OCCURRENCE OF ANTHRACNOSE DISEASE OF ALOEVERA (*Aloe vera* L.) CAUSED BY *Colletotrichum gloeosporioides* AND ITS MANAGEMENT

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CERTIFICATE

*This is to certify that, the thesis entitled, "**OCCURRENCE OF ANTHRACNOSE DISEASE OF ALOEVERA (Aloe vera L.) CAUSED BY Colletotrichum gloeosporioides 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 (M.S.) IN PLANT PATHOLOGY embodies the result of a piece of bona fide research work carried out by **MD. AL-AMIN ISLAM, Registration No.: 17-08198** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: 16 June, 2019
Place: Dhaka, Bangladesh

(Abu Noman Faruq Ahmmed)
Associate Professor & Supervisor

Dedicated to

My

Beloved Parents

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OCCURRENCE OF ANTHRACNOSE DISEASE OF ALOEVERA (*Aloe vera* L.) CAUSED BY *Colletotrichum gloeosporioides* AND ITS MANAGEMENT

ABSTRACT

Experiments were conducted in Dhaka, Kathalbaria and Kholabaria villages of Laxsmipur union of Natore district, Bangladesh to identify the causal agents of anthracnose disease of Aloevera (*Aloe vera* L.) and for its field management during 2017-18. The causal organism *Colletotrichum gloeosporioides* was isolated by tissue plating method and identified based on morphological and cultural characteristics that was confirmed by pathogenicity test. Eleven treatments including chemicals, botanicals, bio agents and traditional practices viz. Folicur 25 EC (Tebuconazole @ 0.1%), Tilt 250 EC (Propiconazole @ 0.1%), Autostin 50 WP (Carbendazim @ 0.2%), Dithane M 45 (Mancozeb @ 0.2%), Companion (Mancozeb+Carbendazim @ 0.2%), Bordeaux mixture ($CuSO_4$+CaO @ 01 %), Lime (CaO @ 0.5%), *Trichoderma harzianum* (Bio agent @ 0.2%), Garlic bulb extract 1:1 (w/v) (*Allium sativum* @ 1 %), Neem leaf extract 1:1 (w/v) (*Azadirachta indica* @ 1%) and Alamanda leaf extract 1:1 (w/v) (*Allamanda cathartica* @ 1 %) were considered for the management of anthracnose disease of Aloevera. An infested farmer's field was selected in Kholabaria and Kathalbaria villages of Laxmipur union of Natore district in rainy season for this experiment. The experiment was conducted by following RCBD design with three replications. Among the treatments, Bordeaux mixture gave best result against this disease. Moreover, Tilt 250 EC and Folicur 25 EC and Garlic bulb extract showed better effect against the disease than the other treatments. Lime also has moderate effect against anthracnose disease of Aloevera. In 2017, after 4^{th} spray, the the lowest plant incidence was recorded in Bordeaux mixture (58.33%) which was statistically identical with Folicur (64.58%), Tilt (64.58%) and Garlic bulb extract (66.67%). Similarly, the lowest disease severity was found in Bordeaux mixture (3.55) followed by Folicur (5.67%), Tilt (6.67%) and Garlic bulb extract (7.67%). Similar result also found in 2018. After 4^{th} spray, the lowest plant incidence was recorded in Bordeaux mixture (38.58%) which was statistically identical with Lime (41.66%) and Garlic bulb extract (45.83%). Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). Considering the overall performance of the treatments, garlic bulb extract could be used as eco-friendly approach. Moreover, use of Bordeaux mixture is better than the traditional use of lime. From chemical pesticides, Tilt 250 EC and Folicur 25 EC could be used for controlling the disease as the last option.

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CHAPTER 1
INTRODUCTION

More than 500 plants species have been reported to be available in Bangladesh having medicinal values (Yusuf *et al*., 1994). In Bangladesh, more than 300 Ayurvedic and Unani pharmaceuticals and significant number of beauty care industries use these plants (Akhter *et al*., 2008). According to Dixie *et al*. (2003), the total size of the medicinal plant market at wholesale price was estimated to US$ 14 million per annum. According to FAO (2007), 121505 tone of medicinal plants and aromatic products extracted globally out of which 90181 tones are collected from Asia. This figure is expanding by 15 to 20 percent annually (Subrat, 2002). Very recently, commercial cultivation of medicinal plant is gaining momentum in Bangladesh as a mean of livelihood because of its potential market both nationally and internationally.

Medicinal plants naturally synthesized and accumulate some secondary metabolites, like alkaloids, sterols, volatile oils etc. (Rattan, 2010). According to the World Health Organization (WHO), a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis (Shahriar *et al.*, 2014). Aloevera is a perennial, succulent, monocotyledonous plant having average height of 60-100 cm. Aloevera belongs to a large group of plants known as xeroids as it has the ability to close its stomata to avoid loss of water and help in retaining a large amount of water in its tissue (Akinyele and Odiyi, 2007). The margin of the leaves has serrated soft and small teeth. It is commonly referred as a miracle plant and has a long history of economics and medicinal uses that spans thousands of years (Daodu, 2000). Its gel is very important for the treatment

of wound, skin cancer, cold constipation, asthma, ulcer, diabetes and fungal infection (Joseph and Justin, 2010). Though it has therapeutic and antimicrobial potential, Aloevera is susceptible to numerous fungal diseases. Leaf spot is the major one which not only affects the leaf texture but also deteriorate the quality and quantity of mucilaginous gel used for medicinal and commercial uses.

Aloe vera L. a member of the family Liliaceae is a popular plant species in Bangladesh, is a perennial, drought resistance, succulent medicinal herb belongs to the family 'Aloeaceae' (Barcroft and Myskja, 2003). Among the cultivated medicinal plants in Bangladesh, Gritokumari/Aloevera (*Aloe vera* L.) got the highest position in terms of production and cultivable areas. Aloevera is now commercially cultivated in Laxsmipur union of Natore district. Due to the traditional knowledge on medicinal plants and unplanned cultivation of such plant posed a threat to the existence of these plants.

However, commercial cultivation of Aloevera is hampered by some pest and disease. Leaf spot, Anthracnose disease, leaf rust, crown rot, root rot, tip die back and slime mold usually found on *Aloe vera* (Anonymous, 2005). Among them, anthracnose disease is the most devastating disease of *Aloe vera.* It is now wide spread problem of Aloe growers in Bangladesh (Anonymous, 2004). The most common symptoms of anthracnose are the circular or oval shape having deep sunken lesion.

There are very limited research works on diseases of Aloevera in Bangladesh and South Asia also. Rajendran and Gnanavel (2011) recorded alternaria leaf spot disease caused by *Alternaria alternata*, Cercospora leaf spot disease caused by *Cercospora* sp. and anthracnose disease caused by *Colletotricum* spp. on *Aloe vera*

in India. They also reported rust spots and basal stem rot disease of *Aloe vera* L. Avasthi, *et al.* (2011) have previously reported anthracnose symptom on the leaf surface of *A. vera* in Gwalior city, India. Based on the symptoms, mycelia and conidial characters, the fungus was identified as *Colletotrichum gloeosporioides* which was further confirmed at Indian Agricultural Research Institute, New Delhi. Some instances of anthracnose disease of *Aloe vera* caused by *Colletotrichum* sp. was also reported from Lucknow (Alam *et al*., 2007). Avasthi *et al*. (2015) reported occurrence of leaf spot diseases on *Aloe vera* (L.) caused by *Curvularia* species i.e. *Curvularia lunata* and *Curvularia ovoidea* from Madhya Pradesh, India. Jat *et al.* (2013) also reported severe form of leaf spot disease on *Aloe vera* caused by *C. lunata*. Khadka and Rawal (2014) found the association of *Fusarium oxysporum* species with Aloe vera leaf and basal rot disease in Western Terai of Nepal. Zhai *et al.* (2013) first reported that leaf spots in *Aloe vera* caused by *Nigrospora oryzae* in China.

Rukhsana *et al*. (2010) reported that leaf spot of *Aloe vera* was incited by *Alternaria* a*lternata* that can cause economic losses in the cultivation of the *Aloe vera* in Pakistan. Bijwa *et al*. (2010) first reported leaf spot disease of *Aloe vera* caused by *Alternaria alternata* in Pakistan. Leaf spot disease was found (caused by *Alternaria alternata*) in *Aloe barbadensis* in India (Kamalakannan *et al*., 2008). Ghosh and Banerjee (2014) first reported *Aloe vera* leaf spot disease caused by *Alternaria brassicae* in West Bengal, India. *Aloe vera* is the new host of *A. brassicae* and this disease is present in moderate to severe form throughout the year causing crop damage in West Bengal.

Rajendran and Gnanavel (2011) reported Alternaria leaf spot disease caused by *Alternaria alternata*, Cercospora leaf spot disease caused by *Cercospora* sp.and

anthracnose disease caused by *Colletotrichum* sp. Chavan and Korekar (2011) reported leaf spot disease of Aloevera caused by *Alternaria alternata, A. tenuissima, Fusarium* spp. in Osmanabad District, India during the years 2008 and 2009.Infection of *Aloe vera* by *Alternaria brassicae* not only reduces the crop loss and market value but it may reduce antioxidant property and other medicinal efficacy of the herb (Pritam and Kale, 2007). Leaf spot disease caused by *C. lunata* and *C. ovoidea* affects the quality and quantity of *Aloe vera* leaf gel (Avasthi *et al.*, 2015).

Shutrodhar and Shamsi (2013) recorded 8 fungal species viz.*Alternaria pluriseptata, Aspergillus flavus, Aspergillus niger, Cladosporium oxysporum, Colletotrichum gloeosporioides, Nigrospora oryzae, Penicillium* sp. and *Pestalotiopsis guepinii* associated with anthracnose and leaf spot disease of *Aloe vera* L. in Bangladesh. The frequency of *C. gloeosporioides* was the maximum. Pathogenicity test revealed that *C. gloeosporioides* causes anthracnose disease in Aloevera.

Ghosh *et al.* (2016) reported that, fungal pathogens causing the leaf spot disease of *Aloe vera* have been isolated from infected leaves, collected from Birbhum and Burdwan districts of West Bengal, India. They found, commercially available fungicide Mancozeb was effective at low concentration (100 µg/ml) to control the pathogen whereas it can tolerate 1000 µg/ml or more concentrations of Bavistin. Two plant growth promoting rhizobacterial strains, viz. *Burkholderia cenocepacia* VBC7 and *Pseudomonas poae* VBK1 were able to produce prominent zones of inhibition against the pathogen in dual culture overlay plates.

Sharma *et al.* (2010) conducted field and laboratory studies in Punjab, India,

during 2008 & 2009, to determine suitable control measures for the leaf spot disease of *Aloe vera*, caused by *Alternaria alternata*. In the field, 15-day-old *A. vera* seedlings were planted and 3 sprays of Indofil M-45 (0.3%), Tilt 25EC (0.1%), Score 25EC (0.1%), Amistar 25SC (0.1 %), neem oil (0.5%), tulsi oil (0.5%), ginger oil (0.5%), garlic oil (0.5%), *D. metel* extract (5.0%), turmeric rhizome extract (5.0%), neem extract (5.0%) and *M. arvensis* extract (5.0%) were given at 15-day intervals. Inoculation was done by spraying the *A. alternata* spore suspension to the pin-pricked leaves of the plants. The percent disease intensity was then calculated and the yield was recorded. Results showed that all the tested fungicides were significantly effective in reducing the disease intensity in both years. Among the plant oils, garlic oil was the most effective, followed by ginger and tulsi oil in reducing the disease intensity. The turmeric rhizome extract also showed promising results in controlling the disease in both years.

Kumar *et al.* (2017) conducted an experiment in India during 2014 and 2015 and found, chemical pesticide Propiconazole 25 EC control the maximum percent disease followed by Mancozeb 75 WP and Carbendazim 50 WP. Among the botanicals maximum percent disease control were recorded in Neem leaf extract @ 5% followed by Garlic bulb extract @ 5% and Tulsi leaf extract @ 5% for leaf rot of Aloevera caused by *Fusarium oxysporum*.

Use of chemical pesticides is discouraged in Aloevera due to its residual effect in leaf. In most cases, aloevera gel is used to prepare traditional juice in Bangladesh. Thus,spraying of lime solution in the leaves is a common practice to control leaf spot disease of aloevera in Bangladesh. But, sometimes lime produce heat and toxicity in the plant that reduce production of aloevera gel in plant. Considering this issue, application of botanical or plant extracts may be a good alternative.

Regmi *et al.* (2014) evaluated leaf extracts of six plants *viz*, *Jatropa curcas*, *Datura strumarium, Azadirachata indica*, *Moringa oleifera, Calotropis gigantean* and *Morus alba* @ 50% by food poison techniques against the fungus *Alternaria alternata* causing leaf spot disease of Aloevera. Neem (*Azadirachata indica*) leaf extracts inhibited the mycelia growth of the fungus. Thus, Neem leaf extracts control the leaf spot disease of Aloevera. Sohag *et al.* (2017) conducted a survey to evaluate the efficacy of Garlic bulb extract, Bion, Bavistin DF (Carbendazim) and proud for controlling leaf spot disease of Taro (*Colocasia esculenta*). Botanicals showed enhanced result against leaf spot disease of Taro (*C. esculenta*). Mausuduzzaman *et al.* (2008) studied Alamanda leaf extract and separated compounds to determine the efficiency against leaf spot disease pathogen viz. *Phomopsis vexans, Fusarium sp.* Alamanda leaf extracts inhibited the mycelia growth of the pathogen *Phomopsis vexans, Fusarium sp.* are responsible for leaf spot disease.

Pareek *et al*. (2012) tested the efficacy of bio-control agent's viz. *Trichoderma harzianum, T. viride and Aspergillus niger* against leaf spot pathogen (*Alternaria alternata*).The most effective antagonist was *T. harzianum* against leaf spot pathogen (*A. alternata*).

The most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently affect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of diseases (Tapwal *et al*., 2011).

Natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides (Kim *et al*., 2005). The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance (Mahadevan, 1982). Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987).

Leaf spot is one of the major threats to expand the commercial cultivation of Aloevera in the county. However, very limited findings are available regarding this disease and its management in Bangladesh. So, this is an urgent issue to detect and identify this disease. It is also essential to measure the severity and incidence of diseases to find out the yield loss. From the farmer interest, it is also need to develop farm applicable preventive measures to control this disease and reduce the yield loss of this medicinal plant.

Considering the above facts and points this research work was designed to achieve the following objectives:

1. To identify the causal organism of anthracnose disease of Aloevera, and
2. To find out field management practices for anthracnose disease of Aloevera in Bangladesh.

CHAPTER 2

REVIEW OF LITERATURE

So far our knowledge goes; no research work directly has been carried out on the management of diseases of Aloevera (*Aloe vera* L.) in Bangladesh. There is also a very limited significant research works on diseases of medicinal plant in the South Asia. However, some research works are found regarding diseases of few medicinal plants in the world. This chapter is to review the previous studies that are related to the present study. The review of some related studies are described below:

2.1. Anthracnose disease of Aloevera

Shutrodhar and Shamsi (2013) reported that Aloevera plants were found to be infected with severe leaf spot and anthracnose symptoms under natural and inoculated conditions. The symptoms appeared on the leaves, as dark brown spots and the leaves become dried. Spots were sub circular 2 - 6mm in diameter. In severe case infection started from the leaf edge and the affected leaves shrinked and dried from the tip.

Avasthi *et al*. (2011) observed a typical anthracnose symptom on the leaf surface of *Aloe vera* during the survey of various nurseries of Gwalior city, India, in 2010. The symptoms of anthracnose were begun with a small round to oval, water-soaked dark green area about 1-2 mm in diameter. These area increase into circular spots with tan to light brown center bordered by water soaked tissue. As these spots expand, center of the lesion became reddish brown to brown color. The average diameter of the spots was 3-30 mm and the size of the necrotic areas

increases as spots coalesce. The acervuli on infected leaves produced black colored spore mass under high humid condition. In the advance stage of infection, spots appeared on both the surfaces of the leaves. The affected area lost the mucilaginous gel and leads the death of infected leaves.

2.2. Causal organisms of anthracnose disease of Aloevera

Shutrodhar and Shamsi (2013) recorded 8 fungal species viz. *Alternaria pluriseptata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium oxysporum*, *Colletotrichum gloeosporioides*, *Nigrospora oryzae*, *Penicillium* sp. and *Pestalotiopsis guepinii* associated with anthracnose and leaf spot disease of *Aloe vera* L. in Bangladesh. The frequency of *C. gloeosporioides* was the maximum. Pathogenicity test revealed that *C. gloeosporioides* causes anthracnose disease in Aloevera.

Kadam *et al.* (2012) observed leaf spot disease of Ghee Kumar (*Aloe vera*) is caused by *Colletotrichum sp.*

Avasthi *et al.* (2011) observed a typical anthracnose symptom on the leaf surface of *Aloe vera* during the survey of various nurseries of Gwalior city, India, in 2010. Based on the symptoms, mycelia and conidial characters, the fungus was identified as *Colletotrichum gloeosporioides* which was further confirmed at Indian Agricultural Research Institute, New Delhi, India.

Rajendran and Gnanavel (2011) recorded Alternaria leaf spot disease caused by *Alternaria alternata*; Cercospora leaf spot disease caused by *Cercospora* sp. and anthracnose disease caused by *Colletotrichum* sp. in *Aloe vera*. They also reported rust spots and basal stem rot disease.

2.3. Leaf spot disease of Aloevera

Parthasarathy *et al.* (2014) found that Aloe plant is infected by Alternaria leaf spot, Aloe rust, Sooty mold and Basal stem rot.

Anonymous, (2005) reported that commercial cultivation of Aloevera is hampered by leaf spot disease.

Anonymous, (2004) reported that leaf spot disease is the most devastating disease of *Aloe vera.* It is now wide spread problem of Aloevera growers in Bangladesh.

2.4. Causal organisms of leaf spot disease of Aloevera

Alam and Rehman (2017) observed leaf spot disease of Aloevera is caused by *Alternaria alternata* in Pakistan.

Chen and Zhong (2017) reported that leaf spot disease of Aloevera is caused by *Alternaria alternata* in China.

Avasthi *et al*. (2016) observed, leaves of *Aloe vera* were infected with leaf spot disease in rainy season of 2010 and 2011. Based on its morphological and cultural characteristics, the pathogen was identified as *Phomopsis* sp.

Lal *et al.* (2016) did pathogenicity test and found leaf spot disease of Aloevera (*Aloe vera*) is caused by *Fusarium solani* (Mart) sacc".

Avasthi *et al.* (2016) conducted a survey on leaf spot disease of *Aloe vera* in various nurseries and botanical gardens during 2010 and 2011 and identified the causal organism as *Cladosporium sphaerospermum*.

Avasthi *et al*. (2015) reported occurrence of leaf spot diseases on *Aloe vera* (L.) caused by *Curvularia* species from Madhya Pradesh, India. On the basis of morphological and microscopic characteristics of the fungus, two species of *Curvularia* i.e. *Curvularia lunata* and *Curvularia ovoidea*, were found to be associated with the leaf spot diseases. Leaf spot disease caused by *C. lunata* began in the form of circular, water soaked lesions on the tip and abaxial surface of the leaves. Gradually the size of the lesion expanded and became light red in color bordered by water soaked tissues. As the disease progressed, the lesions were sunken, maroon color with an average size of 0.5-1.4 × 0.4-1.0 cm. In later stages, lesions became dry, necrotic and turned into dark maroon in color. The disease was observed during both the rainy and winter seasons. In case of *C. ovoidea symptoms* appeared as elongated, water soaked lesions, generally occurred on the spiny margins and abaxial surface of the leaves. Progressively, these lesions became sunken and light brown in color. Later, these lesions were enlarged, center of the lesions dark brown in color with maroon red margins, and measured about 0.7-1.7 × 0.6-1.3 cm in size. In severe infection, the spiny margin of the leaves was twisted inside due to necrosis of the tissues. Interestingly, the disease was observed only in the winter season.

Rahman (2015) reported that leaf spot disease of Aloevera is caused by *Alternaria* sp. And *Curvularia* sp. in Bangladesh.

Khadka and Rawal (2014) found the association of *Fusarium oxysporum* species with *Aloe vera* leaf and basal rot disease in Western Terai of Nepal.

Firoz (2014) found that *Alternaria* sp. is responsible for causing leaf spot disease in Aloevera in Bangladesh

Abkhoo and Sabbagh (2014) observed leaf spot and leaf blight disease on *Aloe vera* plants as small, circular to oval dark brown necrotic sunken spots on leaves and the pathogen was identified as *Alternaria alternata* on the basis of morphological and cultural characteristics.

Ghosh and Banerjee (2014) first reported *Aloe vera* leaf spot disease in West Bengal, India. They reported the causal fungal pathogen of this disease was *Alternaria brassicae*. The symptoms appeared on the leaves in form of small dark brown necrotic spots on both sides which gradually enlarge to cover up an area of 2-8 cm in diameter. The infected area transforms from dark brown to black. Gradually the leaf surface is covered with numerous such lesions which become rotten and dried within 4-7 days with a central cup shaped depression having a depth of 5-8 mm. These disease symptoms indicate that it is leaf spot. The disease intensity was peak from April to July (83.28-95.71%) in four places of their survey. While the minimum percentage of disease intensity was recorded in December (25.71-50%) of four study areas. This work not only globally establishes Aloevera as a new host of *Alternaria brassicae* but also this disease of *Aloe vera* is first reported from the district of West Bengal.

Zhai *et al.* (2013) observed the first report of leaf spots in *Aloe vera* caused by *Nigrospora oryzae* in China.

Avasthi *et al.* (2013) found Leaf spot disease of *A. vera* in nurseries of Gwalior city in India after the post-rainy season and causal agent was identified as *Phoma betae* A.B. Frank.

Silva and Singh (2012) found *A. vera* plants infected with leaf spots in USA and the causal organism produced conidia with longitudinal and transverse septa, and

was morphologically identified as an *Alternaria alternata*.

Chavan and Korekar (2011) reported leaf spot disease of *Aloe vera* caused by *Alternaria alternata, A. tenuissima, Fusarium* spp. and for root disease caused by *Aspergillus verocosa, Fusarium oxysporum.* They found leaf spot disease in winter and rainy season but not in summer season and root disease of Aloevera was found in summer, winter and rainy season. Their survey was conducted in Osmanabad District, India during the years 2008 and 2009.

Avasthi *et al.* (2011) noticed leaf spot disease of *Aloe vera* plants growing in nurseries and botanical gardens of Gwalior, Madhya Pradesh, India and based on the morphological and cultural characteristics, fungus was identified as *Polyrostrata indica.*

Rajendran and Gnanavel (2011) reported black or sooty mold diseases of *Aloe vera* in India. Whitefly, mealy bugs or aphids, any insect that feeds on the sap of the plant leaves are carry this fungus. Control of these insects reduces that disease. Bijwa *et al*. (2010) first reported the leaf spot disease of *Aloe vera* caused by *Alternaria alternata* in Pakistan. On the basis of morphological characteristics, the fungus was identified as *Alternaria alternata* by the Fungal Culture Bank of Pakistan (FCBP).

Sharma *et al.* (2007) found Indian Aloe (*Aloe barbadensis* Mill.), an important medicinal plant suffers from leaf spot disease caused by *Alternaria alternata* (Fr.) Keissler.

Pritam and Kale (2007) reported that infection of *Aloe vera* by *Alternaria brassicae* not only reduces the yield and market value but it may reduce antioxidant property and other medicinal efficacy of the herb.

According to Mandal and Maiti (2005), a new leaf rot disease of *Aloe vera* (*Aloe barbadensis*) was observed for the first time in Gujarat, India, in 2000. The disease was serious when abundant moisture was available. Symptoms started as water-soaked lesions at the leaf base.The whole plant died after 2-3 days. As rotting progressed, the leaf epidermis bulged out due to gas formation and the leaf content was converted to a slimy mass which was eventually released.

2.5. Management of leaf spot disease of Aloevera

2.5.1. Management of leaf spot disease by chemical pesticides

Kumar *et al.* (2017) conducted an experiment in India during 2014 & 2015 and found, chemical pesticide Propiconazole 25 EC control the maximum percent disease followed by Mancozeb 75 WP and Carbendazim 50 WP. Among the botanicals maximum percent disease control were recorded in Neem leaf extract @ 5% followed by Garlic bulb extract @ 5% and Tulsi leaf extract @ 5% for leaf rot of Aloevera caused by *Fusarium oxysporum.*

Ghosh *et al.* (2016) reported that, fungal pathogens causing the leaf spot disease of *Aloe vera* have been isolated from infected leaves, collected from Birbhum and Burdwan districts of West Bengal, India. They found, commercially available fungicide Mancozeb was effective at low concentration (100 µg/ml) to control the pathogen whereas it can tolerate 1000 µg/ml or more concentrations of Bavistin. Two plant growth promoting rhizobacterial strains, viz. *Burkholderia cenocepacia* VBC7 and *Pseudomonas poae* VBK1 were able to produce prominent zones of inhibition against the pathogen in dual culture overlay plates.

Ashwani *et al*. (2011) stated that, the most important method of protecting the plants against the fungal attack is the use of fungicides. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently affect the humans through the food chain. The development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of disease.

Sharma *et al.* (2010) conducted field and laboratory studies in Punjab, India, during 2008 & 2009, to determine suitable control measures for the leaf spot disease of *Aloe vera*, caused by *Alternaria alternata*. In the field, 15-day-old *A. vera* seedlings were planted and 3 sprays of Indofil M-45 (0.3%), Tilt 25EC (0.1%), Score 25EC (0.1%), Amistar 25SC (0.1 %), neem oil (0.5%), tulsi oil (0.5%), ginger oil (0.5%), garlic oil (0.5%), *D. metel* extract (5.0%), turmeric rhizome extract (5.0%), neem extract (5.0%) and *M. arvensis* extract (5.0%) were given at 15-day intervals. Inoculation was done by spraying the *A. alternata* spore suspension to the pin-pricked leaves of the plants. The percent disease intensity was then calculated and the yield was recorded. Results showed that all the tested fungicides were significantly effective in reducing the disease intensity in both years. Among the plant oils, garlic oil was the most effective, followed by ginger and tulsi oil in reducing the disease intensity. The turmeric rhizome extract also showed promising results in controlling the disease in both years.

2.5.2. Management of leaf spot disease by botanicals or plant extracts

Sohag *et al.* (2017) conducted a survey to evaluate the efficacy of Garlic bulb extract, Bion, Bavistin DF (Carbendazim) and proud for controlling leaf spot disease of Taro (*Colocasia esculenta*). Botanicals showed enhanced result against leaf spot disease of Taro (*C. esculenta*).

Regmi *et al.* (2014) evaluated leaf extracts of six plants *viz*, *Jatropa curcas*, *Datura strumarium*, *Azadirachata indica*, *Moringa oleifera*, *Calotropis gigantean* and *Morus alba* @ 50% by food poisn techniques against the fungus *Alternaria alternata* causing leaf spot disease of Aloevera. Neem (*Azadirachata indica*) leaf extracts inhibited the mycelia growth of the fungus. Thus, Neem leaf extracts control the leaf spot disease of Aloevera.

Mausuduzzaman *et al.* (2008) studied Alamanda leaf extract and separated compounds to determine the efficiency against leaf spot disease pathogen viz. *Phomopsis vexans*, *Fusarium sp.* Alamanda leaf extracts inhibited the mycelia growth of the pathogen *Phomopsis vexans*, *Fusarium sp.* Responsible for leaf spot disease.

Kim *et al*. (2005) observed natural products seem to be a viable solution to the environmental problems caused by the synthetic pesticides and many researchers are trying to identify the effective natural products to replace the synthetic pesticides.

Mahadevan (1982) found that the presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance.

Singh and Dwivedi (1987) reported that such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases.

2.5.3. Management of leaf spot disease by bio-agents (*Trichoderma harzianum*)

Pareek *et al*. (2012) tested the efficacy of bio-control agent's viz. *Trichoderma harzianum, T. viride and Aspergillus niger* against leaf spot pathogen (*Alternaria alternata*). The most effective antagonist against leaf spot pathogen (*A. alternata*) was *T. harzianum.*

Galletti *et al*. (2008) reported that a way of reducing chemical inputs could be to use bio-control agents (*Trichoderma harzianum*) to replace or supplement fungicide treatments against cercospora leaf spot disease of sugar beet.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experiment 1: Identification of causal organisms of anthracnose disease of Aloevera

3.1.1. Experimental site

The laboratory experiments related to isolation and identification of disease causing organisms was carried out in Plant Disease Clinic, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. Moreover, Pathogenicity test was done in glass house of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.1.2. Experimental period

The experiment was carried out during the period from January 2017 to June 2017.

3.1.3. Test materials

The commercially cultivated Aloevera or Ghritakumari (Aloe or Indian aloe), *Aloe vera* L. belongs to the family Liliaceae was used for the experiment.

3.1.4. Sample collection

Diseased leaves exhibited different types of typical symptoms were collected from Kholabaria and Kathalbaria villages of Natore district in Bangladesh. The samples were carried to the Plant Pathology Laboratory of SAU in individual snap locked plastic bags. The collected samples were preserved in refrigerator at 4°C before investigation. In the laboratory the samples were examined for visible symptoms as well as for microscopic examination and isolation of causal organism(s).

3.1.5. Sample selection

Diseased leaves (anthracnose) with some healthy portion were selected as sample for isolation and identification of the causal organism.

3.1.6. Isolation and identification of causal organism of anthracnose disease of Aloevera

a) By visual symptom observation (Visual assessment)

The diseased leaves were carefully examined visually by magnifying glass to observe the disease symptom development, characteristics of symptoms, sign of the pathogen. Idea about causal organisms was taken from the previous literature (Pernezny *et al*. 2008; Mullen, 2007; Waller *et al*., 1998; Shutleff and Averre, 1997; Putnam, 1995; Hensen and Wick, 1993).

b) By direct microscopic observation (Microscopic study)

The diseased leaves of medicinal plants were collected and kept in polythene bags and tagged. The samples were then taken to the laboratory. The collected sample was observed under stereoscopic microscope. The slides were prepared from the diseased samples to observe under compound microscope. The causal pathogens were identified according to reference materials and CMI Description (Mathur and Kongsdal, 2003; Riley, 2002; Carlile *et al*., 2001; Ellis, 1971; Booth, 1971).

c) By incubation in blotting paper (Incubation method)

The diseased leaves were placed on the sterile moist blotting paper in the petridish. The plates containing leaves were incubated at 22±1 °C temperature with alteration of 12 hours cycle of light and darkness for seven days. When the fungus grew well and sporulated, the organisms were examined under stereoscopic

microscope. Then slides were prepared from pathogenic structures and observed under microscope and identified with the help of relevant literature and CMI Description (Mathur and Kongsdal, 2003; Riley, 2002; Carlile *et al*., 2001; Ellis, 1971).

d) Isolation by tissue plating method

The diseased leaves were cut into pieces (5 mm diameter) and surface sterilized with $HgCl_2$ (1: 1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and placed on to acidified PDA medium in Petri dish. The plates containing leaf pieces were incubated at room temperature for seven days. When the fungus grew well and sporulated, the organism was re-cultured by tip culture method to obtain pure culture. Then slides were prepared from pathogenic structures and observed under microscope and identified with the help of relevant literature and CMI description (Agrios, 2005; Mathur and Kongsdal, 2003; Barnett and Hunter, 1972; Ellis, 1971; Booth, 1971).

e) By pathogenicity test

Pathogenicity test was performed to confirm the pathogen as per prescribed procedure (Agrios, 2005). At first pathogen was isolated from diseased leaves and cultured in PDA medium for 5 days as pure culture. Hyphal mat from 5 days old potato broth cultures of the pathogens were scraped aseptically onto a fine cheese cloth, filtered and washed in several changes of sterile distilled water to remove traces of stalling materials. The mats were then transferred aseptically into 200ml of distilled water containing 5 ml glucose solution in warring blender and homogenized for one minute at low speed in order to get the inoculum ready for pathogenicity test on test plant (Awale, 2019). Plant leaves were inoculated with fungal spore suspension containing concentration of 10^5 spores/ml by pin prick

method. Three leaves of each plant were inoculated. Plants were covered with polythene bag after inoculation for 24 hours to maintain suitable moisture condition.

3.1.7. Inoculums preparation and inoculation

Inoculums preparation and inoculation was done following the protocol of Awale (2019).

a. 100 ml of distilled water with 10 drops of Tween 20 was taken in a beaker.
b. 10 ml of water solution was putted on fungal plate and the spores were scraped using spatula.
c. The spore mixture was placed in a small beaker and stir was done for 10 minutes.
d. The mixture was filtered using cheese cloth and funnel and the spores were quantified using Hemacytometer and compound microscope.
e. The spore suspension was adjusted to ideal concentration of 10^5 spores/ml.
f. Three healthy plants were grown in plot. The leaves of selected plants were cleaned by sterile water.
g. The selected leaves were injured by sterilized tooth pick.
h. Then prepared inoculum suspension was placed on injured area of leaves by micropipette.
i. The whole plant was sprayed by sterile water and covered by polythene bag to maintain relative humidity.
j. The plants were watered once a day while it was being waited to show the symptoms.
k. After seven days evaluation was done from the inoculation.

3.2. Experiment 2: Development of management practices for anthracnose disease of Aloevera

3.2.1. Experimental site

The field experiments were conducted at Kholabaria and Kathalbaria villages of Laxsmipur Union of Sadar Upazilla of Natore district in Bangladesh as this villages are very popular for commercial cultivation of Aloevera in Bangladesh.

3.2.2. Experimental period

The experiment was carried out during the period from July 2017 to August 2018 in rainy season as disease incidence and severity remains high at this time (Firoz, 2014 and Rahman, 2015).

3.2.3. Experimental set up

In 2017, the experiment was conducted in heavily infested farmer's field of Aloevera in Kholabaria in rainy season under natural epiphytic condition. To confirm the findings, the experiment was again re-tested in 2018 at Kathalbaria in another highly infested farmer's field in rainy season under natural epiphytic condition. Aloevera is commercially cultivated in Natore from last 25 years. Rainy season is very favorable for anthracnose disease of Aloevera. Thus, disease incidence and severity is comparatively high in rainy season. In addition, due to continuous monoculture, amount of disease is gradually increasing day by day in that region. Considering this point, the field experiment was conducted in Kathalbaria and Kholabaria villages in rainy season for management of anthracnose disease of Aloevera. The experiments were conducted in farmer's field under natural epiphytic condition. A naturally highly infested Aloevera field was selected for management of anthracnose disease.

3.2.4. Treatments

The management approaches was taken against anthracnose disease of Aloevera by considering preventive curative and eco-friendly components. Farmers are using lime with heavy doses to control this disease. Thus, lime was added at both the field experiment with botanicals, chemicals and bio-agents. In 2017, eleven treatments including five chemical fungicides viz. Folicur 25 EC, Tilt 250 EC, Autostin 50 WP, Dithane M 45, Companion; one hand made chemical fungicides viz. Bordeaus mixture; one traditional chemical practice viz. Lime; on bio-agent viz. *Trichoderma harzianum* and three botanicals viz. Garlic bulb extract, Neem leaf extract, Alamanda leaf extract were used to check their efficacy against anthracnose disease. However, nine treatments were used at Kathalbaria field experiment in 2018. Companion and Neem leaf extracts were excluded due their fewer efficacies against anthracnose disease of Aloevera. Further Alamanda leaf extract was included at the replacement of Neem leaf extract based on review of literature. The details of the used treatments are given in Table 1.

Table 1. Details of the treatments used for management of anthracnose disease of Aloevera

Sl. No.	Trade Name/ Name	Common Name / Active Ingredient	Rate of Application (%)
1.	Folicur 25 EC	Tebuconazole	0.1
2.	Tilt 250 EC	Propiconazole	0.1
3.	Autostin 50 WP	Carbendazim	0.2
4.	Dithane M 45	Mancozeb	0.2
5.	Companion	Mancozeb + Carbendazim	0.2
6.	Bordeaus mixture	$CuSO_4$+CaO	01
7.	Lime	CaO	0.5
8.	*Trichoderma harzianum*	Bio agent	0.2
9.	Garlic bulb extract 1:1 (w/v)	*Allium sativum*; Botanicals	01
10.	Neem leaf extract 1:1 (w/v)	*Azadirachta indica* Botanicals/ plant extract	01
11.	Allamanda leaf extract 1:1 (w/v)	*Allamanda cathartica* Botanicals/ plant extract	01

3.2.5. Preparation of fungicidal suspension /Solution

Suspensions were prepared at recommended doses by mixing thoroughly of requisite quantity of fungicide with normal clean water. For example, two gram Dithane M-45 was mixed in one liter water to prepare 0.2% doses. For liquid fungicides, 1ml Tilt 250EC was mixed with one liter of water to prepare 0.1% solution. In case of lime, 25 gram lime powder was mixed with five liter water.

3.2.6. Preparation of Bordeaux mixture

Copper Sulphate, lime and water were mixed in the ratio of 1:1:100 to prepare one per cent Bordeaux mixture. In order to prepare 100 liter of Bordeaux mixture, one kg Copper Sulphate was dissolved in 50 L water in a plastic bucket. In another plastic bucket, one kg fresh quick lime powder was mixed with 50 L water. Then the Copper Sulphate solution is poured into lime solution and stirring the mixture. The prepared Bordeaux mixture should be sieved through a cloth before spraying. Bordeaux mixture suspension was prepared at the day of spraying

3.2.7. Preparation of Plant extract/botanicals

The extracts were prepared by using the method of Ashrafuzzaman and Hossain, 1992. For preparation of extracts, collected leaves and bulbs were weighted in an electric balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:1 (w/v) ratio 100 ml of distilled water was added with 100 g plant parts. Botanical solutions were also prepared in the day of spraying.

3.2.8. Preparation of Bio-agent suspension

Trichoderma suspension was collected from Ispahani Biotech Ltd. for this experiment. Two ml *Trichoderma* solution was mixed with one liter water to prepare 0.2% solution. The bio-agent suspension was prepared just before spraying.

3.2.9. Spray schedule

Spray was done in standing plant of the field. In 2017, four sprays of the selected treatments were done at afternoon at 10 days interval in rainy season. First spray was made on 6 May and 2nd, 3rd and 4th sprays were made on 16 May, 26 May and 5 June of 2017, respectively. On the other hand, in 2018 three sprays with selected ten treatments were done at 10 days interval. First spray was made on 2 August and 2nd and 3rd sprays were made on 12th and 22 August of 2018, respectively.

3.2.10. Data collection

Disease incidence and severity were recorded for each experiment before spray. Ten plants of a plot were considered for measuring plant incidence. Five plants for each plot were selected randomly for measurement of disease incidence and severity. Data on the following parameter was recorded:

1. Disease Incidence (%)
    a. Diseased plant incidence
    b. Diseased leaf incidence
2. Disease severity (%)

3.2.11. Measurement of Plant Diseases:

Disease was measured in terms of intensity. Disease intensity can be expressed in Disease Incidence and Disease Severity (Horsfall and Barratt, 1945).

a) Incidence of disease

Disease incidence was calculated in the number of proportion of the plant units diseased in relation to the total number of units examined. Plant units mean the leaves, stems, fruits, etc. that show any symptoms. Disease incidence was calculated by following formula (Nutter *et al*., 2006; Agrios, 2005; Kranz, 1988; James, 1974; Large, 1966):

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

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

b) **Severity of disease**

Disease severity was calculated in the proportion of amount of plant tissues infected in relation to the total amount of tissue examined. Disease severity data was collected on the following parameters (Nutter *et al*., 2006; Agrios, 2005; Kranz, 1988; James, 1974):

$$\text{Disease severity (\%)} = \frac{\text{Area of tissues infected} \times 100}{\text{Area of tissues inspected}}$$

3.2.12. Design of the experiment

Field experiment was conducted following by Randomized Complete Block Design (RCBD) with three replications. Each plot size was 1.5 meter length with 1 meter width. Plot to plot distance was 0.5 meter and block to block distance was 1 meter.

3.2.13. Statistical Analysis

The collected data was analyzed statistically by Statistics 10 computer package program. Analysis of variance (ANOVA) was used to find out the variation of result from experimental treatments. Treatment means were compared by Least Significant Difference (LSD).

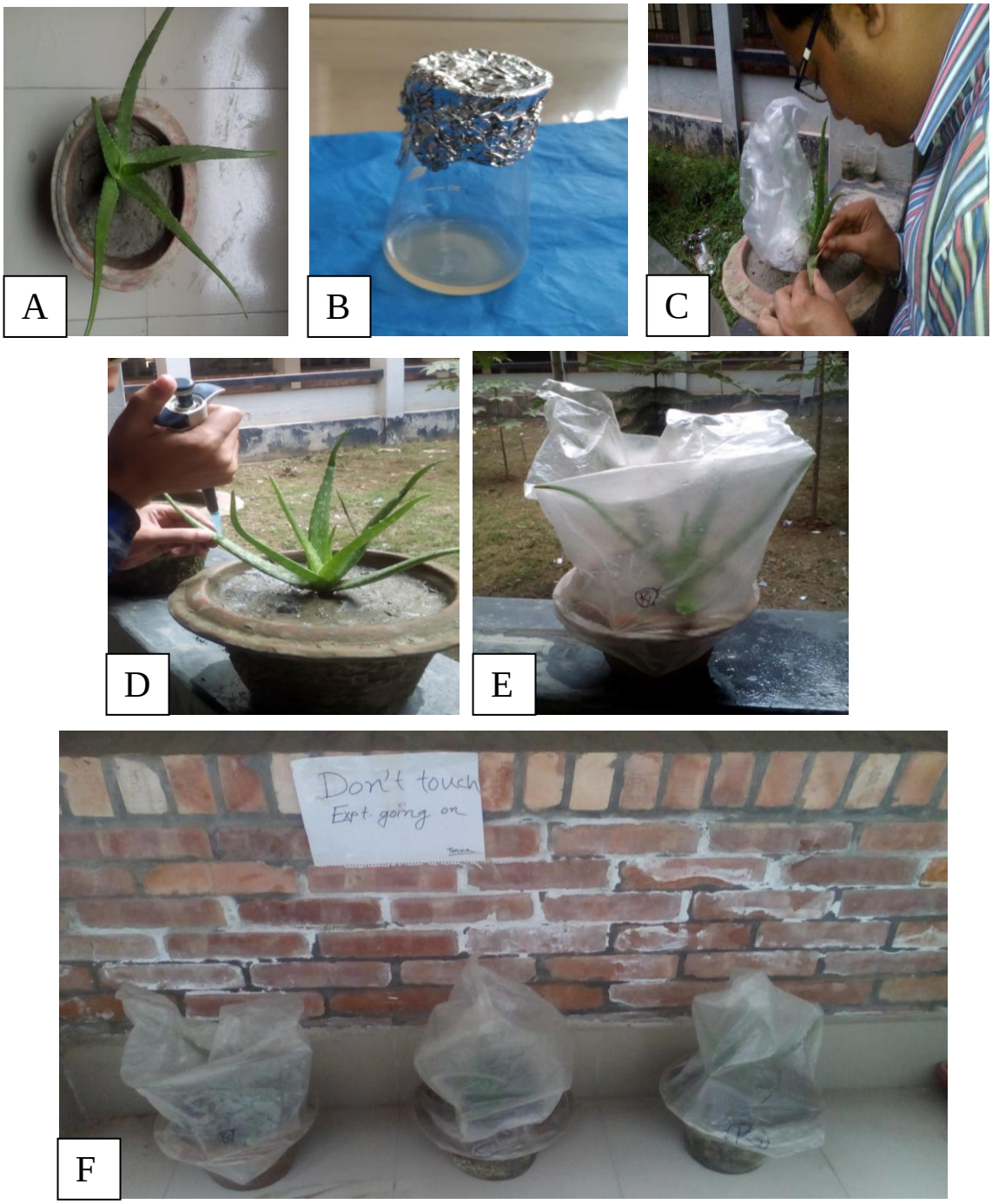

Plate 1: Pathogenicity Test (Inoculation and incubation of Aloevera plant); (A) Healthy plant; (B) Prepared spore suspension of *Colletotrichum gloeosporioides*; (C) Leaves injured by sterile tooth pick; (D) Inoculum suspension placed on injured leaves by micropipette; (E&F) Covering whole plant by polythene bag

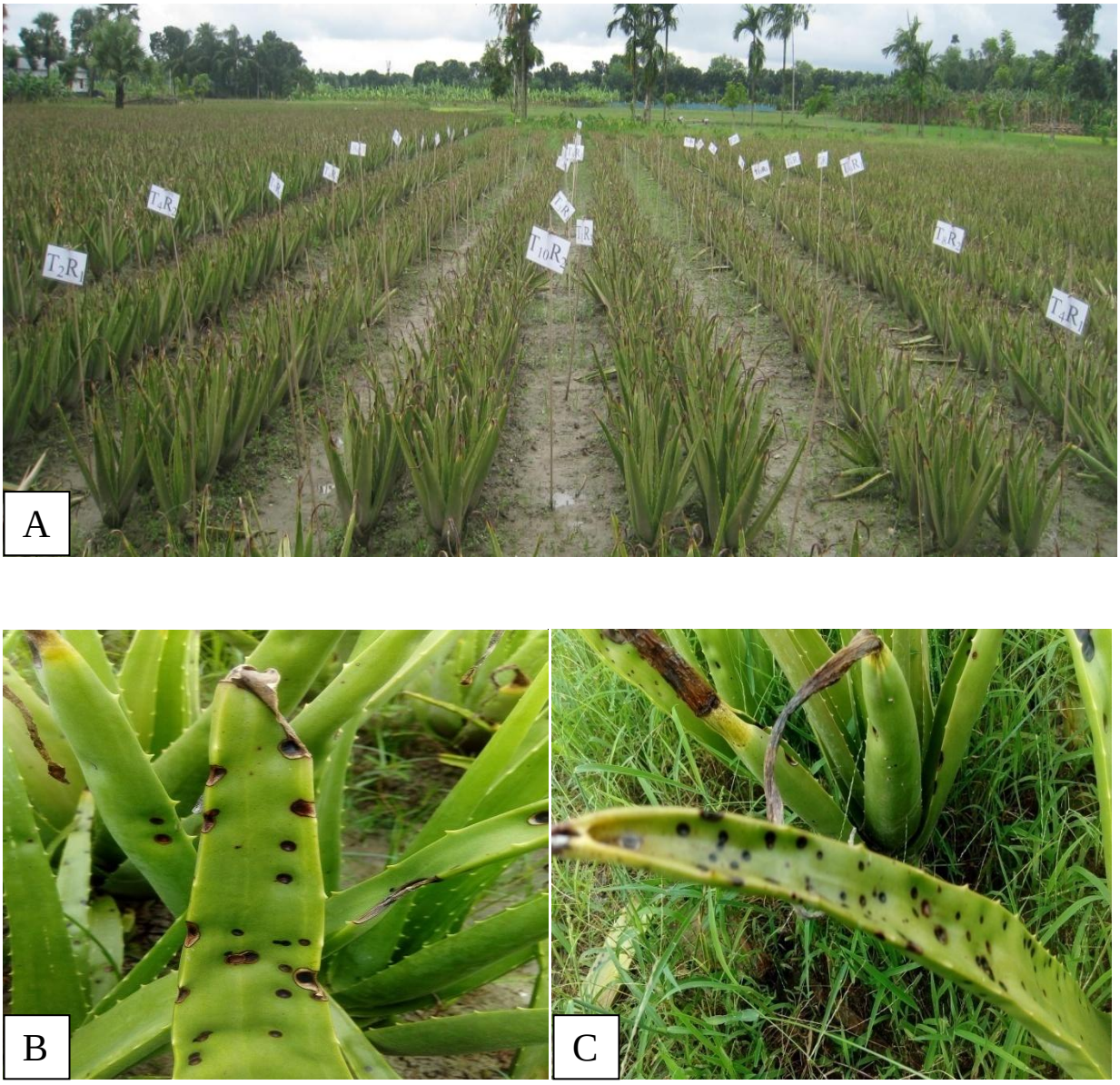

Plate 2. Field experiment conducted at Kholabaria in 2017; (A) Experimental site at Kholabaria village of Natore district in 2017; (B&C) Severe attack of anthracnose disease on aloevera

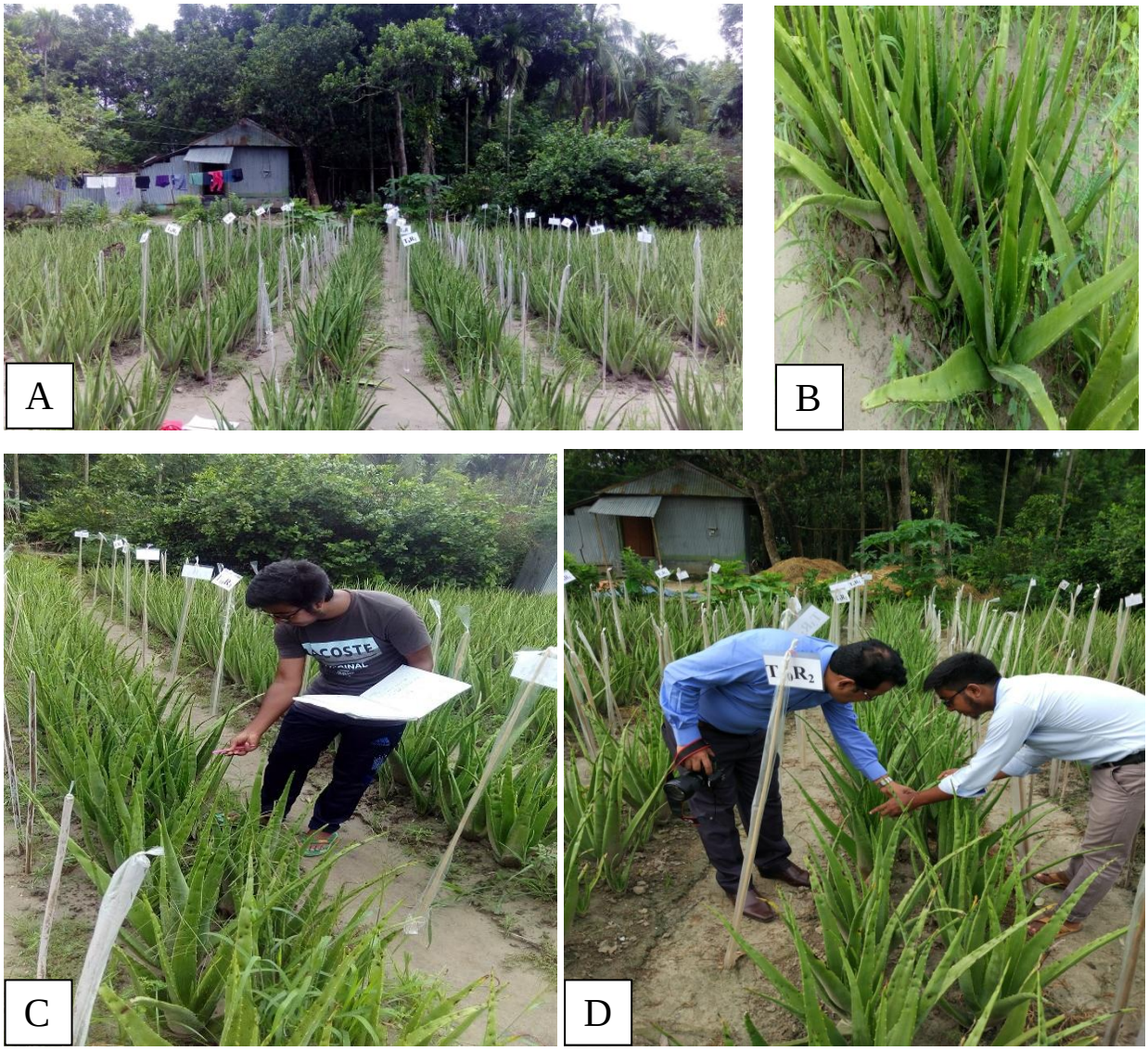

Plate 3. Field experiment conducted at Kathalbaria in 2018; (A) Experimental site at Kathalbaria village of Natore district in 2018; (B) Healthy leaves of Aloevera; (C&D) Measurement of diseases and Data collection

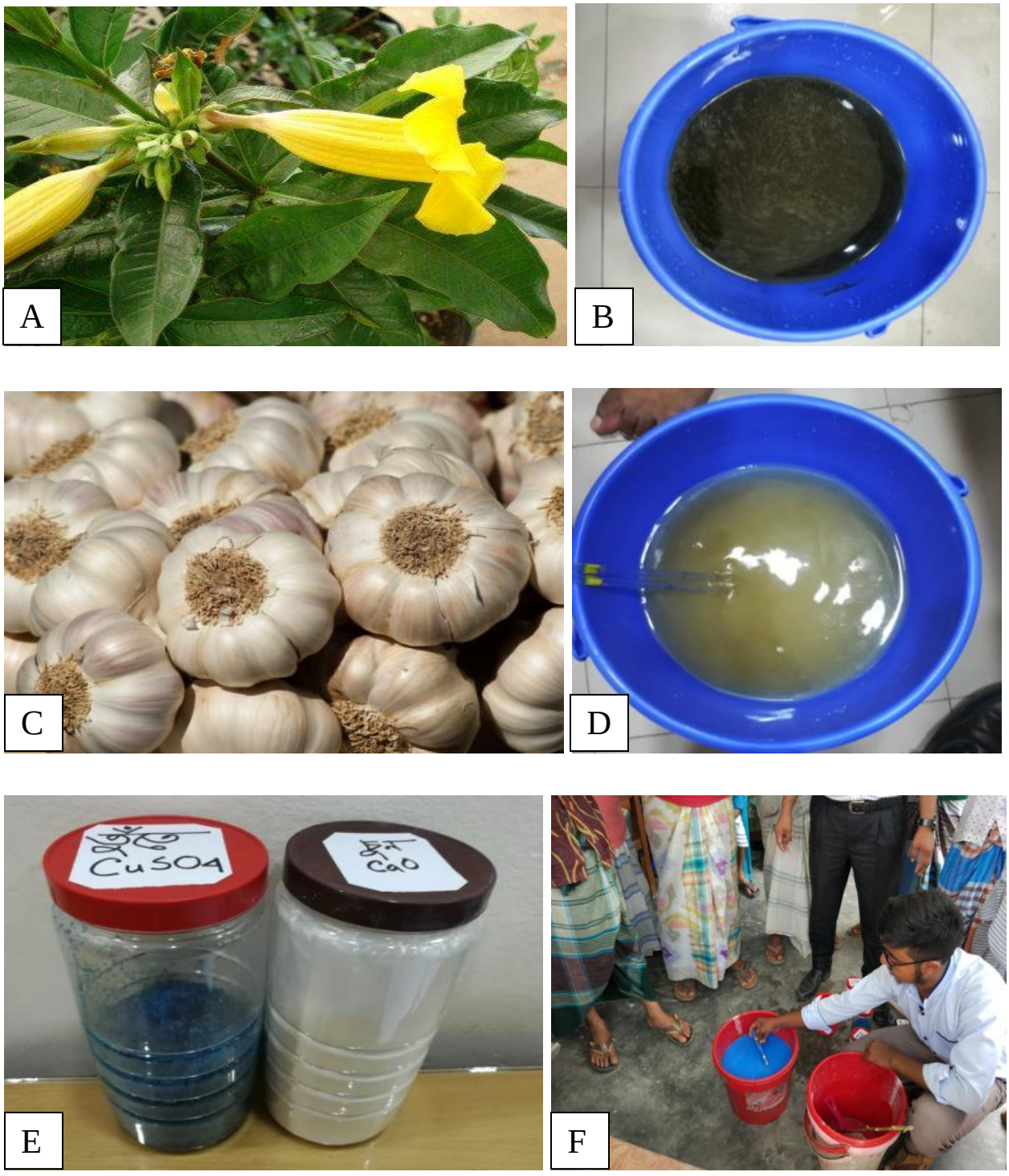

Plate 4. Preparation of treatments; (A&B) Alamnada leaf extract; (C&D) Garlic leaf extract; (E&F) Bordeaux mixture

CHAPTER 4

RESULTS

4.1 Experiment 1: Identification of causal organism of anthracnose disease of Aloevera

4.1.1 Anthracnose disease of Aloevera

A. Occurrence and symptoms

During the survey of various fields of Natore district, a typical anthracnose symptom on the leaf surface of *A. vera* was observed in August, 2017. Small round to oval, water-soaked dark green area of about 1-2 mm in diameter were found on leaves. The area increased into circular spots with tan to light brown center bordered by water soaked tissue. As these spots expanded, center of the lesion became reddish brown to brown color (Plate 5. A). Average diameter of the spots were 3-30 mm and the size of the necrotic areas increased by coalescing several spots (Plate 5. B&C). The acervuli on infected leaves produced orange-pink to brownish spore masses on older lesions under high humid condition (Plate 6. B). In the advance stage of infection, spots appeared on both the surfaces of leaf and affected area lost the mucilaginous gel and leads the death of infected leaves.

B. Isolation of the causal organism

Isolation of the pathogen was done from collected diseased plant samples from various fields. Leaves showing the typical symptoms were thoroughly washed in tap water and cut into small pieces. These pieces were surface sterilized with 1% silver chloride ($HgCl_2$) for 2 min and washed 3-4 times in sterile distilled water. The surface sterilized leaf pieces were then aseptically transferred to petriplates containing Potato Dextrose Agar media. Plates were incubated at 25±2ºC for 4 to

5 days and the isolate was purified by single spore isolation and maintained on PDA medium to keep the cultures viable. The fungal colony appeared on inoculated tissue were white to grey with puffy mycelium and dark orange color, surrounded by white zone on the reverse side (Plate 6. A). Acervuli were formed after15-20 days of inoculation.

C. Identification of causal organism

The identified causal organism was *Colletotrichum gloeosporioides*. The fungus produces spores within an acervulus (fungal fruiting structure). The disk or cushion shaped acervuli break through the surface of host tissue. Short, simple, colorless conidiophores produce abundant conidia. Long, black setae may be produced among conidiophores (Plate 6. B). Conidia are hyaline when viewed alone, but it may appear pink or salmon colored en mass. Spores are short, ovoid to cylindrical, and single celled. Based on the symptoms, mycelia and conidial characters, the fungus was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

D. Pathogenicity Test

Pathogenicity test for *C. gloeosporioides* was carried out by Pin Prick method on plant leaves. Small 4-5 month old plants of *Aloe vera* were planted in pots filled with fertilized soil and cultivated for 6-8 weeks in a glass house. Healthy leaves were selected and a suspension (10^5 conidia/ml) of 7-8 days old culture of *C. gloeosporioides* was sprayed onto pinpricked leaves. Pinpricked leaves sprayed with sterile distilled water were served as control. After 6-7 days, the symptoms were appeared in the inoculated leaves (Plate 7. B). The pathogen was appeared on the inoculated leaves and the fungus was consistently re-isolated from infected leaf onto Potato Dextrose Agar media. No symptoms were observed in control leaves.

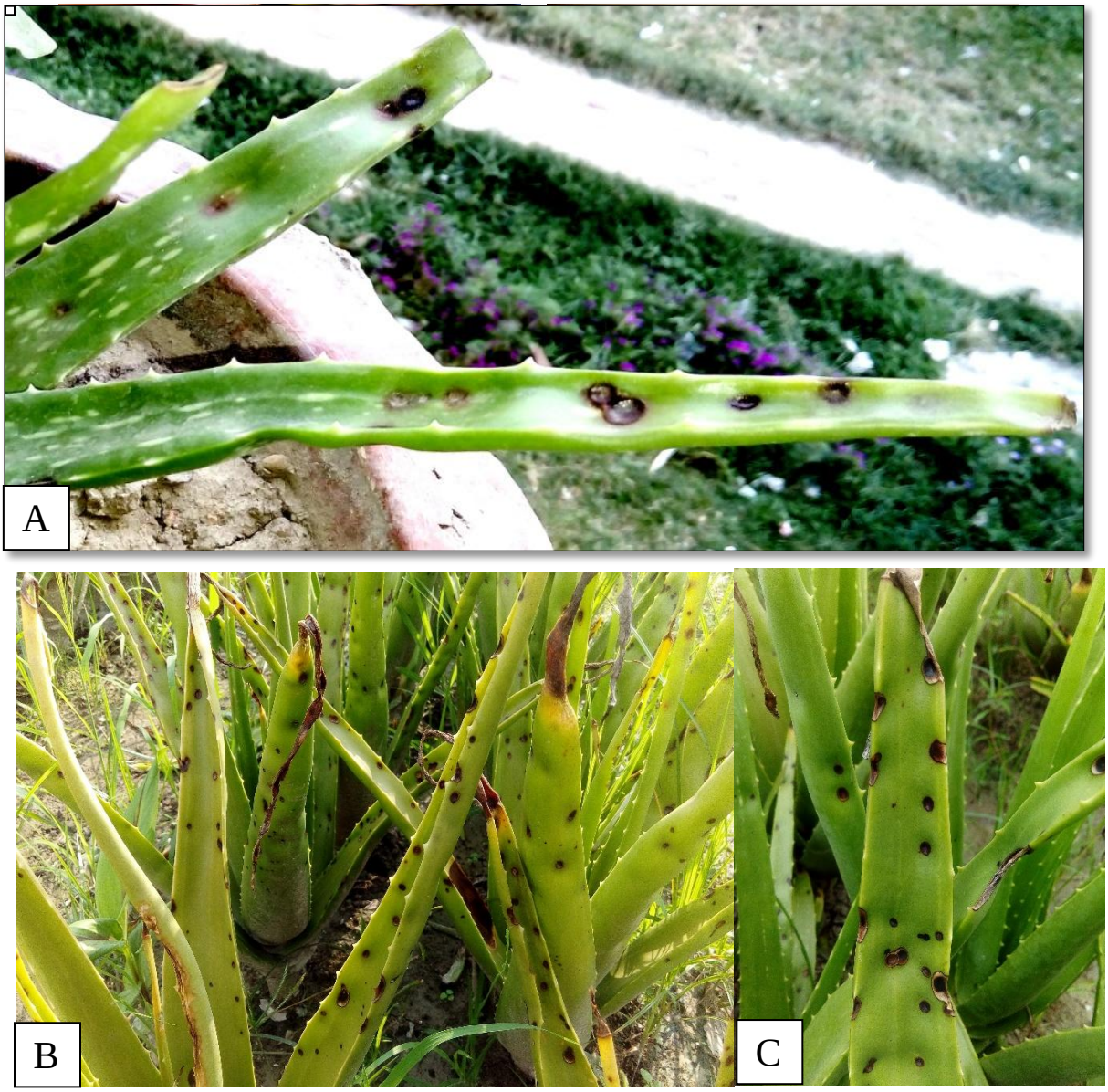

Plate 5. Symptoms of anthracnose disease of Aloevera; (A-C) Different symptoms of anthracnose disease on Aloevera leaf

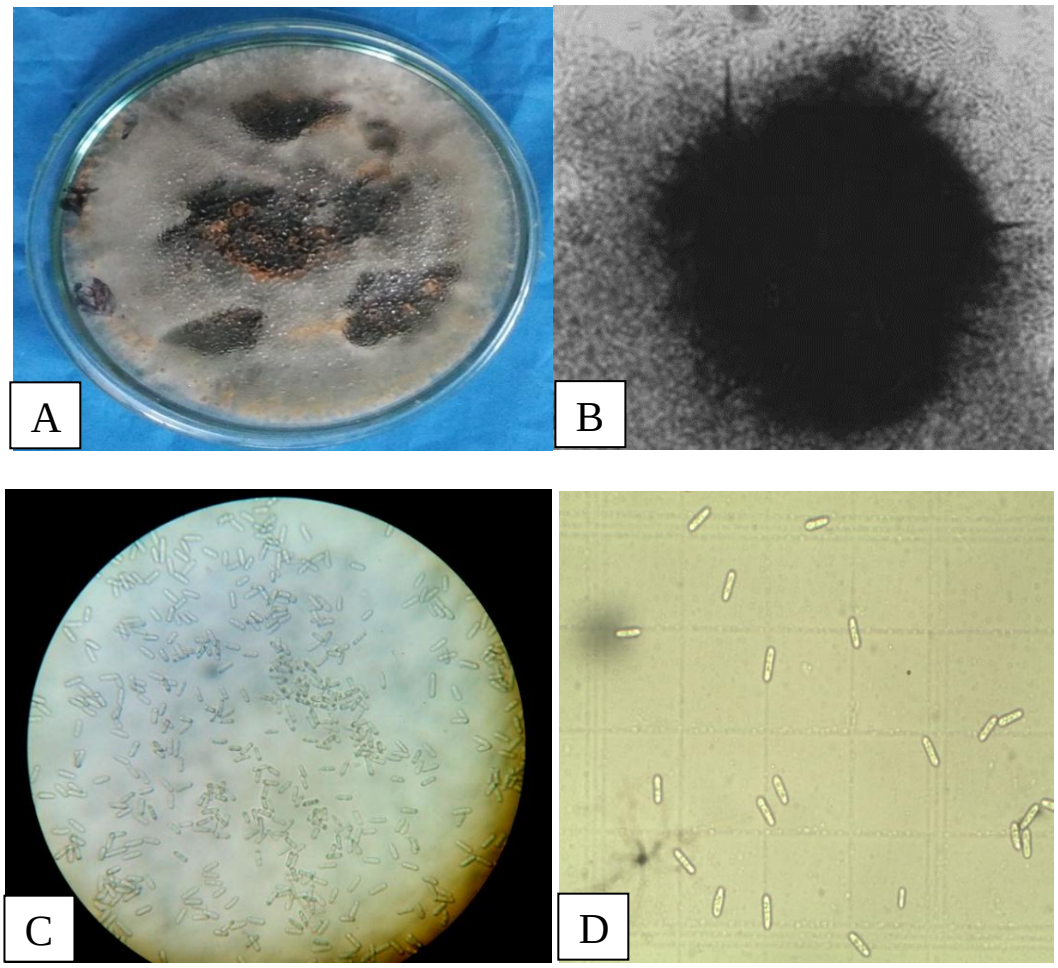

Plate 6. Causal organism of anthracnose disease of Aloevera; (A) Pure Culture of *Colletotrichum gloeosporioides* (B) Acervulus and conidia of *C. gloeosporioides* (C) Conidia of *C. gloeosporioides* (10X) (D) Conidia of *C. gloeosporioides* (40X)

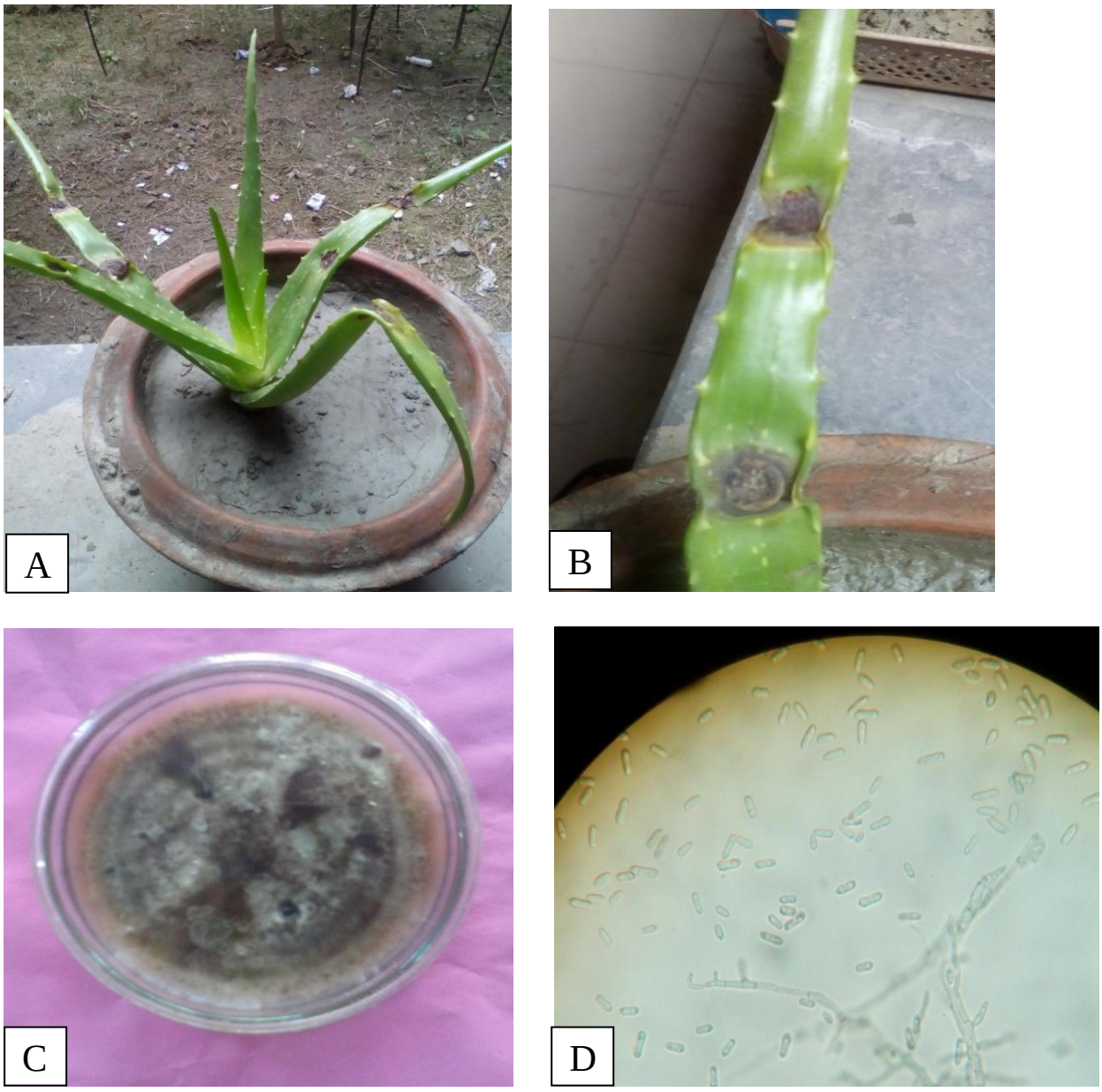

Plate 7. Pathogenicity test showing typical symptoms of anthracnose disease of Aloevera (A&B) Leaves of Aloevera plant showing anthracnose disease (C) Pure Culture of *Colletotrichum gloeosporioides* (D) Conidia of *C. gloeosporioides*

4.2. Experiment 2: Management of anthracnose disease of Aloevera by selected treatments

4.2.1. Efficacy of different treatments on anthracnose disease of Aloevera at Kholabaria in 2017

4.2.1.1. Incidence & severity of anthracnose disease of Aloevera before spray

The experiment was conducted in rainy season in a highly infested field of Kholabaria, Natore under natural epiphytic condition. Before spraying, plant incidence, leaf incidence and disease severity were measured under each treatment plot. The plant incidence, leaf incidence and disease severity were varied from 79.16 to 95.83%, 59.26 to 81.48% and 12 to 13%, respectively (Table. 2).

Table 2. Disease incidence and severity of anthracnose disease of Aloevera before spray at Kholabaria in 2017

Treatments	Disease Incidence (%)		Disease Severity (%)
	Diseased Plant Incidence (%)	Diseased Leaf Incidence (%)	
T_1 (Folicur 25 EC)	87.50 a	70.37 ab	12.67 a
T_2 (Tilt 250 EC)	87.50 a	59.26 b	12.33 a
T_3 (Autostin 50 WP)	91.67 a	66.67 ab	12.67 a
T_4 (Dithane M 45)	91.67a	77.78 ab	12.67 a
T_5 (Companion)	87.50 a	66.67 ab	13.00 a
T_6 (Bordeaux mixture)	79.16 a	70.37 ab	12.00 a
T_7 (Lime)	87.50 a	81.48 a	12.67 a
T_8 (*Trichoderma harzianum*)	91.66 a	81.48 a	12.33 a
T_9 (Garlic bulb extract)	87.50 a	70.37 ab	12.67 a
T_{10} (Neem leaf extract)	95.83 a	81.48 a	12.67 a
T_{11} (Control)	91.67 a	81.48 a	12.67 a
LSD (0.05)	17.605	19.269	3.1022
CV (%)	11.56	15.47	14.39

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.1.2. Incidence and severity of anthracnose disease of Aloevera after first spray

After first spry, the effect of eleven treatments against anthracnose disease of Aloevera are presented in Table (Table. 3). The efficacy of the treatments was assessed based on different parameters like leaf incidence and disease severity. In ten days after 1st spray, the effect of different treatments in terms of disease incidence and severity was not differed significantly in most cases comparison to control However, Bordeaux mixture and Tilt 250 EC showed better effect than the other treatments.

Table 3. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 1st spray at Kholabria in 2017

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Folicur 25 EC)	66.66 a-d	10.67 ab
T_2 (Tilt 250 EC)	50.61 d	10.67 ab
T_3 (Autostin 50 WP)	60.49 b-d	11.67 ab
T_4 (Dithane M 45)	70.37 a-c	11.67 ab
T_5 (Companion)	64.19 b-d	12.00 ab
T_6 (Bordeaux mixture)	58.02 cd	9.67 b
T_7 (Lime)	75.31 ab	11.33 ab
T_8 (*Trichoderma harzianum*)	82.71 a	12.33 ab
T_9 (Garlic bulb extract)	62.96 b-d	12.00 ab
T_{10} (Neem leaf extract)	76.54 ab	12.67 a
T_{11} (Control)	82.71 a	12.67 a
LSD (0.05)	16.557	2.7561
CV (%)	14.45	14.01

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

4.2.1.3. Incidence and severity of anthracnose disease of Aloevera after second spray

The effect of treatments differed significantly for leaf incidence and severity at 10 days after 2nd spray (Table 4). Leaf incidence was varied significantly to different treatments from 46.92% to 80.24%, where the lowest leaf incidence was recorded in Bordeaux mixture (46.92%) that was statistically identical with Tilt 250 EC (44.44%). Similarly, disease severity was ranged from 8 to 12.33% and the lowest severity was observed in Bordeaux mixture. The highest incidence and severity was recorded in control plot.

Table 4. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 2nd spray at Kholabria in 2017

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Folicur 25 EC)	56.78 cd	9.00 de
T_2 (Tilt 250 EC)	44.44 d	9.67 c-e
T_3 (Autostin 50 WP)	55.55 cd	11.33 a-c
T_4 (Dithane M 45)	64.19 bc	11.33 a-c
T_5 (Companion)	59.26 cd	11.00 a-d
T_6 (Bordeaux mixture)	46.92 d	8.00 e
T_7 (Lime)	70.37 a-c	10.00 b-e
T_8 (*Trichoderma harzianum*)	80.24 a	12.33 a
T_9 (Garlic bulb extract)	57.89 cd	10.33 a-d
T_{10} (Neem leaf extract)	75.31 ab	12.00 ab
T_{11} (Control)	80.24 a	12.33 a
LSD (0.05)	15.091	2.3062
CV (%)	14.40	12.80

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.1.4. Incidence and severity of anthracnose disease of Aloevera after third spray

Significant effects were observed among the treatments in controlling anthracnose disease of Aloevea at 10 days after 3rd spray. The treatments effects were differed significantly in terms of disease and severity (Table 5). The highest effect against the disease was observed in case spraying of Bordeaux mixture followed by Tilt 250 EC, Folicur 25 EC and Garlic bulb extract. The highest disease incidence and severity was recorded in Control treatment followed by neem leaf extract and *Trichoderma harzianum.* The lowest leaf incidence and disease severity was found in spraying of Bordeaux mixture that was 33.33% and 4.33%, respectively.

Table 5. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 3rd spray at Kholabria in 2017

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Folicur 25 EC)	46.91 cd	7.00 c
T_2 (Tilt 250 EC)	37.03 d	8.33 bc
T_3 (Autostin 50 WP)	73.15 ab	10.33 ab
T_4 (Dithane M 45)	69.52 ab	10.00 ab
T_5 (Companion)	54.31 b-d	10.17 ab
T_6 (Bordeaux mixture)	33.33 d	4.33 d
T_7 (Lime)	64.19 a-c	9.33 ab
T_8 (*Trichoderma harzianum*)	77.78 a	11.33 a
T_9 (Garlic bulb extract)	45.67 cd	8.33 bc
T_{10} (Neem leaf extract)	70.70 ab	11.33 a
T_{11} (Control)	80.24 a	12.33a
LSD (0.05)	22.183	2.1503
CV (%)	22.58	13.85

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.1.5. Incidence and severity of anthracnose disease of Aloevera after 4th spray

A remarkable effect was observed among the treatments in controlling anthracnose disease of Aloevera at 10 days after 4th spray. The treatments effects were differed significantly in terms of disease incidence and severity (Table 6). The highest effect against leaf incidence was also observed in case of Bordeaux mixture (27.15) which was statistically similar with Tilt 250 EC (34.56%). Similarly, the lowest disease severity was found in Bordeaux mixture (3.55) followed by Folicur (5.67%), Tilt (6.67%) and Garlic bulb extract (7.67%). In all cases, the highest disease incidence and severity were recorded in Control treatments which were statistically similar with neem leaf extract and *Trichoderma harzianum.*

Table 6. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 4th spray at Kholabaria in 2017

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Folicur 25 EC)	37.03 de	5.67 e
T_2 (Tilt 250 EC)	34.56 ef	6.67 de
T_3 (Autostin 50 WP)	45.678 cd	10.33 ab
T_4 (Dithane M 45)	51.85 bc	10.00 ab
T_5 (Companion)	48.14 c	9.50 a-c
T_6 (Bordeaux mixture)	27.15 f	3.50 f
T_7 (Lime)	58.02 b	9.00 bc
T_8 (*Trichoderma harzianum*)	72.83 a	11.33 a
T_9 (Garlic bulb extract)	38.27 de	7.67 cd
T_{10} (Neem leaf extract)	67.89 a	11.00 a
T_{11} (Control)	77.78 a	12.33a
LSD (0.05)	9.2372	1.8447
CV (%)	11.19	12.70

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.2. Efficacy of different treatments on anthracnose disease of Aloevera at Kathalbaria in 2018

4.2.2.1. Incidence and severity of leaf anthracnose of Aloevera before spray

Another field experiment was conducted at Kathalbaria in Nature district in 2018. The efficacy of the treatments was assessed based on different parameters viz. plant incidence, leaf incidence and disease severity. The experiment was conducted in rainy season in a naturally infested field. Before spraying, plant incidence, leaf incidence and disease severity were measured and found varied from 93.75 to 100.0%, 90.41 to 98.03% and 1.33 to 1.63%, respectively (Table 7).

Table 7. Disease incidence and severity of anthracnose disease of Aloevera before spray at Khathalbaria in 2018

Treatments	Disease Incidence (%)		Disease Severity (%)
	Diseased Plant Incidence (%)	Diseased Leaf Incidence (%)	
T_1 (Autostin 50 WP)	97.92 a	92.59 a	1.36 a
T_2 (Dithane M 45)	97.92 a	94.45 a	1.53 a
T_3 (Tilt 250 EC)	97.92 a	94.44 a	1.33 a
T_4 (Folicur 25 EC)	97.92 a	98.03 a	1.46 a
T_5 (Bordeaux mixture)	95.83 a	92.48 a	1.53 a
T_6 (Lime -CaO)	100.00 a	92.59 a	1.46 a
T_7 (*Trichoderma harzianum)*	95.83 a	96.29 a	1.43 a
T_8 (Garlic bulb extract)	95.83 a	90.41 a	1.56 a
T_9 (Allamanda leaf extract)	95.83 a	96.29 a	1.63 a
T_{10} (Control)	93.75 a	96.30 a	1.43 a
LSD (0.05)	7.6925	8.7598	0.3349
CV (%)	4.64	5.41	13.22

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.2.2. Incidence and severity of anthracnose disease of Aloevera after first spray

Ten days after 1st spray, the effect of different treatments in terms of disease incidence and severity was not differed significantly in most cases comparison to control (Table 8). However, Bordeaux mixture and Garlic bulb extract showed comparatively better effect than the other treatments.

Table 8. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 1st spray at Kathalbaria in 2018

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Autostin 50 WP)	85.18 a-c	1.26 a
T_2 (Dithane M 45)	88.89 ab	1.43 a
T_3 (Tilt 250 EC)	90.74 ab	1.26 a
T_4 (Folicur 25 EC)	92.26 a	1.36 a
T_5 (Bordeaux mixture)	81.15 bc	1.33 a
T_6 (Lime)	81.47 bc	1.26 a
T_7 (*Trichoderma harzianum*)	90.73 ab	1.36 a
T_8(Garlic bulb extract)	78.86 c	1.46 a
T_9 (Allamanda leaf extract)	84.96 a-c	1.43 a
T_{10} (Control)	94.33 a	1.43 a
LSD (0.05)	9.781	0.325
CV (%)	6.56	13.90

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.2.3. Incidence and severity of anthracnose disease of Aloevera after second spray

From the below data, it was examined that the effect of treatments differed significantly for disease incidence and severity at 10 days after 2nd spray (Table 9). Leaf incidence was varied significantly to different treatments ranged from 66.01 to 92.37%, where the lowest leaf incidence was recorded in Bordeaux mixture (66.01%) that was statistically similar with spraying of Garlic bulb extract (67.31%). The lowest disease severity was observed in spraying of Bordeaux mixture (1%) that was statistically similar with Lime (1.03%).

Table 9. Disease incidence and severity of anthracnose disease of Aloevera after 10 days of 2nd spray at Kathalbaria in 2018

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Autostin 50 WP)	81.48 b	1.20 ab
T_2 (Dithane M 45)	83.33 ab	1.33 ab
T_3 (Tilt 250 EC)	84.96 ab	1.16 ab
T_4 (Folicur 25 EC)	79.30 bc	1.16 ab
T_5 (Bordeaux mixture)	66.01 d	1.00 b
T_6 (Lime)	70.37 cd	1.03 b
T_7 (*Trichoderma harzianum*)	85.18 ab	1.30 ab
T_8(Garlic bulb extract)	67.31 d	1.26 ab
T_9 (Allamanda leaf extract)	77.77 bc	1.26 ab
T_{10} (Control)	92.37 a	1.40 a
LSD (0.05)	9.096	0.345
CV (%)	6.73	16.61

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

4.2.2.4. Incidence and severity of anthracnose disease of Aloevera after third spray

Significant effect was observed among the treatments in controlling anthracnose disease of Aloevera at 20 days after 3rd spray but the treatments effects were differed quite significantly in terms of disease incidence and severity (Table 10.) The highest effect against leaf incidence was also observed in case of Bordeaux mixture (18.51%) which is statistically similar with Lime (18.75%) and Garlic bulb extract (24.61%) too. Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). The highest disease incidence and severity were recorded in Control plot which is statistically similar with Autostin 50 WP and Dithane M 45.

Table 10. Disease incidence and severity of anthracnose disease of Aloevera after 20 days of 3rd spray at Kathalbaria in 2018

Treatments	Disease Incidence (%)	Disease Severity (%)
	Diseased Leaf Incidence (%)	
T_1 (Autostin 50 WP)	34.18 ab	0.50 ab
T_2 (Dithane M 45)	29.48 bc	0.53 ab
T_3 (Tilt 250 EC)	30.28 bc	0.43 bc
T_4 (Folicur 25 EC)	22.65 c-e	0.37 cd
T_5 (Bordeaux mixture)	18.51 e	0.20 de
T_6 (Lime)	18.75 de	0.25 e
T_7 (*Trichoderma harzianum*)	28.21 bc	0.50 ab
T_8(Garlic bulb extract)	24.61 c-e	0.36 cd
T_9 (Allamanda leaf extract)	26.47 b-d	0.38 cd
T_{10} (Control)	41.39 a	0.56 a
LSD (0.05)	7.831	0.130
CV (%)	16.63	19.22

Means followed by the same letters in a column do not differ at 5% level of significance by LSD.

CHAPTER 5

DISCUSSION

Aloe vera is the most familiar and highly usable medicinal plants in Bangladesh. It is a perennial, succulent, monocotyledonous plant of the family Liliaceae. Among the cultivated medicinal plants in Bangladesh, Gritokumari/Aloevera (*Aloe vera* L.) got the highest position in terms of production and cultivable areas. Aloevera is now commercially cultivated in Laxsmipur village of Natore district. Due to the traditional knowledge on medicinal plants and unplanned cultivation of such plant posed a threat to the existence of these plants.

Cultivation of Aloevera is disturbed by some pest and disease. Some diseases viz. leaf rot, leaf spot, anthracnose disease, leaf rust, crown rot, root rot, tip die back and slime mold usually found on *Aloe vera* of Aloevera plant reduces the production of yield (Anonymous, 2005). Among them, anthracnose disease is the most devastating disease of *Aloe vera* It is now wide spread problem of Aloevera growers in Bangladesh (Anonymous, 2004). The most common symptoms of anthracnose are the circular or oval shape having deep sunken lesion.

Colletotrichum *gloeosporioides* were successfully isolated by tissue planting method from infected leaf of anthracnose disease of Aloevera collected from different fields of Natore district. However, pathogenicity test was successful for *Colletotrichum gloeosporioides*. The previous literatures indicate that several pathogens are associated with this complex disease.

Similar result was also found by some researcher's viz. Shutrodhar and Shamsi (2013), Avasthi *et al*. (2011) and Alam *et al*. (2007). Shutrodhar and Shamsi (2013) recorded 8 fungal species viz. *Alternaria pluriseptata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium oxysporum*, *Colletotrichum gloeosporioides*, *Nigrospora oryzae*, *Penicillium* sp. and *Pestalotiopsis guepinii* associated with anthracnose and leaf spot disease of Aloevera in Bangladesh. The frequency of *C. gloeosporioides* was the maximum. Pathogenicity test revealed that *C. gloeosporioides* causes anthracnose disease in Aloevera. Avasthi, *et al.* (2011) reported anthracnose symptom on the leaf surface of *A. vera* in India and identified the causal organism as *Colletotrichum gloeosporioides*. Some instances of anthracnose disease of *Aloe vera* caused by *Colletotrichum* sp. was also reported from Lucknow (Alam *et al*., 2007).

In 2017, the experiment was conducted in heavily infested farmer's field of Aloevera in Kholabaria in rainy season under natural epiphytic condition. To confirm the findings, the experiment was again re-tested in 2018 at Kathalbaria in another highly infested farmer's field in rainy season under natural epiphytic condition. Aloevera is commercially cultivated in Natore from last 25 years. Kathalbaria and Kholabaria villages of Laxsmipur Union of Sadar Upazila of Natore district are very popular for commercial cultivation of Aloevera in Bangladesh. Moreover, rainy season is very favorable for anthracnose disease of Aloevera. Thus, disease incidence and severity is comparatively high in rainy season (Firoz, 2014; Rahman, 2015). In addition, due to continuous monoculture, amount of disease is gradually increasing day by day in that region. Considering this point, the field experiment was conducted in Kathalbaria and Kholabaria villages in rainy season for management of anthracnose disease of Aloevera. The experiments were conducted in farmer's field under natural epiphytic condition.

Eleven treatments including five commercial fungicides, one homemade fungicide (Bordeaux mixture), one traditional practice (Lime), one bio agent (*Trichoderma harzianum*) and two botanicals were tested against the disease in field condition in 2017. However, nine treatments including four commercial fungicides, one homemade fungicide (Bordeaux mixture), one traditional practice (Lime), one bio agent (*Trichoderma harzianum*) and two botanicals (Garlic bulb extract and Alamanda leaf extract) were tested against the anthracnose disease of Aloevera in field condition in 2018.

At Kholabaria, four spay was done with selected treatments in an interval of 10 days. After first spray no significant difference was found among the treatments. However, Bordeaux mixture (disease severity - 9.67%) showed better effect than the other treatments. After second spray, statistically no significant difference was found for disease incidence and severity. Bordeaux mixture (disease severity 8.00%) gave better performance similar as previous result. After third spray, significant difference was observed in terms of disease incidence and disease severity. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC and Garlic bulb extract with disease severity 4.33%, 7.00%, and 8.33% respectively. After the fourth and final spray at Kholabaria field of Aloevera, a remarkable effect of the treatments was observed against the disease. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC, Tilt 250 EC and Garlic bulb extract with disease severity 3.50%, 5.67%, 6.67% and 7.67% respectively. The highest effect against leaf incidence was also observed in case of Bordeaux mixture (27.15%) which is statistically similar with Tilt 250 EC (34.56%).

On the other hand, at Kathalbaria three sprays of the selected treatments with an interval of 10 days were done in the field of Aloevera. After first spay no

significant difference was found among the treatments. Moreover, statistically no significant difference was also observed after second spray. But Bordeaux mixture and Garlic bulb extract showed better result. After third and final spray at Kathalbaria field experiment, the highest effect against leaf incidence was observed in case of Bordeaux mixture (18.51%) which is statistically similar with Lime (18.75%) and Garlic bulb extract (24.61%) too. Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). The highest disease incidence and severity were recorded in Control plot which is statistically similar with Autostin 50 WP and Dithane M 45. Lime also has moderate effect against anthracnose disease of Aloevera.

Similar result was also found by Sohag *et al.* (2017) and Regmi *et al.* (2014). Sohag *et al.* (2017) conducted a survey to evaluate the efficacy of Garlic bulb extract, Bion, Bavistin DF (Carbendazim) and proud for controlling leaf spot disease of Taro (*Colocasia esculenta*). Garlic bulb extract showed enhanced result against leaf spot disease of Taro (*C. esculenta*). Regmi *et al.* (2014) evaluated leaf extracts of six plants *viz, Jatropa curcas, Datura strumarium, Azadirachata indica, Moringa oleifera, Calotropis gigantean* and *Morus alba* @ 50% by food poison techniques against the fungus *Alternaria alternata* causing leaf spot disease of Aloevera. Botanicals inhibited the mycelia growth of the fungus.

CHAPTER 6

SUMMARY AND CONCLUSION

Medicinal plants naturally synthesized and accumulate some secondary metabolites, like alkaloids, sterols, volatile oils etc. Aloevera is a perennial, succulent, monocotyledonous plant belongs to a large group of plants known as Xeroids as it has the ability to close its stomata to avoid loss of water and help in retaining a large amount of water in its tissue. The margin of the leaves has serrated soft and small teeth. Aloevera is susceptible to numerous fungal diseases. Anthracnose is the major one which not only affects the leaf texture but also deteriorate the quality and quantity of mucilaginous gel used for medicinal and commercial uses. This research work was designed to identify the causal organism of anthracnose disease of Aloevera and to find out field management practices for anthracnose disease of Aloevera in Bangladesh.

Experiments were conducted at Sher-e-Bangla Agricultural University, Dhaka and Kathalbaria and Kholabaria villages of Natore district of Bangladesh to identify the causal agents of anthracnose disease of Aloevera (*Aloe vera* L.) and for its management during 2016-17 and 2017-2018. The laboratory experiments related to isolation and identification of disease causing organisms was carried out in the central laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The field experiments were conducted in commercially growing areas of Kathalbaria and Kholabaria villages of Natore district.

Diseased leaves of Aloevera with typical symptoms were collected from cultivated fields to isolate pathogens. Identification of causal organisms was done by direct observation, microscopic study, growing on blotter paper, growing on culture

medium and pathogenicity test. Disease intensity was measured in terms of disease incidence and severity. Disease incidence and disease severity of anthracnose disease of Aloevera were measured in different fields of Kathalbaria and Kholabaria villages of Natore district from April 2016 to June 2017 and July 2017 to August 2018 where Aloevera is commercially cultivated.

The field experiment for management of anthracnose disease of aloevera was conducted in Kholabaria village of Natore district by following RCBD design with three replications. Eleven treatments including chemicals, botanicals, bio agents and traditional practices were considered for the management of anthracnose disease. The treatments were Folicur 25 EC, Tilt 250 EC, Autostin 50 WP, Dithane M 45, Companion, Bordeaux mixture, Lime, *Trichoderma harzianum*, Garlic bulb extract 1:1 (w/v), Neem leaf extract 1:1 (w/v) and Alamanda leaf extract 1:1 (w/v). The experiment was conducted in rainy season, as the disease incidence and severity is high in that season. The experiments were conducted in farmer's field under natural epiphytic condition. A natural highly infested Aloevera field was selected for management of anthracnose disease. Three plants for each plot were selected randomly for measurement of leaf incidence and severity. However, five plants of a plot were considered for measuring plant incidence. Four sprays of the selected treatments were done at 10 days interval from 6 May to 5 June in 2017 in Kholabaria.

In 2018 three sprays of the selected treatments were done at 10 days interval from 2nd August to 22 August in 2018. The collected data was statistically analyzed by Statistics 10 computer package program. Analysis of variance (ANOVA) was used to find out the variation of result from experimental treatments. Treatment means were compared by Least Significant Difference test (LSD).

Colletotrichum gloeosporioides were successfully isolated by tissue planting method from infected leaf of anthracnose disease of Aloevera collected from different fields of Natore district. Moreover, pathogenicity test was successful for *Colletotrichum gloeosporioides*. The previous literatures indicate that several pathogens are associated with this complex disease.

For disease management, four spay was done with selected treatments in Kholabaria in 2017 at an interval of 10 days. After first spray no significant difference was found among the treatments. However, Bordeaux mixture (disease severity - 9.67%) showed better effect than the other treatments. After second spray, statistically no significant difference was found for disease incidence and severity. Bordeaux mixture (disease severity 8.00%) was gave better performance similar as previous result. After third spray, significant difference was observed in terms of disease incidence and disease severity. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC and Garlic bulb extract with disease severity 4.33%, 7.00%, and 8.33%, respectively. After the fourth and final spray at Kholabaria field of Aloevera, a remarkable effect of the treatments was observed against the disease. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC, Tilt 250 EC and Garlic bulb extract with disease severity 3.50%, 5.67%, 6.67% and 7.67% respectively. The highest effect against leaf incidence was also observed in case of Bordeaux mixture (27.15%) which is statistically similar with Tilt 250 EC (34.56%).

In 2018, at Kathalbaria three spray of the selected treatments with an interval of 10 days were done in the field of Aloevera. After first spay no significant difference was found among the treatments. After second spray no significant

difference was observed statistically. However, Bordeaux mixture and Garlic bulb extract showed better result. After third and final spray at Kathalbaria field experiment, the highest effect against leaf incidence was observed in case of Bordeaux mixture (18.51%) which is statistically similar with Lime (18.75%) and Garlic bulb extract (24.61%) too. Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). The highest disease incidence and severity were recorded in Control plot which is statistically similar with Autostin 50 WP and Dithane M 45. Lime also has moderate effect against anthracnose disease of Aloevera.

So, finally it could be said that among the treatments, Bordeaux mixture gave best result against this disease. Moreover, Tilt 250 EC and Folicur 25 EC and Garlic bulb extract showed better effect against the disease than the other treatments. Lime also has moderate effect against anthracnose disease of Aloevera. Considering the overall performance of the treatments, garlic bulb extract could be used as eco-friendly approach. However, cost benefits analysis of garlic bulb extract required before advice to the farmers. Use of Bordeaux mixture is better than the traditional use of lime. From the chemical fungicides, Tilt 250 EC and Folicur 25 EC could be used for controlling the disease as the last option.

Medicinal plants are the blessing for the mankind. Every creature of the world is directly or indirectly depends on medicinal plants. Aloevera is one of the most important medicinal plant in aspect Bangladesh. It is not only used for its medicinal value but also used as an aesthetic and toiletries purpose. This research is a primary research about anthracnose disease of Aloevera. These types of findings may pave the way of higher research in future.

CHAPTER 7

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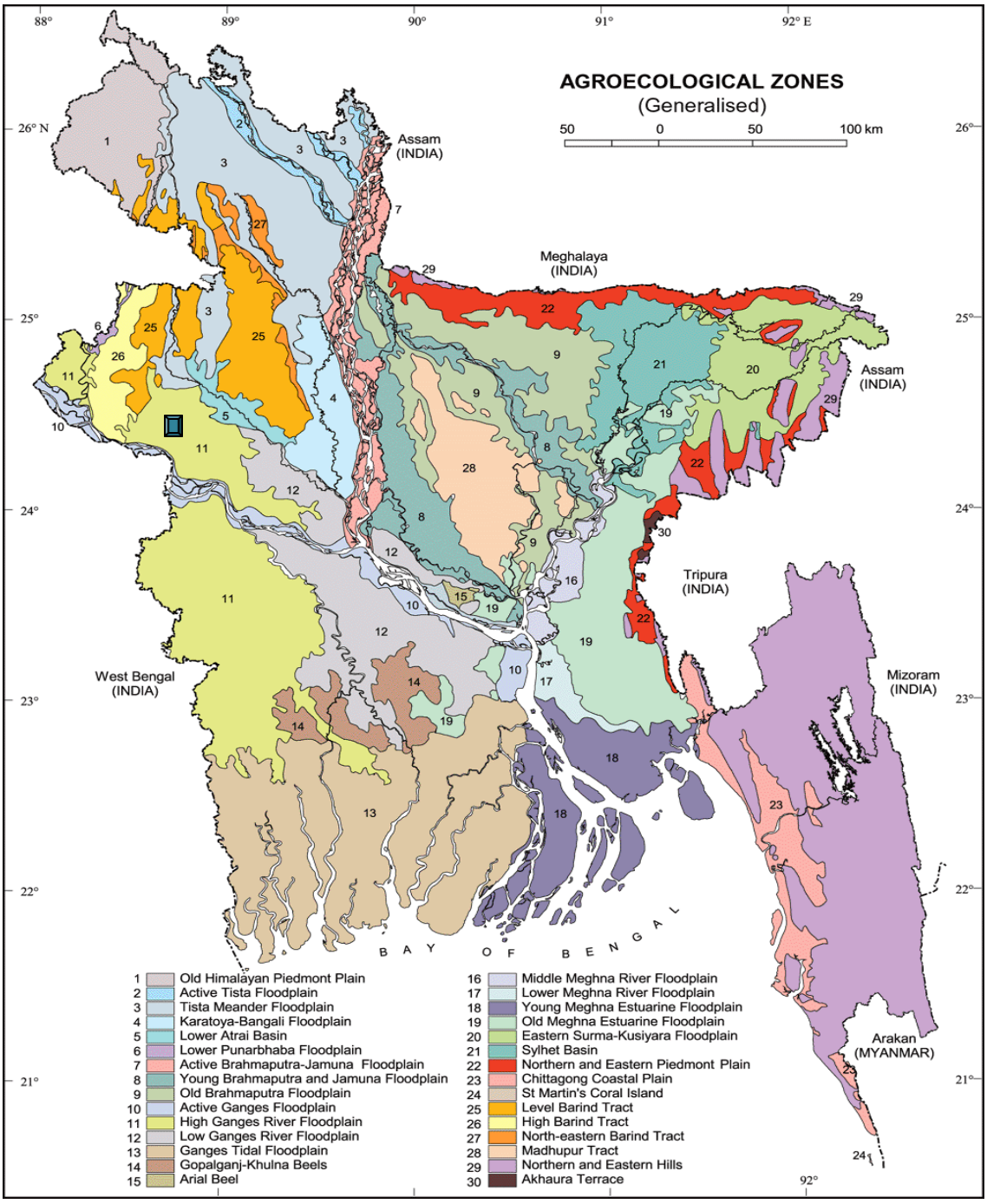
CHAPTER 8

APPENDICES

Appendix I. Map showing the experimental site under the study

The field experimental site (Natore) under study



Appendix II. Composition of PDA media

Material	**Volume**
Distilled water	1000 ml
Potato	200 g
Dextrose	20 g
Agar	20g