



Survey and Identification of Pathogenic Diseases Affecting Gerbera Plants

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Abstract

This comprehensive study aimed to investigate the causal organisms, disease incidence and pathogenicity of microorganisms affecting gerbera plants in the Dhaka zone of Bangladesh. Nine sites of Agargaon, Savar, Gazipur, and Narayanganj were surveyed to identify the diseases based on symptomological studies. Diseases caused by fungi including cercospora leaf spot (*Cercospora* sp.), alternaria leaf spot (*Alternaria alternata*), sclerotinia wilt (*Sclerotinia sclerotiorum*), anthracnose (*Colletotrichum capsici*), powdery mildew (*Erysiphe* sp.), blight (*Curvulariageniculata*), graymold (*Botrytis* sp.), stem rot (*Fusarium oxysporum*), and viral diseases such as leaf curl (*Leaf curl virus*) and mosaic (*Mosaic virus*) were identified. Among all the identified diseases, the highest disease incidence (66.67%, Gazipur) was exhibited by alternaria leaf spot and the lowest disease incidence (5.56%, Narayanganj) was shown by graymold. The rate of reduction of plant height due to cercospora leaf spot, alternaria leaf spot, blight, anthracnose, graymold, sclerotinia wilt, stem rot, powdery mildew, mosaic, and leaf curl diseases was 28.1%, 28.42%, 12.47%, 12.69%, 28.36%, 30.67%, 19.14%, 28.08%, 12.89%, and 22.06%, respectively. The rate of reduction of other plant parts of gerbera was also recorded. To confirm pathogenicity, *Curvulariageniculata*, and *Erysiphe* sp. were tested on healthy gerbera plants following Koch's postulates. The plants exhibited the disease symptoms of *Curvulariageniculata* and *Erysiphe* sp. after 9 and 6 days of inoculation, respectively, while the control plants remained symptom-free. Thus, the pathogenicity of *Curvulariageniculata* and *Erysiphe* sp. was confirmed.

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Introduction

Gerbera plants (*Gerbera jamesonii*) belong to the Asteraceae family and are highly valued for their vibrant and diverse range of colours, making them a beloved choice for floral displays and used as cut flowers or decorative garden flowers widely (Kanwar and Kumar, 2008). Some cultivars often have very attractive bicoloured flowers and the plant was named after Traugott Gerber, the German naturalist and botanist. Gerbera is also known as African Daisy, Barberton Daisy and Transvaal Daisy (Nagrle *et al.*, 2013). Gerbera is one of the most charming and demanding cut flowers, which are cultivated under protective conditions in Bangladesh. It is ranked fifth among the top ten cut flowers and is considered one of the most valuable and promising cut flowers next to the rose (Saikia and Talukdar, 2017). Gerbera is used for the manufacturing of perfumes by extracting essential oils (Mohanty, 2014). Besides, these flowers are also used for flavoring food and for decoration.

In Bangladesh, large-scale commercial cultivation of gerbera started in the mid-eighties in Jessore district when commercial floriculture flourished on account of its economic potential (Sultana, 2003). Dhaka, the capital city of Bangladesh, is a significant hub for Gerbera traders and users, where reportedly around 1600 retail flower shops are located. Bangladesh exports a large amount of ornamental and cut flowers to the world market without missing a year (Mou, 2012). Gerbera is very popular and has a great demand in markets, but they are prone to various pathogenic diseases such as leaf blight, leaf spot, gray mould, anthracnose, soft rot, root rot, bacterial leaf spot, powdery mildew, etc., which can significantly affect their growth and quality (Bose and Yadab, 1989).

Understanding the prevalence, identification of causal organisms, disease incidence, and confirmation of pathogenicity is crucial for effective disease management strategies for Gerbera cultivation. It aims to conduct a comprehensive survey to evaluate the range of diseases affecting gerbera plants, their respective incidence rates and pathogenicity confirmation. Accurate identification of causal

organisms is vital for effective disease management strategies. The study employed symptomological observations and laboratory analysis to identify the pathogens responsible for the observed diseases. In addition to identifying the diseases and causal organisms, the study also aimed to determine the incidence rates of each disease. By quantifying the disease incidence, the researchers can assess the severity and impact of specific diseases on gerbera plants in the Dhaka region.

The findings of this study will contribute to a comprehensive understanding of the pathogenic diseases affecting gerbera plants in the Dhaka region. By identifying the causal organisms, determining disease incidence rates, and confirming pathogenicity, the research will provide valuable insights for developing effective disease management strategies, ensuring the health and productivity of gerbera crops. Consequently, the present work was focused on assessing the prevalence of pathogenic diseases and identifying the causal organisms associated with these diseases in the Dhaka region.

Methodology

Survey and sample collection sites

A total of nine nurseries were surveyed to study different diseases of the gerbera plants surrounding the Dhaka region (Table 1). Infected leaves and other plant parts that exhibited different types of typical symptoms of diseases were collected from the survey areas. The diagnosis was carried out at the Disease Diagnostic Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

Identification of disease

Symptoms of different pathogenic diseases of gerbera plants were studied and marked out in the field and laboratory conditions based on visible signs of infection, the pattern of symptoms, physical abnormalities, morphological appearances, and diagnostic tests of infected plant parts. Morphological characteristics of the causal organisms were studied under both compound and stereomicroscopes.

Isolation and pure culture preparation of the pathogen

The infected plant parts that showed the signs and symptoms of diseases were aseptically cut into small pieces and thoroughly washed in running tap water, and after that, the surface was sterilized with 3% Clorox for 30-60 seconds and washed thrice in sterile distilled water. The surface sterilized cut pieces of stem and leaf were then placed into each plastic petri dish containing blotter paper and incubated at room temperature for 5-7 days. After the incubation period, the samples were investigated under a stereomicroscope to identify the pathogens. The fungi were filed by observing their growth characteristics on the infected plant parts following the articles of Ellis (1960), Booth (1971), Sutton (1980), and Mathur and Kongsdal (2003). The mycelial growths from blotter paper were aseptically transferred to sterile Potato Dextrose Agar (PDA) petri plates, where 3 Petri plates were taken for each isolated mycelial growth and incubated at 25±1°C. After 7-9 days of incubation, the pure mycelial cultures of the causal organisms have been observed.

Identification of the pathogens

A bit of fungal hyphae was taken on a clean slide from 15-20 days old culture and examined under a stereomicroscope to study the morphological characteristics. Finally, the morphological characteristics like the colour, shape and size of mycelium, conidiophores, conidia, and branching habit of hyphae were compared with the original description of fungal morphology.

Pathogenicity test

A native variety of gerbera plants (*Gerbera jamesonii*) was used to conduct the pathogenicity test. The healthy plants used for running the test were raised in earthen pots. Plants ranging from sixty to seventy days were first sprayed with distilled water and for the next 24 hours, they were kept covered using polythene bags. To inoculate the plants with the pathogenic fungi, slight injury was made on the leaves of the healthy plants and then sprayed with the suspension containing mycelia and spores of the

identified fungi. In the case of fungi causing powdery mildew disease, the pathogenicity test was performed by placing the white mycelium directly into the healthy leaf from the infected one. Only sterile distilled water was sprayed on the control plants.

After completion of spraying, all of the plants were enveloped with polythene bags and placed inside a protective glass house. Observation of the occurrence of disease symptoms was recorded regularly.

A completely randomized design (CRD) comprising three replications was followed to carry out this experiment.

Assessment of plant height reduction percentage

The measurement of plant height both in healthy and infected gerbera plants was taken by centimeter scale. The percentage of reduction of plant height was attained by using the following formula:

$$\text{Plant height reduction \%} = \frac{\text{Healthy plant height} - \text{Infected plant height}}{\text{Healthy plant height}} \times 100$$

Assessment of percent disease incidence

The plants that showed symptoms typical of the infections caused by pathogens were considered diseased plants. The number of infected plant units and the total number of inspected plant units were counted. For different diseases, disease incidence was estimated as a percentage of the individual plant expressing typical symptoms and signs of disease in field conditions. In this manner, the percentage of disease incidence in all locations was calculated during the survey. Then, disease incidence was attained by using the following formula (Greer and Webster, 2001):

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

Statistical analysis

The data were analyzed using the statistical software Statistix 10, by subjecting them to one-way analysis of variance (ANOVA). The comparisons of mean were conducted by using Fisher's Least Significant Difference (LSD) test, where $P \leq 0.05$ was considered significant.

Results

Survey on disease incidence

The survey revealed alternaria leaf spots as the most common disease of gerbera plants in the nurseries. Besides, these plants were also affected with anthracnose, cercospora leaf spot, stem rot, grey mould, sclerotinia wilt, powdery mildew, mosaic and leaf curl diseases in moderate to acute form at all the surveyed locations (Table 2).

Study on the symptomology of plant diseases

During the survey, the symptoms observed to identify the causal organisms responsible for many diseases in gerbera plants were recorded which is shown in Table 3. and Fig.1.

Study on cultural characteristics of the causal organism

Alternaria alternata

The pure culture of *A. alternata* was obtained within 7-9 days of incubation on PDA media (Fig.2). The mycelium was short in size, hyaline, septed and branched. The conidiophore was short in size, coloured, septed and bore several conidia at the tip.

Under a compound microscope, conidia were dark in color, short-beaked, muriform shaped, multi-celled and borne at the tip of conidiophores either singly or in chains. Under the stereomicroscope, a chain-like structure of conidia was observed (Fig.3).

Cercospora sp.

Mycelium was well-developed, slender, septed, branched, intercellular, and brown in color. Conidiophores were dark in color, septed and arise in tufts from stomata. Conidia were hyaline or pale yellow, obclavate, and each conidium left a small scar at the place of its attachment on liberation from conidiophore (Fig.3).

Colletotrichum capsici

The colony of *C. capsici* was white to grey with a dark green centre on the blotter paper. The fungus produced dark brown coloured globus and saucer-shaped acervuli containing numerous setae. Conidia and conidiophores were born in acervuli. The conidia produced from acervuli were hooked-shaped, having an oil globule at the centre of the spore. Under the stereomicroscope, a thorn-like structure was recorded (Fig.3).

Table 1. Nurseries in Dhaka region for survey and pathogenic diseases affecting gerbera plants collection.

Location	Sl. No.	Name of Nurseries
Agargaon	1	Mayer Doa Nursery
	2	KrishibidUpakaran Nursery
	3	Sobuj Bangla Nursery
Savar	4	Rangdhanu Nursery
	5	Ananda Nursery
Narayanganj	6	Sharif Nursery
	7	Mukta Nursery
	8	Sopno Jatra nursery
Gazipur	9	BADC nursery

Curvularia geniculata

The pure culture of *C. geniculata* was obtained within 7 days of incubation on PDA media (Fig.2). The fungus formed dark, long, straight or flexuous conidiophores either single or in groups carrying dark, shiny conidia. Conidia were curved, smooth-walled, 5 celled, the middle cell was the largest, the two septa at each end close to each other, basal cell truncated or

round, the middle cell was dark brown in colour, end cells comparatively light brown in colour (Fig.3).

Sclerotinia sclerotiorum

Mycelium formed a dense network of hyaline branches. These thread-like structures were seen throughout the infected plant tissue (Fig.3).

Botrytis sp.

The pure culture of *Botrytis* sp. produced a whitish cottony colony within 7 days of incubation on PDA media (Fig.2). A grey mass of spores was observed.

Thread-like branched hyphal structures were observed with brown tree-like conidiophores. Globose-shaped conidia were seen, which were hyaline and aseptate (Fig.3).

Table 2. Survey on disease incidence (DI) of gerbera plants grown in the Dhaka zone.

Sl. No.	Disease Name	Causal Organism	Percentage of disease incidence (DI) in different locations			
			Agargaon	Savar	Narayanganj	Gazipur
1	Alternaria leaf spot	<i>Alternaria alternata</i>	38.89 a	58.34 a	27.78 b	66.67 a
2	Cercospora leaf spot	<i>Cercospora</i> sp.	22.23 b	-	33.33 a	50 b
3	Blight	<i>Curvulariageniculata</i>	11.11 d	-	16.67 c	50 b
4	Anthracnose	<i>Colletotrichum capsici</i>	-	-	-	33.33 c
5	Stem rot	<i>Fusarium oxysporum</i>	-	-	11.11 d	16.67 d
6	Gray mold	<i>Botrytis</i> sp.	16.67 c	-	5.56 e	33.33 c
7	Sclerotinia wilt	<i>Sclerotinia sclerotiorum</i>	-	-	-	16.67 d
8	Powdery mildew	<i>Erysiphe</i> sp.	11.11 d	8.34 b	-	16.67 d
9	Mosaic	<i>Mosaic virus</i>	-	8.34 b	16.67 c	50 b
10	Leaf curl	<i>Leaf curl virus</i>	-	-	-	33.33 c

Erysiphe sp.

The conidia were hyaline in color and thin-walled. The shape of the conidia was observed as oblong in the young stage and cylindrical in the matured stage (Fig.3).

the fungus, namely micro conidia, macro conidia, and chlamydospore. The micro and macro conidia were born externally on hypha-like conidiophores. Chlamydospores were formed from normal hypha, which undergone increased growth and thickening of their cell wall (Fig.3).

Fusarium oxysporum

The pure culture of *F. oxysporum* was obtained within 7 days of incubation on PDA media (Fig.2). The color of mycelium was delicate white to pink and often purple. Three types of spores were produced by

Mosaic virus

The mosaic virus was identified as the causal organism of the mosaic disease of gerbera plants by observing its symptoms only (Table 3).

Table 3. Study on the symptomology of identified diseases in gerbera plants.

Disease Name	Infected Parts	Symptoms observed during the survey			
		Size	Shape	Colour	Texture & Distribution
Alternaria leaf spot	Leaves, flowers	Small-medium	Round or irregular	Brown to dark with concentric ring pattern	Water-soaked, corky lesions scattered; several spots coalesced; flowers distorted; plant development suppressed
Cercospora leaf spot	Leaves	Small-large	Circular, oval	Reddish brown	Smooth or slightly sunken lesions; coalesced together, found short holes on spots
Blight	Leaves, stem, flower	Large	Irregular patches and streak	Yellow to brown	Lesions start from tip to down; necrosis occurred; flower reduced
Anthracnose	Leaves, stem, flower	Small	Circular, irregular	Rusty dark brown	Scabby lesion on stem; occurred necrosis; unbroken, raised lesions margin; coalesced together
Stem rot	Shoot, root	-	-	Yellowing leaves	Water-soaked, discoloured, mushy, soft stem; dropping lower leaves; stunted plant growth
Grey mould	Any part but most in tender tissue	Large	Irregular	Grey to brown	Water-soaked, petals withered and blighted, dieback, presence of black resting spores, petals matted and stuck together
Sclerotinia wilt	Stem, leaves, flower	-	-	White mould, brown lesion	Fluffy mycelial growth; wilted leaves; softening stem; eventually lead to plant death
Powdery mildew	Any part	Small-large	Irregular	White spore, yellow leaves	Fluffy, powdery appearance of spores; leaves withered; flower distorted; plant stunted
Mosaic	Leaves	-	Irregular	Yellow patches or streak	Leaf curled, puckered and stunted; veins lighter; flowers deformed; plant dwarfed
Leaf curl	Leaves	-	-	Yellowing new leaves	Downward rolling and crinkling leaves; leathery and brittle older leaves; severe stunted plant

Leaf curl virus

Leaf curl virus was identified as the causal organism of the leaf curl disease of gerbera plants by monitoring the symptoms only (Table 3).

Plant height reduction percentage

The gerbera plant height reduction significantly varied based on different disease infections (Table 4).

The highest plant height reduction was observed in sclerotium wilt disease condition (30.67%), followed by alternaria leaf spot (28.42%), graymold (28.36%), cercospora leaf spot (28.1%) and powdery mildew (28.08%). However, the lowest plant height reduction was observed in blight disease (12.47%), which is statistically similar to anthracnose (12.69%) and mosaic (12.89%) diseases.

Table 4. Assessment of plant height reduction percentage of gerbera plant due to disease infection.

Sl. No.	Name of the disease	Plant height		
		Infected plant (cm)	Healthy plant (cm)	Reduction (%)
1	Alternaria leaf spot	27.2 g	38 e	28.42 b
2	Cercospora leaf spot	30.2ef	42 c	28.1 b
3	Anthracnose	33 cd	37.8 e	12.69 e
4	Blight	36.5 b	41.7 c	12.47 e
5	Sclerotinia wilt	31.2 de	45 b	30.67 a
6	Gray mold	39.4 a	55 a	28.36 b
7	Powdery mildew	29.2efg	40.6 cd	28.08 b
8	Stem rot	28.3fg	35 f	19.14 d
9	Mosaic	34.5bc	39.6 de	12.89 e
10	Leaf curl	32.5 cd	41.7 c	22.06 c

*Pathogenicity test**Curvularia leaf blight*

Among the inoculated plant, (3 inoculated, 1 infected) 33.33% initially developed pale yellow to light brown

spot-like symptoms at 9 days after inoculation (DAI) and matured in 25 (DAI). The symptoms gradually increased in size with every passing day. No such symptoms were observed in the control plant (Fig.4).

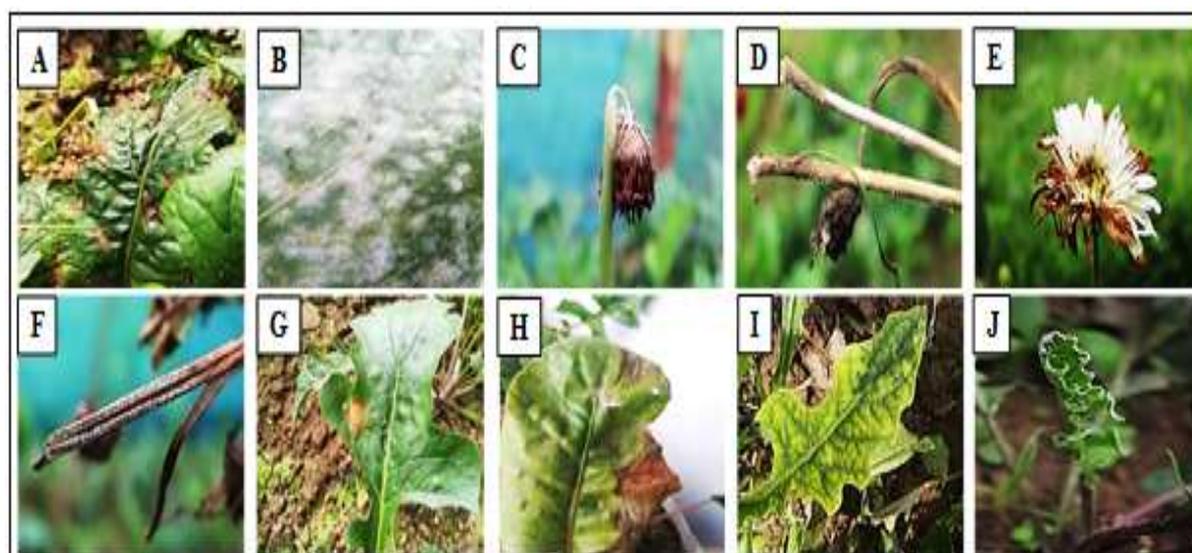


Fig. 1 Symptoms of different diseases observed during the survey of Gerbera plants in the Dhaka zone; (A) Alternaria leaf blight, (B) Powdery mildew, (C) Stem rot, (D) Sclerotium wilt, (E) Grey mold, (F) Anthracnose, (G) Cercospora leaf spot, (H) Blight, (I) Mosaic, (J) Leaf curl.

Powdery mildew

In the case of *Erysiphe* sp., among the inoculated plant, (3 inoculated, 2 infected) 66.67% initially developed powdery mildew symptoms at 6 days after inoculation. No such symptoms were observed in the control plant (Fig.4).

Discussion

The findings of this study present insightful knowledge on the causal organisms, disease incidence, reduction rates in plants, and pathogenicity of diseases affecting gerbera plants in the Dhaka zone.

In accordance with this study, the most prevalent disease in gerbera plants is alternaria leaf spot (66.67% DI, Gazipur) caused by *Alternaria alternata*. Farhood and Hadian (2012) reported the same disease and causal pathogen (small, brown, scattered spots with typical symptoms) which were further supported by Bhat *et al.* (2013); Shamala and Janardhana (2015). Some other researchers also reported an association of *Alternaria* with gerbera plants but with different species (Baker and Davis, 1950; Yeasmin and Shamsi, 2013; Nayeb *et al.*, 2019).

Cercospora leaf spot was identified as a pathogenic disease in some economically important floriculture crops, especially cut flowers like roses and gerbera by Borah (2019), based on both visual inspection and microscopic observation.

Powdery mildew is the most common foliar disease of gerbera daisies, but they are also plagued by graymold (*Botrytis* sp.) and alternaria leaf spot. Brishco and Hausbeck (2018) also reported that gerbera plants are susceptible to root and crown rot diseases, including those caused by *Pythium* *Phytophthora* and *Fusarium*.

Anthracnose disease of gerbera plants was noticed only in BADC nursery, Gazipur (33.33% DI) eventuated on the stem and leaves of gerbera. Numerous brown to black, unbroken, circular, scabby lesions were found on the stem and also on the flower

stalk. The fungus produced dark brown coloured, globuse, saucer shaped acervuli with prominent and numerous setae found under the stereomicroscope. The species was confirmed as *Colletotrichum capsici* from the compound microscopic slide view.

In addition to these, the colony of the fungus growing on the blotter paper was white to grey with a dark green centre. Due to anthracnose disease, the rate of reduction of stem length was 10.94% and of plant height was 12.69%. *C. capsici* in the gerbera plant was also isolated and identified by Swagatika *et al.* (2015).

Another study conducted by Yeasmin and Shamsi (2013) also revealed that several species of *Colletotrichum* were associated with gerbera disease in Bangladesh.

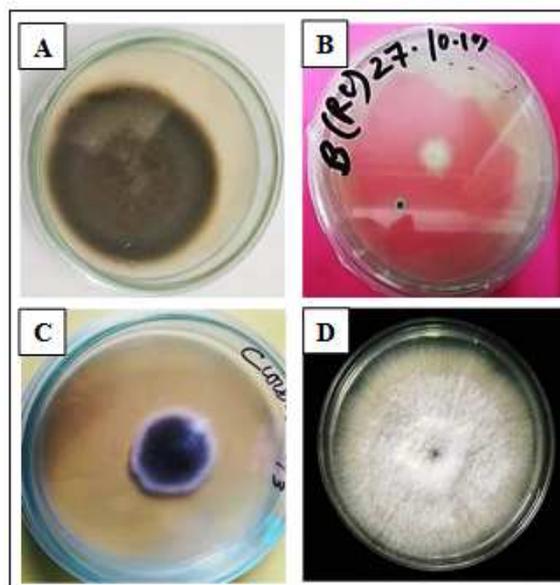


Fig. 2. Pure culture of identified pathogens on PDA Petri-plates; (A) *Alternaria alternata*, (B) *Botrytis* sp., (C) *Curvulariageniculata*, (D) *Fusarium oxysporum*.

Stem rot disease caused by *Fusarium oxysporum* was recognized from the infected shoot and root samples collected from BADC, Gazipur (16.67% DI) and Narayanganj (11.11%). Microconidia and macroconidia were found from the mycelial growth and conidial study and the morphology of the pure culture was white cottony growth with irregular margins. *Fusarium oxysporum* was well represented among the rhizospheric microflora.

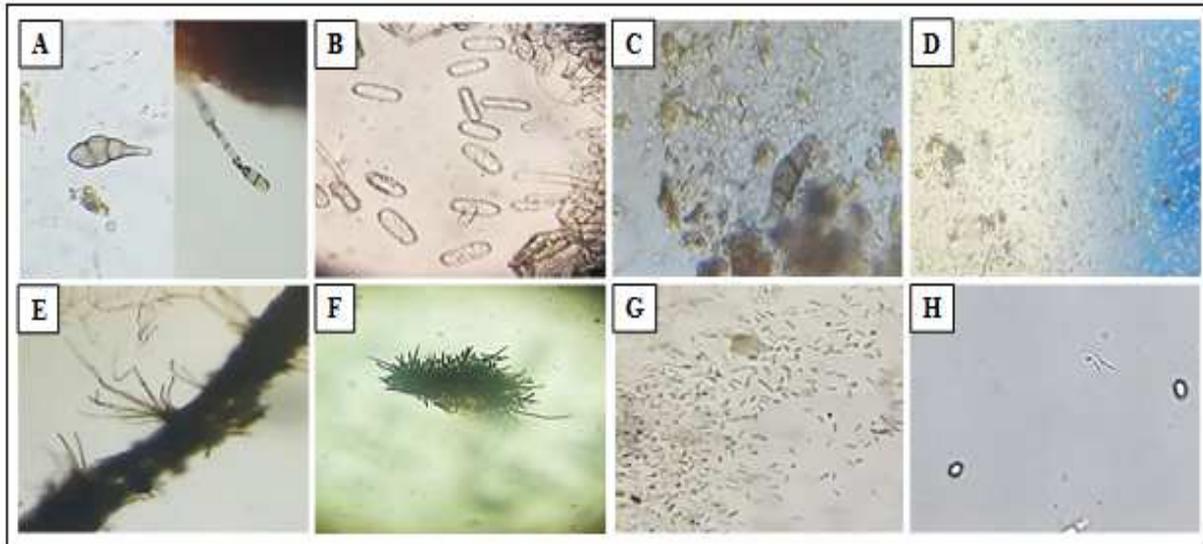


Fig. 3. Structure of different fungal pathogens under a compound microscope (40X); (A) *Alternaria alternate*, (B) *Erysiphe* sp. (C) *Curvulariageniculata*, (D) *Fusarium oxysporum*, (E) *Cercospora* sp., (F) *Colletotrichum capsici*, (G) *Sclerotinia sclerotiorum*, (H) *Botrytis* sp.

This saprophytic fungus is well known for root rot, stem rot and wilt. *F. oxysporum* was isolated from vascular tissues of infected gerbera plants by Garibaldi *et al.* (2007). They also conducted the pathogenicity test by inoculating healthy gerbera plants with the fungus which developed wilt symptoms and vascular discoloration in the roots, veins and crown. Minuto *et al.* (2007) also observed

F. oxysporum in infected plants of gerbera. Another research by Garibaldi *et al.* (2008) showed that *Fusarium* sp. was responsible for wilt disease of gerbera plants in several greenhouses.

According to Kaewruang *et al.* (1988); Minuto *et al.* (2007), *F. oxysporum* was noticed to have a strong pathogenic association with gerbera plants.



Fig. 4. The pathogenicity test of curvularia leaf blight powdery mildew diseases on gerbera plants.

Sclerotinia wilt disease caused by *Sclerotinia sclerotiorum* was observed from BADC nursery, Gazipur. The reduction rate of the stem length (34.22%) and the plant height (30.67%) were recorded from the field due to sclerotinia wilt diseases. Mosaic, one of the viral diseases of gerbera plants, was noticed in Gazipur, Savar and Narayanganj. The Symptoms that occurred mostly in leaves were irregular leaf molting (light and dark green or yellow patches). Plants were often dwarfed, and flowers stunted deformed and fewer than usual. The highest disease incidence was recorded in Gazipur (50%) and the lowest in Savar (8.34%). In the Agargaon area, no mosaic symptoms were recorded. On average, the rates of reduction due to mosaic disease in gerbera plants were also recorded as 12.89%, 34.78% and 24.43% reduction in plant height, leaf width and leaf length, respectively. Verma and Singh (1980) and Verma *et al.* (2004) also reported mosaic disease in gerbera plants. In addition, it was reported by Kitajima (1988) that virus particles affect ornamental plants like daisy, hibiscus, dahlia, and periwinkle. Another important viral disease, named leaf curl of gerbera was observed in gerbera plants. The leaf curl disease of gerbera was only observed in Gazipur and disease incidence was recorded 33.33%.

The reduction rates in plant height due to the identified diseases highlight the severity and impact of the infections. These reductions in plant growth indicated the detrimental effects of the diseases on plant development and vigor. It is important to note that different diseases affect various plant parts to varying degrees. From our study, we can confirm that the most important disease for gerbera plant reduction is sclerotium wilt because it causes higher plant height reduction. While anthracnose, blight and mosaic diseases can be considered as less destructive for plant growth reduction.

Koch's postulate is a standard method for determining the causal relationship between a pathogen and a disease was followed to confirm the pathogenicity of two fungal diseases, blight

(*Curvularia geniculata*) and powdery mildew (*Erysiphe* sp.) The successful fulfillment of Koch's postulates by inducing disease symptoms in healthy gerbera plants inoculated with *Curvulariageniculata* and *Erysiphe* sp. provided conclusive evidence of their pathogenicity. This confirmation contributes to understanding the diseases affecting gerbera plants in the Dhaka zone and can guide future research and disease management efforts.

Conclusion

Gerbera flowers are not only visually stunning with their vibrant colours and intricate petals but also hold great significance in various cultural and symbolic contexts. Throughout history, gerbera flowers have been cherished in the world as well as in Bangladesh for their beauty and have become a popular choice in floral arrangements, bouquets, and even garden landscapes. As gerbera is not the main cut flower of Bangladesh, their allure and versatility make them a beloved choice among flower enthusiasts, florists, and gardeners alike. However, these findings contribute to a better understanding of the diseases affecting Gerbera plants in the Dhaka zone, which can aid in the development of effective management strategies to mitigate their impact and protect Gerbera cultivation in the region. Further research and implementation of proper disease control measures are warranted to ensure the health and productivity of gerbera plants in this area. Thus, farmers, business owners, and anybody else who depend on the production of gerberas for their livelihood are able to profit from it and have a solid future.

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Author contributions

SH proposed, designed and performed the experiment, analyzed the data and prepared the

manuscript. MK performed the experiment and drafted the manuscript. NNT supervised the experiment. All authors have read and permitted the published version of the manuscript.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Approval

Not applicable.

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