OCCURRENCE OF DIFFERENT DISEASES ASSOCIATED WITH GERBERA (*GERBERA JAMESONII*) IN DHAKA DIVISION

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CERTIFICATE

This is to certify that the thesis entitled, "***Occurrence of Different Diseases Accociated with Gerebra (Gerbera jamesonii) in Dhaka Division***" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by **MAHBUBA KHANAM Registration No. 18-09128** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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DEDICATED TO

MY BELOVED PARENTS

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LIST OF SYMBOL AND ABBREVIATIONS

ABBREVIATIONS	FULL WORD
%	Percentage
PDA	Potato Dextrose Agar
g	Gram
ml	Milliliter
L	Liter
^{o}C	Degree celcius
^{o}F	Degree Fahrenheit
psi	Per square inch
cm	centimeter
µm	Micron
et al.	and others (at el)
CRD	Complete Randomized Design
DAI	Days After Inoculation
ISTA	International Seed Testing Association
sp.	Species
m^2	Square meter
etc.	Et cetera
PDI	Percent Disease Incidence
viz	Videlicet (namely)

OCCURRENCE OF DIFFERENT DISEASES ASSOCIATED WITH GERBERA (*GERBERA JAMESONII*) IN DHAKA DIVISION

ABSTRACT

The study was conducted to survey and identification of pathogenic diseases of gerbera plant in and around Dhaka and isolation of associated causal organism and confimation their pathogenicity during the period of April to October 2019, in the central laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. For survey total nine sites were visited of Agargaon, Savar, Gazipur and Narayanganj. Based on symptomological study fungal diseases such as Alternaria leaf spot (*Alternaria alternata*), Cercospora leaf spot (*Cercospora* sp.), Anthracnose (*Colletotrichum capsici*.), Blight (*Curvularia geniculata*), Sclerotinia wilt (*Sclerotinia sclerotiorum*), Gray mold (*Botryti*s sp.), Powdery mildew (*Erysiphe* sp.), Stem rot (*Fusarium oxysporum*.) and the viral diseases such as Mosaic (*Mosaic virus*), Leaf curl (*Leaf curl virus*) were identified. Among all identified diseases, Alternaria leaf spot showed the highest disease incidence (66.67%, Gazipur) and Gray mold showed lowest disease incidence (5.56%, Narayanganj). The reduction rate of plant height due to Alternaria leaf spot, Cercospora leaf spot, Anthracnose, Blight, Sclerotinia wilt, Gray mold, Powdery mildew, Stem rot, Mosaic and Leaf curl disease were 28.42%, 28.1%, 12.69%, 12.47%, 30.67%, 28.36%, 28.08%, 19.14%, 12.89% and 22.06% respectively. Reduction rate of other plant part due to Alternaria leaf spot (leaf length 42.62%, leaf width 26.79%), Cercospora leaf spot (leaf length 15.27%, leaf width 15.69%), Anthracnose (stem length 10.94%), Blight (leaf length 22%, leaf width 33.33%), Sclerotinia wilt (stem length 34.22%), Gray mold (flower size 42.86%), Powdery mildew (leaf length 19.17%, leaf width 18.75%), Stem rot (leaf length 17.42%, leaf width 17.65%, flower stalk length 44.78%), Mosaic (leaf length 24.43%, leaf width 34.78%) and leaf curl (leaf length 63.08%, leaf width 60.47%) were recorded. Following Koch's prostulate, pathogenicity test of *Curvularia geniculata* and *Erysphe* sp. were done on healthy gerbera plants. Inoculated plant showed symptoms of Blight disease and Powdery mildew disease after 9 and 6 days inoculation respectively. Whereas controlled plant showed no symptoms of diseases. That confirmed the pathogenicity of *Curvularia geniculata* and *Erysiphe* sp.

CHAPTER I

INTRODUCTION

The popularity of floricultural crops has increased in horticultural industry throughout the world due to its diverse uses in different events in our life. It can be used as one's own interest and happiness or manufacturing purpose in commercial industry. Essential oil extracted from flowers is use for manufacturing of perfumes (Mohanty, 2014). Besides this, flowers are used for decoration, flavoring food. The flowers are symbols of beauty, love and tranquility. Flowers are presented as a show of affection. For centuries, flowers have also played an important part in our physical and mental well being too. Most of the people prefer cut flower for its long lasting longevity.

Floriculture has become one of the important commercial trade in agriculture. Many people involved in flower cultivation for their livelihood. There is great demand of floriculture in global market. In Bangladesh, commercial floriculture has developed for its economic potential. In Bangladesh, Floriculture brought into limelight by some innovative farmers in late seventies with tuberose on a small scale basis (Mou, 2012). Large-scale commercial production started from mid eighties in Jhikargacha upazila of Jessore district (Sultana, 2003). Later it speeded largely in Jessore, Savar, Chuandanga, Mymensingh and Gazipur which turned to be the major flower production in Bangladesh. Cultivation of flower is reported to give 3-5 times and 1.5-2 times more returns than obtained from rice and vegetable cultivation, respectively (Dadlani, 2003). The area coverage under commercial flower cultivation is approximately 10,000 hectares of land while commercial nurseries have covered approximately 2,000 to 2,500 hectares of land (Momin, 2006). At present, flower-marketing is not fully organized in Bangladesh. Major

traders for flowers can be found in Shahbag, Farmgate and Gulshan in Dhaka. Substantial trade can also be found in Chittagong and other big cities. There are reportedly around 4000 retail shops of flowers in the country. Forty per cent of the retail shops are located in Dhaka, while Chittagong and Sylhet having 25 per cent each and the remaining 10 per cent of the shops are in other district towns. At a wholesale flower market (in Dhaka), some 700 traders do flower business worth at least $16,000 every day (Mou, 2012). Every year Bangladesh exports a large amount of flower by different intermediaries in the world market, which include mainly cut flowers and ornamental foliage (Mou, 2012).

Gerbera is one of the most demanding and charming cut flower being grown under protective condition in Bangladesh. It is considered one of the promising and valuable cut flower next to rose, ranks fifth among the top ten cut flowers (Saikia *et al.,* 2017). Gerbera belongs to the family Asteraceae and it has wide range of attractive colors including yellow, orange, cream-white, brick red, red and various other intermediate colors. The double cultivars sometimes have bicoloured flowers, which are very attractive It was named in honour of the German botanist and naturalist Traugott Gerber. Gerbera is also known as Transvaal Daisy, Barberton Daisy & African daisy (Nagrale *et al.,* 2013). The genus *Gerbera* consists of about forty species of hardy and perennial flowering plants. Out of which only *Gerbera jamesonii* is under cultivation. The gerbera daisy has large vibrant flowers, which has made it a popularity with flower lovers. On an average, the annual flower is 20-35 flower stick per plant and it directly related to the cultivar (Li Zhang *et al.,* 2008). The flower stalks are long thin leafless. This characteristics made gerbera very popular and is of great demand in market for preparation of bouquets. Yield of gerbera flower is greatly influenced by microclimate or

environment in polyhouse, cultivars and management practices followed during the cultivation. The yield of gerbera under controlled condition is around 200-250 flowers/m2/year and 120-150 flowers/m2/year under open condition. Gerbera is ideal for flowerbeds, borders, pots and rock gardens. It is also used in the preparation of traditional Chinese medicine: tuer-feng, curing cold with cough and for rheumatism (Pandav, 2012).

However, gerbera is subjected to infection by many pathogens results in serious reduction in yield. Those pathogens may be fungal, bacterial or virus. The fungal diseases usually occurred in gerbera are leaf spot, leaf blight, anthracnose, gray mold, root rot, soft rot, powdery mildew etc. Bacterial leaf spot mainly occur in gerbera. Gerbera mosaic virus, cucumber mosaic virus seem to occur in gerbera (Bose and Yadab, 1989).

Alternaria leaf spot disease is caused by *Alternaria* sp. Among all the species of *Alternaria*, *A. alternata* was noticed causing leaf spot on all the varieties of gerbera in greenhouse (Ghosh *et al*., 2002). Alternaria leaf spot is a serious disease in Bangladesh, causing considerable losses in greenhouse conditions. The symptoms of the disease on the leaves were characterized by the development of brown, small, scattered dots, which gradually enlarged and coalesced to form large, oval, circular or irregular, brown to black lesions with concentric rings (Mircova and Konstantantinova, 2003).

Anthracnose disease mainly occur in leaves and flower stalk of gerbera. The fungus produced dark brown color globus and saucer shaped acevuli with numerous number of satae (Swagatika *et al.,* 2015). The infected area of plant shows burn like apprearence.

Stem rot disease is also occurred on gerbera in greenhouse farm. Affected plants were stunted and developed yellow leaves unilaterally with initially brown and eventually black streaks in the vascular system. The first symptoms occurred 2 months after

transplanting during the hottest period of the summer with an average air temperature of 27ºC. Vascular streaks in the yellow leaves were continuous with a brown discoloration in the vascular system of the crown and upper taproot and occasionally turned red (Garibaldi *et al*., 2008).

Cercospora leaf disease caused by *Cercospora* sp. is also appeared on gerbera plant. The symptoms consists of small round water soaked lesions developed on leaves and stems. Later those spots coalesced and blighted the entire leaf (Borah *et al*., 2019).

Blight symptoms consists of brown color oval to circular spot surrounded by yellow halo. Later the spot emerged and necrotic lesions were observed on leaves. Casual organism of the disease was *Curvularia* spp. (Torres *et al.,* 2013).

Sclerotinia wilt disease on gerbera is caused by *Sclerotinia sclerotiorum*. It produces white, fluffy mycelium growth on infected area of plant. Usually this is preceded by pale to dark brown lesions on the stem at the soil line (Folk and Tusnádi, 1985).

Powdery mildews are caused by obligate pathogens; therefore, these pathogens need living hosts to reproduce and grow. The species of powdery mildew fungi are often host specific. Common disease symptoms and signs include white powdery looking mycelium and spores mostly seen on the upper side of leaves; leaves may also become distorted, wilted, and eventually blighted. Environmental conditions that favor this disease are high humidity at night, low humidity during the day, and temperatures around 70 to 80ºF. Spores are carried by wind to the leaves where they will germinate. Water on the leaves inhibits spore germination (Moorman, 2014).

Gray mold is a common caused by *Botrytis* sp. that can affect many kinds of plants. Common disease symptoms include brown spots on petals that increase in size over time,

leading to flower death and sometimes death of the entire plant, flower buds that do not open, irregularly shaped leaves with dead areas that form a bull's eye pattern, and stem cankers. *Bortytis cinerea* survives on dead plant tissue and the reproductive spores travel by wind or water onto living plants. High humidity or wet conditions are favorable for the disease; the spores quickly die in dry conditions (Hudson and Schraufnagel, 2004).

Bacterial leaf spot disease initially develops water soaked lesion at the edge of the leaf, mid vein or randomly across the leaf. These lesions progressively turn black or brown and may be surrounded by yellow halos (Kawarabayashi and Suyama, 1990).

Mosaic disease is most common disease occurred in almost all varieties of plants. This virus disease is considered everywhere as one of the most important pathological problems for gerbera cultivation. Symptoms include chlorotic mottle and vein yellowing on the leaves, deformed flowers and stunting; in some cases the plants are asymptomatic but less vigorous (Bertaccini *et al.*, 1996). The prevalence of high summer temperature and the absence of infective aphids may possibly be correlated with the lack of infection (Tompkins, 1937).

Now-a-days leaf curl disease was observed on gerbera. Disease plant showed typical symptoms of vein thickening, severe inward curling and a reduction in leaf size and stunting. The pathogen responsible was transmitted to healthy plant by grafting of infected scions and through the whitefly vector (Mahesh *et al*., 2010).

Pathogenicity refers to the ability of an organism to cause disease to its host. This ability represents a genetic component of the pathogen and the over damage done to the host is a properly of the host pathogen interactions. Pathogenicity test of pathogen done for identification of pathogen suspected of being the agents of a plant disease. It is mainly

done by Koch's prostulate method which formulated by Robert Koch and Friedrich Loeffler in 1884.

Gerbera is economically important in Bangladesh. But due to disease, there is huge loss in gerbera production. Research work related diseases and their pathogenicity associated with gerbera is few. Considering above facts, this research work is designed to achieve the following objectives:

1. To identify diseases associated with gerbera
2. To characterize of isolated pathogen associated with gerbera
3. To study the pathogenicity of the isolated pathogen

CHAPTER II
REVIEW OF LITERATURE

2.1 Survey of Diseases of Ornamentals

Chudali *et al.* (2020) explored the prevalence of insect pests and diseases in gerbera and their management practices adopted in Kathmandu valley. The study revealed that most of the respondants thought that major loss of gerbera production due to diseases rather than insect infestation. Major diseases included Powdery mildew (87.5%), Root rot (50%), Fusarium wilt (37.5%) and Crown rot (25%). Economically less detrimental diseases included Bacterial blight (18.75%) and *Botrytis* (18.75%).

Borah *et al.* (2019) identified pathogenic diseases of economically important floriculture crops based on visual inspection and microscopic observation carried out during 2017-18 in around Jorhat district of Assam. During the investigation, 3 diseases of chrysanthemum (Alternaria leaf spot, Sooty mould and Mosaic); 3 diseases of gladiolus (*Fusarium* infection, Alternaria leaf spot, Curvularia leaf spot and Corm rot); 5 diseases of gerbera (Alternaria leaf spot, Cercospora leaf spot, Damping off, *Stemphylium* infection and coniothyrium infection); 2 diseases of rose (Alternaria leaf spot and Cercospora leaf spot) and a fungal disease (Alternaria leaf spot) of marigold was identified.

Shamala and Janardhana (2015) surveyed the prevalence and incidence of leaf blight disease in chrysanthemum in six major growing districts of South Karnataka during 2012-13. The pathogen associated with leaf blight was isolated and identified as *Alternaria alternata*. The varieties Chandini and Karnel were more susceptible to blight

showing highest disease incidence (100%) and severity (100%) recorded from Mandya and lowest disease incidence, severity, PDI of 18.40%, 38.68% and 24.89% were recorded on Rajawhite variety in Bangalore region. In vitro evalution of four fungicides on *A. alternata* revealed that Carbendazim+ Mancozeb was most effective, followed by Carbendazim, Mancozeb and Metalaxyl+Mancozeb in inhibition of mycelium at 2.0% concentration.

Bhat *et al*. (2013) observed Alternaria leaf blight disease on gerbera which developed spots on leaves were circular and brown with somewhat irregular margins; the lesion later turned dark brown and also mentioned disease incidence and intensity ranged from 50.7 to 67.5% and 19.2 to 28.2% respectively in Kashmir valley. The pathogen associated with Alternaria leaf blight of gerbera was *Alternaria alternata*.

Milleza *et al.* (2013) surveyed viruses infecting *Rosa spp*. present in New Zealand. The detected viruses from rose samples were prunus necrotic ringspotvirus, rose spring dwarf associated virus, rose yellow vein virus, rose cryptic virus, arabis mosaic virus. Viral symptoms included leaf chlorosis and chlorotic line patterns; leaf mottling and puckering; vein clearing and banding; misshapen and balled (rosette) leaves and flower break.

Moury *et al*. (2001) investigated for viruses in rose propagated in Europe resulted in detection of only prunus necrotic ringspot virus (PNRSV) among seven viruses screened.

Bellardi *et al.* (2000) surveyed old and new viruses associated with lily in Italy. Previously lily symptomless carlavirus (LSV), cucumber mosaic virus were detected. In 1999 some lilies of a pink color cultivar showing color breaking on the petals, were

infected by a potyvirus like isolate serologically only related to turnip mosaic potyvirus (TuMV).

2.2 About Fungal Diseases and Their Pathogenicity

Mahadevakumar *et al* (2019) identified an association of Anthracnose disease which was characterized by dark concentric sunken necrotic lesions with spore mass in the acervuli on leaves and peduncles of tuberose observed in field survey. The fungal colony on PDA was grayish to dark gray and conidia were falcate, one-celled, hyaline. The identified pathogen was *Colletotrichum truncatum.* Koch's postulates were conducted on 45 days old tuberose by foliar application of conidial suspension of *C. truncatum*. The pathogen's identity was confirmed by re-isolation and identification.

Nayeb *et al.* (2019) observed typical symptoms of Alternaria leaf necrosis on gerbera daisy and isolated pathogen from infected leaf tissues was identified as *Alternaria gaisen* .The fungus was further differentiated from closely related species of genus *Alternaria* by phylogenic analysis and pathogenicity of the isolated pathogen was verified following Koch's pathogenicity postulates.

Swagatika *et al.* (2015) isolated and identified *Colletotrichum capsici* on gerbera plant. The pathogen mainly infected the leaves and flower stalks of gerbera. The fungus produced dark brown color globus and saucer shaped acevuli with numerous number of satae. The pathogenicity was also carried out and confirmed the pathogen was *C. capsici*.

Swett and Uchida (2015) characterized *Fusarium* diseases on commercially grown orchids in Hawaii. The major symptoms of decline include root degradation, foliar blight, pseudobulb rot and sheath rot. The main five species associated with diseased orchids

were Fusarium proliferatum, F. solani, F. oxysporum and previously undescribed species which similar to *F. subglutinans a*nd were designated FS-A and FS-B.

Nagale *et al.* (2013) described the morphological and cultural characteristics of *Alternaria alternata* which produce profuse mycelium on PDA with an average width of 4.42 µm in diameter and suggested synthetic media viz. Leonions's agar, Glucose-peptone agar and Sabourand's agar and non-synthetic media, Oat meal agar and PDA were excellent for the mycelial growth and conidial production of *A. alternata*.

Yeasmin and Shamsi (2013) isolated several fungal pathogen that caused disease on gerbera such as *Alternaria citrii, A. tennuisssima, Aspergillus niger, As. flavus, As. fumigatus, As. Terreus*, *Bipolaris hawaiiensis, Chaetomella raphigera, Cladosporium cladosporoides, Colletotrichum capsici, C. dematium, C. coffeanum, C. lindemuthianum, C. musae, C. orbiculare*. The frequency percentage of association of *Colletotrichum* spp. was higher than any other fungi. *B. hawaiiensis* is new record for Bangladesh. Among the isolated fungi *A. citrii, A. tennuissima, C. capsici, C. dematium, C. coffeanum* and *Culvularia clavata* were found to be pathogenic to gerbera plant.

Farhood and Hadian (2012) reported Alternaria leaf spot on gerbera in North of Iran which was caused by *Alternaria alternata* and conducted pathogenicity test that produce typical symptoms of *A. alternata (*brown, small, scattered spots on the leaves that gradually become round or irregular spots coalesce to affect large areas of leaves) after two weeks of inoculation on healthy plants.

Olson and Benson (2011) collected isolates of *phytophthora* in floriculture crops from North Carolina. In all, 163 isolates from 13 host species at 11 locations were identified primarily as *P. nicotianae, P. drechsleri, P. cryptogea* and *P. tropicalis*.

Baiswar *et al.* (2010) observed the symptoms of Powdery mildew on the leaves of gerbera during april 2008 in an experimental field of ICAR Research Complex in India. Symptoms were more prominent on upper leaves, which later turned nectrotic and the presence of ectophytic mycelium and indistinct aprressoria which confirmed by scanning electron microscopy (SEM). Catenate conidia with fibrosin bodies were also observed and foot cell of conidiophores were followed by two or three shorter, terminated with ellipsoidal conidia produced in chains and no perfect stage was found to be associated with this fungus. These characteristics confirm the identity as the anamorphic *Podosphaera* sp.

Troisi *et al*. (2010) reported *Golovinomyces cichoracearum* which is caused Powdery mildew on gerbera plant by covering leaf surface with white mycelium and conidia and as the disease progressed, infected leaves turned yellow and died. They also conducted pathogenicity test by inoculating pathogen on healthy plant which infested 8 days later after inoculation.

Wolcan (2010) collected typical symptoms of Downy mildew from the leaves of gerbera in Buenos Aires province, Argentina. Based on symptoms and the morphology of the fungus, the pathogen was identified as *Bremia lactucae*. This is the first report of Downy mildew on gerbera in Argentina.

Garibaldi *et al*. (2008) exhibited symptoms of a Wilt disease caused by *Fusarium* sp. on gerbera in the greenhouse farms in the region of Guarapuava, Parana, Brazil. Affected plants were stunted and developed yellow leaves unilaterally with initially brown and eventually black streaks in the vascular system. The first symptoms occurred 2 months after transplanting during the hottest period of the summer with an average air temperature of 27^{o}C.Vascular streaks in the yellow leaves were continuous with a brown discoloration in the vascular system of the crown and upper taproot and occasionally turned red. *Fusarium* sp. was consistently and readily isolated onto PDA and colonies were identified as *Fusarium oxysporum* on the basis of colony and conidia morphology.

Garibaldi *et al.* (2007) isolated the *Fusarium oxysporum* from vascular tissue of infected gerbera plant (yellow leaves were continuous with a brown discoloration in the vascular system of the crown and upper taproot) and colonies *F. oxysporum* were identified subculturing on PDA media . Then they conducted pathogenicity test by inoculating the *F. oxysporum* on healthy plant which developed wilt symptoms and vascular discoloration in the roots, crown and veins within 30 days.

Minuto *et al.* (2007) obtained five isolates of *Fusarium oxysporum* from infected gerbera (*Gerbera jamesonii*), chrysanthemum (*Chrysanthemum morifolium*), paris daisy (*Argyranthemum frutescens*) and African daisy (*Osteospermum* sp.) and conducted pathogenicity test on compositae hosts and found out the isolates of *F. oxysporum* were virulent.

Mirkova and Konstantinova (2003) reported *Alternaria* spp. that causes Alternaria leaf disease on gerbera in Bulgaria and characterized the symptoms by the development of

brown, small, scattered dots which gradually enlarged and coalesced to form large, oval, circular or irregular, brown to black lesions with concentric rings.

Gaag *et al*. (2001) reported Phytophthora root and crown rot disease associated with Saintpaulia, Gerbera and Spathiphyllum pot plants. The pathogens that causing phytophthora root and crown rot were *Saintpaulia ionantha, P. nicotianae, P. cryptogea* and *Spathiphyllum wallssii.*

Eisa *et al.* (2000) revealed a considerable occurrence of Root-rot, Wilt and Damping off infection in carnation, gerbera and marigold. Based on symptomatology, morphological and cultural characteristics the fungal agents *Fusarium spp., Rhizoctonia solani* and *Pythium sp.* (carnation) as well as *Fusarium spp.* and *Phytophthora spp*. were identified. Characteristic features of wilt symptoms on the infected plants characterized with dark brown discoloration on vascular system & often accompanied with reduction in root system formation and the infection by fungi causing root and collar rots were usually associated with yellowing, browning or dryness leaves.

Vazquez *et al.* (1997) detected white erumpent sori on the undersurfaces of a bed of African daisy (*Gerbera jamesonii*) and produced chlorotic spots on the upper leaf surfaces. When the sub-epidermal sori on gerbera plants were examined microscopically erumpent chains of round to cylindrical, hyaline to pale yellow sporangiospores borne on short club-shaped sporangiophores were found. It was concluded that the white rust found on gerbera in Mexico , was caused by *Albugo tragopogonis*. This is the first report of white rust of gerbera caused by *A. tragopogonis* in North America.

Jee *et al.* (1996) found out that *Phytophthora cryptogea* causing the foot rot of *Gerbera jamesonii* in Korea. Infected plants showed symptoms of wilting due to decay of the main rooting systems which led to their eventual death. A total of 47 isolates were collected from roots and petioles of infected plants and identified as *P. cryptogea b*ased on morphological, cultural and physiological characteristics. *P. cryptogea* has not been reported as a causal pathogen of foot rot gerbera or any other hosts in Korea Republic previously.

Kaewruang *et al.* (1988) reported that in Western Australia the pathogen *Phytophthora cryptogea* was more pathogenic on gerbera than *Fusarium oxysporum* which was found to be pathogenic in earlier study.

Folk and Tusnádi (1985) isolated *Sclerotinia sclerotiorum* from gerbera plants growing in gardens with vegatables. Pathogenicity was confirmed by inoculation of potted gerbera plants.

Scholten (1970) found out that in most cases *Phytophthora cryptogea* appeared to be the cause of Wilt disease of gerbera in green house area in Netherland and also mentioned *Verticillium albo-atrum* and *V. dahlinae* causeing wilt disease in gerbera.

Baker and Davis (1950) reported that *Alternaria* and *Stemphylium* caused some disease on ornamental plant in California. Among them *A. porri* isolated from gerbera plant and the observational evidence indicated that this fungus is seed-borne on gerbera in California. Also found out that *Stemphylium* caused leaf spot on gladiolus.

2.3 About Bacterial Diseases and Their Pathogenicity

Shamala and Janardhana (2018) observed Bacterial blight of chrysanthemum which was caused by *Pseudomonas putida*. Symptoms includes dark brown spots and blotches on leaves extending beyond plant leaves with water-soakes lesions on stems, darkening and death of buds and stem terninals. Affected plants showed brown to black decay at the base. Pathogenicity was proved by Koch's postulates.

Marques *et al*. (2016) collected leaf samples of gerbera plants showing symptoms of Bacterial blight. Seven isolates obtained were subjected to phenotypic and molecular characterization including pathogenicity tests and partial sequencing of the 16s rdna gene, all isolates were gram-negative, aerobic, oxidase positive, produced fluorescent pigment, induced hypersensitivity in tobacco leaves. The results of tests and analysis of the sequences of rdna showed 100% identity with *Pseudomonas cichorii*.

Hernandez *et al*. (2000) reported bacteria present in the rhizosphere of *Gerbera jamesonii, Dianthus caryophyllus, D. barbatus*. The rhizobacteria studied (*Pseudomonas cepacia, P. fluorescens and Azospirillum brasilense*) exhibited chemoattraction to root exudates. *Pseudomonas* was dominant over *Azospirillum, Bacillus* and *Streptomyces. P. cepacia* exhibited maximum chemotactic responses to root exudates of both genera after 14 days and 40 minutes of exposure.

Kawarabayashi and Suyama (1990) found out bacterial disease in *Gerbera jamesonii* at Atsugi farm, Kanagawa prefecture. The symptoms of the bacterial disease was described and causal bacterium was isolated and identified as *Pseudomonas cichorii* and its

pathogenicity confirmed by needle-prick and spray inoculation. This is the first report of a bacterial disease of *G. jamesonii* caused by *P. Cichorii* in Japan.

2.4 About Virus Diseases

Marys *et al*. (2014) reported Tomato spotted wilt virus on gerbera and chrysanthemum in Venezuela. Symptomatic plants were showed concentric rings, irregular chlorotic blotches and deformation on leaves. Tomato spotted wilt virus was identified on both gerbera and chrysanthemum by ELISA test.

Stanković *et al.* (2011) observed displayed chlorotic oak-leaf patterns followed by necrosis and distortion of leaves within a greenhouse-grown gerbera in Serbia. Symptomatic leaves were tested for the presence of Tomato spotted wilt virus (TSWV), Impatiens necrotic spot virus (INSV), and Chrysanthemum stem necrosis virus (CSNV) by ELISA diagnostic kits. All tested plants were negative for INSV and CSNV and positive for TSMV. This is the first report of Tomato wilt virus on gerbera in Serbia.

Verma *et al.* (2004) first reported the Cucumber mosaic disease on gerbera in India which is caused by Cucumber mosaic virus. They observed color break symptoms on the petals, asymmetrical ray florets and deformed flowers as the symptoms of mosaic on *Cucumber sativus, Nicotiana benthamiana, N. clevelandii, N. glutinosa and N. tabacum*. The virus was transmitted nonpersistently by *Myzus persicae* and *Aphis gossypii* and identified as Cucumber mosaic virus (CMV) using enzyme-linked immunosorbent assay (ELISA) with CMV- specific antibodies.

Kaminska (1992) studied the response of gerbera to infection with Tomato spotted wilt virus. The susceptibility of gerbera cultivars to Tomato spotted wilt virus was observed. The presence of the virus in plant tissues was determined by ELISA test.

Kitajima (1988) described virus and virus-like pathogens affecting ornamental plants. The identified diseases were infectious chlorosis of malvaceae (in Hibiscus); cucumber mosaic virus (in orchid, periwinkle, Salvia); dahlia mosaic virus; cauliflower mosaic virus in Mathiola; orchid viruses (orchid ringspot); Cassia mottle virus in some *Cassia spp*.; tobacco streak virus in dahlia; tomato spotted wilt virus in daisy; bean yellow mosaic virus in gladiolus; pepper ringspot virus in Transvaal daisy and mycoplasmalike organisms in daisy, periwinkle.

Verma and Singh (1980) studied transmission (by grafting) and host range of a virus, named gerbera mosaic virus, causing vein clearing of leaves, flower distortion and color break and plant stunting at IARI, New Delhi.

CHAPTER III
MATERIALS AND METHODS

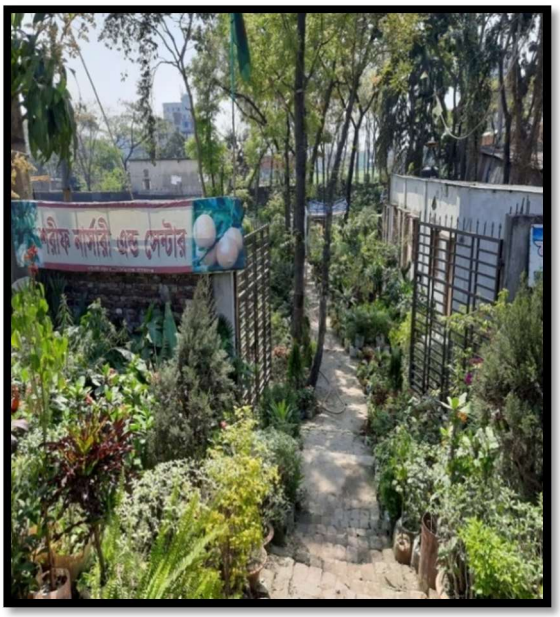
In this chapter all the materials and methods related to the experiment are described.

3.1 Experimental site

For the study of different disease of gerbera different nurseries around Dhaka were surveyed. The experiment was conducted at the Plant Disease Diagnostic Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

Table 1: Experimental sites under surveyed around Dhaka

Location	Name of Nurseries
Agargaon	1. Mayer Doa Nursery
	2. Krishibid Upakaran Nursery
	3. Sobuj Bangla Nursery
Savar	1. Rangdhanu Nursery
	2. Ananda Nursery
Narayanganj	1. Sharif Nursery
	2. Mukta Nursery
	3. Sopno Jatra nursery
Gazipur	1. BADC nursery



Photograph 1. Surveyed nurseries for different diseases of gerbera

3.2 Time of Experiment

The study was conducted during the period of April 2019 to October 2019.

3.3 Collection of samples

Diseased leaves and other infected plant parts exhibited different types of typical symptoms were collected from infected Gerbera plants of different Gerbera fields of Dhaka for recording field diseases (Photograph 6). Then the samples were carried to the Plant Disease Clinic of Sher-e-Bangla Agricultural University in individual brown paper envelop and tagged for later convenience. 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).

3.4 Identification of disease

Symptoms of different pathogenic diseases of gerbera plants were studied and identified in the field and laboratory condition. Sampling survey conducted in different areas by observing symptom development on the plant based on morphological apprearence, physical abnormalities of gerbera plant parts diseases were identified.

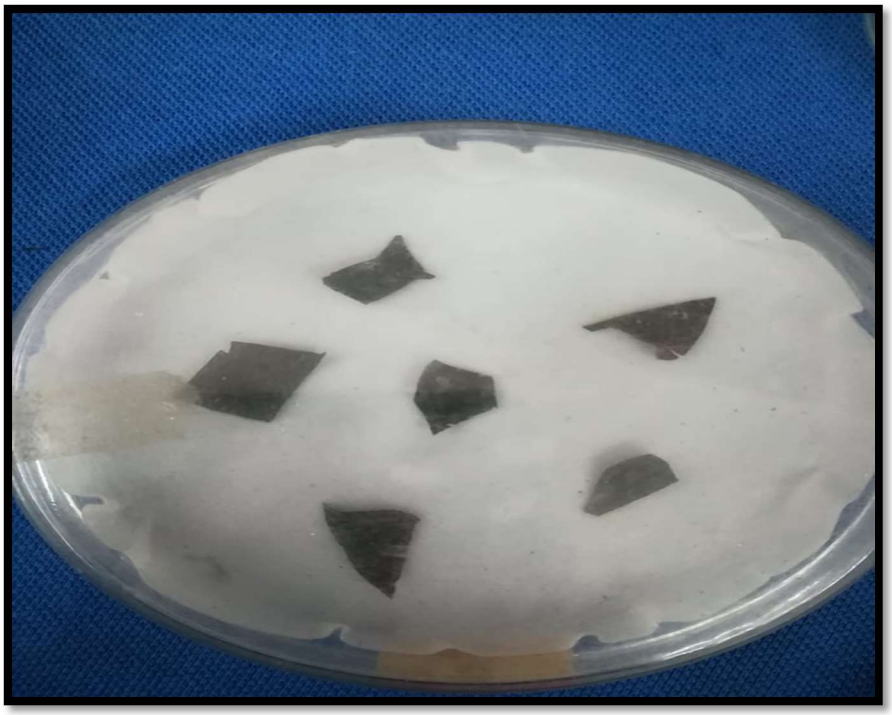
3.5 Isolation of fungal pathogen

Plant parts showing the typical disease sign and symptoms were cut into small pieces aseptically, washed thoroughly in running tap water, then surface sterilized with chlorox (3%) for 30 seconds to one minute and washed three times in sterile distilled water. The samples was dried by sterilize blotting paper. The surface sterilized leaf/ stem pieces were then placed into the blotter paper for further study.

3.6 Blotter method

For the pathogenic confirmation diseased parts or infected leaves incubated in plastic petridishes in blotter paper following the ISTA (1999) rules. Three piece of blotter paper were soaked in sterilized water and place at the bottom of 9 cm labeled plastic petridishes. Five or six disease samples were selected from each sample and placed in

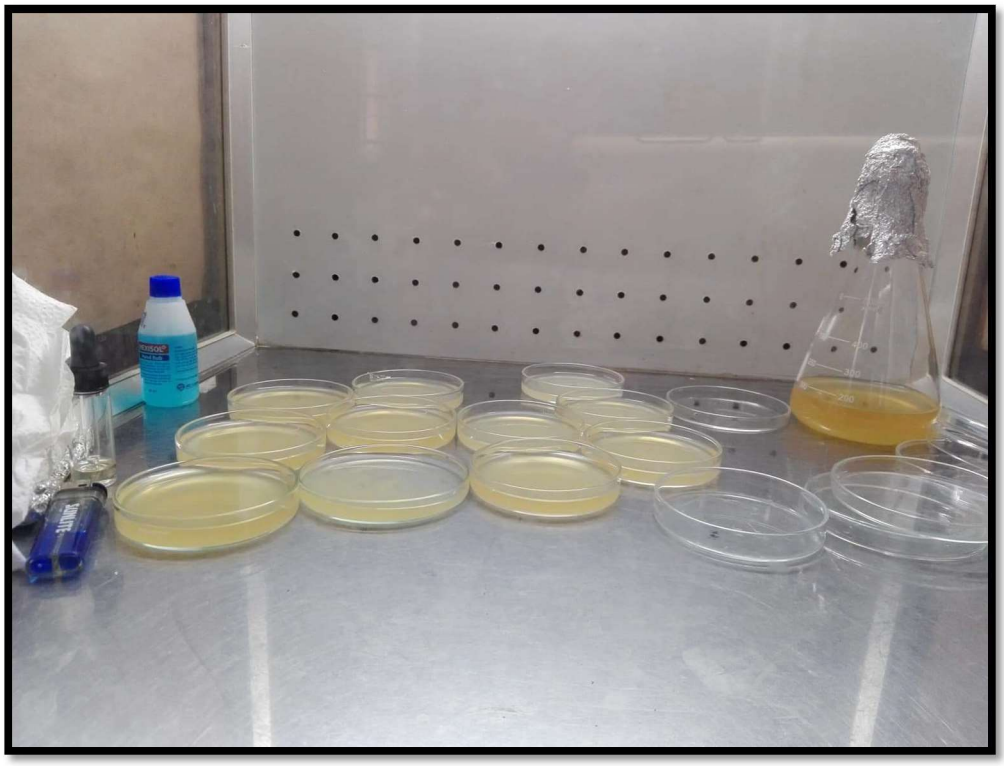
each plastic petridish using a pair of forceps. The lid of each petridish was held in place with gummy cello tape (Photograph 2).Then the petri dishes were incubated at room temperature for 5-7 days for growth of Pathogen (fungal). After incubation samples were examined under stereomicroscope to identified the pathogen. The fungi were recorded by observing their growth characters on the infected samples following the keys of Booth (1971), Ellis (1960), Mathur and Kongsdal (2003) and Sutton (1980).



Photograph 2 : Surface sterilize leaf sample placed over moistened blotter paper

3.6 Preparation of PDA media and pure culture of causal organism

Potato dextrose agar (PDA) were prepared by 200g potato extract, 1000 ml distilled water, 17g dextrose, 17g agar in a conical flask and autoclaved at 121° C under 15psi for 30 minutes. After autoclaved the media was kept few minutes for cool and then under laminar air flow cabinet 25-30 drops of lactic acid were added to media. After that media poured to sterile petridishes (Photograph 3). Then pure colonies from Blotter method were transferred to PDA media. Seven to nine days after inoculation pure culture of causal organism were found (Photograph 4).



Photograph 3: Preparation of PDA media in laminar air flow

Photograph 4. Pure culture of *Fusarium oxysporum*

3.7 Identification of the culture

The culture obtained was compared with the original description of the fungus for morphological characters. A bit of fungal hypha was taken from 15-20 days old culture on a slide and teased gently by the help of two sterilized pricking needle. Then it was stained with glycerin , covered with glass cover slips and examined under microscopes to study the morphological characters like size, shape, color of mycelium conidiophores, conidia and branching habit of hyphae. Microphotograph of the mycelium, conidia were taken.

3.8 Pathogenicity test

Pathogenicity test was done on local variety of gerbera plant (*Gerbera jamesonii*). The healthy plants were raised in earthen pots. Sixty to seventy days old plants were sprayed with distilled water then they were covered with polythene bags for 24 hours. For inoculation of fungal pathogen leaves of the plants were injured slightly by pricking with the help of the sterilized needle. The leaves were then sprayed with suspension containing mycelia bits and spores of the fungus which was prepared in sterilized water. The control plants were sprayed with only sterilized distilled water. After spraying, all the plants were covered with polythene bags and kept inside a glass house.

In case of powdery mildew pathogen, pathogenicity test was done by directly placing white mycelium from infected leaf to healthy leaves with a help of sterile toothpick. After inoculation it was covered with polythene bag.

Observation on occurrence of disease symptom was recorded regularly.

3.9 Data recorded

The data were collected with three replication. The following parameters were considered during data collection:

1. Infected plant parts
2. Distribution of disease
3. Status of disease
4. Reduction of plant parts due to disease
5. Disease incidence (%)
6. Pure culture growth

7. Disease appearance after inoculation
8. Symptomological study

A)Reduction percentage of plant parts

The measurement of healthy plant parts and infected plant parts of gerbera were taken by centimeter scale. Reduction percentage of plant parts were obtained by using following formula:

$$\text{Reduction percentage} = \frac{\text{Healthy plant part} - \text{infected plant part}}{\text{Healthy plant part}} \times 100$$

B)Disease Incidence

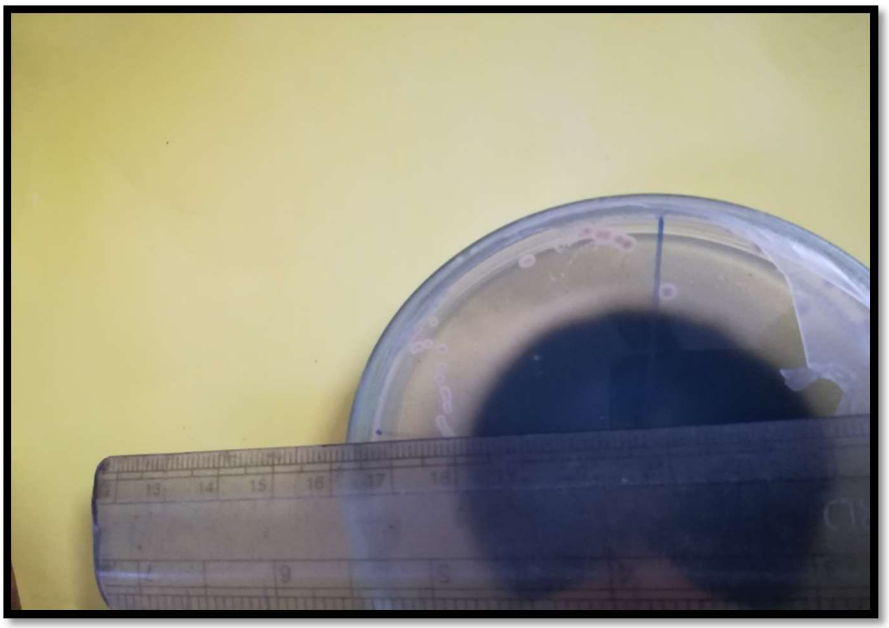
The number of infected plant unit and number of total inspected plant unit were counted. For different disease incidence was estimated as a % of individual plants expressing typical symptoms and sign of a disease within the field section. Thus % disease incidence was calculated in all locations surveyed. Then disease incidence were obtained by using following formula:

$$\text{Disease Incidence} = \frac{\text{The number of infected plant unit}}{\text{Total number of inspected plant unit}} \times 100$$

The plant showing typical symptoms by the pathogenic infection were considered as disease plant. Disease incidence was calculated by the number of proportion of the plant units diseased in relation to the total number of unit examined (Agrios, 2005).

C)Pure Culture Growth

The growth of pure culture of causal organism were measured with scale. The measurement were taken 7-9 after inoculation.



Photograph 5: Measurement of pure culture growth

3.10 Statistical Analysis of Data

The data for characters under the present study were statistically analyzed by following Completety Randomized Design (CRD).

Photograph 6 : Sample collection from nurseries

CHAPTER IV
RESULTS AND DISCUSSION

The experimental results regarding to studies of diseases of gerbera and their pathogenicity are presented below:

4.1 Survey of Disease Incidence

A study was conducted in 2019 in different nurseries of Dhaka to monitor the incidence of the diseases affecting gerbera grown under protection cultivation. The survey revealed that the most common disease in nurseries was the Alternaria leaf spot. Besides, gerbera plants were also affected with Cercospora leaf spot, Anthracnose, Gray mold, Stem rot, Powdery mildew, Sclerotinia wilt, Mosaic and Leaf curl diseases in moderate to severe form at all the locations surveyed.

Table 2: Survey on incidence of fungal diseases of gerbera grown under protected condition

Sl no..	Disease name	Causal organism	Disease incidence (%) on different locations			
			Agargaon	Savar	Narayanganj	Gazipur
1.	Alternaria leaf spot	*Alternaria alternata*	38.89	58.34	27.78	66.67
2.	Cercospora leaf spot	*Cercospora* sp.	22.23	-	33.33	50
3.	Blight	*Curvularia geniculata*	11.11	-	16.67	50
4.	Anthracnose	*Colletotrichum capsici.*	-	-	-	33.33
5.	Stem rot	*Fusarium oxysporum*	-	-	11.11	16.67
6.	Gray mold	*Botrytis* sp.	16.67	-	5.56	33.33
7.	Sclerotinia wilt	*Sclerotinia sclerotiorum*	-	-	-	16.67
8.	Powdery mildew	*Erysiphe* sp.	11.11	8.34	-	16.67

Table 3: Survey on incidence of virus diseases of gerbera grown under protected condition

Sl no.	Disease name	Causal organism	Disease incidence (%) on different locations			
			Agargaon	Savar	Narayanganj	Gazipur
1.	Mosaic	Mosaic virus	-	8.34	16.67	50
2.	Leaf curl	Leaf curl virus	-	-	-	33.33

4.2 Dieases of gerbera

4.2.1.Alternaria leaf spot of gerbera

4.2.1.1 Symptomological study

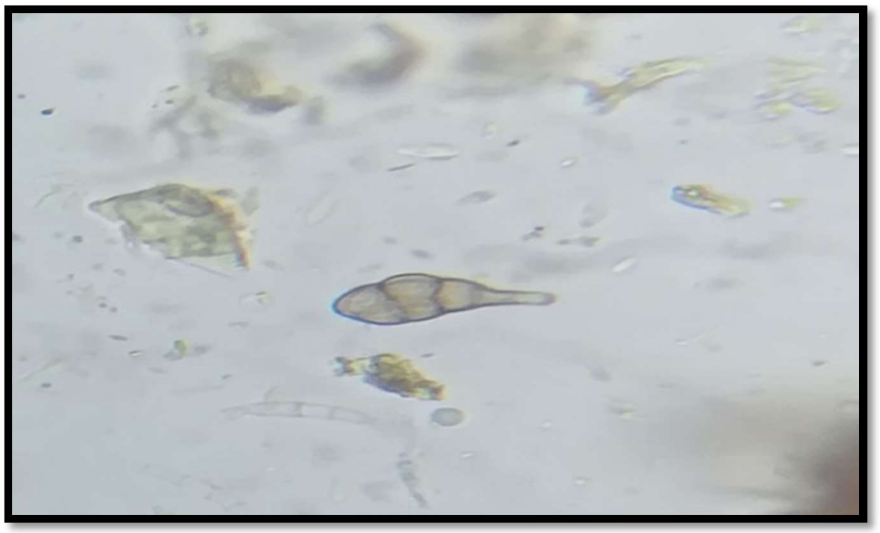
Small water soaked brown scattered spots on the leaves that gradually become round or irregular were found on gerbera leaves (Photograph 7A). Spots coalesce to affect large areas of leaves and causes defoliation (Photograph 7B). Affected plants showed lower vitality, suppressed development and fewer, smaller, distorted in shape flowers.

4.2.1.2 Identification of causal organism

The identified causal organism associated with gerbera plant was *Alternaria alternata.* The mycelium was short, septated, hyaline and branced. The conidiophore was short, septated colored and beared several conidia at the top. Conidia were dark, short beaked, multicelled and muriform (both longitudinal and transverse septum were present) (Photograph 7C) and borne at the tip of conidiophores singly or in chain (Photograph 7D). These characteristics were identified under compound microscope. In stereomicroscope, a chain like structure of conidia was seen (Photograph 7E). Pure culture of *A. alternata* was grown on PDA media within 7-9 days (Photograph 7F).

7(A)

7(B)



7(C)

7(D)

7(E)

7(F)

Photograph 7. Symptoms and causal organism of Alternaria leaf spot of gerbera (A-E). A) Initial stage of leaf spot on gerbera B) Advance stage of leaf spot C) Conidia of *Alternaria alternata* under compound microscopre (40x) D) Chain of conidia of *A. alternata* under compound microscope (40x) E) Chain like structure of conidia under stereomicroscope of *A. alternata* F) Pure culture of *A. alternata.*

4.2.1.3 Reduction rate due to Alternaria leaf spot disease

By visual observation of typical symptoms of Alternaria leaf spot on gerbera, infected leaf length, width, plant height were recorded and compared with the leaf length, width and plant height of healthy plant of gerbera.

Table 4. Measurement of reduction rate due to Alternaria leaf spot on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction percentage
Leaf length	14	24.4	**42.62**
Leaf width	8.2	11.2	**26.79**
Plant height	27.2	38	**28.42**

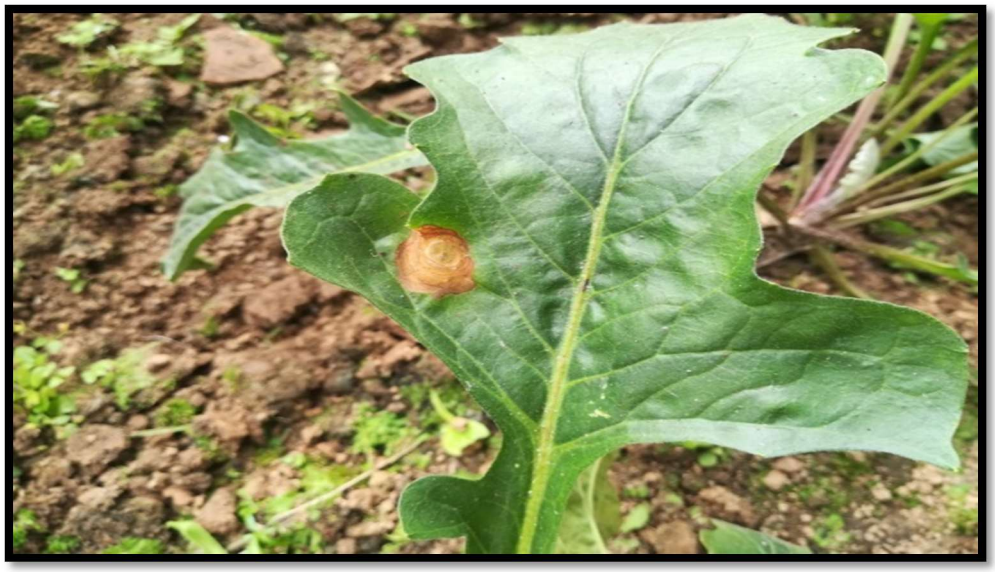
4.2.2. Cercospora leaf spot of gerbera

4.2.2.1. Symptomological study

The typical symptoms were characterized by the development of brown, small, scattered dots, which gradually enlarged and coalesced to form large , oval, circular or irregular brown to reddish brown lesions with concentric rings (Photograph 8A). In severe cases short hole created on gerbera leaves. Affected plants suffered from nutrient deficiency.

4.2.2.2. Identification of causal organism:

The identified causal organism that causes Cercospora leaf spot on gerbera was *Cercospora* sp. Mycelium was well developed , septated, branched, slender, intercellular and brown colored. Conidiophores were septated, dark colored stuctures coming out in tufts from stomata (Photograph 8B). Conidia were hyaline or pale yellow, obclavate and on liberation from conidiophore , each conidium leaves a small scar at the place of its attachment.



8(A)

8(B)

Photograph 8. Symptoms of Cercospora leaf spot of grbera (A-B). A) Initial stage of Cercospora leaf spot on gerbera plant. B) Microscopic view of tuft of conidiophore of *Cercospora* sp. on gerbera under compound microscope (40x).

4.2.2.3. Reduction rate due to Cercospora leaf spot disease:

By visual observation of typical symptoms of Cercospora leaf spot on gerbera , the infected leaf length, width, plant height were recorded and compared with the leaf length, width and plant height of healthy plant of gerbera.

Table 5. Measurement of reduction rate due to Cercospora leaf spot on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Leaf length	22.2	26.2	**15.27**
Leaf width	8.6	10.2	**15.69**
Plant height	30.2	42	**28.1**

4.2.3. Anthracnose disease on gerbera

4.2.3.1. Symptomological study

Infection generally occurred on stem and leaves of gerbera. Minute, brown to black rust colored, unbroken, circular, scabby lesion appeared on stem (Plate 9A). The margin of the affected area become raised. The scab later infect the flower and these causes great economic losses.

4.2.3.2. Identificaton of causal organism

The identified causal organism that caused Anthracnose disease on gerbera was *Colletotrichum capsici*. The fungus produced dark brown colored globus and saucer shaped acervuli with numerous number of setae (Photograph 9B). Conidia and conidiophore produced in acervuli (a plate-like stroma on which conidia and conidiophore are borne). The conidia were hooked shaped produced from acervuli, having an oil globule at the centre of the spore (under microscope). In stereomicroscope,

thorn like structure was observed (Photograph 9C). On the blotter paper its colony was white to grey with dark green center.

9(A)

9(B)

9(C)

Photograph 9. Symptoms of Anthracnose disease of gerbera (A-C). A) Severe stage of infection due to *Colletotrichum capsici*. B) Microscopic view of setae and acervuli of *C. capsici.* under compound microscope (40x) C) Stereomicroscopic view of *C. capsici*.

4.2.3.3. Reduction rate due to Anthracnose disease:

By visual observation of typical symptoms of Anthracnose on gerbera, the infected stem length, plant height were recorded and compared with the stem length and plant height of healthy plant of gerbera.

Table 6. Measurement of reduction rate due to Anthracnose disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Stem length	12.2	13.7	**10.94**
Plant height	33	37.8	**12.69**

4.2.4. Blight disease on gerbera

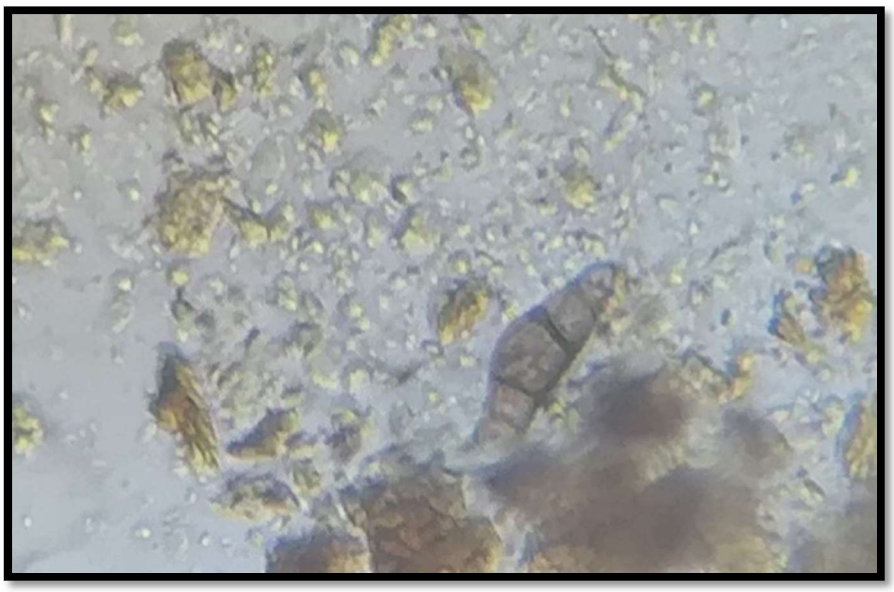
4.2.4.1. Symptomological study

Typically the blight symptoms occurred on the leaves of gerbera. Leaves become yellow and then become brown from the leaf tip down (Photograph 10A). Irregular patches and streaks may also occurred. The infected plant drastically reduced flower size and it caused great economic losses.

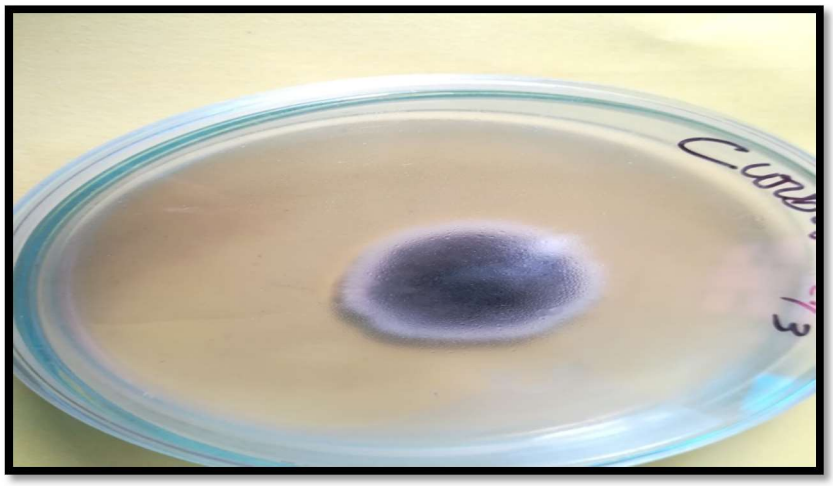
4.2.4.2. Identification of causal organism

The identified causal organism that caused Blight disease of gerbera was *Curvularia geniculata*. The fungus forms long, dark, straight or flexuous conidiophores either single or in groups bearing dark, shiny conidia. Conidia smooth-walled, curved, 4 septated (Photograph 10B), 5 cells, middle cell usually the largest, the two septa at each end close to each other, middle cell were dark brown color, end cells comparatively light brown color, basal cell truncate or round. Pure culture of *Curvularia geniculata* was grown on PDA media within 7 days (Photograph 10C).

10(A)



10(B)



10(C)

Photograph 10. Symptoms of Blight disease of gerbera caused by *Curvularia geniculata* (A-C) A)Typical symptoms of blight disease on gerbera B) Conidia of *Curvularia geniculata* under compound microscope C).Pure culture of *Curvularia geniculata*

4.2.4.3. Reduction rate due to Blight disease:

By visual observation of typical symptoms of Blight disease on gerbera infected leaf length, width, plant height were recorded and compared with the leaf length, width and plant height of healthy plant of gerbera.

Table 7. Measurement of reduction rate due to Blight disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Leaf length	19.5	25	**22**
Leaf width	5.2	7.8	**33.33**
Plant height	36.5	41.7	**12.47**

4.2.5. Sclerotinia wilt disease on gerbera

4.2.5.1. Symptomological study

The first visible of white mold formed on stems, leaves and flower of gerbera plant (Photograph 11A). The first symptom of the disease was a brown lesion shortly followed by a characteristic fluffy, white growth (mycelium) of the fungus on infected host plants. Resting bodies (globular, flattened, elongated or irregular in shape) called sclerotia were produced in the white mycelium growth. When the fungus is onset in the field by favorable environmental conditions, losses can be great and control measures should be considered.

4.2.5.2. Identification of causal organism

The identified causal organism was *Sclerotinia sclerotiorum* that caused Sclerotinia wilt disease on gerbera. Several minute spore can observed under compound microscope (Photograph 11B).

11(A)

11(B)

Photograph 11. Symptoms of Sclerotinia wilt of gerbera (A-B). A) Severe stages of Sclerotnia wilt of gerbera caused by *Sclerotinia sclerotiorum* B) Microscopic view of *S. sclerotiorum* under compound microscope (100x).

4.2.5.3. Reduction rate due to Sclerotinia wilt disease

By visual observation of typical symptoms of Sclerotinia wilt disease on gerbera, the infected stem length, plant height were recorded and compared with the stem length and plant height of healthy plant of gerbera.

Table 8. Measurement of reduction rate due to Sclerotinia wilt disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Stem length	12.3	18.7	**34.22**
Plant height	31.2	45	**30.67**

4.2.6. Gray mold disease on gerbera

4.2.6.1. Symptomological study

Symptoms of Gray blight include flower blight, bud rot, stem rot and leaf blight. The damage can be occurred almost any plant part, but prefers tender tissues such as petals and buds (Photograph 12A). Infection first appeared as a water soaking and browning regardless of the tissue affected. Irregular, enlarged, tanish and water soaked spots were appreared. Infected petals wither, turn tannish brown and blighted. Flag to roundish, black resting bodies of the fungus appeared on infected plant parts. The petals of badly infected flowers often become matted and stick together. Flower can also become infected while still in the bud.

4.2.6.2. Identification of causal organism

The identified causal organism that caused Gray mold disease of gerbera was *Botrytis* sp. Gray mass of spore were observed (Photograph 12B). Threadlike branched hyphal structures were seen with brown tree like conidiophore. Globuse Conidia were found which were hyaline and non-septate. Pure culture of *Botrytis* produced whitish cottony colony on PDA culture media within 7 days (Photograph 12C).

12(A)

12(B)

12(C)

Photograph 12. Symptoms of Botrytis blight of gerbera (A-C). A) Petal infection due to botrytis blight disease B) Microscopic view of *Botrytis* sp. under compound microscope (40x) C) Pure culture of *Botrytis* sp. on PDA media.

4.2.6.3. Reduction rate due to gray mold disease

By visual observation of typical symptoms of Gray mold disease on gerbera, infected flower size, plant height were recorded and compared with the flower size and plant height of healthy plant of gerbera.

Table 9. Measurement of reduction rate due to Gray mold disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Flower size	4	7	**42.86**
Plant height	39.4	55	**28.36**

4.2.7. Powdery mildew disease of gerbera

4.2.7.1.Symptomological study

Powdery mildew disease occur worldwide that causes a powdery growth on the surface of leaves , buds, young shoots, fruit and flowers. The white powdery appearance is due to large numbers of microscopic spores borne in chain (Photograph 13A and B). In severe stage of this disease , the mildewed plant parts may be stunted and distorted. Leaves commonly turn yellow and wither, flower are distorted or fewer in number and quality are reduced.

4.2.7.2. Identification of causal organism

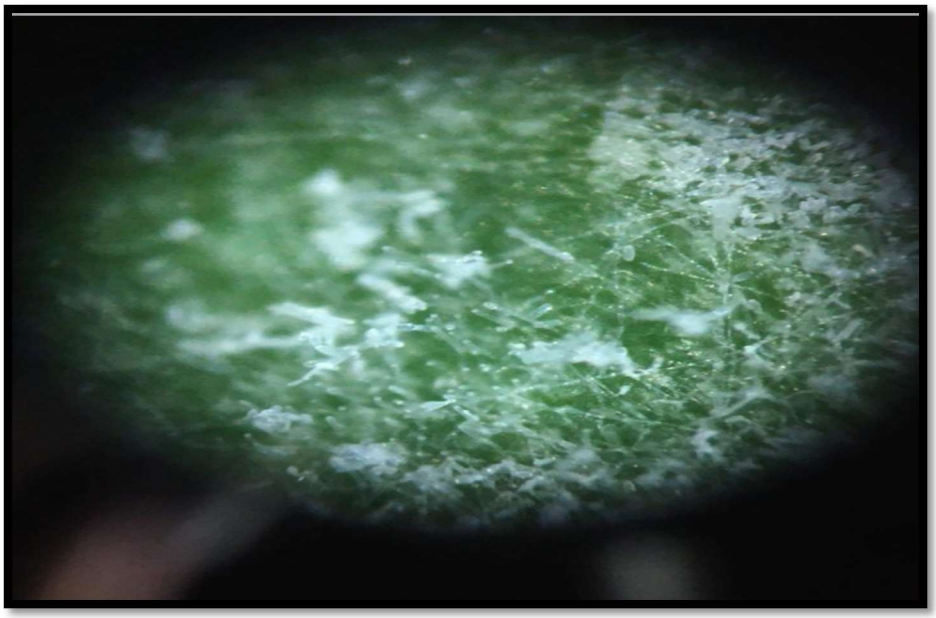
The identified causal organism that causes Powdery mildew disease on gerbera was *Erysiphe* sp. The color of conidia of *Erysiphe* sp. was observed hyaline and thin-walled.

The shape of conidia was varied from oblong (young) to cylindrical (matured) conidia (Photograph 13C).

13(A)

13(B)

13(C)



13(D)

Photograph 13. Symptoms of Powdery mildew on gerbera (A-D) A) Early stage of infection by Powdery mildew disease caused *Erysiphe* sp. B) Severe stage of infection C) Conidia of *Erysiphe* sp. under compound microscope(40x) D) Stereomicroscopic view of *Erysiphe* sp.

4.2.7.3. Reduction rate due to Powdery mildew disease

By visual observation of typical symptoms of Powdery mildew on gerbera, infected leaf length, width, plant height were recorded and compared with the leaf length, width and plant height of healthy plant of gerbera.

Table 10. Measurement of reduction rate due to powdery mildew disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Leaf length	15.6	19.3	**19.17**
Leaf width	6.5	8	**18.75**
Plant height	29.2	40.6	**28.08**

4.2.8. Stem rot disease on gebera

4.2.8.1. Symptomological study

This symptom often occurs on one side of plant on one shoot or root. The first indication of this disease is a yellowing and drooping lower leaves. Successive leaves yellow, wilt and die often before the plant reaches maturity (Photograph 14A). If the main stem is cut, dark brown streaks may be seen running lengthwise throughout the stem.

4.2.8.2. Identification of the causal organism

The identified causal organism of Stem rot disease on gerbera was *Fusarium oxysporum.* The mycelium of *Fusarium* were delicate white to pink, often with purple in color. The fungus produces three types spores. These are micro-conidia, macro-conidia, and chlamydospores. The micro and macro- conidia formed externally on hypha-like

conidiophores. Chlamydospores formed from normal hypha which undergoes increased growth and thickening of their cell wall. Pure culture of *Fusarium* was grown in PDA media within 7 days (Photograph 14D).

14(A)

14(B)

14(C)

14(D)

Photograph 14. Symptoms of Stem rot disease of gerbera (A-D). A) Rotting of stem of gerbera B) White mycelium on flower stalk C) White to greenish mycelium on soil line of gerbera plant D) Pure culture of *Fusarium oxysporum.*

4.2.8.3. Reduction rate due to Stem rot disease

By visual observation of typical symptoms of stem rot disease on gerbera, infected leaf length and width, flower stalk size, plant height of infected plant were recorded and compared with the leaf length and width, flower stalk size and plant height of healthy plant of gerbera.

Table 11. Measurement of reduction rate due to Stem rot disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction Percentage
Leaf length	17.5	21.2	**17.42**
Leaf width	7	8.5	**17.65**
Flower stalk size	16.4	29.7	**44.78**
Plant height	28.3	35	**19.14**

4.2.9. Mosaic disease of gerbera

4.2.9.1. Symptomological study

This symptoms are mostly occur on leaves. Irregular leaf mottling (light and dark green or yellow patches or streaks) was seen (Photograph 15). Leaves were commonly stunted , curled or puckered, veins may be lighter than usual or banded with dark green or yellow. Plants are often dwarfed and flowers are fewer than usual, deformed and stunted.

4.2.9.2. Identification of causal organism

The identified causal organism of Mosaic disease of gerbera was *Mosaic virus*.

Photograph 15. Symptoms of Mosaic disease on gerbera leaveas.

4.2.9.3. Reduction rate due to mosaic disease

By visual observation of typical symptoms of Mosaic disease on gerbera, infected leaf length and width, plant height of infected plant were recorded and compared with the leaf length and width and plant height of healthy plant of gerbera.

Table 12. Measurement of reduction rate due to Mosaic disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction percentage
Leaf length	13.3	17.6	**24.43**
Leaf width	6	9.2	**34.78**
Plant height	34.5	39.6	**12.89**

4.2.10. Leaf curl disease of gerbera

4.2.10.1. Symptomological study

The symptoms were characterized by severe stunting of the plants with downward rolling and crinkling of the leaves (Photograph 16). The newly emerging leaves exhibit slight yellow coloration and later they also show curling symptoms. Older leaves become leathery and brittle.

4.2.10.2. Identification of causal organization

The identified causal organism of Leaf curl disease was *Leaf curl virus*.

Photograph 16. Leaf curl disease of gerbera.

4.2.10.3. Reduction rate due to Leaf curl disease disease

By visual observation of typical symptoms of Leaf curl disease on gerbera, infected leaf length and width, plant height of infected plant were recorded and compared with the leaf length and width and plant height of healthy plant of gerbera.

Table 13. Measurement of reduction rate due to Leaf curl disease on gerbera

Diameter	Infected plant (cm)	Healthy plant (cm)	Reduction percentage
Leaf length	7.2	19.5	**63.08**
Leaf width	3.4	8.6	**60.47**
Plant height	32.5	41.7	**22.06**

4.3. Pathogenicity test

4.3.1.Curvularia leaf blight

For pathogenicity test of *Curvularia*, mycelium were taken from pure culture of *Curvularia geniculata* and mixed with 1 L water to prepare spore suspension. Then suspension was sprayed to leaves of selected gerbera plant (Photograph 17) and covered with polythene bag (Photograph 18). Some plant were sprayed with sterilized distill water and considered them as control.

Table 14: Pure culture growth of *Curvularia geniculata*

Days after inoculation (DAI)	Length of pure culture (cm)	Width of pure culture (cm)
7	0.8	0.9
10	1.1	1.3
12	1.2	1.4
15	2.3	2.4
20	2.7	2.8

4.3.1.1. Symptomology of Blight

Inoculated plant developed a little yellow to light brown spot like symptoms initially 9 days after inoculation (Photograph 19). Later the symptoms gradually increased in size (Photograph 20). There was no symptoms in control plant (Photograph 21).

Table 15: Pathogenicity test of *Curvularia geniculata* on gerbera under protected area

Sl. No.	Inoculation technique	No. of leaves/Plants (mean)		Percentage
		Inoculated	Infected	
1.	Spraying spore suspension	3	1	33.33
2.	Control (spraying sterilized distilled water on leaves)	3	0	0

.

Photograph 17. Inoculation *Curvularia geniculata* was done by spraying method

Photograph 18. Inoculated plant covered with polythene bag

Photograph 19. Symptoms of Blight disease appeared after 9 days of

Photograph 20. Symptoms of Blight disease appeared 25 days after inoculation

Photograph 21. No symptoms of Blight appeared in controlled plant

4.3.2.Powdery mildew

For pathogenicity test, mycelium were taken from infected leaves of gerbera (Photograph 22) and placed or dusting on healthy leaves of gerbera with the help of tooth pick (Photograph 23). Then inoculated plants were covered with polythene bag. For comparing the pathogenicity some plants were sprayed with sterilized distill water and considered them as control. Symptom of powdery mildew was developed 6 days after inoculation (Photograph 24). Controlled plant did not show any symptoms (Photograph 25).

Table 16: Pathogenicity test of powdery mildew under protected area

Sl. No.	Inoculation technique	No. of leaves/Plants (mean)		Percentage
		Inoculated	Infected	
1.	Inoculated by tooth pick	3	2	66.67
2.	Control (spraying sterilized distilled water on leaves)	3	0	0

Photograph 22. Mycelium picked from previously infected leaf

Photograph 23. Inoculated leaf of gerbera

Photograph 24. Symptoms of Powdery mildew appeared 6 days after

Photograph 25. No symptoms of Powdery mildew developed in controlled

Gerbera is one of the most promising and valuable cut flower in Bangladesh. On the basis on its demands and charm, it is considered as astonishing next to rose. Gerbera ranks fifth among the top ten cut flowers Saikia *et al.,* (2017).

Field diseases of gerbera were investigated in four locations of Dhaka district covering nine sites of cultivation. Disease samples were collected from investigated areas and causal organisms were isolated and identified.

For detecting fungi, blotter method following the ISTA (1999) rules were used. After incubation for 5-7 days pathogen were identified under compound and stereomicroscope by observing mycelial growth and fruiting structures following the keys of Booth (1971), Ellis (1960), Mathur and Kongsdal (2003) and Sutton (1980). So far pathogenic species viz. *Alternaria* sp., *Cercospora* sp., *Colletotrichum* sp., *Curvularia* sp., *Erysiphe* sp., *Fusarium* sp., *Sclerotinia* sp. and *Botryti*s sp. were identified and confirmed through blotter paper. For the pathogenicity confirmation test, pure culture of the identified pathogens were prepare on PDA media from the pure colonies from blotter method. Seven to nine days after incubation pure culture of *Alternaria alternata, Fusarium oxysporum, Curvularia geniculata* and *Botrytis* sp. were found.

According to this study, most common disease found in gerbera is Alternaia leaf spot caused by *Alternaria alternata* (Photograph 7). Farhood and Hadian (2012) reported the same disease and causal pathogen (brown, small, scattered spots with typical symptoms) are also supported by Bhat *et al*. (2013) and Shamala and Janardhana (2015). More researchers also reported association of *Alternaria* with gerbera but with different species, Baker and Davis (1950), Yeasmin and Shamsi (2013) and Nayeb *et al*. (2019).

Cercospora leaf spot (Photograph 8), a pathogenic disease identified in some economically important floriculture crops specially cut flower like gerbera and rose by Borah *et al*. (2019), based on visual inspection and microscopic observation. In gerbera, powdery mildew is the most common foliar disease of gerbera daisies, but they are also plagued by Gray mold (*Botrytis* sp.) and Alternaria leaf spot. Moreover, Brisco and Hausbeck (2018) also reported that gerbera is also susceptible to a host of root and crown rot diseases, including those caused by *Fusarium, Phytophthora* and *Pythium* spp.

Anthracnose disease of gerbera (Photograph 13) were found only BADC nursery, Gazipur (33.33% DI) (Table 2) eventuated on stem and leaves of gerbera. Numerous brown to black circular, unbroken, scabby lesion were found on stem and also flower stalk. The fungus produced dark brown colored globus saucer shaped acervuli with prominent and numerous number of setae found under stereomicroscope. Compound microscopic slide view were confirmed the species was *Colletotrichum capsici*. In addition, on the blotter paper its colony was white to grey with dark green center. Reduction rate due to Anthracnose disease were 10.94% stem length and plant height 12.69% (Table 6) respectively. Swagatika *et al.* (2015) also isolate and identified *C. capsici* on gerbera plant. Another study of Mahadevakumer *et al.* (2019) identified an association of Anthracnose disease also confirmed by re-isolation and identification. Besides Yeasmin and Shamsi (2013) also reported several species of *Colletotrichum* were associated with gerbera disease in Bangladesh.

Stem rot disease caused by *Fusarium oxysporum* (Photograph 14) were identified from the infected shoot and root samples collected from BADC, Gazipur (16.67%, DI) and Narayanganj (11.11%, DI) (Table 2). From the mycelial growth and conidial study

microconidia and macroconidia were found and the morphology of the pure culture was white cottony growth with irregular margin. *Fusarium oxysporum* was well represented among the rhizosphere microflora. This saprophytic fungus is well known for stem rots, root rots including wilts. *F. oxysporum* was isolated from vascular tissues of infected gerbera plants and conducted pathogenicity test by inoculating on healthy gerbera plants which developed wilt symptoms and vascular discoloration in the roots, crown and veins Garibaldi *et al.* (2007). Minuto *et al*. (2007) also found *F. oxysporum* from infected gerbera plants. On the other research of Garibaldi *et al.* (2008) found wilt diseases on gerbera in the several greenhouse *Fusarium sp.* was the causal pathogen. According to Kaewruang *et al.* (1988) and Minuto *et al*. (2007), F. oxysporum found as strong pathogenic association with gerbera.

Sclerotinia wilt disease was caused by *Sclerotinia sclerotiorum* (Photograph 11) found on BADC nursery, Gazipur. Stem length (34.22%) and plant height (30.67%) (Table 8) reduction were recorded from the field due to Sclerotinia wilt diseases.

The Mosaic, a viral disease of gerbera was recorded in Savar, Narayanganj and Gazipur. The Symptoms are mostly occur in leaves. Irregular leaf molting (light and dark green or yellow patches) (Photograph 15). Plants are often dwarfed and flowers are fewer than usual, deformed and stunted. The highest disease incidence was recorded in Gazipur (50%) and lowest in Savar (8.34%) (Table 2). No Mosaic symptoms were recorded in Agargaon area. On an average, reduction rate due to Mosaic disease on gerbera were also recorded and found 12.89% plant height reduction and 24.43% and 34.78% reduction in leaf length and leaf width (Table12) respectively. Verma *et al.* (2004) and Verma and Singh (1980) also found Mosaic disease on gerbera. Additionally Kitajima (1988)

reported that virus particle affecting ornamental plants like hibiscus, dahlia, daisy and periwinkle.

Another viral disease, Leaf curl of gerbera (Photograph 16) was observed in gerbera plant. The leaf curl of gerbera was only found in Gazipur and disease incidence was recorded 33.33% (Table2).

Extremely limited information available about the prevalence of pathogenic diseases in flower crops specially in gerbera of Bangladesh. Besides, as the capital city of a developing country and main economic zone for the floriculture industry in and around Dhaka was choosen for this study. Therefore, the study was conducted to survey in different nursery and identified and recorded the pathogen associated with gerbera plants.

CHAPTER VI

SUMMARY AND CONCLUSION

Gerbera is one of the most attractive cut flower throughout the world. In our country it also become popular among all types people for its colorful look. The flowers are suit very well in different floral arrangements. Now a days many of diseases have been observed on gerbera which limits its quality and production. But there is very few research work regarding the diseases of gerbera in Bangladesh. Considering this fact, the present investigations were carried out on various aspects to generate scientific information on pathological problem.

A survey was done in and around Dhaka for observation of diseases of gerbera. Based on survey and symptomological study many fungal and viral disease of gerbera were identified. The production of cut flowers of gerbera under the protected cultivation is threatened by many fungal diseases such as Alternaria leaf spot (*Alternaria alternata*), Cercospora leaf spot (*Cercospora* sp.), Anthracnose (*Colletotrichum capsici*), Blight (*Curvularia geniculata*), Sclerotinia wilt (*Sclerotinia sclerotiorum*), Gray mold (*Botryti*s sp.), Powdery mildew (*Erysiphe* sp.), Stem rot (*Fusarium oxysporum*) and the viral diseases such as Mosaic (Mosaic virus), Leaf curl (Leaf curl virus). The survey revealed that the most common disease in nurseries was the Alternaria leaf spot. Besides, gerbera plants were also affected with Cercospora leaf spot, Anthracnose, Gray mold, Stem rot, Powdery mildew, Sclerotinia wilt, Mosaic and Leaf curl diseases in moderate to severe form at all the locations surveyed.

Alternaria leaf spot is characterized by development of brown, small dots which gradually enlarged to form brown to black concentric rings. Anthracnose includes reddish to black tiny spore on stem. Blight consists of brown, oval to circular spot, later the spot emerged and necrotic lesion were observed on leaves. Cercospora leaf spot disease consists of small round water soaked lesion and later those spots coalesced and blighted the entire leaf. Sclerotinia wilt disease produces white, fluffy mycelium growth on infected area of gerbera. Powdery mildew symptoms consist of white powdery looking mycelium and spores mostly seen upper side of leaves and leaf may also become distorted, wilted and eventually blighted. Gray mold includes brown spots on petals that increase in size over time. Stem rot includes yellowing and drooping lower leaves, successive leaves yellow, wilt and die and if main stem is cut, dark brown streaks seen running lengthwise. Mosaic symptoms include chlorotic mottle and vein yellowing on the leaves and deformed flowers and stunting. Plant with Leaf curl disease showed typical symptoms of vein thickening, severe inward curling and a reduction in leaf size and stunting.

Tissue isolation, compound microscopy and stereomicroscopic examination and culture preparation were done to identify the associated causal organism. Conidia of *Alternaria alternata* were dark, short beaked, multicelled and muriform and borne at the tip of conidiophores singly or in chain (under microscope) and produce chain like conidia (under stereomicroscope). In case of *Cercospora* sp., conidiophores were septated, dark colored structures coming out in tuft from stromata and conidia were hyaline ,obclavate (under microscope). *Colletotrichum capsici* was identified by dark brown colored globus and saucer shaped acervuli with numerous number of setae and conidia were

hooked shaped (microscopic view). Conidia of *Curvularia geniculata* were smooth-walled, curved, 4 septated, middle cell usually the largest, the two septa at the end and middle were dark color, end cells comparatively light color, basal cell truncate or round (microscopic view) and pure culture of C*urvularia geniculata* was black to brown in color. Conidia of *Botrytis* sp. were globuse, hyaline and non septate (under microscope) and produced whitish cottony colony on PDA media within 7 days. The color of conidia of *Erysiph*e sp. was hyaline and thin-walled and the shape varied from oblong to cylindrical. *Fusarium oxysporum* produces three types of spores such as micro-conidia, macro-conidia and chlamydospores (under microscope) and produced white to pink mycelium on PDA media within 7-9 days.

For confirmation of *Curvularia geniculata* and *Erysiphe* sp. which cause blight and powdery mildew disease respectively, pathogenicity test were done on gerbera plant following Koch's prostulate method. After inoculation of pathogen into plant, the inoculated plant showed symptoms 7-9 days after inoculation. Whereas the uninoculated plant did not show any symptoms. That proved the pathogenicity of *Curvularia geniculata* and *Erysiphe* sp.

As gerbera is not main crop in Bangladesh, research work regarding this crop is very few. Our study only focus the survey, identification, isolation of diseases and their pathogenicity associated with gerbera in and around Dhaka. Further study should be done in molecular basis for identification of advance species of causal organism and their management to prevent the diseases including more diversified area and location countrywide. So our entrepreneur and farmer and whoever deal with gerbera production to their livelihood will be benefitted and secured their future.

CHAPTER VI

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APPENDIX

Department of Plant Pathology

Sher-e-Bangla Agricultural University

Plant Disease Survey for Gerbera

Name of grower/famer: **Date of data collection:**

Address: Village: **Union:** **Upazilla:** **District:**

Host common name: **Scientific name:** **Age of plant/crop**:

Disease name/ symptoms	% stem length reduction	% leaf length reduction	% leaf width reduction	% Flower size reduction	Total plant inspected	Disease symptoms found in inspected plant	% disease incidence
Alternaria leaf spot							
Cercospora leaf spot							
Anthracnose							
Blight							
Gray mold							
Sclerotinia wilt							
Powdery mildew							
Stem rot							
Mosaic							
Leaf curl							

Symptomological Study

Symptoms	Alternaria leaf spot	Cercospora leaf spot	Anthracnose	Blight	wilt	Stem rot	Gray mold	Powdery mildew	Mosaic
Size									
Shape									
Margin									
Yellow halo									
Appearence									
Scattered									
Surface of leaves									
Shunken/ raised									
Sign									
Age of leaves									
others									
Figures									

Name and Signature of Surveyor

Date:

Name and Signature of Supervisor

Date: