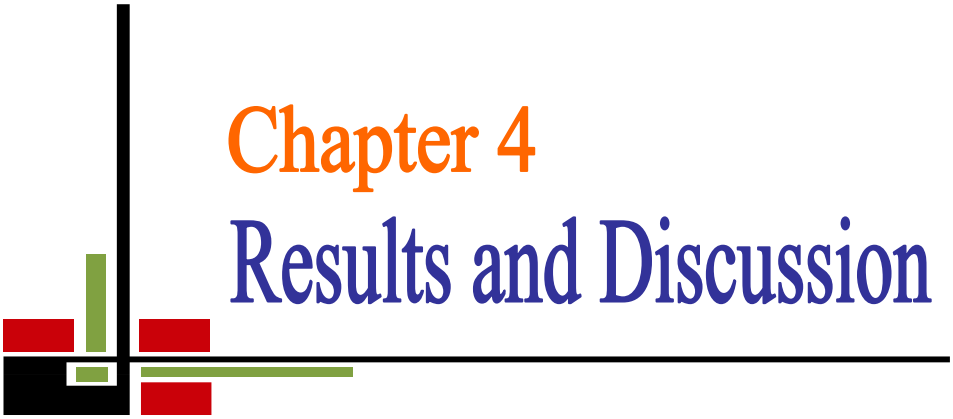
Cercospora leaf spot disease incidence was estimated as per 3.2.9.

3.3.12 Assessment of *Cercospora* disease severity

Cercospora leaf spot disease incidence were estimated as per 3.2.9.

3.4 Statistical analysis

The data obtained from both experiments were statistically analyzed to observe the significant difference among the means. The mean values of all the characters were calculated and analysis of variance was performed by using MSTAT-C software. The significance of the difference among the varieties treatment means was estimated by the Duncan Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).



Chapter 4
Results and Discussion

CHAPTER 4

RESULTS

Two field experiments were conducted in two consequetive growing to evaluate selected varieties of Indian spinach against *Cercospora* leaf spot and its management using bio-pesticides and selected botanicals. For both the experiments, analyses of variance (ANOVA) of the data on disease incidence, and severity at early, mid and late growth stage, number of *Cercospora* leaf spots/leaf, stem length, leaf area infection and yield of Indian spinach are presented in Appendix IV-XIII. The results have been presented in the tabulated and graph and possible interpretations given under the following headings and sub-headings:

4.1 Experiment-I: Evaluation of Selected Varieties of Indian Spinach (*Basella Alba* L.) against *Cercospora* Leaf Spot at Natural Condition

4.1.1 Disease incidence (%) and disease severity (%) at different growth stages of Indian spinach

There were significant differences found in number of healthy, infected leaves, disease incidence (%) and disease severity (%) at early, mid and late growth stage. But total leaves/plant showed non-significant differences in selected for different varieties of Indian spinach under the study.

4.1.1.1 At early growth stage

At early growth stage, the highest number of healthy leaves/plant (18.70) was recorded in BARI-2 variety which was statistically similar (18.47) with BARI-1 variety, whereas the lowest number (11.45) was found in local variety of Indian spinach. The lowest number of infected leaves/plant (10.18) was recorded in BARI-2 which was statistically similar (10.58) with BARI-1, while the highest number (16.67) was recorded in local variety. The highest number of total leaves/plant (29.05) was recorded in BARI-1 variety and the lowest number (28.12) was found in local variety.

The lowest *Cercospora* leaf spot disease incidence (35.25%) was estimated in BARI-2 variety which was statistically similar (36.36%) with BARI-1 variety, whereas the highest disease incidence (59.36%) was recorded in local variety of Indian spinach.

The lowest *Cercospora* leaf spot disease severity (21.60%) was observed in BARI-2 variety which was statistically similar (23.05%) with BARI-1 variety, whereas the highest disease severity (45.25%) was recorded in local variety of Indian spinach.These results presented in Table 2

Table 2. Disease incidence (%) and severity (%) among selected Indian spinach varieties at early growth stage against *Cercospora* leaf spot

Indian Spinach Variety	At early growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
BARI-1	18.47 a	10.58 b	29.05	36.36 b	23.05 b
BARI-2	18.70 a	10.18 b	28.88	35.25 b	21.60 b
Local	11.45 b	16.67 a	28.12	59.36 a	45.25 a
CV(%)	5.56	6.64	4.74	5.06	5.74

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

4.1.1.2 At mid growth stage

In context of mid growth stage, the highest number of healthy leaves/plant (30.68) was observed with BARI-2 variety which was statistically similar (30.00) in BARI-1 variety and the lowest number (16.80) was found in local variety of Indian spinach.. On the other hand, the lowest number of infected leaves/plant (17.93) was found in BARI-2 followed by BARI-1 (18.90), whereas the highest number (30.42) was recorded in local variety. The highest number of total leaves/plant (48.90) was observed in BARI-1 variety, while the lowest number (47.22) was recorded in local variety of Indian spinach.

The lowest disease incidence (36.92%) was estimated in BARI-2 variety which was statistically similar with BARI-1 variety (38.71%), whereas the highest disease incidence (64.49%) was recorded in local Indian spinach variety.

The lowest disease severity (25.85%) was found in BARI-2 variety which was statistically similar with BARI-1 variety (27.12%), whereas the highest disease severity (52.45%) was observed in local Indian spinach variety. are presented in Table 3.

Table 3. Disease incidence (%) and severity (%) among different Indian spinach varieties at mid growth stage against *Cercospora* leaf spot

Indian Spinach Variety	At mid growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
BARI-1	30.00 a	18.90 b	48.90	38.71 b	27.12 b
BARI-2	30.68 a	17.93 c	48.62	36.92 b	25.85 b
Local	16.80 b	30.42 a	47.22	64.49 a	52.45 a
CV(%)	7.71	4.69	4.79	4.57	7.51

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

4.1.1.3 At late growth stage

From Table 4 it was revealed that at late growth stage, the highest number of healthy leaves/plant (39.28) was found in BARI-2 variety which was statistically similar with BARI-1 (37.35) variety and the lowest number (19.33) was recorded in local Indian spinach variety. The lowest number of infected leaves/plant (28.30) was calculated in BARI-2 which was statistically similar with BARI-1 (29.75), while the highest number (47.77) was found in local variety. The highest number of total leaves/plant (67.58) was recorded in BARI-2 variety and the lowest number (67.10) was found in local variety of Indian spinach.

The lowest disease incidence (41.95%) was recorded in BARI-2 variety which was statistically similar (44.35%) with BARI-1 variety and the highest disease incidence (71.37%) was found in local Indian spinach variety.

The lowest disease severity (34.85%) was recorded in BARI-2 variety which was statistically similar (35.40%) in BARI-1 variety and the highest disease incidence (62.50%) was found in local Indian spinach variety.

Table 4. Disease incidence (%) and severity (%) among different Indian spinach varieties at late growth stage against *Cercospora* leaf spot

Indian Spinach Variety	At late growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
BARI-1	37.35 a	29.75 b	67.10	44.35 b	35.40 b
BARI-2	39.28 a	28.30 b	67.58	41.95 c	34.85 b
Local	19.33 b	47.77 a	67.10	71.37 a	62.50 a
CV(%)	10.96	5.00	5.79	6.11	4.68

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

4.1.2 Prevalence of disease incidence and severity at different growth stage

Considering percent disease incidence and severity data, it was revealed that percent disease incidence and severity increased with the increasing of time among all varieties of Indian spinach. Among the variety the highest disease incidence and severity was recorded in local variety at all growth stage, whereas the lowest incidence was found in BARI-2 followed by BARI-1 variety (Figure 3 and 4).

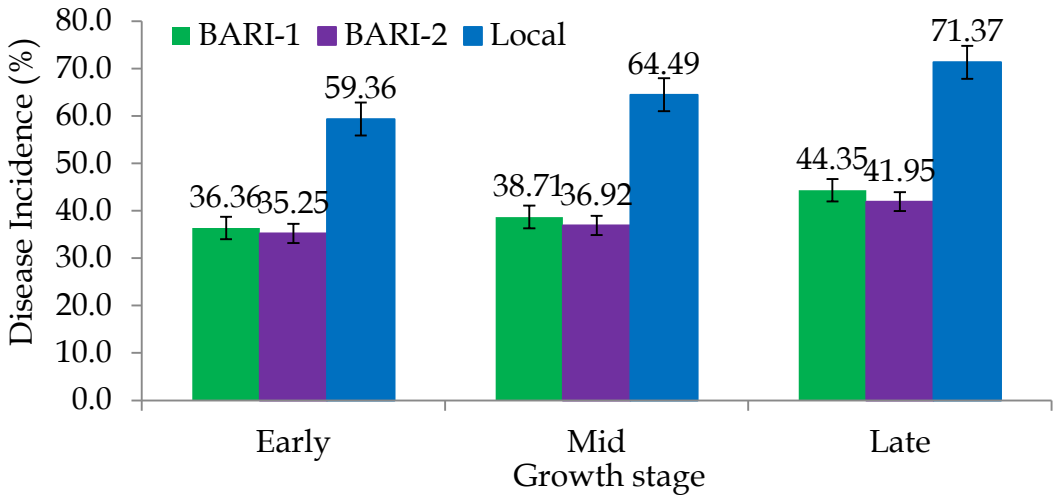


Figure 3. disease incidence (%) of *Cercospora* leaf spot among the selected varieties of Indian spinach.

Vertical bars represent LSD value at 0.01 level of probability.

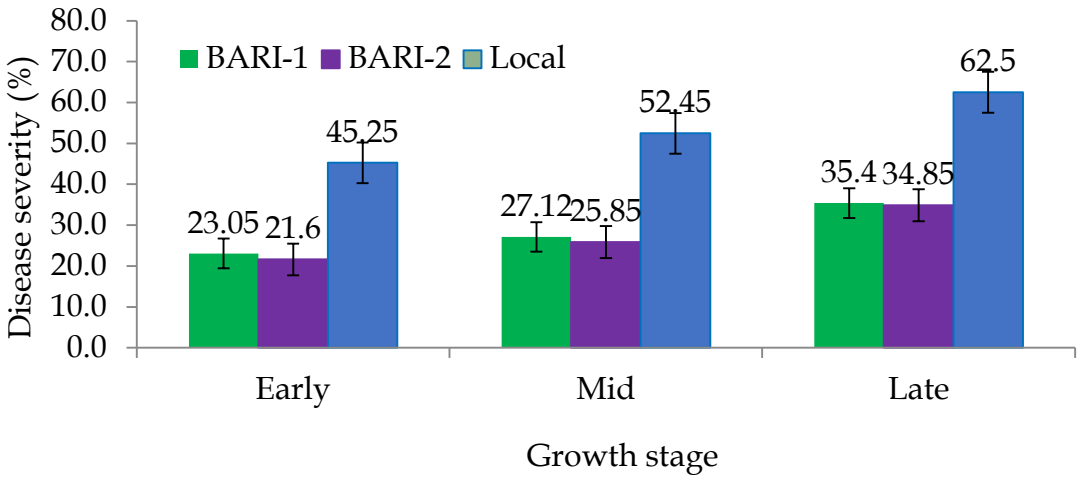


Figure 4. disease severity (%) of *Cercospora* leaf spot among different variety of Indian spinach.

Vertical bars represent LSD value at 0.01 level of probability.

4.1.3 Number of *Cercospora* leaf spots/leaf, stem length (cm) and leaf area infection (%) of Indian spinach at different growth stage

Mean performance of number of *Cercospora* leaf spots/leaf, stem length (cm) and leaf area infection (%) of Indian spinach at different growth stage under untreated condition were varied significantly. The results are shown in table 5.

Number of *Cercospora* leaf spots/leaf

Statistically significant variation was found in terms of number of *Cercospora* leaf spots/leaf at early, mid and late growth at field condition.

At early growth stage, the highest number of *Cercospora* leaf spots/leaf (13.07) was observed in local variety and the lowest number (6.68) was found in BARI-2 which was statistically similar (7.48) to BARI-1 Indian spinach variety.

On the other hand, at mid growth stage, the highest number of *Cercospora* leaf spots/leaf (19.87) was found in local variety, while the lowest number (11.76) was observed in BARI-2 which was statistically similar (12.18) to BARI-1 Indian spinach variety.

Data revealed that at late growth stage, the highest number of *Cercospora* leaf spots/leaf (26.12) was recorded in local variety, while the lowest number (15.85) was observed in BARI-2 which was statistically similar (16.45) to BARI-1

Indian spinach variety. In considering the total *Cercospora* leaf spot for the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (59.06) was observed in local variety, while the lowest number (34.29) was recorded in BARI-2 which was statistically similar (36.11) to BARI-1 Indian spinach variety. Data revealed that the local variety of Indian spinach exhibited the highest number of *Cercospora* leaf spot compare to the other varieties.

Stem length (cm)

Different varieties of Indian spinach varied significantly in terms of stem length. The longest stem (75.44 cm) was found in BARI-2 variety followed (72.77 cm) by BARI-1 variety and the shortest stem (66.90 cm) was observed in local variety of Indian spinach.

Leaf area infection (%)

Different varieties of Indian spinach varied significantly in terms of leaf area infection. The highest leaf area infection (74.05%) was recorded in local variety, whereas the lowest number (42.04%) was found in BARI-2 which was statistically similar (45.28%) to BARI-1 Indian spinach variety.

Table 5. Number of *Cercospora* leaf spots/leaf at different growth stage and total, stem length and leaf area infection of different Indian spinach varieties

Indian Spinach Variety	Number of *Cercospora* leaf spots/leaf at different growth stage				Stem length (cm)	Leaf area infection (%)
	Early	Mid	Late	Total		
BARI-1	7.48 b	12.18 b	16.45 b	36.11 b	72.77 b	45.28 b
BARI-2	6.68 b	11.76 b	15.85 b	34.29 c	75.44 a	42.04 b
Local	13.07 a	19.87 a	26.12 a	59.06 a	66.90 c	74.05 a
CV(%)	13.02	6.38	3.97	4.20	4.41	8.14

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

4.1.4 Yield (t/ha) due to *Cercospora* leaf spot

Statistically significant differences were recorded in terms of yield of Indian spinach due to different varieties. The highest yield (23.79 t/ha) was recorded in BARI-2 variety which was followed (22.16 t/ha) by BARI-1 variety, whereas the lowest yield (19.48 t/ha) in local variety of Indian spinach (Table 6). Data revealed that the highest yield (23.79 t/ha) was recorded in BARI-2 which was followed (22.16 t/ha) by BARI-1 variety, whereas the lowest yield (19.48 t/ha) was found in local variety of Indian spinach (Table 6).

Table 6. Mean performance of yield of different Indian spinach varieties due to *Cercospora* leaf spot

Indian Spinach Variety	Yield (kg/ha)	Yield (t/ha)
BARI-1	22160.00 b	22.16 b
BARI-2	22790.00 a	23.79 a
Local	19480.00 c	19.48 c
CV(%)	7.39	7.39

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

4.1.5 Relationship between disease severity (%) and yield (t/ha) of Indian spinach

The data on yield of Indian spinach were regressed against disease severity% and a negative linear relationship was obtained between the parameters. It was evident in the Figure 5 that the equation y = -0.1285 + 27.497 gave a good fit to the data, and the co-efficient of determination (R^2 = -0.8716) indicates that, fitted regression line had a significant negative regression co-efficient. It is evident, yield of Indian spinach decreased with increased of disease severity.

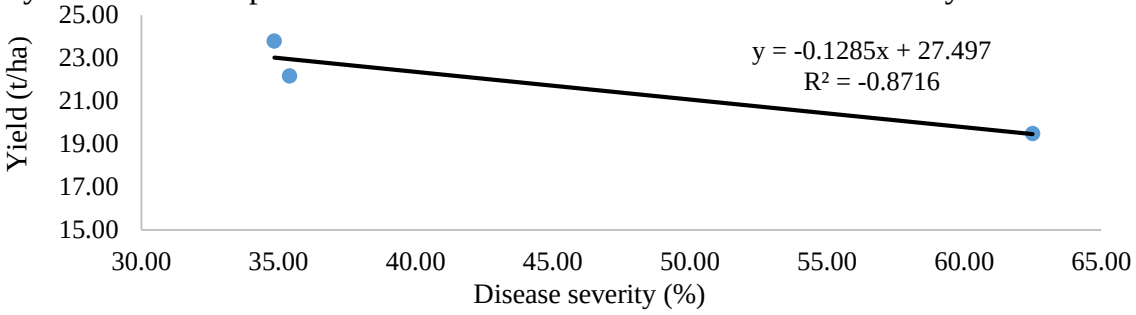


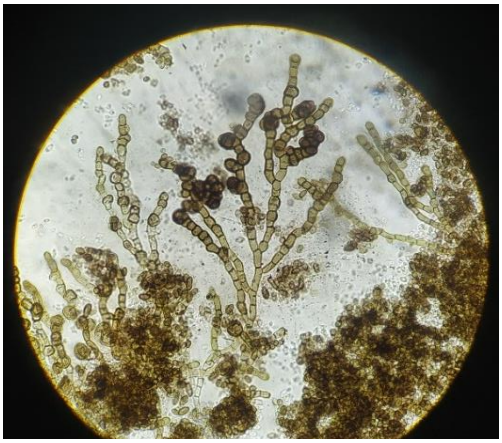
Figure 5. Relationship between disease severity (%) and yield (t/ha) of Indian spinach.

4.1.6 Identification of the Causal Organism

Microscopic study of the whitish-gray lesions revealed, the presence of small and black stromata within the leaf substomatal cavities. The stromata produced loose fascicles of conidiophores, up to 15 conidiophores in a fascicle. Conidiophores were simple, one to four septate, golden brown but subhyaline at the apex, straight, geniculate, having distinctive spore scars, and unbranched. Conidia were solitary, hyaline, filiform, straight to slightly curved, with obtuse to subacute at the apex and subtruncate bases, multiseptate. On the basis of morphological characteristics, the causal organism was identified as *Cercospora beticola* Sacc.

A. Growth of *Cercospora beticola* in CDA media

B. *Cercospora beticola* from infected leaf (Compound Microscopic view, 10X)



C. *Cercospora beticola* from pure culture (compound Microscopic view, 10X)

Plate 7. *Cercospora beticola* from (A) CDA media (B) infected leaf (C) pure culture

4.2 Experiment- II: Test of Efficacy of Bio-pesticides for the Control

4.2.1 Effect of selected bio-pesticides and botanicals on percent disease incidence and severity in Indian spinach at different growth stage against *Cercospora* leaf spot

Statistically significant variation was found in terms of number of healthy, infected, total leaves/plant, disease incidence (%) and disease severity (%) at early, mid and late growth stage on local variety of Indian spinach.

4.2.1.1 At early growth stage

At early growth stage, the highest number of healthy leaves/plant (30.47) was counted in T_2 (Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval) treatment which was statistically similar (28.60) to T_4 (Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval) treatment and followed (27.00) by T_5 (Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval) treatment. On the other hand, the lowest number (12.47) was recorded in T_0 (Untreated control) treatment which was followed by (25.20, 25.80 and 26.80, respectively) by T_6 (Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval), T_3 (Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval) and T_1 (Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval) treatment and they were statistically similar (Table 7).

In case of infected leaves, the lowest number of infected leaves/plant (1.07) was found in T_2 treatment which was statistically similar (1.13 and 1.20) to T_4 and T_5 treatment followed (2.27, 2.40 and 2.73) by T_1, T_3 and T_6 treatment, while the highest number (16.73) was observed in T_0 treatment. The highest number of total leaves/plant (31.53) was recorded in T_2 treatment which was followed by other treatments but the lowest number (29.20) was observed in T_0 treatment.

For disease incidence of *Cercospora* leaf spot, the lowest disease incidence (3.38%) was observed in T_2 treatment which was statistically similar (3.81% and

4.26%) to T_4 and T_5 and followed (7.97%, 8.51% and 9.78%) by T_1, T_3 and T_6, while the highest disease incidence (57.31%) was found in T_0 treatment.

In case of disease severity of *Cercospora* leaf spot at early growth stage, the lowest disease severity (3.18%) was observed in T_2 treatment which was statistically similar (3.45% and 3.82%) to T_4 and T_5 treatment and followed (6.24% and 7.32%) by T_1 and T_3, while the highest disease severity (44.86%) was found in T_0 treatment which was followed (8.15%) by T_6 treatment.

Table 7. Effect of selected bio-pesticides and botanicals on percent disease incidence and severity in Indian spinach at early growth stage against *Cercospora* leaf spot

Treatments	At early growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
T_0	12.47 d	16.73 a	29.20 b	57.31 a	44.86 a
T_1	26.20 c	2.27 b	28.47 b	7.97 b	6.24 c
T_2	30.47 a	1.07 c	31.53 a	3.38 c	3.18 d
T_3	25.80 c	2.40 b	28.20 b	8.51 b	7.32 bc
T_4	28.60 ab	1.13 c	29.73 ab	3.81 c	3.45 d
T_5	27.00 bc	1.20 c	28.20 b	4.26 c	3.82 d
T_6	25.20 c	2.73 b	27.93 b	9.78 b	8.15 b
CV(%)	4.57	10.03	4.03	9.72	8.78

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.0n level of probability

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

4.2.1.2 At mid growth stage

At mid growth stage, the highest number of healthy leaves/plant (50.93) was counted in T_2 treatment which was statistically similar (48.47) to T_4 treatment

and followed (45.73, 46.13 and 46.80) by T_3, T_1 and T_5 treatment which are statistically similar, whereas the lowest number (17.13) was found in T_0 treatment (Table 8). For infected leaves, the lowest number of infected leaves/plant (2.53) was recorded in T_2 treatment which was statistically similar (3.07 and 3.27) to T_4 and T_5 treatment and followed (4.07) by T_1 treatment, whereas the highest number (30.60) was found in T_0 treatment. The highest number of total leaves/plant (53.47) was recorded in T_2 treatment which was statistically similar (51.53, 50.60, 50.20 and 50.07, respectively) to T_4, T_3, T_1 and T_5, whereas the lowest number (47.73) was found in T_0 which was followed (48.73) by T_6 treatment.

In case of disease incidence of *Cercospora* leaf spot, the lowest disease incidence (4.74%) was recorded in T_2 treatment which was statistically similar (5.95% and 6.53%) to T_4 and T_5 treatment, whereas the highest disease incidence (64.10%) was found in T_0 treatment.

For disease severity of *Cercospora* leaf spot at mid growth stage, the lowest disease severity (3.95%) was observed in T_2 treatment which was statistically similar (5.16% and 5.38%) to T_4 and T_5 treatment and followed (7.38% and 8.24%) by T_1 and T_3 treatment, while the highest disease severity (53.11%) was found in T_0 treatment which was followed (9.22%) by T_6 treatment.

Table 8. Mean performance of different bio-pesticides and botanicals on percent disease incidence and severity on Indian spinach at mid growth stage against *Cercospora* leaf spot

Treatments	At mid growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
T_0	17.13 d	30.60 a	47.73 c	64.10 a	53.11 a
T_1	46.13 bc	4.07 cd	50.20 a-c	8.12 cd	7.38 c
T_2	50.93 a	2.53 e	53.47 a	4.74 e	3.95 d
T_3	45.73 bc	4.87 bc	50.60 a-c	9.65 bc	8.24 bc
T_4	48.47 ab	3.07 e	51.53 ab	5.95 e	5.16 d
T_5	46.80 bc	3.27 de	50.07 a-c	6.53 de	5.38 d
T_6	43.53 c	5.20 b	48.73 bc	10.67 b	9.22 b
CV(%)	4.38	6.85	3.54	6.96	6.20

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

4.2.1.3 At late growth stage

At late growth stage, the highest number of healthy leaves/plant (68.73) was found in T_2 treatment which was followed (64.80, 64.60 and 62.60) by T_4, T_5 and T_1 treatment which are statistically similar, while the lowest number (19.07) was recorded in T_0 treatment (Table 9). In consideration of infected leaves, the lowest number of infected leaves/plant (3.80) was observed in T_2 treatment which was statistically similar (4.67) to T_4 treatment and followed (5.33) by T_5 treatment, whereas the highest number (47.40) was found in T_0 treatment. The highest number of total leaves/plant (72.53) was observed in T_2 treatment which was statistically similar (69.93 and 69.47, respectively) to T_5 and T_4 treatment and closely followed (68.67, 68.53 and 68.13) by T_1, T_6 and T_3, whereas the lowest number (66.47) in T_0 treatment.

For disease incidence of *Cercospora* leaf spot, the lowest disease incidence (5.24%) was found in T_2 which was statistically similar (6.72%) to T_4 treatment, whereas the highest disease incidence (71.31%) was recorded in T_0 treatment.

In case of disease severity of *Cercospora* leaf spot at late growth stage, the lowest disease severity (4.65%) was observed in T_2 treatment which was statistically similar (5.95%) to T_4 treatment and followed (6.34%) by T_5 treatment, while the highest disease severity (61.56%) was found in T_0 treatment which was followed (10.07% and 9.18%) by T_6 and T_3 treatment.

Table 9. Mean performance of different bio-pesticides and botanicals on percent disease incidence and severity on Indian spinach at late growth stage against *Cercospora* leaf spot

Treatments	At late growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
	Healthy	Infected	Total		
T_0	19.07 e	47.40 a	66.47 c	71.31 a	61.56 a
T_1	62.60 b-d	6.07 cd	68.67 bc	8.83 cd	7.84 c
T_2	68.73 a	3.80 f	72.53 a	5.24 f	4.65 e
T_3	61.07 cd	7.07 bc	68.13 bc	10.37 bc	9.18 bc
T_4	64.80 b	4.67 ef	69.47 a-c	6.72 ef	5.95 de
T_5	64.60 bc	5.33 de	69.93 ab	7.64 de	6.34 d
T_6	60.67 d	7.87 b	68.53 bc	11.49 b	10.07 b
CV(%)	3.33	6.29	2.51	6.24	5.19

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

4.2.2 Comparison of disease incidence (%) and disease severity (%) at different growth stage under treated condition

In consideration of disease incidence data revealed that in time being from early to late growing stage disease incidence of *Cercospora* leaf spot of Indian spinach increase slowly in all the bio-pesticides and botanicals treatment. The results revealed that in all treatments significantly could reduce the *Cercospora* leaf spot disease incidence and severity. The present study showed that, BAU-fungicides with different botanicals performed the best in controlling *Cercospora* leaf spot than the other treatment and also untreated control condition (Figure 6-7).

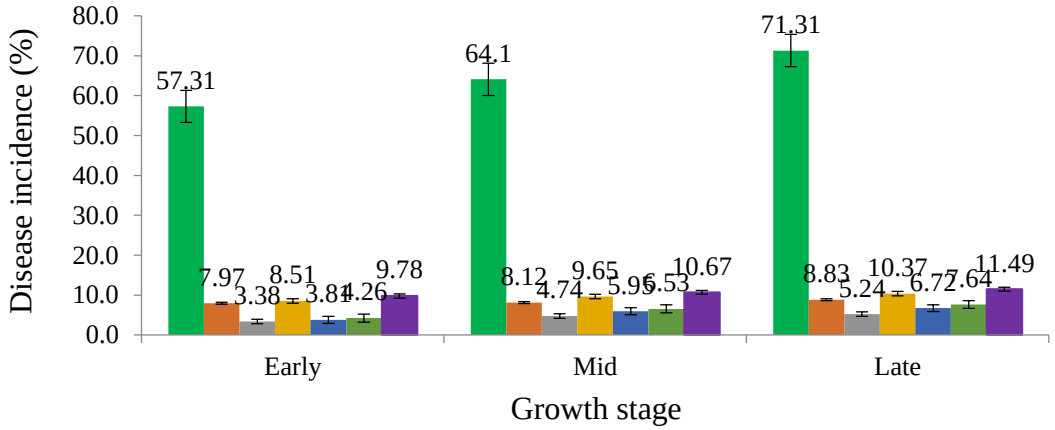


Figure 6. Disease incidence (%) of *Cercospora* leaf spot of Indian spinach in different growth stage treated with bio-pesticides and botanicals.

Vertical bars represent LSD value at 0.01 level of probability.

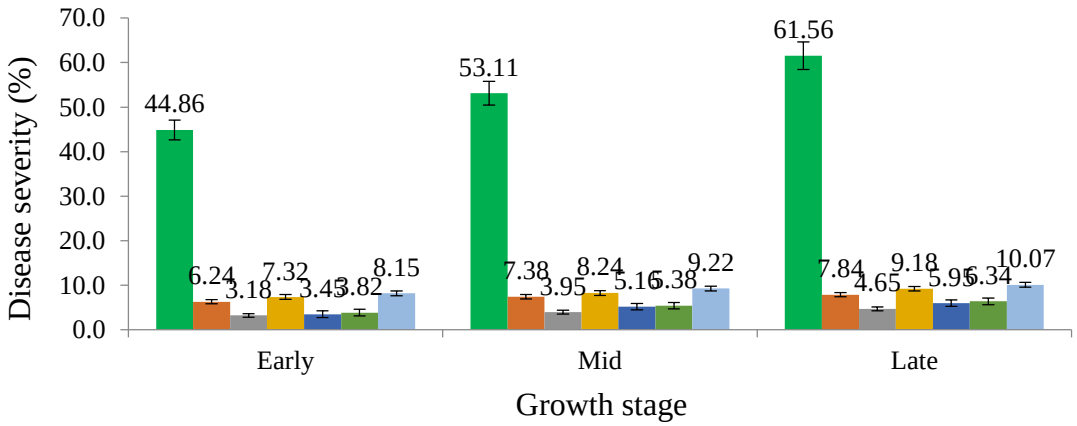


Figure 7. Disease severity (%) of *Cercospora* leaf spot of Indian spinach in different growth stage treated with bio-pesticides and botanicals.

Vertical bars represent LSD value at 0.01 level of probability.

4.2.3 Mean performance of number of *Cercospora* leaf spots/leaf, stem length (cm) and leaf area infection (%) of Indian spinach at different growth stage under treated condition

Mean performance of number of *Cercospora* leaf spots/leaf, stem length (cm) and leaf area infection (%) of Indian spinach at different growth stage under treated condition were varied significantly. The results are presented in table 10.

Number of *Cercospora* leaf spots/leaf

Number of *Cercospora* leaf spots/leaf of Indian spinach at early, mid and late growth stage showed statistically significant differences due to bio-pesticides and botanicals. At early growth stage, the highest number of *Cercospora* leaf spots/leaf (13.47) was found in T_0 treatment, whereas the lowest number (1.27) was found in T_2 treatment which was followed (2.07 and 2.13) by T_4 and T_5 treatment (Table 10). At mid growth stage, the highest number of *Cercospora* leaf spots/leaf (20.53) was recorded in T_0 treatment, while the lowest number (2.07) was found in T_2 treatment which was followed (2.93, 3.20 and 3.60) by T_4, T_5 and T_1 treatment. At late growth stage, the highest number of *Cercospora* leaf spots/leaf (26.73) was found in T_0 treatment, whereas the lowest number (2.60) was recorded in T_2 treatment which was followed (3.53 and 3.87) by T_4 and T_5 treatment. In case of total number the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (60.73) was observed in T_0 treatment, while the lowest number (5.94) was recorded in T_2 treatment which was followed (8.53 and 9.20) by T_4 and T_5 treatment.

Stem length (cm)

Statistically significant variation was recorded in terms of stem length of Indian spinach due to different bio-pesticides and botanicals. The longest stem (75.78 cm) was measured in T_2 treatment which was statistically similar (74.18 cm, 73.73 cm, 72.22 cm and 71.49 cm, respectively) to T_4, T_5, T_1 and T_3 treatment and followed (70.59 cm) by T_6, while the shortest stem (67.28 cm) was measured in T_0 treatment.

Leaf area infection (%)

Statistically significant variation was recorded in terms of leaf area infection of *Cercospora* leaf spot of Indian spinach due to different bio-pesticides and botanicals. The highest leaf area (74.58%) was observed from T_0 treatment, whereas the lowest infection (5.26%) was recorded in T_2 treatment which was statistically similar (6.15% and 7.82%) to T_4 and T_5 treatment and followed (8.56%) by T_1 treatment.

Table 10. Mean performance of number of *Cercospora* leaf spots/leaf, stem length (cm) and leaf area infection (%) of Indian spinach at different growth stage under treated condition

Treatments	Number of *Cercospora* leaf spots/leaf at the different stage				Stem length (cm)	Leaf area infection (%)
	Early	Mid	Late	Total		
T_0	13.47 a	20.53 a	26.73 a	60.73 a	67.28 c	74.58 a
T_1	2.67 c	3.60 b-d	4.53 cd	10.80 cd	72.22 a-c	8.56 cd
T_2	1.27 e	2.07 e	2.60 f	5.94 f	75.78 a	5.26 e
T_3	2.87 bc	4.13 bc	4.87 bc	11.87 bc	71.49 a-c	10.25 bc
T_4	2.07 d	2.93 de	3.53 e	8.53 e	74.18 ab	6.15 de
T_5	2.13 d	3.20 cd	3.87 de	9.20 de	73.73 ab	7.82 c-e
T_6	3.27 b	4.33 b	5.53 b	13.13 b	70.59 bc	12.34 b
CV(%)	6.75	8.76	5.46	5.89	3.58	9.39

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed byAllamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

4.2.4 Mean performance of yield under treated condition

Yield per hectare of Indian spinach showed statistically significant variation . The highest yield (23090.00 kg/ha) was observed in T_2 which was statistically similar with other treatment and the lowest yield (19660.00 kg/ha) was recorded in T_0 treatment (Table 11). The highest yield (23.09 t/ha) was observed in T_2 which was statistically similar with other treatment and the lowest yield (19.66 t/ha) was recorded in T_0 treatment. In consideration of yield increase over control the highest value (17.45%) was recorded in T_2 and the lowest increase (9.72%) was recorded in T_6 treatment.

Table 11. Effect of different bio-pesticides and botanicals on disease severity, stem length and yield of Indian spinach

Treatments	Yield (kg/ha)	Yield (t/ha)	Yield increase over control (%)
T_0	19660.00 b	19.66 b	--
T_1	21860.00 a	21.86 a	11.19
T_2	23090.00 a	23.09 a	17.45
T_3	21660.00 a	21.66 a	10.17
T_4	22510.00 a	22.51 a	14.50
T_5	22380.00 a	22.38 a	13.84
T_6	21570.00 a	21.57 a	9.72
CV(%)	4.79	4.79	--

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

T_0 = Untreated control (without any treatment)

T_1 = Seed treated with BAU-biofungicide followed by Allamonda leaf extract and spray at 7 days interval

T_2 = Seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval

T_3 = Seed treated with BAU-biofungicide followed by Mustard oil cake and spray at 7 days interval

T_4 = Seed treated with BAU-biofungicide followed by Garlic and spray at 7 days interval

T_5 = Seed treated with BAU-biofungicide followed by Biskatali leaf extract and spray at 7 days interval

T_6 = Seed treated with Allamonda leaf extract followed by Neem leaf extract and spray at 7 days interval

DISCUSSION

Indian spinach (*Basella alba* L.) commonly known as Puishak, belongs to the family Basellaceae, is a popular tropical leafy green vegetable is widely cultivated for its edible leaves (Reddy *et al*., 2014; Kamruzzaman *et al*., 2015). It is a popular summer leafy vegetable widely cultivated and commonly found in the home gardens in Bangladesh. Indian spinach has been reported as having potential antiulcer, cytotoxic, antibacterial, anti-inflammatory, nephron-protective and wound healing properties and functioning as a central nervous system depressant (Kumar *et al*., 2013). Almost all parts of this plants are used in traditional medicine, which include as a laxative, rubefacient, demulcent, an astringent; febrifuge, diuretic, and to treat conjunctivitis, dysentery, diarrhoea, indigestion, constipation, boils and sores and as an antidote to poison (Encyclopedia of Life, 2017; National Parks Board, 2020). In Bangladesh, Indian spinach is cultivated in around 10358.74 ha. of land and the average yield of 7.3 t ha^{-1} with the production of 79,093 Metric ton (BBS, 2018).

So far sixteen different diseases of Indian spinach have so far been reported from different parts of the world (Hossain, 2007; Shova *et al*., 2020). In Bangladesh, only four diseases viz. leaf spot caused by *Alternaria* sp., *Gloesporium* sp., and *Cercospora* sp., foot rot caused by *Sclerotium rolfsii* Sacc.; anthracnose caused by *Colletotrichum* sp., Macrophomina leaf spot and stem rot caused by *Macrophomina phaseolina* have been reported in different countries of the world (Sarker *et al*., 2017).

Among the diseases, leaf spot is a major disease of Indian spinach causes red spots and holes in leaves. The causal agent *Cercospora* Sp. mainly is seed born, however; the pathogen is also able to survive for at least one year in plant debris and soil also. 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). *Cercospora* leaf spot of Indian spinach is caused by *Cercospora beticola* which affect mainly on leaf. The typical symptoms appear as circular to oval shaped, purple color pinhead spots with a necrotic gray centre surrounded

by a purple to brown border (Recardo *et al*., 2015). The present investigation was undertaken to evaluate different collecting Indian spinach variety against *Cercospora* leaf spot and to determine the effect of bio-fungicide and botanicals, in reducing the incidence of *Cercospora* leaf spot of Indian spinach. The following objectives were considered for this experiment.

Two experiments were comprised to fulfill the objectives and they were-

Evaluation of Different Varieties of Indian Spinach (*Basella Alba* L.) against *Cercospora* Leaf Spot at Natural Condition

At early, mid and late growth stage, the highest number of healthy leaves/plant (18.70, 30.68 and 39.28, respectively) was recorded in BARI-2 variety, whereas the lowest number (11.45, 16.80 and 19.33, respectively) in local variety of Indian spinach. At early, mid and late growth stage, the lowest number of infected leaves/plant (10.18, 17.93 and 28.30, respectively) was recorded in BARI-2, while the highest number (16.67, 30.42 and 47.77, respectively) in local variety. At early, mid and late growth stage, the highest number of total leaves/plant (29.05, 48.90 and 67.58, respectively) was recorded in BARI-1 variety and the lowest number (28.12, 47.22 and 67.10) in local variety. At early, mid and late growth stage, the lowest *Cercospora* leaf spot disease incidence (35.25%, 36.92% and 41.95%, respectively) was observed in BARI-2 variety, whereas the highest (59.36%, 64.49% and 71.37%) in local variety of Indian spinach. At early, mid and late growth stage, the lowest *Cercospora* leaf spot disease severity (21.60%, 25.85% and 34.85%, respectively) was observed in BARI-2 variety, whereas the highest disease severity (45.25%, 52.45% and 62.50%, respectively) was recorded in local variety of Indian spinach. Similar findings also reported by Hasan *et al*. (2016), They showed that local variety of Indian spinach is more susceptible to *Cercospora* leaf spot than BARI spinach 1 and the highest disease incidence (58.90 to 53.19) and the greater disease severity (35.00 to 52.20) in local variety and maximum disease incidence was found in BARI spinach 1 in compare with local variety. It was also similar with

the findings in context of *Cercospora* leaf spot disease incidence and severity of this study. Sarker *et al*. (2017) reported disease incidence 64.3% in BARI puishak-2 in control condition in the agro-climatic condition of Sylhet Agricultural University, Sylhet, Bangladesh which also similar with the findings of this study.

At early, mid and late growth stage, the highest number of *Cercospora* leaf spots/leaf (13.07, 19.87 and 26.12, respectively) was observed in local variety and the lowest number (6.68, 11.76 and 15.85, respectively) was found in BARI-2. In considering the total *Cercospora* leaf spot for the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (59.06) was observed in local variety, while the lowest number (34.29) in BARI-2. The longest stem (75.44 cm) was found in BARI-2 variety and the shortest stem (66.90 cm) in local variety of Indian spinach. The highest leaf area infection (74.05%) was recorded from local variety, whereas the lowest number (42.04%) from BARI-2. The highest yield (23.79 t/ha) was recorded in BARI-2 variety, whereas the lowest yield (19.48 t/ha) in local variety of Indian spinach. Hasan *et al*. (2016) recorded higher plant weight in BARI spinach 1 compared to the local variety in a earlier experiment.

In this study considering the yield and yield attributes both the BARI varieties performed better than the local variety and probably it was happened due to the highest disease incidence and disease severity of local variety due to the *Cercospora* leaf spot disease.

Microscopic study of the whitish-gray lesions revealed the presence of small and black stromata within the leaf substomatal cavities. Conidiophores were simple, one to four septate, golden brown but subhyaline at the apex, straight, geniculate, having distinctive spore scars, and unbranched. Conidia were solitary, hyaline, filiform, straight to slightly curved, with obtuse to subacute at the apex and subtruncate bases, multiseptate (Kirarei *et al*., 2019). On the basis of

morphological characteristics, the causal organism was identified as *Cercospora beticola*.

Management of *Cercospora* Leaf Spot of Indian Spinach Using Seleced Bio-pesticides and Botanicals in Field under Treated Condition

The experiment comprised of three selected bio-pesticides and three selected botanicals including an untreated control as treatment.

At early, mid and late growth stage, the highest number of healthy leaves/plant (30.47, 50.93 and 68.73, respectively) was observed in T_2 and the lowest number (12.47, 17.13 and 19.07, respectively) in T_0 treatment. In case of infected leaves, the lowest number of infected leaves/plant (1.07, 2.53 and 3.80, respectively) was found in T_2, while the highest number (16.73, 30.60 and 47.40, respectively) was observed in T_0 treatment. The highest number of total leaves/plant (31.53, 53.47 and 72.53, respectively) was recorded in T_2 and the lowest number (29.20, 47.73 and 66.47, respectively) in T_0 treatment. For disease incidence of *Cercospora* leaf spot, the lowest disease incidence (3.38%, 4.74% and 5.24%, respectively) was observed in T_2, while the highest disease incidence (57.31%, 64.10% and 71.31%, respectively) in T_0 treatment. In case of disease severity of *Cercospora* leaf spot at early growth stage, the lowest disease severity (3.18%, 3.95% and 4.65%, respectively) was observed in T_2, while the highest disease severity (44.86%, 53.11% and 61.56%, respectively) in T_0 treatment. The alternate approaches of chemical control using of different bio-fungicides and botanicals also found to be environmentally sound and effective against the pathogen (Uddin *et al*., 2013; Maketon *et al*., 2008).

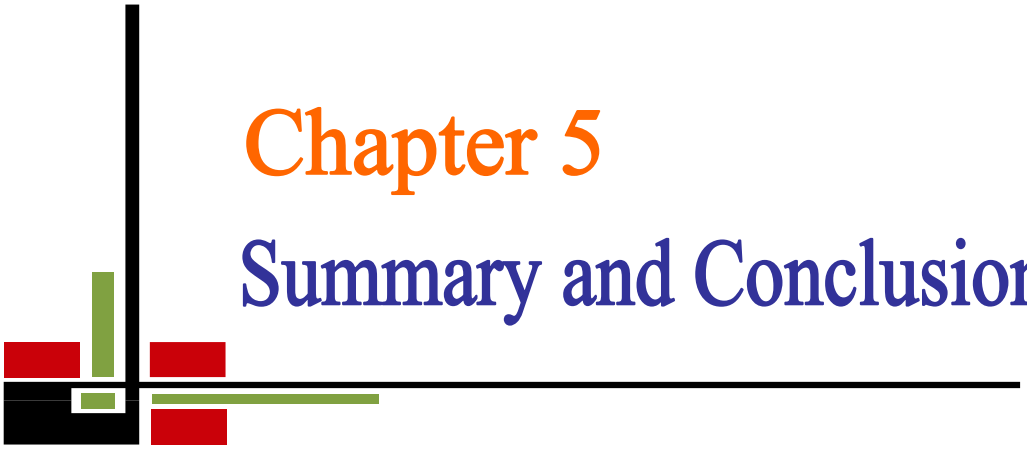
At early, mid and late growth stage, the highest number of *Cercospora* leaf spots/leaf (13.47, 20.53 and 26.73, respectively) was found in T_0, whereas the lowest number (1.27, 2.07 and 2.60, respectively) in T_2 treatment. In case of total number the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (60.73) was observed in T_0, while the lowest number (5.94) in T_2 treatment. The longest stem (75.78 cm) was found in T_2, while the shortest stem (67.28 cm) in

T_0 treatment. The highest leaf area infection (74.58%) was observed from T_0, whereas the lowest number (5.26%) in T_2 treatment. The highest yield (23.09 t/ha) was observed in T_2 and the lowest yield (19.66 t/ha) in T_0 treatment. In consideration of yield increase over control the highest value (17.45%) was recorded in T_2 and the lowest increase (9.72%) in T_6 treatment. Hasan *et al*. (2016) reported that seed treating with Carbendazim followed by foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim significantly reduced disease incidence of Indian spinach. Sarker *et al*. (2017) reported the lowest disease incidence (28.9%) in the application of treatments Trichocompost. In this study, it was revealed that BAU-fungicides with different botanicals treated plot showed best performance in reducing the severity of the disease through its Induced Systemic Resistance (ISR). Hasan *et al*. (2016) reported that foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim significantly reduced severity of Indian spinach.

Data revealed that the highest number of *Cercospora* leaf spots/leaf of Indian spinach was recorded in untreated control condition compare to the other treatments. Hasan *et al*. (2016) reported in an earlier experiment that foliar spray with Carbendazim, seed coating with BAU bio-fungicide followed by foliar spray with Carbendazim, only foliar spray with Carbendazim significantly reduced the number of *Cercospora* leaf spot/leaf of Indian spinach. Among the treatments Seed treated with BAU-biofungicide + Neem leaf extract and spray at 7 days interval performed the best then the second best one was the Seed treated with BAU-biofungicide + Garlic and spray at 7 days interval and then Seed treated with BAU-biofungicide + Biskatali leaf extract and spray at 7 days interval. Some researchers also found the efficacy BAU-fungicides with different botanicals on controlling disease incidence in Indian spinach and other plants (Uddin *et al*., 2013; Maketon *et al*., 2008).

Due to *Cercospora* leaf spot disease, photosynthetic process is disturbed and leaves becomes deformed resulting weakens the plant with premature defoliation which ultimately resulting the lowers yield and also the lowest market value (Hasan *et al*., 2016).

Data revealed that in time being from early to late growing stage disease incidence (%) and disease severity (%) of *Cercospora* leaf spot of Indian spinach increasing slowly with increasing of time for all the bio-pesticides and botanicals. The results revealed that all selected treatments significantly could reduce the *Cercospora* leaf spot disease incidence and severity. The present study also showed that, BAU-fungicides with different botanicals performed the best in controlling *Cercospora* leaf spot than the other treatment and also untreated control condition (Maketon *et al*., 2008; Uddin *et al*., 2013; Hasan *et al*., 2016).



Chapter 5

Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

Consequently, two experiments were conducted in the experimental field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka to evaluate different varieties of Indian spinach (*Basella Alba* L.) against *Cercospora* leaf spot and its management using selected bio-pesticides and botanicals in field.

The 1st experiment was conducted during the period of April to June, 2018. Total 3 (three) varieties of Indian spinach as BARI-1, BARI-2 and one local variety were selected. The single factor experiment was laid out in Randomized Complete Block Design (RCBD) with twelve replications. Data were collected on number of healthy and infected leaves/plant, disease incidence (%), disease severity (%) and number of *Cercospora* spot at early, mid and late growth stage, stem length, leaf area infection (%) and yield of Indian spinach and statistically significant variation was recorded for selected varieties.

At early, mid and late growth stage, the highest number of healthy leaves/plant (18.70, 30.68 and 39.28, respectively) was recorded in BARI-2 variety, whereas the lowest number (11.45, 16.80 and 19.33, respectively) in local variety of Indian spinach. At early, mid and late growth stage, the lowest number of infected leaves/plant (10.18, 17.93 and 28.30, respectively) was recorded in BARI-2, while the highest number (16.67, 30.42 and 47.77, respectively) in local variety. At early, mid and late growth stage, the highest number of total leaves/plant (29.05, 48.90 and 67.58, respectively) was recorded in BARI-1 variety and the lowest number (28.12, 47.22 and 67.10) in local variety. At early, mid and late growth stage, the lowest *Cercospora* leaf spot disease incidence (35.25%, 36.92% and 41.95%, respectively) was observed in BARI-2 variety, whereas the highest (59.36%, 64.49% and 71.37%) in local variety of Indian spinach. At early, mid and late growth stage, the lowest *Cercospora* leaf spot disease severity (21.60%, 25.85% and 34.85%, respectively) was recorded in

BARI-2 variety, whereas the highest disease severity (45.25%, 52.45% and 62.50%, respectively) was recorded in local variety of Indian spinach.

At early, mid and late growth stage, the highest number of *Cercospora* leaf spots/leaf (13.07, 19.87 and 26.12, respectively) was observed in local variety and the lowest number (6.68, 11.76 and 15.85, respectively) was found in BARI-2. In considering the total *Cercospora* leaf spot for the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (59.06) was observed in local variety, while the lowest number (34.29) in BARI-2. The longest stem (75.44 cm) was found in BARI-2 variety and the shortest stem (66.90 cm) in local variety of Indian spinach. The highest leaf area infection (74.05%) was recorded from local variety, whereas the lowest number (42.04%) from BARI-2. The highest yield (23.79 t/ha) was recorded in BARI-2 variety, whereas the lowest yield (19.48 t/ha) in local variety of Indian spinach.

Microscopic study of the whitish-gray lesions revealed the presence of small and black stromata within the leaf substomatal cavities. Conidiophores were simple, one to four septate, golden brown but subhyaline at the apex, straight, geniculate, having distinctive spore scars, and unbranched. Conidia were solitary, hyaline, filiform, straight to slightly curved, with obtuse to subacute at the apex and subtruncate bases, multiseptate. On the basis of morphological characteristics, the causal organism was identified as *Cercospora beticola*.

The 2nd experiment was conducted during the period of February to April, 2019. Local variety of Indian spinach were used as the test crop in this experiment. The experiment comprised of three selected bio-pesticides and three selected botanicals including an untreated control as treatment. Data were collected on number of healthy and infected leaves/plant, disease incidence (%), disease severity (%) and number of *Cercospora* spot at early, mid and late growth stage, stem length, leaf area infection (%) and yield of Indian spinach and statistically significant variation was recorded for bio-pesticides and botanicals treatments.

At early, mid and late growth stage, the highest number of healthy leaves/plant (30.47, 50.93 and 68.73, respectively) was observed in T_2 and the lowest number (12.47, 17.13 and 19.07, respectively) in T_0 treatment. In case of infected leaves, the lowest number of infected leaves/plant (1.07, 2.53 and 3.80, respectively) was found in T_2, while the highest number (16.73, 30.60 and 47.40, respectively) was observed in T_0 treatment. The highest number of total leaves/plant (31.53, 53.47 and 72.53, respectively) was recorded in T_2 and the lowest number (29.20, 47.73 and 66.47, respectively) in T_0 treatment. For disease incidence of *Cercospora* leaf spot, the lowest disease incidence (3.38%, 4.74% and 5.24%, respectively) was observed in T_2, while the highest disease incidence (57.31%, 64.10% and 71.31%, respectively) in T_0 treatment. In case of disease severity of *Cercospora* leaf spot at early growth stage, the lowest disease severity (3.18%, 3.95% and 4.65%, respectively) was observed in T_2, while the highest disease severity (44.86%, 53.11% and 61.56%, respectively) in T_0 treatment.

At early, mid and late growth stage, the highest number of *Cercospora* leaf spots/leaf (13.47, 20.53 and 26.73, respectively) was found in T_0, whereas the lowest number (1.27, 2.07 and 2.60, respectively) in T_2 treatment. In case of total number the entire growth stage, the highest number of *Cercospora* leaf spots/leaf (60.73) was observed in T_0, while the lowest number (5.94) in T_2 treatment. The longest stem (75.78 cm) was found in T_2, while the shortest stem (67.28 cm) in T_0 treatment. The highest leaf area infection (74.58%) was observed from T_0, whereas the lowest infection (5.26%) in T_2 treatment. The highest yield (23.09 t/ha) was observed in T_2 and the lowest yield (19.66 t/ha) in T_0 treatment. In consideration of yield increase over control the highest value (17.45%) was recorded in T_2 and the lowest increase (9.72%) in T_6 treatment.

Findings of the experiments revealed that local variety was more susceptible to *Cercospora* leaf spot diseases and the causal organism of these leaf spot was identified as *Cercospora beticola*. Among the selected bio-pesticides and botanicals seed treated with BAU-biofungicide + Neem leaf extract and spray at 7 days interval was the best compare to the others treatments of the study.

Considering all things, the following conclusion may be as follows-

1. At three growth stages, the highest incidence and severity was found in local variety (59.36%, 64.49% 71.37% and 45.25%, 52.45%, 62.50%), respectively. Whereas the lowest DI and DS in BARI 2 (35.25%, 36.92%, 41.95% and 21.60%, 25.85%, 34.85%), respectively in untreated condition.

2. The highest number of *Cercospora* leaf spots/leaf was observed in local variety (13.07, 19.87 and 26.12, respectively) and the lowest number was found in BARI-2 (6.68, 11.76 and 15.85, respectively), at three growth stages. On the other hand, the highest leaf area infection was recorded from local variety (74.05%), whereas the lowest number was found from BARI-2 (42.04%).

3. The highest yield was recorded in BARI-2 variety (23.79 t/ha), whereas the lowest yield was found in local variety of Indian spinach (19.48 t/ha).

4. For isolation of *Cercospora* sp., Blotter, PDA, CDA media are used where only fungal growth found in only CDA media. In microscopic view, Conidiophores were simple, one to four septate, golden brown but subhyaline at the apex, straight, geniculate, having distinctive spore scars, and unbranched. Conidia were solitary, hyaline, filiform, straight to slightly curved, with obtuse to subacute at the apex and subtruncate bases, multiseptate were found.

5. In treated condition, at three growth stages, the highest incidence and severity was found in treatment T_0 (57.31%, 64.10%, 71.31% and 44.86%, 53.11%, 61.56%), respectively. Whereas the lowest DI and DS in treatment T_2 (3.38%, 4.74%, 5.24% and 3.18%, 3.95%, 4.65%), respectively.

6. At three growth stages, the highest number of *Cercospora* leaf spots/leaf was found in T_0 (13.47, 20.53 and 26.73, respectively), whereas the lowest number was observed in T_2 treatment (1.27, 2.07 and 2.60, respectively). The

highest leaf area infection was observed from T_0 (74.58%), whereas the lowest infection was found in T_2 treatment (5.26%).

7. The highest yield was observed in T_2 treatment (23.09 t/ha) and the lowest yield was found in T_0 treatment (19.66 t/ha).

RECOMMENDATIONS

Considering the results of the present experiments, the following recommendations can be drawn/ may be suggested:

1. BARI 2 variety showed best performance in case of percent disease incidence and severity
2. Among the selected bio-pesticides and botanicals, seed treated with BAU-biofungicide followed by Neem leaf extract and spray at 7 days interval was the best.
3. Such study is needed to be repeated in different agro-ecological zones (AEZ) of Bangladesh for the evaluation of regional adaptability, and
4. Other control measures may be used for further study.

References

REFERENCES

Adhikari, H.N., Kumar, N. and Shruthi, S.D. (2012). A Review on medicinal importance of *Basella alba*. *Intl. J. Pharmacal. Sci. & Drug Res*. **4**: 110-114.

Akpan, E.A. and Andrew, D.E. (2018). Application of botanical foliar spray on the control of fungal diseases of *Vigna radiata* (mung bean) in Uyo, South-South Nigeria. *J. Res. Ecol*. **6**(1): 1377-1383.

Akter, S. and Shamsi, S. (2013). Mycoflora associated with *Basella* spp. and their pathogenic potentiality. *Bangladesh J. Sci. Res*. **26**(1&2): 83-87.

Asaduzzaman, M., Hossain, M.M., Parvez, M.N., Islam, M.A. and Meah, M.B. (2008). Effect of dose and frequency of garlic tablet spray on the incidence of cercospora leaf spot of chilli. *J. Agrofor. Environ*. **7**(1): 119-122.

Basunia, A.K., Hossain, M.M., Islam, M.A., Akter, M.M. and Akter, M.M. (2020). Influence of bioslurry on the growth, yield and nutritional status of Indian Spinach. *J. Bangladesh Agril. Uni*. **18**(2): 379-387.

BBS (Bangladesh Bureau of Statistics). (2018). The Yearbook of Agricultural Statistics of Bangladesh. Statistics Div., Minis. Plan. Govt. peoples Repub. Bangladesh, Dhaka, p. 271.

Bdliya B.S. and Gwio-Kura, K.K. (2007). Efficacy of some fungicides in the management of *Cercospora* leaf spot of groundnut in the Sudan savanna of Nigeria. *J. Plant Protection Res*. **47**: 243-254.

Chotangui, A.H., Betigne, M., Mandou, M.S., Kamaleu, N.G.F. and Kouam, E.B. (2020). Effect of phyto-extracts of neem (*Azadirachta indica*) and garlic (*Allium sativum*) on leaf spot disease of groundnut (*Arachis hypogaea* L.). *Open Agric*. **5**: 441-449.

Das, S., Alam, M.N., Batuta, S., Ahamed, G., Fouzder, C., Kundu, R., Mandal, D. and Begum, N.A. (2017). Exploring the efficacy of *Basella alba* mucilage towards the encapsulation of the hydrophobic antioxidants for their better performance. *Process Biochem*, **61**: 178-188.

Debele, S. and Ayalew, A. (2015). Integrated management of Cercospora leaf spots of groundnut (*Arachis hypogaea* L.) through host resistance and fungicides in Eastern Ethiopia. *African J. Plant Sci*. **9**(2): 82-89.

Dey, S., Haque, A.H.M.M., Hasan, R., Biswas, A. and Sarker, S. (2017). Efficacy of botanicals and chemicals to control *Cercospora* leaf spot disease of country bean in field condition. *J. Appl. Life Sci. Intl*. **11**(3): 1-7.

Diemeleou, C.A., Zoue, L.T. and Niamke, S.L. (2014). *Basella alba* seeds as a novel source of non-conventional oil with beneficial qualities. *Romanian Biotech. Letters*, **19**(1): 8966-8978.

Encyclopedia of Life. (2017). Encyclopedia of Life. In: Encyclopedia of Life. http://www.eol.org.

Ernst, M. (2017). Malabar spinach. CCD-CP-130. Center for Crop Diversification, University of Kentucky, Lexington.

FAO (1988). Production Year Book, Food, Agricultural Organization of the United Nation, Rome Italy. **42**: 190-193.

FRG (2018). Fertilizer Recommendation Guide, Bangladesh Agricultural Research Council (BARC), Dhaka. p. 99.

Gomathinayagam, S., Balasubramanian, N., Shanmugaiah, V., Rekha, M., Manoharan, P.T. and Lalithakumari, D. (2011). Molecular Characterization of Carbendazim Resistance of Plant Pathogen (*Bipolaris oryzae*), Fungicides - Beneficial and Harmful Aspects, Dr. Nooruddin Thajuddin (Ed.), ISBN: 978-953-307-451-1.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for agricultural research (2ed.). John Wiley and Sons, New York. pp. 188-240.

Hanelt, P. (2017). Mansfeld's World Database of Agricultural and Horticultural Crops. In: Mansfeld's World Database of Agricultural and Horticultural Crops. Gatersleben, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). p. 185.

Hasan, M.M., Islam, N.B., Naznin, S., Islam, M.M. and Kishowar-E-Mustarin. (2016). Management of Cercospora Leaf Spot of Indian Spinach (Basella alba L.) with BAU Bio-fungicide and a Plant Growth Promoting Hormone. *Universal J. Plant Sci*. **4**(4): 43-49.

Hossain I. (2007). Research on crop disease management at Bangladesh Agricultural University. Advances in Plant pathology researches in Bangladesh. Plant Pathology Division, BARI. **2007**: 251-282.

Hossain, M.H. and Hossain, I. (2013). Screening of different plant extracts against leaf spot (*Cercospora arachidicola* and *Cercosporidium personatum*) of groundnut (*Arachis hypogaea* L.). *Bangladesh J. Agril. Res*. **38**(3): 491-503.

Hossain, M.H. and Hossain, I. (2014). Evaluation of three botanicals, bavistin and BAU-biofungicide for controlling leaf spot of groundnut caused by *Cercospora arachidicola* and *Cercosporidium personatum*. *The Agriculturists*, **12**(1): 41-49.

Kamruzzaman, M., Khatun, S., Rakib, A., Hoque, M.I. and Ranil, M.H. (2015). Temporal variation in seed quality of Indian spinach preserved in different containers. *Intl. J. Agril. Res. Innova. & Technol.* **10**(5):50-53.

Khan, M.F. and Smith, L.J. (2005). Evaluating fungicides for controlling Cercospora leaf spot on sugar beet. *Crop Prot*. **24**(1): 79-86.

Khare, C.P. (2004). Indian medicinal plants: an illustrated dictionary. Springer, Berlin, p 83.

Kirareia, E.K., Kipsumbaib, P.K. and Kiprop, E.K. (2019). The occurrence and characterization of pathogen causing leaf spots disease of spinach in Nandi and Uasin Gishu Countries, Kenya. *Africa Env. Review J.* **3**(2): 122-133.

Kumar, S., Prasad, A.K., Iyer, S.V. and Vaidya, S.K. (2013). Systematic pharmacognostical, phytochemical and pharmacological review on an ethno medicinal plant Indian spinach, *Basella alba* L. *J. Pharmaco. & Phyto*. **5**(4): 53-58.

Kumar, S.S., Manoj, P. and Giridhar, P. (2015). Nutrition facts and functional attributes of foliage of Basella spp. *LWT Food Sci. Technol*. **64**: 468-474.

Kumar, S.S., Manoj, P., Giridhar, P., Shrivastava, R. and Bharadwaj, M. (2017). Fruit extracts of *Basella rubra* that are rich in bioactives and betalains exhibit antioxidant activity and cytotoxicity against human cervical carcinoma cells. *J. Functional Foods*, **15**: 509-515.

Maketon, M., Apisitsantikul, J. and Siriraweekul, C. (2008). Greenhouse evaluation of *Bacillus subtilis* AP-01 and *Trichoderma harzianum* AP-001 in controlling tobacco diseases. *Brazil. J. Microbiol*. **39**(2): 296-300.

Mitra, A. and Das, S.K. (2015). Fabric dyeing with natural dye extracted from *Basella alba* fruit and spectroscopic analysis of the extract at different conditions *J. Chem. Pharm. Res*. **7**(12): 1117-1124.

Mohammed, B.M. (2012). Bio-control of Cercospora Leaf Spot Disease of Groundnut (*Arachis Hypogeae*) Using Extracts of *Moringa Oleifera* Lam and Jatropha Curcas L. A Thesis submitted to the Postgraduate School Federal University of Technology, Owerri. p. 115.

National Parks Board. (2020). Flora and fauna web. In: Flora and fauna web, Singapore: National Parks Board. http://florafaunaweb.nparks.gov.sg/Home.aspx.

Neindow, M. (2017). Effectiveness of Desert date seed extract (DDSE), Neem seed extract (NSE), Jatropha seed extract (JSE) and Tobacco leaf extract (TLE) for the control of CLS disease of groundnut. *J. Agric. Biotech. Sustain. Dev*. **10**(9): 170-177.

Nousraat, E., Datta, J., Hasan, R., Asad-Ud-Doullah, and Mahfuzul, A.H.M.H. (2016). Performance of chemical and botanicals against *Cercospora* leaf spot (CLS) of mungbean. *Asian Res. J. Agric.* **2**(3): 1-6.

Oladele, O. (2011). Microorganisms associated with the deterioration of fresh leafy Indian Spinach in storage. *J. Plant Pathol Microbiol*. **11**: 45-51.

Poornima, P. Yashoda, R., Prashanthi, S.K., Nargund, V.B. and Venugopal, C.K. (2011). Antifungal effect of botanicals against *Cercospora beticola*, the incitant of leaf spot of palak. *Karnataka J. Agril. Sci*. **24**(4): 575-576.

Ramesh, M.A. and Zacharia, S. (2017). Efficacy of Bio-agents and Botanicals against leaf Spot (*Cercospora arachidicola* Hori) of Groundnut (*Arachis hypogaea* L.). *J. Pharmacol. & Phytochem*. **6**(5): 504-506.

Recardo, B., Luiz, F.C., Costa, M.E.B. and Piero, R.M.D. (2015). Mode of action of chitosan and ASM for the control of *Cercospora* leaf spot on table beet. *Tropical Plant Pathol*. **40**: 176-183.

Reddy, M.T., Begum, H., Sunil, N., Rao, P.S., Sivaraj, N. and Kumar, S. (2014). Preliminary characterization and evaluation of landraces of Indian spinach (*Basella* spp. L.) for agro-economic and quality traits. *Plant Breeding & Biotech*. **2**: 48-63.

Sarker, S., Haque, A.H.M.M., Islam, M., Dey, S. and Biswas, A. (2017). Evaluation of different botanicals and biological agents to control Cercospora leaf spot of Indian Spinach in field condition. *J. App. Life Sci. Intl*. **13**(3): 1-8.

Shova, N.J., Shamsi, S. and Bashar, M.A. (2020). Prevalence and pathogenic potentiality of fungi associated with leaf spot of *Basella alba* and *B. Rubra*. *Dhaka Univ. J. Biol. Sci.* **29**(2): 183-189.

Singh, A., Dubey, P.K., Chaurasiya, R., Mathur, N., Kumar, G., Bharati, S. and Abhilash, P.C. (2018). Indian spinach: an underutilized perennial leafy vegetable for nutritional security in developing world. *Energ. Ecol. Environ*. **3**(1): 45-51.

Singh, R. and Singh, V.K. (2014). Effect of different management options viz., carbedazim, neem leaf extract, intercropping with seasmum and their integration in integrated disease management of sesame. *The Bioscan*, **9**(1): 107-110.

Srivastava, S. and Nelson, S. (2012). *Cercospora* leaf spot of eggplant. *Plant Disease*. **82**: 1-5.

Toshiyuki, M., Kazuhiro, H. and Masayuki, Y. (2001) Structures of new oleanane-type triterpene oligoglycosides, Basella saponins A, B, C, and D, from the fresh aerial parts of *Basella rubra* L. *Chem Pharm Bull.* **49**: 776-779.

Uddin, M.N., Bakr, M.A., Islam, M.R., Hossain, M.I. and Hossain, A. (2013). Bioefficacy of plant extracts to control cercospora leaf spot of mungbean (*Vigna radiata*). *Intl. J. Agric. Res, Innov. & Tech*., **3**(1): 60-65.

Uddin, M.N. (2007). Management of *Cercospora* leaf spot of Mungbean (Vigna radiata) through botanicals, MS Thesis, Dept. of Plant Pathology, Sher-e Bangla Agricultural University. P. 72.

Useful Tropical Plants. (2017). Useful tropical plants database. In: Useful tropical plants database: K Fern.http://tropical. theferns. info/

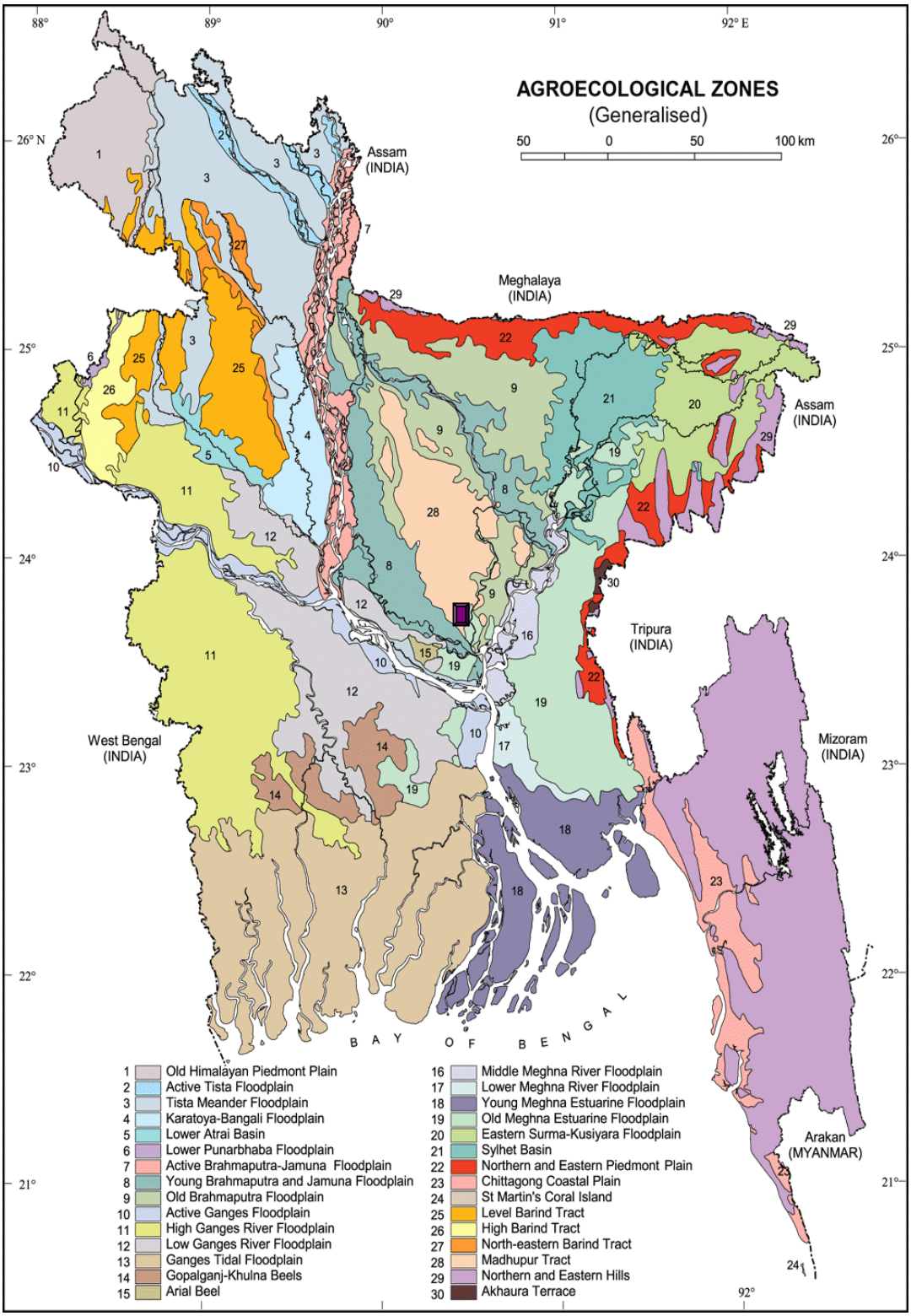
Yadav, R.S., Dikshit, A. and Mishra, R.K. (2015). Application of chemicals, bio-agents and botanicals for their antifungal efficacy against the growth of *Alternaria* spp, incitant of alternaria leaf spot of Cabbage (*Brassica olaracea* var. capitata L.). *J. Che. Bioche. Sci*. **1**(10): 14-19

Yashoda, P., Hegde, R., Prashanthi, S.K., Nargund, V.B. and Venugopal, C.K. (2011). Antifungal effect of botanicals against *Cercospora beticola,* the incitant of leaf spot of palak. *Karnataka J. Agril. Sci*. **24**(4): 575-576.

Appendices

APPENDICES

Appendix I. The Map of the experimental site



Appendix II. Soil characteristics of experimental field as per the Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Experimental field , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. Physical and chemical properties of the initial soil

Characteristics	Value
% Sand	26
% Silt	43
% clay	31
Textural class	Sandy loam
pH	5.9
Catayan exchange capacity	2.64 meq 100 g/soil
Organic matter (%)	1.15
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	45

Appendix III. Monthly record of air temperature, relative humidity, rainfall, and sunshine (average) of the experimental site during the period from April to June, 2018 and February to April, 2019

Month	Air temperature (^{0}c)		Relative humidity (%)	Rainfall (mm)	Sunshine (hr)
	Maximum	Minimum			
April, 2018	33.4	23.2	67	78	6.9
May, 2018	34.7	25.9	70	185	7.8
June, 2018	32.4	25.5	81	228	5.7
February, 2019	27.9	17.4	67	43	6.8
March, 2019	29.7	18.9	70	13	6.9
April, 2019	33.6	22.7	69	86	7.1

Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka–1207

Appendix IV. Analysis of variance of the data on disease incidence (%) and severity (%) among different Indian spinach varieties at early growth stage against *Cercospora* leaf spot

Source of variation	Degrees of freedom	Mean square				
		At early growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	11	0.622	0.543	1.755	2.513	2.140
Variety	2	203.701**	158.401**	2.973	2223.917**	2108.37**
Error	22	0.813	0.686	1.846	4.886	2.954

**: Significant at 0.01 level of significance

Appendix V. Analysis of variance of the data on disease incidence (%) and severity (%) among different Indian spinach varieties at mid growth stage against *Cercospora* leaf spot

Source of variation	Degrees of freedom	Mean square				
		At mid growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	11	3.865	0.740	5.921	3.381	6.684
Variety	2	734.908**	578.803**	9.748	2856.679**	2701.90**
Error	22	3.964	1.104	5.336	4.565	6.972

**: Significant at 0.01 level of significance

Appendix VI. Analysis of variance of the data on disease incidence (%) and severity (%) among different Indian spinach varieties at late growth stage against *Cercospora* leaf spot

Source of variation	Degrees of freedom	Mean square				
		At late growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	11	8.745	3.053	8.296	10.369	4.249
Variety	2	1453.187**	1411.308**	0.941	3202.317**	2999.22**
Error	22	12.282	3.115	15.180	10.320	4.291

**: Significant at 0.01 level of significance

Appendix VII. Analysis of variance of the data on number of *Cercospora* leaf spots/leaf at different growth stage and total, stem length and leaf area infection of different Indian spinach varieties

Source of variation	Degrees of freedom	Mean square					
		Number of *Cercospora* leaf spots/leaf at growth stage of				Stem length (cm)	Leaf area infection (%)
		Early	Mid	Late	Total		
Replication	11	1.011	0.777	0.350	0.393	8.308	13.408
Variety	2	145.12**	250.40**	398.42**	253.99**	39.19*	3725.75**
Error	22	1.397	0.869	0.598	0.365	10.005	19.150

**: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance

Appendix VIII. Analysis of variance of the data on yield of different Indian spinach varieties due to *Cercospora* leaf spot

Source of variation	Degrees of freedom	Mean square	
		Yield (kg/ha)	Yield (t/ha)
Replication	11	19472.48	1.947
Variety	2	569404.85**	56.940**
Error	22	25993.592	2.599

**: Significant at 0.01 level of significance

Appendix IX. Analysis of variance of the data on disease incidence (%) and severity (%) of *Cercospora* leaf spot disease in Indian spinach at early growth stage due to different bio-pesticides and botanicals

Source of variation	Degrees of freedom	Mean square				
		At early growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	2	0.373	0.093	0.093	1.096	0.003
Variety	6	102.986**	96.969**	4.844*	1135.030**	680.265**
Error	12	1.316	0.156	1.367	1.741	0.933

**: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance

Appendix X. Analysis of variance of the data on disease incidence (%) and severity (%) of *Cercospora* leaf spot disease in Indian spinach at mid growth stage due to different bio-pesticides and botanicals

Source of variation	Degrees of freedom	Mean square				
		At mid growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	2	1.282	0.177	0.693	0.625	0.516
Variety	6	396.593**	309.846**	10.369**	1380.604*	939.372**
Error	12	3.500	0.275	3.182	1.189	0.671

**: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance

Appendix XI. Analysis of variance of the data on disease incidence (%) and severity (%) of *Cercospora* leaf spot disease in Indian spinach at late growth stage due to different bio-pesticides and botanicals

Source of variation	Degrees of freedom	Mean square				
		At late growth stage No. of leaves/plant			Disease incidence (%)	Disease severity (%)
		Healthy	Infected	Total		
Replication	2	0.602	0.434	0.213	0.936	0.192
Variety	6	877.817**	747.393**	10.497*	1710.638**	1270.624**
Error	12	3.642	0.545	3.007	1.175	0.612

**: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance

Appendix XII. Analysis of variance of the data on number of *Cercospora* leaf spots/leaf at different growth stage and total, stem length and leaf area infection of Indian spinach due to different bio-pesticides and botanicals for controlling *Cercospora* leaf spot disease

Source of variation	Degrees of freedom	Mean square					
		Number of *Cercospora* leaf spots/leaf at growth stage of				Stem length (cm)	Leaf area infection (%)
		Early	Mid	Late	Total		
Replication	2	0.025	0.143	0.025	0.041	0.580	1.183
Variety	6	53.95**	127.88**	221.19**	124.84**	23.17*	1894.44**
Error	12	0.071	0.261	0.163	0.114	6.695	2.812

**: Significant at 0.01 level of significance; *: Significant at 0.05 level of significance

Appendix XIII. Analysis of variance of the data on yield of Indian spinach due to different bio-pesticides and botanicals for controlling *Cercospora* leaf spot disease

Source of variation	Degrees of freedom	Mean square	
		Yield (kg/ha)	Yield (t/ha)
Replication	2	620.89	0.062
Variety	6	35891.45*	3.589*
Error	12	1090.45	1.090

*: Significant at 0.05 level of significance