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Alternaria Leaf Blight on Gerbera: Assessment and *in vitro* Control in Bangladesh

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ABSTRACT

Background and Objective: Alternaria leaf blight is a major constraint in Gerbera (*Gerbera jamisonii*) cultivation, causing significant yield losses. This study aimed to assess the incidence and intensity of the disease in Jhikargacha, Jashore, Bangladesh, and evaluate the efficacy of botanical and chemical control measures against *Alternaria alternata*. **Materials and Methods:** A survey was conducted across five farms to record disease incidence and intensity before and during the flowering stage. Pathogenicity tests confirmed *A. alternata* as the causal organism. *In vitro* assays evaluated six botanicals (Garlic, Neem, Ashoka, Turmeric, Tulsi, and Peppermint) at 10% concentration and six fungicides (Dithane M 45, Blitox 50 WP, Jazz 80 WP, Ridomil Gold MZ 68 WG, Hexagen 5 EC, and Tilt 250 EC) at 500, 1000, and 2000 ppm against mycelial growth. Data were analyzed using ANOVA in MSTAT-C 5.4, and means were compared via DMRT at $p<0.05$. **Results:** Before the flowering stage, mean disease incidence and intensity were recorded at 35.24 and 18.46%, increasing to 44.86 and 24.11% during flowering. Garlic clove extract exhibited the highest mycelial growth inhibition (97.33%), followed by Peppermint leaf extract (94.07%). Among fungicides, Tilt 250 EC completely inhibited mycelial growth at 2000 ppm, while Dithane M 45 showed 93.33% inhibition at the same concentration. **Conclusion:** Alternaria leaf blight is prevalent in Gerbera cultivation, with increasing severity during flowering. Garlic and Peppermint extracts, along with Tilt 250 EC and Dithane M 45, proved effective in suppressing *A. alternata*, suggesting their potential for integrated disease management.

KEYWORDS

Plant extracts, *Alternaria alternata*, fungicide, disease incidence, disease intensity

INTRODUCTION

Gerbera (*Gerbera jamesonii* L.) is connected to the close family Asteraceae, a well-known cut flower grown worldwide in various weather circumstances. It is famously known as the 'Barberton daisy' or 'Transvaal daisy'. Genus *Gerbera* L., comprises 30 varieties, which are of Asiatic and Southern African-American sources. Among the different varieties, Gerbera jamesonii is the only varieties under farming[1].

Fungal diseases, particularly leaf blight, leaf spot, botrytis blight, powdery mildew, fusarium stem rot, Rhizoctonia crown rot, sclerotium rot, thielaviopsis root rot, white rot, and rust, are prevalent under Bangladeshi climatic conditions, affecting the production quality of cut flowers, especially during early

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growth and flowering stages[2]. The production of gerbera is hindered by many factors, and among all disease is the main problem. Alternaria leaf blight is of serious nature reducing the plant vigor, flower quality, and market value MIrkova[3,4].

While the use of synthetic chemicals has undoubtedly boosted food grain production, achieving self-sufficiency and even becoming an exporter, concerns about environmental degradation and human health have arisen[5]. As a result, organic farming methods have garnered interest, prompting researchers to investigate efficient fungicidal management approaches for diseases attributed to *Alternaria* species[6,7] This study aims to develop an eco-friendly disease management strategy by surveying the incidence and intensity of Alternaria leaf blight in Gerbera and evaluating *in vitro* control methods using plant extracts and chemical fungicides in Jhikargacha Upazila, Jashore District, Bangladesh.

MATERIALS AND METHODS

Experimental site and duration: An extensive survey was conducted in five Gerbera farms in Jhikargacha Upazilla under the Jashore District of Bangladesh to know the status of Alternaria leaf blight of Gerbera. The laboratory experiment was carried out to evaluate the efficiency of some selected botanicals and chemical fungicides against *Alternaria alternata* causing Alternaria Blight of Gerbera from November, 2018 to May, 2019.

Experimental design and materials: The study was conducted in five Gerbera farms in Jhikargacha, Jashore, Bangladesh, to assess Alternaria leaf blight caused by *Alternaria alternata*. Disease incidence and intensity were calculated before and during flowering stages using standard formulas. Pathogen isolation and identification were performed using potato dextrose agar (PDA) medium and morphological characterization. Pathogenicity tests confirmed Koch's postulates. (Maiti, C.K). *In vitro* evaluations included six botanicals (Garlic, Neem, Ashoka, Turmeric, Tulsi, and Peppermint) at 10% concentration were collected locally from available sources, and six fungicides (Dithane M 45, Blitox 50 WP, Jazz 80 WP, Ridomil Gold MZ 68 WG, Hexagen 5 EC, and Tilt 250 EC) at 500, 1000, and 2000 ppm. were obtained from commercially available chemical fungicide shops.

The poisoned food technique was used for efficacy testing using a Completely Randomized Design (CRD) with three replications.

Percent disease incidence: The disease incidence of Alternaria leaf blight caused by *Alternaria alternata* was determined by the survey conducted. Ten plants were selected before the flowering stage and another ten plants were selected during the flowering stage from each of the five farms for recording the incidence of Alternaria leaf blight of Gerbera. Percent disease incidence was calculated by the following formula[8]:

$$\text{Percent disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves examined}} \times 100$$

Percent disease intensity (PDI): The intensity of Alternaria leaf blight was assessed using a 0 to 5 rating scale[9], classifying disease severity based on the percentage of leaf area affected (Table 1). This scale categorizes infection levels into six distinct groups, ranging from no visible infection to severe infection, facilitating standardized assessment and comparison. Each category represents a specific disease severity level, as illustrated in Fig. 1, which visually depicts infected leaves under different categories of disease intensity.

Fig. 1: Visually represents infected Gerbera leaves categorized according to the 0 to 5 rating scale

Table 1: 0 to 5 rating scale for measuring disease intensity

Category	Numerical value	Leaf area infected (%)
I	0	Disease free
II	1	0.1-10.0
III	2	10.1-25.0
IV	3	25.1-50.0
V	4	50.1-75.0
VI	5	>75

Each category represents a specific disease severity level:

- **Category I (0):** No visible infection (healthy leaf)
- **Category II (1):** Very mild infection (0.1-10% of leaf area affected)
- **Category III (2):** Mild infection (10.1-25% of leaf area affected)
- **Category IV (3):** Moderate infection (25.1-50% of leaf area affected)
- **Category V (4):** Severe infection (50.1-75% of leaf area affected)
- **Category VI (5):** Very severe infection (>75% of leaf area affected)

The numerical values assigned to each category quantify disease severity in a standardized manner. These values enable mathematical calculations, such as determining the percent disease intensity (PDI), which provides a comprehensive measure of disease severity across multiple leaves or plants.

Percent disease intensity (PDI) was calculated by using the following formula[10]:

$$PDI = \frac{\sum(n \times v)}{N \times G} \times 100$$

Where:

Σ = Summation
n = Number of leaves in each category
v = Numerical value of each category
N = Total Number of leaves examined
G = Maximum numerical value

Isolation and identification of pathogen: Five Gerbera leaves showing typical blight symptoms were collected for isolation purposes. The infected portion of leaves showing such typical symptoms was cut into small bits and surface sterilized with 0.1% mercuric chloride solution for 30 sec. The bits were washed repeatedly thrice in sterile distilled water and transferred to a sterile petri dish (90 mm diameter) containing 20 m PDA (added 1 mL lactic acid to prevent bacterial growth) After identification, they were transferred to PDA slants and incubated at 25±1°C for further use. The culture was purified by a single spore technique[11]. The pure culture was maintained by repeated sub-culturing at an interval of 21 days for further studies. The stock culture in PDA slants was stored at 4°C in the refrigerator.

The pathogen causing leaf spots in Gerbera were identified through cultural and morphological characterization using a compound microscope (Carl Zeiss Primo Star; Manufacturer: Carl Zeiss AG; Location: Oberkochen, Baden-Württemberg, Germany), along with pathogenicity studies.

Pathogenicity test: The pathogenicity test of the isolated fungus was conducted on 5 detached leaves of gerbera[12]. Healthy leaves were removed from 45 days old plants grown in the Department of Plant Pathology, PSTU, washed with sterilized distilled water, and placed in sterilized petri plates, with their petioles inserted in moist cotton. These leaves were kept for observation at room temperature for 4 days to rule out any latent infection present in the leaves. Inoculation with a spore suspension of the isolated fungus was done by placing four drops of spore suspension at four equidistant places on the upper surface of the leaf, control was also maintained. The leaves were then placed in humid chambers and humidity was maintained by keeping moist cotton in the chambers. These chambers were placed in diffused sunlight in the laboratory at room temperature till the appearance of typical disease symptoms. The Re-isolation was made from the lesions produced by artificial inoculation and a comparison was made with inoculated fungus. The pathogenicity was confirmed after satisfying Koch's postulates[13].

***In vitro* evaluation of botanicals:** Six commonly available plants (Garlic, Neem, Ashoka, Turmeric, Tulsi, and Peppermint) were collected and evaluated against *Alternaria alternata* fungi isolated from Gerbera. Leaf extracts were prepared from 100 g washed leaves of each plant species in 100 mL distilled water and filtered through double-layered cloth. The filtrates obtained were further filtered through Whatman No. 1 filter paper and used as standard plant extracts.

Six plant extracts were evaluated *in vitro* against *Alternaria alternata*, applying the poisoned food technique[14] and using potato dextrose agar as basal culture medium. An appropriate quantity of each standard plant extract was separately mixed thoroughly with PDA medium in the bottle to obtain 10% of each treatment. Then 20 mL of PDA medium was poured into sterile glass Petri dishes. All the treatments and control plates were aseptically inoculated by placing a 5 mm mycelial disc of pure culture of *Alternaria alternata* and all these plates were then incubated at 25±1°C temperature. Observations on the colony diameter of the test fungus were recorded. Percent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Maiti *et al*.[15]:

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where:

I = Percent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicides/botanicals)

***In vitro* evaluation of fungicides:** The efficacy of six fungicides namely; Dithane M 45, Blitox 50 WP, Jazz 80 WP, Ridomil Gold MZ 68 WG, Hexagen 5 EC, and Tilt 250 EC was evaluated *in vitro* at various concentrations against *A. alternata*, applying poisoned food technique[16] and using potato dextrose agar (PDA) as basal culture medium at the rate 500, 1000 and 2000 ppm. All the plates were inoculated aseptically by placing in the center a 5 mm culture disc obtained from actively growing 7 days old pure culture of *A. alternata* and incubated in an inverted position at 25±2°C. Observations on radial mycelial growth/colony diameter were recorded at an interval of 24 hrs and continued till untreated control plates were fully covered with the mycelial growth of the test pathogen. Percent inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula[17]:

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where:

C = Growth of the test fungus in untreated control plates

T = Growth of the test fungus in treated plates

Data analysis: Data were analyzed by Analysis of Variance (ANOVA) using the MSTAT-C 5.4 program and means were compared according to Duncan's Multiple Range Test (DMRT) at a significance level of $p<0.05$[18].

RESULTS AND DISCUSSION

Isolation and Identification of *Alternaria alternata*: The fungal pathogen *Alternaria alternata* was obtained from Gerbera leaves exhibiting characteristic leaf blight symptoms. The conidiophores appeared straight, branched, and pale brown. The conidia, which were light brown, formed long, branched chains and were obclavate to pyriform in shape, with either a short conidial beak at the tip or none at all. Based on these characteristics, the causal agent of Gerbera leaf blight was identified as *A. alternata*. These findings align with the observations reported by Perveen and Bokahri[19].

The microscopic structures of *A. alternata*, including its conidiophores and conidia, are illustrated in Fig. 2, which provides a detailed visual representation of the pathogen under a compound microscope.

Symptomatology: Gerbera leaves infected by *Alternaria alternata* initially developed small, brown, scattered spots that progressively expanded and merged, forming larger lesions. These lesions were oval, circular or irregular in shape, ranging from brown to black, and often displayed concentric rings. Over time, the affected leaves turned brown and withered. Similar symptoms have been documented in studies by Nagrale *et al.*[20].

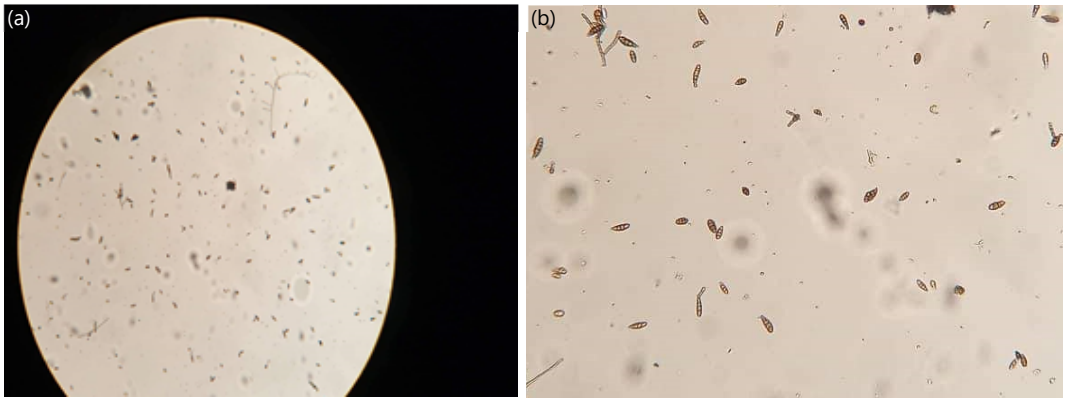

Fig. 2(a-b): Microscopic image of *A. alternata* showing characteristic conidia under a compound microscope

Table 2: Incidence of Alternaria leaf blight of gerbera before the flowering stage

Farm No.	Total No. of leaves collected	No. of infected leaves	Percent disease incidence
1	98	20	20.40
2	82	30	36.59
3	74	30	40.54
4	110	40	36.36
5	90	40	44.44
Total	454	160	35.24

Table 3: Incidence of Alternaria leaf blight of Gerbera during the flowering stage

Farm No.	Total No. of leaves collected	No. of infected leaves	Percent disease incidence
1	77	27	35.06
2	85	36	42.35
3	89	42	47.19
4	95	46	48.42
5	102	50	49.02
Total	448	201	44.86

Table 4: Percent disease intensity (PDI) of Alternaria leaf blight of gerbera

			No. of leaves (n)		n×v	
Category	Numeric value (v)	Leaf area infected	Before flowering	During flowering	Before flowering	During flowering
I	0	Disease free	294	247	0	0
II	1	0.1-10.0	30	35	30	35
III	2	10.1-25.0	35	43	70	86
IV	3	25.1-50.0	70	87	210	261
V	4	50.1-75.0	16	22	64	88
VI	5	>75	9	14	45	70
Total			454	448	419	540

Percent disease incidence (PDI) of Alternaria leaf blight: Percent disease incidence was calculated by counting the total number of leaves and the number of infected leaves on randomly selected plants before flowering (Table 2) and during flowering (Table 3).

Before the flowering stage, the highest 44.44% disease incidence was found in farm No. 5 followed by 40.54, 36.59, 36.36, and 20.40% in farms No. 3, 2, 4, and 1, respectively. The overall disease incidence was 35.24%. For conducting this calculation total of 454 leaves were randomly collected from five farms and 160 of those were found infected (Table 2).

During the flowering stage, the highest 49.02% disease incidence was found in farm No. 5 followed by 48.42, 47.19, 42.35, and 35.06% in farms No. 3, 2, 4, and 1, respectively. The overall disease incidence was 44.86%. For conducting this calculation total of 448 leaves were randomly collected from five farms and 201 of those were found infected (Table 3). This experiment was conducted to compare the percent disease incidence at the flowering stage and before the flowering stage. Similar results were reported about the incidence of leaf blight which ranged from 8.33 to 50.00%. A similar observation was found by Bhat *et al.*[5] in Kashmir. The disease was prevalent in all the flower-growing areas of Kashmir valley.

Percent disease intensity (PDI) of Alternaria leaf blight: The overall scenario about the percent disease intensity of Alternaria leaf blight disease in the village Godkhali, Jhikargacha and Jashore was presented in Table 4:

$$\text{PDI before flowering} = \frac{419}{454 \times 5} \times 100 = 18.458$$

$$\text{PDI during flowering} = \frac{540}{448 \times 5} \times 100 = 24.107$$

Table 5: *In vitro* effect on plant extracts on radial mycelial growth of *Alternaria alternata*

Treatment	Radial mycelial growth (cm)	Percent inhibition
Control	9.00[a]	0.00
Garlic	0.240[e]	97.33
Neem	3.157[b]	64.92
Ashoka	3.190[b]	64.55
Datura	3.967[a]	55.92
Tulsi	2.60[c]	71.11
Peppermint	0.533[d]	94.07

Means followed by the same letter do not differ significantly by DMRT at a 5% level of significance

In this experiment collected leaves were sorted into six different categories according to a 0 to 5 rating scale following different ranges of percentage of infected leaf area (Table 4). Percent disease intensity (PDI) before the flowering stage and during the flowering stage was determined. A total number of 454 leaves were observed to determine PDI. As 18.46% disease intensity was found before the flowering stage and a total number of 448 leaves were observed to determine PDI during the flowering stage. 24.107% disease intensity was found during the flowering stage. The survey program revealed that the percent disease incidence and percent disease intensity were higher at the flowering stage than before the flowering stage. Almost similar results were reported by those who found the highest disease severity of Alternaria leaf blight was 16.00%. A similar observation was found by Bhat *et al*.[5] in Kashmir, who found the overall mean disease intensity ranged from 19.2 to 28.2%.

Pathogenicity test: Inoculated leaves developed brown, small, scattered dots that gradually enlarged and coalesced to form large, oval, circular or irregular brown lesions with concentric rings. These symptoms were observed similarly to the field symptoms. The pathogen was re-isolated from the diseased leaves, and it was morphologically identical to the original isolate, confirming its role as the causative agent of the disease, thus fulfilling Koch's postulates.

***In vitro* evaluation of plant extracts:** The efficacy of six plant extracts viz., Garlic, Neem, Ashoka, Turmeric, Tulsi, and Peppermint were evaluated against *A. alternata* isolated from infected Gerbera plants collected from Jhkargacha, Jashore by poisoned food technique.

Plant extracts significantly reduced the colony growth of the test fungus as compared to the control (Table 5). The lowest (0.240 cm) radial mycelial growth of *A. alternata* was observed in Garlic clove extract with 97.33% inhibition which was followed by Peppermint leaf extract the radial mycelial growth (0.533 cm) with 94.07% inhibition. Tulsi leaf extracts caused (2.60 cm) growth and 71.11% inhibition. The radial mycelial growth of Neem and Ashoka were (3.157 cm) and (3.190 cm), respectively which are statistically similar showing 64.92 and 64.55% inhibition, respectively. Datura showed the least inhibition (55.92%) with maximum mycelial growth (3.967 cm).

Plant extract of garlic was found most effective against the mycelial growth and sporulation of the *A. alternata in vitro* causing leaf spots of papaya[21,22]. Ghule *et al*.[23] reported that *Allium sativum* bulb extracts were most effective against the mycelial growth of *Alternaria carthami* followed by *Azadirachta indica* leaf extract. Extracts of other plants like Neem, Tulsi, and Onion were also found effective against *A. alternata* causing early blight of potatoes. Tapwal *et al*.[24] stated that Neem extract inhibited 58.6% colony growth of *A. alternata*.

***In vitro* evaluation of fungicides:** Effects of six fungicides viz., Dithane M 45, Blitox 50 WP, Jazz 80 WP, Ridomil Gold MZ 68 WG, Hexagen 5 EC, and Tilt 250 EC on radial mycelial growth percent inhibition were assessed against *A. alternata* isolated from Gerbera plants.

Table 6: *In vitro* effect on plant extracts on radial mycelial growth of *Alternaria alternata*

	Radial mycelial growth (cm)			Percent inhibition		
Fungicide	500 ppm	1000 ppm	2000 ppm	500 ppm	1000 ppm	2000 ppm
Dithane M 45	2.0^{d}	1.2^{e}	0.6^{e}	77.77	86.66	93.33
Blitox 50 WP	6.2^{c}	5.6^{c}	3.5^{d}	31.11	37.77	61.11
Jazz 80 WP	7.5^{a}	6.7^{a}	5.0^{b}	16.67	25.55	44.44
Redomil Gold MZ 68 WG	7.3^{b}	6.6^{b}	5.2^{a}	18.88	26.66	42.22
Hexagen 5 EC	6.2^{c}	5.4^{d}	4.0^{c}	31.11	40.00	55.55
Tilt 250 EC	0.4^{e}	0.2^{f}	0.0^{f}	95.55	97.77	100.0
Control	9.0			0.00		

Means followed by the same letter do not differ significantly by DMRT at a 5% level of significance

Results revealed that six fungicides each at three different concentrations viz., 500, 1000, and 2000 ppm were tested *in vitro* by following the poisoned food technique for the mycelial growth of *A. alternata*. The data recorded on the percent inhibition of mycelial growth are presented in Table 6. A perusal of data reveals that the fungicides Tilt 250 EC and Dithane M 45 were superior in inhibiting the growth of fungus over the check. Further, percent mycelial inhibition was increased with an increase in concentrations of the fungicides tested. Irrespective of concentration, Tilt 250 EC was found percent (100%) inhibition at 2000 ppm and was proved to be the most effective fungicide in inhibiting the mycelial growth of *A. alternata* followed by Dithane M 45 (93.33% at 2000 ppm). However, Blitox 50 WP and Hexagen 5 EC proved superior only at 2000 ppm for mycelial inhibition. But they were not proved superior at 500 and 1000 ppm. Minimum inhibition was recorded in Jazz 80 WP at 500 ppm (16.67%) followed by Redomil Gold MZ 68 WG (18.88%). So, the experiment proved the maximum inhibition for the *A. alternata* mycelial growth was shown by Propiconazole (Tilt 250 EC) followed by Mancozeb (Dithane M 45).

These findings identified Propiconazole as the most effective fungicide in suppressing mycelial growth and sporulation of *A. alternata* in pomegranate fruits. Similarly, Kadam *et al*.[25] observed that Propiconazole, at concentrations of 0.1, 0.2, and 0.3%, completely inhibited the mycelial growth of *A. alternata* in pomegranate, outperforming hexaconazole and difenconazole. The efficacy of captan and mancozeb effect in reducing disease severity in sunflowers caused by Alternaria blight. Earlier studies by Jiménez-Reyes *et al*.[26] also highlighted the effectiveness of mancozeb against Alternaria species.

CONCLUSION

The findings of this study indicate that the incidence and severity of the disease were higher during the flowering stage compared to the pre-flowering stage. Under *in vitro* conditions, Tilt 250 EC (a chemical fungicide) and garlic clove extract (a botanical treatment) were found to be the most effective in inhibiting the mycelial growth of *Alternaria alternata*, the causative agent of leaf blight in Gerbera. These results suggest that both Tilt 250 EC and garlic clove extract could serve as effective treatments for managing *A. alternata*-induced leaf blight in Gerbera.

SIGNIFICANCE STATEMENT

Alternaria leaf blight, caused by *Alternaria alternata*, poses a major threat to Gerbera cultivation in Bangladesh. This study highlights the disease's prevalence, showing increased incidence and intensity during the flowering stage. The pathogenicity of *A. alternata* was confirmed, and effective management strategies were evaluated. Among six tested botanicals, garlic extract demonstrated the highest fungal inhibition (97.33%), while peppermint followed closely (94.07%). Additionally, Tilt 250 EC fungicide provided 100% inhibition at 2000 ppm. These findings provide essential insights into controlling *A. alternata*, offering eco-friendly and chemical-based solutions for disease management. This research contributes to sustainable Gerbera cultivation by identifying potent biocontrol agents and fungicides, crucial for Bangladesh's floriculture industry.

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