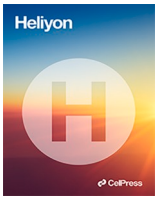
Heliyon 10 (2024) e33165

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Botrytis gray mold of Lilium in Bangladesh: Diagnosis, basic study and control☆,☆☆

Md Mahfuz Alam [a], Khondoker Mohammad Alam [b], Rumana Momotaz [b], Most Arifunnahar [b], Md Mosiur Rahman Bhuyin Apu [c], Shaikh Sharmin Siddique [d,*]

[a] *Crops Division, Bangladesh Agricultural Research Council (BARC), Dhaka-1215, Bangladesh*
[b] *Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701 Bangladesh*
[c] *Floriculture Division, Horticulture Research Centre, BARI, Bangladesh*
[d] *Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh*

ARTICLE INFO

Keywords:
Biological control
Botrytis cinerea (berk.) Cooke
Chemical control
Pathogen identification
In vitro
In planta

ABSTRACT

Bangladesh Agricultural Research Institute (BARI) released two beautiful Lilium varieties in 2020. In the same year the farmers in Gazipur district reported a set of disease symptoms on these flowers and alerted the Plant Pathology Division of BARI. Subsequent investigation confirmed the symptoms as Botrytis gray mold (BGM), caused by *Botrytis cinerea*. The pathogen identity was confirmed through ITS gene sequencing. A series of *in vitro* and *in planta* experiments conducted to understand the symptoms, the optimal growth condition for the pathogen, potential resistant Lilium genotypes, effective chemical treatments and potential of biological control agents to combat the disease. *B. cinerea* exhibited the highest growth in V8 media (88.55 mm) at pH6 (85.32 mm) and temperature between 20 and 25 °C (89.56 mm), and pH6 (85.32 mm). Screening revealed that five oriental-originated genotypes provided lower disease incidence (31.66–41.66 %), and were categorized as moderately resistant to resistant in disease reaction. Six fungicides successfully reduced mycelial growth *in vitro*. Moreover, Ipridione provided the lowest % disease incidence (27.11) and % disease severity (5.33) in the *in planta* nethouse experiment. Therefore, this fungicide was recommended to the farmers initially. Additionally, two fungal biocontrol agents *Epicoccum nigrum* EJS002 and *Trichoderma* ThC003, demonstrated effectiveness in reducing leaf lesion size over control in a detach leaf assessment technique. In conclusion, this study presents BGM of Lilium as a farmers issue for the first time in Bangladesh. It also provides valuable insights into its management, recommending specific chemical fungicides for farmers to use, and two fungal antagonists (*E. nigrum* EJS002 and *Trichoderma* ThC003) as potential disease control agent.

Abbreviations: BGM, Botrytis gray mold; BARI, Bangladesh Agricultural Research Institute; PDA, Potato Dextrose Agar; OMA, Oat Meal Agar; CMA, Corn Meal Agar; NA, Nutrient Agar; CFU, Colony Forming Units.

☆ It is also confirmed that the manuscript has been read and approved by all named authors. Moreover, the order of authors listed in the manuscript has been approved by all named authors.☆☆ The corresponding author declared on the title page of the manuscript will be the contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs\.

* Corresponding author. Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1701, Bangladesh.

E-mail address: siddiquess@bsmrau.edu.bd (S.S. Siddique).

https://doi.org/10.1016/j.heliyon.2024.e33165
Received 30 July 2023; Received in revised form 13 June 2024; Accepted 14 June 2024
Available online 15 June 2024

1. Introduction

The *Liliaceae* family includes around 250 genera and about 3000 species widespread globally [1,2]. The genus *Lilium* comprises around 100 species and is distributed in the northern hemisphere [1]. The Lilium flower, commonly known as 'Lily', is very popular for its scented nature, attractive colours and shape [3,4]. It is considered as one of the most important cut flowers worldwide after roses, chrysanthemums, and tulips [5].

This lucrative flower has recently been introduced in Bangladesh due to its high demand and profitability. During the growing season 2019–20, two distinct varieties (BARI Lilium-1 and BARI Lilium-2) were introduced for their noticeable productivity and attractiveness [6]. However, a disease infection that affects the flower production and quality has been subsequently reported. It is known that the gray mold caused by *Botrytis elliptica* and *B. cinerea* are major constraint for lilies production [7]. This disease could destroy 10–15 % of the plants of a farm after severe infection, resulting in serious loss to the lily producers [8]. Moreover, it can reduce bulb growth and marketability of cut flowers or pot-grown plants [9]. No research has so far been reported to describe Lilium BGM symptoms or any other disease symptoms in Bangladesh. Therefore, a quick investigation was undertaken by the Plant Pathology Division (BARI) to characterize the symptoms, diagnose the pathogen and identify its favourable growth conditions, and control of the pathogen. In addition, this research aimed to examine the resistance of different varieties/genotypes to gray mold, to evaluate the efficacy of available chemical fungicides and potential biocontrol agents against the pathogen. The objective of this research was to provide an effective control method to the growers to reduce their loss during Lilium production.

2. Materials and methods

2.1. Disease diagnosis and basic study of the pathogen

2.1.1. Disease sample collection, symptoms characterisation

Twenty commercial Lilium growing nethouses were visited (2019-20 growing season) to characterize the disease symptoms in the Gazipur (24.0958°N, 90.4125°E) district of Bangladesh following farmers report. Twenty plants/nethouse were randomly checked. A number of leaves, flowers and stems were collected for pathogen isolation and identification. The symptoms were described extensively.

2.1.2. Pathogenic fungal isolation, Purification, characterisation, and Koch's postulate

Pathogenic fungi were isolated using the tissue isolation method [10]. Thirty isolates were cultured as purified single spore cultures. The cultural characteristics of all the single spore cultures (on Potato dextrose agar media) were similar. Therefore, a single colony was randomly selected for further characterisation and Koch's postulate. The pathogen isolate was examined and identified by light microscopy and molecular method (ITS based gene sequencing). Different morphological characteristics, such as mycelium traits, spore shape and size, were observed under a compound microscope (Olympus, BX41, Japan, with a magnification of 20x/40x).

The pathogenicity of the selected isolated fungi was evaluated by inoculating healthy Lilium leaves. Inoculum was prepared from 10 days old pathogen cultures. Five mm mycelial plugs were placed on the leaves wounded by a sterilised needle. Leaves inoculated with PDA plugs served as the control. Both inoculated and control leaves were kept in a humid (80 % RH) chamber for 48–72 h and then transferred to a room temperature (25±2 °C). Afterward, the leaves were monitored to check the symptoms development. Later (approximately 5d after inoculation), the fungus was re-isolated as described. The cultural and morphological characteristics of the selected isolate were recorded to identify the pathogen at the species level.

2.1.3. Sequencing and molecular identification

Two purified single spore isolates were cultured for 5 d in a PDA medium. The mycelia were scraped, dried, and stored at −80 °C for extraction of the Genomic DNA. The frozen mycelia were grind by mortar and pestle. DNA was purified from tissue powder using a genomic DNA extraction kit (Promega, USA). The extracted DNA was amplified with universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCGG-3′) and ITS4 (5′-TCC GTA GGT GAA CCT GCGG-3′). Amplification reaction mixture comprised of DNA template (2 μL), ITS1 (1 μL), ITS 4 (1 μL), PCR master mix (Promega, USA) 12.5 μL, ddH_2O (double distilled water, 9.5 μL), using 25 μL reaction system. The PCR program was as follows: 3 m pre-denaturation step at 94 °C and 15s denaturation step at 94 °C, annealing at 55 °C for 30s, and extension at 72 °C for 30s. After the 35th cycle, the last step was to incubate at 72 °C for 7 m. The resultant product was immediately cooled to 4 °C and resolved in 1.0 % agarose gel electrophoresis. The PCR amplicon was used for Sanger sequencing after cleaning with a PCR clean-up kit (Promega, USA). The nucleotide sequences were analyzed through a blast search at NCBI data base. A phylogenetic tree was constructed using a known sequence from the NCBI data base using MEGA X software [11].

2.1.4. In vitro optimisation of pathogen growth media, pH, and temperature

Different physical parameters, such as preferable growth medium, pH, and incubation temperature for the growth of the pathogen (*B. cinerea* isolate GBLB01 infecting Lilium), were optimized. Five specific growth media for fungus such as PDA, Oat meal ager (OMA), Corn meal ager (CMA), V8 Agar, and Nutrient agar (NA) were tested. In addition, five pH levels such 5, 6, 7, 8, and 9 were tested (on PDA plates) to optimise the pH for growth of the Lillium gray mold pathogen. Finally, five incubation temperatures (15, 20, 25, 30, and 35 °C) were also evaluated for optimum mycelial growth and spore germination. There were five replications per treatment, and the study was repeated once.

2.2. Disease control

2.2.1. Evaluation of released Lilium varieties and genotypes for resistance against BGM by natural infection in the nethouse

Two recently released varieties (BARI Lilium-1 and BARI Lilium-2) and fourteen Lilium genotypes (collected from Floriculture Division, HRC, BARI) were screened for field resistance to BGM in Bangladesh. Among these sixteen genotypes, 11 were Asiatic, and 5 were oriental in origin. All planting materials were diagnosed and confirmed as BGM-free prior to planting. Two consecutive *in planta* nethouse trial was established during January–May 2020 and 2021 at HRC, BARI in sandy loam soil under irrigated conditions. There were fifteen plants for each genotype, planted with a spacing of 40 cm × 30 cm. The genotypes were grown for 5 months. The intercultural operations (weeding, irrigation, insecticide application) were done as required. At three months following planting, the above ground BGM symptoms on shoots, leaves, and stems were noted. Ten plants were randomly selected for recording the disease data. Three leaves of the second whorl from the apex of each plant were selected for disease scoring. The disease was scored according to a 0–5 rating scale from a previous report [12] with some modification; where 0 = no spot or blighted leaves present, 1 = up to 20 % of the leaves/shoots/stems of the plant were blighted, 2 = 21–40 % leaves/shoots/stems of the plants were blighted, 3 = 41–60 % leaves/shoots/stems of the plant were blighted, 4 = 61–80 % leaves/shoots/stems of the plant were blighted and 5 = >80 % leaves/shoots/stems of the plant were blighted. The percent disease incidence (PDI) for each plant was calculated separately according to the following equation:

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

Disease severity for each plant was calculated according to the following equation

$$\text{Percent disease severity} = \frac{\Sigma\,(n \times v)}{N \times G} \times 100$$

Where n = the number of diseased leaves in each category, v = number of values of the category, N = total number of leaves examined, and G = highest grade value.

The tested genotypes were arbitrarily categorized into six different reaction groups on the basis of percent disease severity by using 0–9 scale (Table 1) according to prior references [3,13] with some modifications.

Five plants were selected randomly for determining different parameters like, plant height (cm), the leaf chlorophyll content (1leafplant^{-1}), and it was measured with a SPAD meter [Chlorophyll meter SPAD-502 plus, Konica Minolta INC. Japan]), and flower diameter (1 flower/plant). The flower diameter (cm) was used as an indirect assessment of yield.

2.2.2. Chemical control of Lilium BGM

2.2.2.1. In-vitro fungicidal efficacy of different fungicides against B. cinerea GBLB01. The antifungal efficacy of six fungicides (Table 2) with different active ingredients was tested against the *B. cinerea* GBLB01 *in vitro*. The specific doses of each fungicide were incorporated with the PDA medium separately. PDA plates without any fungicide were considered as the control. All the PDA plates were inoculated with a 5 mm mycelium plug from a 5d old *B. cinerea* (GBLB01) culture. Immediately after inoculation, all plates were incubated at 25 °C in 16 h light, and 8 h darkness. The fungal growth was measured after 2d of inoculation. The fungal growth measurement was continued until the fungal growth of the control plate reached the edges of the PDA plates. Two diagonal measurements were taken on each plate during the mycelial growth measurement, and the mean mycelial growth was calculated. For each fungicide, the percent growth inhibition was calculated according to the following equation:

$$S = \frac{(A - B)}{A} \times 100$$

Where S = Percent of the mycelial growth inhibition, A = mean diameter of fungus colonies on a medium without fungicide (control), and B = mean diameter of fungus colonies on a medium with fungicide (a specific treatment). There were five replications for each fungicide treatment, and this experiment was repeated once.

2.2.2.2. In-planta antifungal efficacy of selected fungicides against BGM of BARI Lillim-1 under nethouse conditions. BARI Lillium-1 was

Table 1
Disease grading according to percentage of disease severity to measure BGM reaction in *Lilium* genotypes during screening in natural epiphytotic condition.

Disease grade	Disease severity%	Reaction	Symbol
0	No infection	Immune	I
1	1–10	Resistant	R
3	10.1–20	Moderately resistant	MR
5	20.1–30	Moderately susceptible	MS
7	30.1–50	Susceptible	S
9	>50	Highly susceptible	HS

Table 2
List of fungicides for the *in vitro* fungicidal efficacy test against *B. cinerea* (GBLB01).

Trade name	Common Name	Dose
Provax	Carboxin + Thiram	2 gmL^{-1}
Amister Top 325 SC	Azoxystrobin + Difenoconazole	1 mlL^{-1}
Autostin	Carbendazim	2 gmL^{-1}
Rovral	Iprodione	1 mlL^{-1}
Folicure	Tebuconazole	1 mlL^{-1}
Cabrio	Matriam + Pyraclostrobin	1 gmL^{-1}

tested for the *in planta* antifungal efficacy of the selected six fungicides in the nethouse during January–May 2020 and 2021 at HRC, BARI. Each fungicide treatment had three replications, twenty plants/replication, with a spacing of 40 cm × 30 cm, and layout in Randomized Complete Block Design (RCBD). The fungicides were sprayed (according to the dose in Table 2) after the first appearance of the disease symptoms. A total of three sprays were conducted at 15d intervals. After 15d of the last spray, the disease incidence and severity were assessed. Ten plants per replication were assessed for disease and other attributes. The disease intensity was assessed according to 0–5 rating scale, and severity was calculated, as mentioned earlier.

The percent disease reduction over control was calculated according to the following formula:

$$D = \frac{A - B}{A} \times 100$$

Where D = Percent disease reduction over control, A = mean disease severity in the control plot, and B = mean disease severity in a specific treatment applied plot. Other attributes like plant height (cm), and flower diameter (cm) were also recorded from randomly selected three plants $replication^{-1}$. The flower diameter was used as an indirect assessment of yield.

2.2.3. Biological control

2.2.3.1. In vitro antifungal efficacy of different biocontrol agents against B. cinerea GBLB01. Seven antagonists (isolated previously from different regions of Bangladesh) were tested against the selected *B. cinerea* isolate *in vitro* (Table 3). The antifungal efficacy of all the antagonists against *B. cinerea* GBLB01 was studied by dual culture method on a PDA plate. Three days old *Trichoderma* isolates (ThB001, ThL001, ThC003, and Tv003) were tested against 5d old *B. cinerea* GBLB01 *in vitro*. A five mm mycelial plug of a *Trichoderma* isolate was inoculated in a PDA plate at a 40 mm distance from the periphery. Simultaneously, a five mm mycelial plug of *B. cinerea* GBLB01 was inoculated at a 60 mm distance from the inoculated *Trichoderma* plug. Later all the inoculated plates were incubated at 25 °C in an incubation chamber. Four separate *Trichoderma* isolates were inoculated separately and incubated accordingly. Alternatively, after the inoculation of *E. nigram* EJS002 (as mentioned above) the plates were incubated for 5 d (as the growth of this antagonist was slow compared to *B. cinerea*). Afterward, the *B. cinerea* GBLB01 was inoculated, as mentioned earlier. The control PDA plates were inoculated with *B. cinerea* GBLB01 only. Radial mycelial growth of the *B. cinerea* GBLB01 in the dual culture and control plates was measured after 48h of inoculation. The mycelial growth was recorded at 24 h intervals up to 7d of inoculation. The percent inhibition of mycelial growth was calculated as mentioned in section 2.6.

Later, two the bacterial antagonists (*B. subtilis* PTB001, and *P. fluorescens* PPR001) were transferred to King's B Medium agar at 28°C for 48h prior to the *in vitro* test. A 5 mm mycelial plug of *B. cinerea* GBLB01 was inoculated as mentioned earlier. Both the *B. subtilis* PTB001, and *P. fluorescens* PPR001 bacterial strains were streaked through the sterile loop needle at the opposite end (60 mm distance) of the *B. cinerea* separately. A five mm PDA plug from 5d culture of *B. cinerea* GBLB01 was used as the control. Afterward, all the inoculated plates were incubated at 25 °C.

The mycelial growth rate and the percent inhibition rates were calculated, as mentioned earlier. There were five replications for each antagonist, and the experiment was repeated once.

Table 3
List of the antagonists isolated from different regions of Bangladesh.

Isolate No.	Name of the antagonist	District	Cultivated plants and trees	Storage condition	Storage temperature	Prior antifungal activity
ThB001	*Trichoderma harzianum*	Norshindhi	Banana	PDA slant	4 °C	*Fusarium oxysporum*, *Sclerotium rolfsii*,
ThL001	*T. harzianum*	Takurgoan	Litchi	PDA slant	4 °C	*Sclerotia sclerotiorum*
ThC003	*T. harzianum*	Gazipur	Bottle gourd	PDA slant	4 °C	
Tv003	*T. viride*	Pabna	Banana	PDA slant	4 °C	
EJS002	*Epicoccum nigrum*	Jamalpur	Rice	PDA slant	4 °C	
PTB001	*Bacillus subtilis*	Pabna	Tomato	Glycerol 30 %	−80 °C	
PPR001	*Pseudomonas fluorescens*	Ranpur	Potato	Glycerol 30 %	−80 °C	

Note: The morphology of all the biocontrol agents were studied previously.

2.2.3.2. In planta antifungal efficacy of different biocontrol agents against B. cinerea GBLB01. Both curative and preventive *in planta* antifungal efficacy were tested in this study. Five days old isolates of *T. harzianum* (ThB001, ThL001, and ThC003), *T. viride* (Tv003), and *E. nigrum* (ESJ002) isolates along with *B. subtilis* (PTB001) and *P. fluorescens* (PPR001) were used for this evaluation.

For the *in planta* curative evaluation, healthy, fully expanded leaves were used as a single replication. All the leaves were cut into 15 cm pieces of leaflets and surface sterilised by NaOCl (2 %) solution for 90 s followed by rinsing thrice in sterilized water. Leaves were air-dried on a paper towel on the laboratory bench and then spread on the upper surface. A 5d old *B. cinerea* (GBLB01) cultured on PDA plates was used for inoculation. The sterilised leaflets were wounded with a sterile needle, followed by inoculation with a 4 mm mycelial plug of the tested pathogen [14]. Immediately after inoculation, the leaflets were placed on a sterilised 20 cm Petri dish and incubated in a humid chamber (a 170x240 $\times$ 80 mm glass box, containing 5 mL distilled water and closed with a tightly fitted lid). The humid chambers were placed in an incubator at 25 $\pm$ 2 °C, 80 percent RH, 16 h light, and 8 h darkness. Afterward, the PDA plug of *B. cinerea* (GBLB01) was removed, and a 5 mm plug of *T. harzianum* (ThB001) was inoculated at the same point of the leaf. There were five leaflets separately re-inoculated with *T. harzianum* (ThB001) PDA plug. Similarly, three other isolates of *Trichoderma* (ThL001, ThC003, and Tv003), and one isolate of *E. nigrum* (ESJ002) were also re-inoculated as a curative study. Whereas for *B. subtilis* and *P. fluorescens* a 50 µl droplet at10^7 colony forming units (CFU) per milliliter were used for re-inoculation.

In the preventive experiment, all the biocontrol agents (BCAs) were inoculated after wounding the leaflets and incubated in a humid chamber, as mentioned earlier. Later, PDA plugs containing biocontrol agents were removed. For *B. subtilis* and *P. fluorescens*, leaves were washed with sterilised distilled water. Finally, the leaves were inoculated with *B. cinerea* (GBLB01) at the same wounded point. There were two controls for both *in planta* curative and preventive experiments. The leaflets inoculated with *B. cinerea* (GBLB01) containing PDA plugs were served as Control 1, and leaflets inoculated with PDA plugs without any pathogen were served as Control 2.

The disease symptoms development was assessed after 2 d of final inoculation by measuring the length and width of diseased symptom lesions that spread beyond the mycelial plugs. The different biocontrol agents were incubated in a separate chamber to avoid cross-contamination. There were five replications per biocontrol agent, and the experiment was repeated once.

2.3. Data analysis

All *in vitro* and *in planta* lab experiments were repeated once. The *in planta* germplasm screening and fungicide trials were conducted twice, separately, in two successive growing seasons (January to May 2020 and 2021). All the experimental data were analyzed using SPSS Statistics v 22. Two repeat experiments were compared through a repeated measure ANOVA (General Linear Model then Repeated measures). When there were no differences ($P \leq 0.05$) observed between repeat experiments, all results were combined and expressed as the mean of all replicates. The differences between treatments were determined by ANOVA. Duncan's multiple range test was used to separate the means.

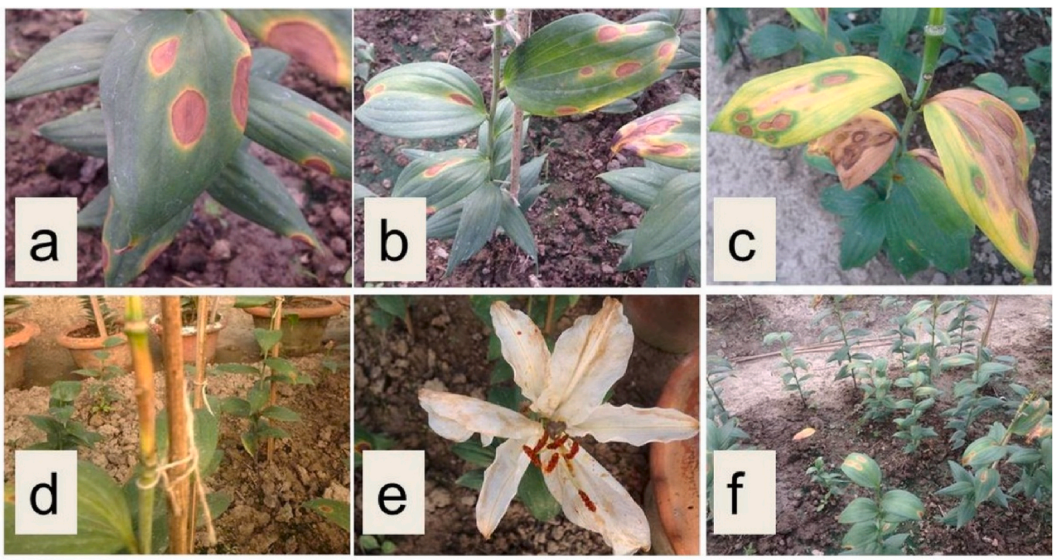


Fig. 1. The symptoms of BGM of Lilium at the field. a and b) initial oval-shaped leaf spot in leaves; c) Several symptoms join together and the leaf became blighted, at the later stage of infection, leaves seem scorched by fire; d) Stem infection and blighted symptoms; e) Flower infection; f) Severely infected field.

3. Results

3.1. Disease diagnosis and basic study of the pathogen

3.1.1. Disease symptoms, cultural characteristics of the pathogen and Koch's postulate

In the fields, disease symptoms were typically visible on the leaves (Fig. 1a–f). The symptoms started as a water-soaked lesion at the early stage. Later the lesions became round, oval, eye or spindle-shaped on the leaves (Fig. 1b). The boundary of the lesion was often covered by chlorotic yellow colour (Fig. 1a and b). With time, the symptoms enlarged and fused together (Fig. 1c). In this stage, the whole leaf became blighted, like scorched by fire (Fig. 1c). In the dry leaves, the symptoms were still prominent, with a grayish lesion covered by a chlorotic greenish-yellow circle (Fig. 1c). The pathogen grew downward towards the base of the plants and caused blight symptoms in the stem (Fig. 1d). The flower petals showed circular necrotic spots that elongated (Fig. 1e) until the whole flowers became blighted and covered with a gray mold. Flower infections could spread towards the basal part of the plants, leading to premature drying and death of entire shoots.

The disease symptoms on Lilium plants was first observed in Gazipur; (23° 99'N, 90° 42′E) of Bangladesh in 2019-20. The disease appeared during mid-January after rising the winter temperature from 17 to 25 °C. The small to medium leaf spot and stem spots were recorded in 13 net-houses. Seven net-houses were completely disease free during Mid January. However, later (last week of March) leaf spots, and infected stem flower^{-1} were recorded at all the net-houses.

The colony of the isolated fungi was creamy white initially but later turned gray with time (Fig. 2 a, and b). Septate hyphae (8–16 μm wide) (Fig. 2 c), with smooth, botryous, ovoid, colourless conidia (6.65–12.75 × 5.55–10.00 μm, the mean value measured from 50 random conidia) (Fig. 2 d and e) recorded during the morphological study. The isolate produced black clump connection on the PDA media (Fig. 2 f) to produce black sclerotia later.

During Koch's postulate, pinpoint water-soaked lesions were established on leaves 72 h after inoculation. Lesions twisted necrotic, increased in size and often merged to form patches within five days (Fig. 3a). Control plant parts did not exhibit any symptoms of the disease. Upon inspection, the characteristics of the disease showed typical symptoms of being infected by gray mold disease and were tentatively identified as *B. cinerea* (Berk.) Cooke. Re-isolation from the artificially inoculated plant parts on PDA consistently generated the inoculated fungus (Fig. 3 b and c). A pathogenic fungus was observed under the microscope and identified to be the pathogen of Lilium gray mold, *B. cinerea* (Fig. 2). All symptomatic characteristics of the artificial inoculations of Lilium gray mold, were similar in appearance to those of the original diseased leaves (Fig. 1) collected from the field.

3.1.2. Sequencing and molecular identification

To identify the pathogen at the species level, genomic DNA was extracted, and PCR amplification was done using universal primers ITS1/ITS4 [15]. The resulting 430 and 441 bp nucleotide sequences of GBLB 01 and GBLB 03, respectively were deposited in Gene Bank (accession No. MN756674 and MN757648, respectively). The nucleotide sequences were BLASTn searched in the NCBI GenB, and the ITS sequence identity of B. cinerea GBLB01 strains MN756674 was 100 % with the sequences of GBLB 01 and GBLB 03 strains

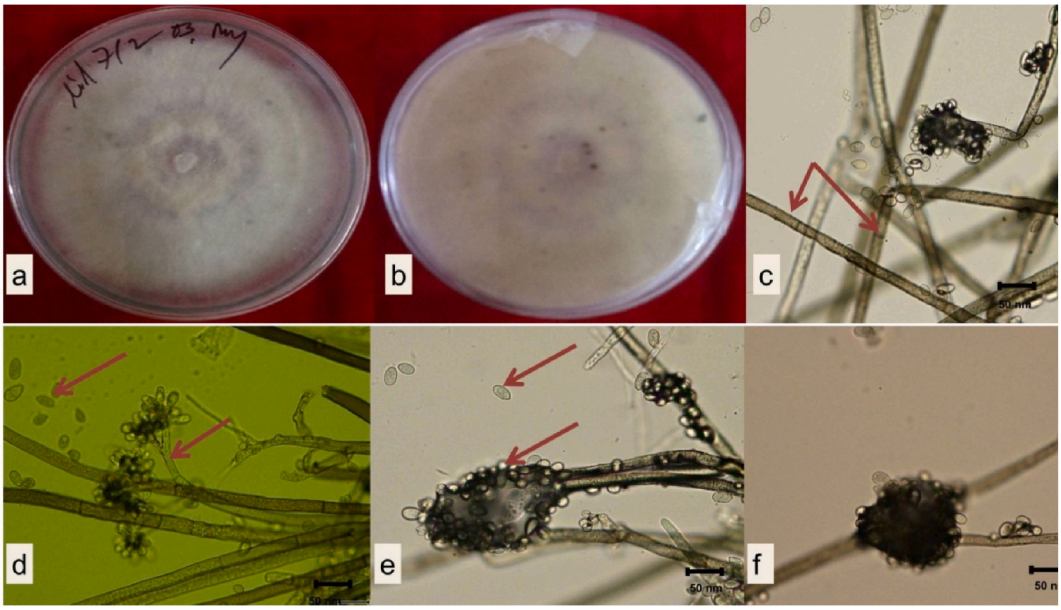


Fig. 2. Morphological characteristics of isolated fungi under a light microscope. a) the front side of the ten days old isolated fungal culture on PDA; b) the reverse side of the ten days old isolated fungal culture on PDA; c) septate mycelia under a compound microscope; d and e) conidiophores with conidia at 20 magnifications under a compound microscope; f) Clump connection of isolated fungi.

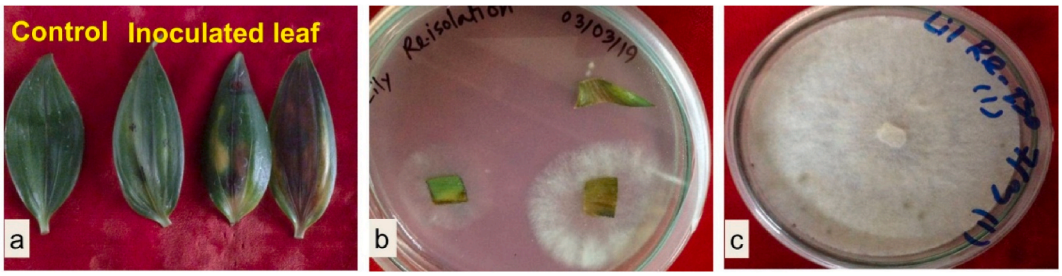


Fig. 3. Koch's postulate to confirm the pathogenicity. a) artificially inoculated leaves showing infection; b) pathogen re-isolation from the artificially infected leaf tissue; c) the front view of a 15 d old re-isolated fungal culture on PDA.

were matched 100 % with the reference sequences B. cinerea KJ685806 and OR544950, respectively. A phylogenetic tree was developed using MEGA11 software; align multiple sequences by ClustalW and analyzed maximum likelihood with 1000 bootstrap values. The phylogenetic analysis indicated that the isolates (GBLB 01 and GBLB 03) clustered with the *B. cinerea* reference strains of GenBank (Fig. 4).

3.1.3. In vitro optimisation of pathogen growth media, pH, and temperature

The highest mycelial growth of *B. cinerea* (88.55 mm) was recorded from V8 agar followed by NA (80.35 mm) and OMA (75.63 mm) media after 5 d of incubation, which is higher than that recorded on PDA (61.77 mm) (Fig. 5a). The isolated *B. cinerea* isolate GBLB01 produced cottony-textured, regular density, and regular mycelial growth on V8 and NA media.

Five different pH significantly affect the growth of the specific *B. cinerea* isolate GBLB01 (Fig. 5b). Among them, pH6 provided the highest colony growth (85.32 mm), after 6 d of incubation (Fig. 5b). The colour, and morphology of the pathogen varied at media with

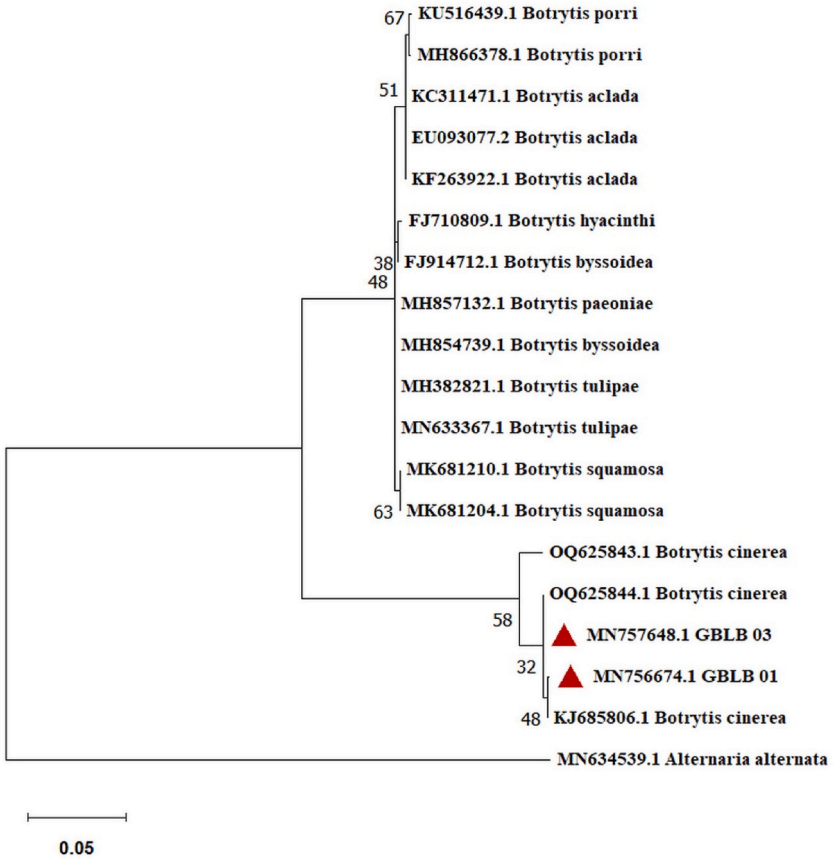


Fig. 4. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [16]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value.

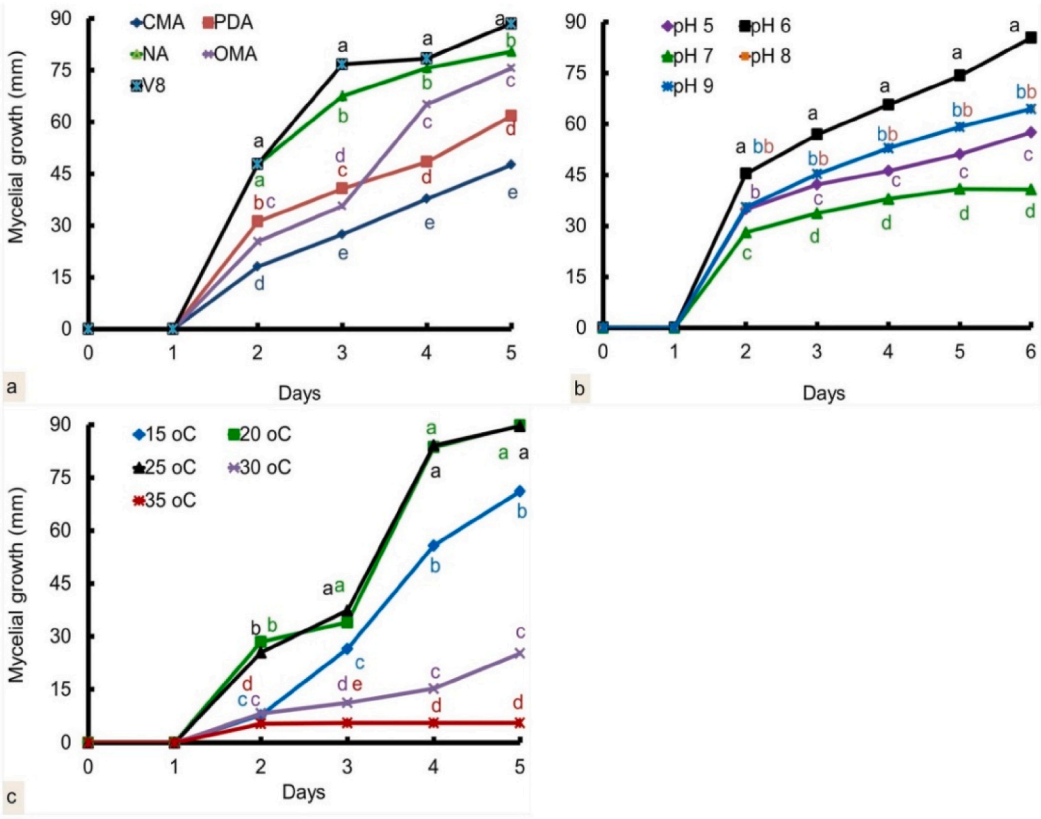


Fig. 5. Effect of different growth media, pH, and incubation temperature on mycelial growth of *B. cinerea* GBLB01 up to 5d of inoculation. a) *B. cinerea* GBLB01 mycelial growth at different growth media. b) *B. cinerea* GBLB01 mycelial growth at different pH. c) *B. cinerea* GBLB01 mycelial growth at different incubation temperatures. Bars indicate the standard error of the mean, and N = 10 (no. of Petridishes).

depending on the tested pH. At pH7 the colonies were dense compared to those developed at pH 6, 8, and 9. The morphology of *B. cinerea* colonies at pH 5 was wavy, round, sticky, and white to gray colour and rose shape. Relatively dense, round, fluffy, border white, center yellowish brown, middle light brown colonies developed at pH6. In addition, irregular round, blackish-gray, fluffy colonies developed at pH7 (Figure not provided).

The mean mycelial growth of *B. cinerea* was significantly influenced by the tested temperatures (Fig. 5c). The highest mycelial growth of *B. cinerea* (90 mm) was recorded at incubation temperatures 20–25 °C on PDA (89.81 and 89.56 mm, respectively) after 5d of

Table 4
Evaluation of BARI *Lilium* varieties/genotypes against the naturally occurring BGM symptoms under field conditions.

Sl no	Variety/Germplasm	Origin	% disease incidence	% disease severity	Disease reaction
1	BARI Lilium-1	Asiatic	58.33 a-e (±6.34)	21.00 de (±2.70)	MS
2	BARI Lilium-2	Asiatic	63.33 a-c (±7.21)	24.00 de (±2.39)	MS
3	L002	Asiatic	66.67 ab (±6.40)	35.67 ab (±4.79)	S
4	L007	Asiatic	58.33 a-e (±6.34)	25.00 de (±3.20)	MS
5	L008	Asiatic	78.33 a (±5.55)	41.33 a (±3.80)	S
6	L011	Asiatic	65.00 ab (±6.15)	26.67 b-e (±3.32)	MS
7	L022	Asiatic	55.00 b-e (±7.37)	20.00 e (±3.13)	MR
8	L025	Asiatic	55.00 b-e (±7.75)	21.33 de (±3.16)	MS
9	L026	Asiatic	60.00 a-d (±7.49)	25.67 c-e (±3.94)	MS
10	L027	Asiatic	70.00 ab (±7.21)	34.67 a-c (±3.77)	S
11	L028	Asiatic	66.67 ab (±6.85)	30.00 b-d (±3.15)	MS
12	L014	Oriental	41.66 c-f (±7.60)	11.00 f (±2.23)	MR
13	L017	Oriental	36.84 ef (±7.16)	8.33 f (±1.59)	R
14	L018	Oriental	31.66 f (±6.61)	6.67 f (±1.53)	R
15	L019	Oriental	33.33 f (±5.92)	8.33 f (±1.59)	R
16	L020	Oriental	40.00 d-f (±6.67)	11.33 f (±1.94)	MR

Note. Data were recorded in the 3rd month after planting. Mean separation was conducted according to DMRT. Numbers inside the parenthesis (±0.00) indicate the standard error of each mean value and N = 20 (total no. of the plant).

incubation (Fig. 5c). Significantly, lower mycelial growth was recorded at 30 °C on PDA (24.87 mm) on the 5th day of incubation. Alternatively, mycelial growth was completely restricted (5.15 mm) at 35 °C after the 5d of incubation. The mycelial colonies at an incubation temperature of 25 °C were comparatively fast-growing and dense. Whereas relatively sparse colonies were noted at 15–25 °C.

3.2. Disease control

3.2.1. Evaluation of released Lilium varieties and genotypes for resistance against BGM natural infection in the nethouse

The susceptibility of all the screened genotypes varied towards naturally occurring BGM in the field (Table 4). Their disease incidence ranged from 31.66 % (L018) to 78.33 % (L008). All five oriental genotypes demonstrated the lower disease incidence (31.66–41.66 %). Genotype L018 recorded the lowest disease severity (6.67 %), and it was statistically similar with L017 and L019 (8.33 %). Therefore, L017, L018, and L019 (oriental) were marked as resistant (R) in disease reaction. The remaining oriental genotypes, L014 and L020, (disease severity 11.00 and 11.33 % respectively) were marked as moderately resistant (MR). Recently released BARI Lilium-1 and BARI Lilium-2 provided higher disease incidence (58.33 % and 63.33 %) and disease severity (21–24.00 %). Consequently, they were marked as moderately susceptible (MS) towards BGM. The asiatic genotype L022 was MR (with 55.00 % disease incidence, and 20.00 % severity). Five other asiatic genotypes (L007, L011, L025, L026, and L028) were MS (severity ranged from 20.00 to 30.00 %). Whereas, rest three asiatic genotypes L002, L008, and L0027 were susceptible in disease reaction (34.67 %–41.33 % disease severity) (Table 4).

The maximum chlorophyll contents were observed in genotypes L018 (46.54), followed by L019 (44.52), and L017 (43.74) (Table 5). Genotype L022 had the tallest plant (75.86 cm) and genotype L007 had the smallest (31.79 cm). The flower diameter among the screened genotypes ranged from 16.58 to 24.26 cm (Table 5). Genotype L020, had the highest flower diameter, followed by Genotypes L028 (19.69 cm) and L011 (19.35 cm). Flower diameter was considerably reduced (16.58–17.33 cm) in genotypes L002, L007, and BARI Lilium-1 (Table 5).

3.2.2. Chemical control of Lilium BGM

3.2.2.1. In-vitro fungicidal efficacy of different fungicides against B. cinerea GBLB01. All the tested fungicides were effective against *B. cinerea* GBLB01 *in vitro* (Fig. 6). Among the tested fungicides Carboxin + Thiram (Provax), produced the lowest mycelial growth (12.21 mm) and Azoxystrobin + Difenoconazole (Amister Top 325 SC) produced the highest mycelial growth (18.76 mm) after 9d of inoculation (Fig. 6). On the basis of this result these six fungicides were selected for controlling the naturally occurring BGM disease of Lilium plants (*in planta*) in nethouse.

3.2.2.2. In-planta antifungal efficacy of selected fungicides against naturally occurring BGM symptoms of BARI Lillim-1 under nethouse condition. All the tested fungicides significantly reduced the disease incidence and severity in compared to control (Fig. 7). The lowest disease incidence (27.11 %), and severity (5.33 %) were recorded from the Iprodione treated plot (Fig. 7 a and b). The disease incidence ranged from 29.441 to 43.884 %, and severity ranged from 7 to 12.44 % for the rest five fungicides. In comparison, the highest disease incidence (63.89 %) and severity (22.11 %) were recorded from the control plot (Fig. 7 a and b). The plant height ranged 63.15–70.55 cm recorded from the six fungicide treated plots. The mean flower diameter varies from 16.20 to 17.17 cm for all the treatment plots. Although, the highest plant height (70.55 cm) and flower diameter (17.17 cm) were recorded from Iprodione and

Table 5
Evaluation of BARI *Lilium* genotypes against the naturally occurring BGM symptoms under field conditions.

Sl no	Variety/Germplasm	Origin	Chlorophyll content	Plant height (cm)	Flower diameter (cm)
1	BARI Lilium-1	Asiatic	28.44 ef (±0.48)	68.63 b (±0.81)	16.96 k (±0.10)
2	BARI Lilium-2	Asiatic	25.39 g (±0.67)	45.86 g (±0.60)	20.12 efg (±0.15)
3	L002	Asiatic	21.59 h (±0.38)	41.82 i (±0.37)	17.33 k (±0.17)
4	L007	Asiatic	27.1 f (±0.38)	31.79 l (±0.70)	16.58 k (±0.19)
5	L008	Asiatic	18.71 i (±0.41)	42.05 i (±0.37)	19.27 hi (±0.250
6	L011	Asiatic	25.36 g (±0.72)	43.61 h (±0.41)	18.63 ij (±0.30)
7	L022	Asiatic	32.22 d (±0.58)	75.86 a (±0.30)	19.63 gh (±0.34)
8	L025	Asiatic	29.62 e (±0.52)	47.88 f (±0.37)	18.28 j (±0.32)
9	L026	Asiatic	29.27 e (±0.58)	51.32 e (±0.20)	20.83 cde (±0.50)
10	L027	Asiatic	22.64 h (±0.47)	64.98 c (±0.24)	22.39 b (±0.24)
11	L028	Asiatic	24.39 g (±0.40)	57.37 d (±0.34)	19.4 ghi (±0.28)
12	L014	Oriental	33.26 d (±0.52)	36.26 k (±0.35)	21.13 cd (±0.43)
13	L017	Oriental	43.74 BCE (±0.51)	39.53 j (±0.27)	20.78 de (±0.43)
14	L018	Oriental	46.54 a (±0.55)	48.74 f (±0.37)	21.72 BCE (±0.35)
15	L019	Oriental	44.52 b (±0.76)	39.78 j (±0.31)	20.33 def (±0.32)
16	L020	Oriental	42.65 c (±0.58)	68.22 b (±0.50)	24.26 a (±0.28)

Note. Data were recorded at the 3rd month after planting. Mean separation was conducted according to DMRT. Numbers inside the parenthesis (±0.00) indicates the standard error of each mean value, and N = 10 (total number of leaves, plants and flowers for estimation of chlorophyll content, plant height and flower diameter).

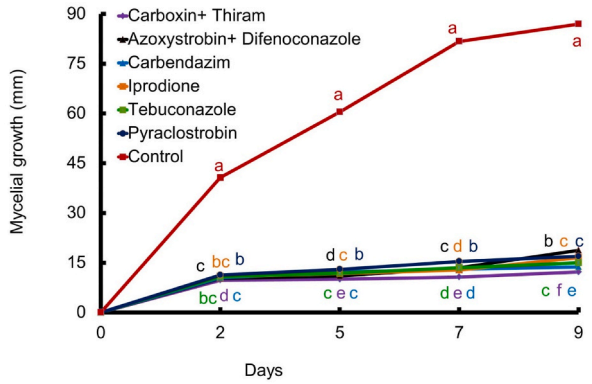


Fig. 6. *In vitro* fungicidal effect of different fungicides against *B. cinerea* GBBL001 grown PDA. Bars indicate the standard error of the mean and N = 10 (no. of Petri dishes).

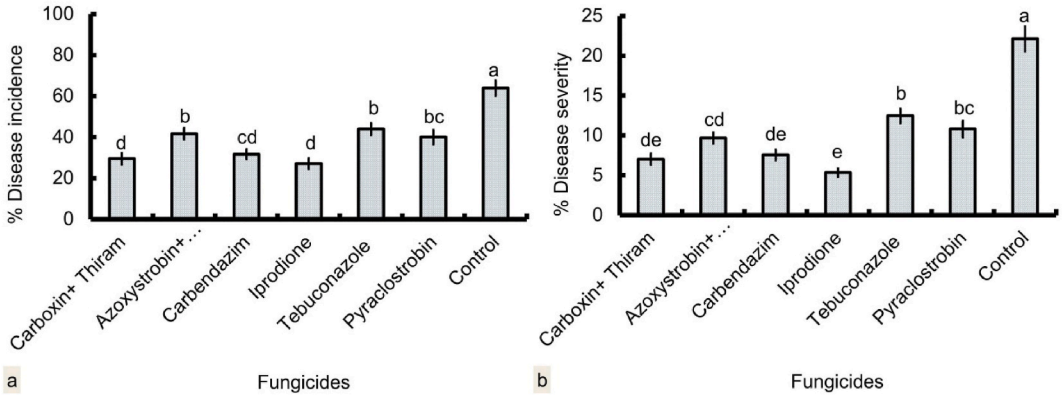


Fig. 7. *In planta* fungicidal efficacy of selected six fungicides against naturally infected BGM symptoms in nethouse. a) % disease incidence, and b) % disease severity. Bars indicate standard error of the mean and N = 60 (no. of plant).

Carboxin + Thiram applied plots, respectively (Table 6). The lowest plant height (56.66 cm) and flower diameter (15.91 cm) were recorded from the control plot (Table 6).

3.2.3. Biological control

3.2.3.1. In vitro antifungal efficacy of different biocontrol agents against B. cinerea GBLB01. All the antagonists significantly decreased the mycelial growth of the pathogenic fungal strain *B. cinerea* GBLB01 compared to the control (Fig. 8). *Trichoderma* isolate ThB001 achieved the maximum growth inhibition percent (54.09 %) (Fig. 8a). Three rest *Trichoderma* isolates (ThL001, ThC003, and T 003) inhibited mycelial growth from 49.16 % to 51.99 %. The lowest percent growth inhibition (44.48 %) was recorded from *E. nigrum* EJS002 (Fig. 8a). *B. subtilis* PTB001 exhibited a higher growth inhibition rate of 49.50 % compared to *P. fluorescens* PPR001(43.42 %) (Fig. 8b).

3.2.3.2. In planta antifungal efficacy of different biocontrol agents against B. cinerea GBLB01. The *in planta* curative experiment demonstrated that all antagonists reduced lesion diameter compared to the control up to 96h after pathogen inoculation (Table 7). *E. nigrum* EJS002 produced the smallest lesion diameter (3.07 mm), and it was statistically equivalent with *Trichoderma* isolate ThC003 (3.16 mm). This result was followed by *Trichoderma* isolates ThL001, ThB001 (lesion diameter were 3.90 and 4.09 mm, respectively), and *B. subtilis* PTB001 (3.91 mm). Control 1 generated the largest lesion (10.07 mm). Likewise, *E. nigrum* EJS002 provided the highest % disease reduction (69.41 %) at 96h, followed by *Trichoderma* isolates ThC003 and ThL001 (68.37 and 61.00 % respectively). Among the bacterial antagonists, the percent disease reduction from *B. subtilis* PTB001 (61.02 %) was higher than the *P. fluorescens* PPR001 (50.03 %).

In addition, all antagonists reduced lesion diameter significantly compared to the control in the *in planta* preventative trial (Table 8). *Trichoderma* ThC003 produced the smallest lesion diameter (3.22 mm) after 96h of pathogen inoculation. *E. nigrum* EJS002 produced statistically similar lesion diameter (3.64 mm) with *Trichoderma* ThC003. The bacterial antagonist *B. subtilis* PTB001 had statistically smaller lesion diameter (3.92 mm) compared to *P. fluorescens* PPR001 (5.19 mm). The largest lesion diameter was

Table 6
Evaluation of fungicides in controlling BGM of Lilium plant.

Fungicides	Plant height (cm)	Flower diameter (cm^2)
Carboxin + Thiaram	69.87 ab (±0.26)	17.176 a (±0.04)
Azoxystrobin + Difenoconazole	63.15 d (±0.21)	16.54 BCE (±0.04)
Carbendazim	69.50 b (±0.24)	16.83 ab (±0.06)
Iprodione	70.56 a (±0.25)	17.14 a (±0.06)
Tebuconazole	67.16 c (±0.26)	16.20 cd (±0.36)
Pyraclostrobin	66.69 c (±0.32)	16.43 BCE (±0.04)
Control	56.66 e (±0.30)	15.91 d (±0.04)

Note. Mean separation was conducted according to DMRT. Numbers inside the parenthesis (±0.00) indicate the standard error of each mean and N = 60 (no of plant and flower).

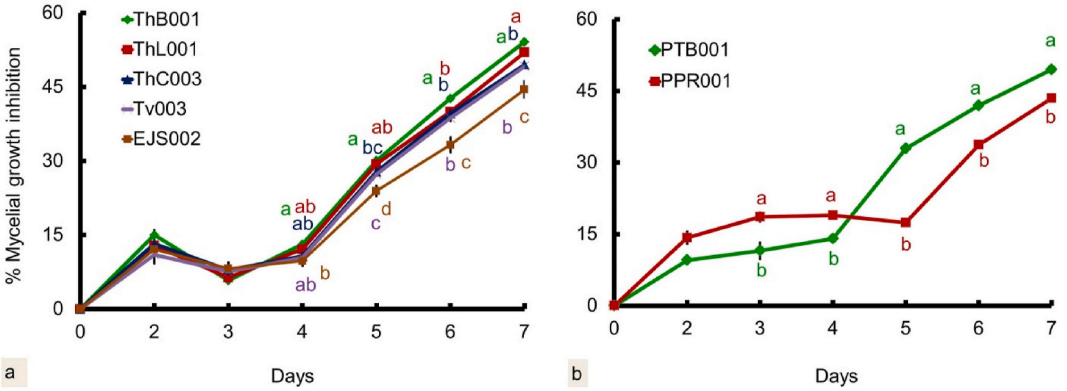


Fig. 8. *In vitro* antifungal effect of different fungal and bacterial antagonists against *B. cinerea* GBBL001 grown PDA. a) antifungal effect of five fungal antagonists; b) antifungal effect of two bacterial antagonists. Bars indicate the standard error of the mean and N = 10 (no. of leaflet).

Table 7
In planta curative antifungal efficacy of all biocontrol agents.

Treatments	Lesion diameter (mm) at different post inoculation hours			Percent disease reduction at 96 h (post inoculation hour) over control
	48h	72h	96h	
EJS002	0.86f (±0.12)	3.20 cd (±0.18)	3.07 e (±0.19)	69.41
ThB001	2.68 cd (±0.19)	2.84 d (±0.48)	4.09 cd (±0.21)	59.00
ThL001	3.36 b (±0.16)	3.69 BCE (±0.16)	3.90d (±0.12)	61.00
ThC003	2.19 d (±0.19)	2.76 d (±0.14)	3.16 e (±0.13)	68.37
Tv003	2.92 BCE (±0.24)	3.27 cd (±0.12)	4.51 BCE (±0.15)	54.94
PTB001	2.5 cd (±0.22)	4.07 b (±0.10)	3.91 d (±0.16)	61.02
PPR001	1.59 e (±0.12)	2.59 d (±0.15)	5.00 b (±0.21)	50.03
Control 1	4.92 a (±0.26)	7.48 a (±0.50)	10.07 a (±0.38)	0
Control 2	0.25 g (±0.03)	0.64 e (±0.11)	1.16 f (±0.064)	

Note. Control 1 = leaflets inoculated with *B. cinerea* (GBBL001) containing PDA plug; Control 2 = leaflets inoculated with PDA plugs without any pathogen. This means bearing the same letter within the same column did not differ significantly at the 5 % level following LSD. Numbers inside the parenthesis (±0.00) indicate the standard error of each mean and N = 10 (no. of leaflets).

produced by the Control 1 (10.18 mm). Similarly, *Trichoderma* ThC003 provided the highest percent disease reduction (68.94), followed by *E. nigrum* EJS002 (64.82). The percent disease reduction for other fungal antagonists was ranged from 56.48 to 62.30 %. The bacterial antagonist *B. subtilis* PTB001 reduced 62.15 % disease over control during *in planta* preventive experiment (Table 8).

4. Discussion

Based on morphological traits, molecular sequencing, and pathogenicity tests, *B. cinerea* was confirmed as the causal agent of the mentioned symptom. This study marks the first report of BGM disease affecting Lilium in Bangladesh. Gray mold symptoms appeared bilaterally on the leaves during artificial inoculation. Subsequent ITS gene sequencing and phylogenetic analysis verified that the fungal strain detected was *B. cinerea*. Although *B. elliptica* has also been reported to cause gray blight in Lilium [7], this study did not identify *B. elliptica* in Bangladesh.

The gray mold pathogen can grow on artificial media due to its extensive host range [5]. The pathogen grew faster on V8, and NA

Table 8
In planta preventive antifungal efficacy of all biocontrol agents.

Treatments	Lesion diameter (mm) at different post inoculation hours			Percent disease reduction at 96 h (post inoculation hour) over control
	48h	72h	96h	
EJS002	0.87e (±0.06)	3.22d (±0.16)	3.64de (±0.20)	64.82
ThB001	2.68c (±0.09)	2.88de (±0.09)	4.13cd (±0.13)	60.15
ThL001	3.33b (±0.11)	3.62c (±0.08)	3.91d (±0.06)	62.30
ThC003	2.20d (±0.08)	2.61e (±0.14)	3.22e (±0.14)	68.94
Tv003	2.83c (±0.22)	3.28cd (±0.15)	4.51c (±0.08)	56.48
PTB001	2.64c (±0.13)	4.14b (±0.15)	3.92d (±0.16)	62.15
PPR001	1.88d (±0.19)	2.61e (±0.08)	5.19b (±0.22)	49.93
Control 1	4.87a (±0.19)	7.61a (±0.25)	10.40a (±0.17)	0
Control 2	0.26f (±0.023)	0.65f (±0.061)	1.29f (±0.23)	–

Note. Control 1 = leaflets inoculated with *B. cinerea* (GBBL001) containing PDA plugs; Control 2 = leaflets inoculated with PDA plugs without any pathogen. This means bearing the same letter within the same column did not differ significantly at 5 % level following LSD. Numbers inside the parenthesis (±0.00) indicate the SE of each mean and N = 10 (no. of leaflets).

media than PDA in this study. Previous research in Bangladesh found that CDA (chickpea dextrose agar) and LDA (lentil dextrose agar) had higher mycelial growth and conidia generation of the same pathogen (isolated from chick pea) than PDA [17]. Similarly, rapid growth of *B. cinerea* (isolated from Lilium) on PDA, PSA, Oats, Lily leaf, Lily flower, Czapek, Rechard, and Agar media was also reported from China [18].

This study also reported the mycelial growth rate and colony colour variation of the selected pathogen on media with different pH levels. Previous reports demonstrated that the same pathogens mycelial growth differed on media with pH ranging from 4 to 11 [18]. The *B. cinerea* isolate grew slowly at pH 4, 5, 9, and 11; faster at pH 10; and grew evenly at pH 6, 7, and 8 [18,19]. The *B. cinerea* isolate GBLB01 from Bangladesh thrived better at pH 6. Thus, media and pH preferences may differ depending on *B. cinerea* isolate, disease-causing host, growing area, or ambient temperature (growing season).

Temperature greatly affects the disease symptom development and fungus mycelial growth. *B. cinerea* grows best at 20–25 °C [20], but temperature above 30 °C may inhibit or cease the mycelial growth [21]. *B. cinerea* isolate GBLB01 from Lilium grew best at 20–25 °C and slowly at higher or lower temperatures. Notably, Lilium is cultivated in Bangladesh from October to March, which coincides with this optimal temperature range. The field symptoms appeared around mid-January when the temperature increased from 17 to 25 °C. The favourable condition for Lilium cultivation also promotes gray mold pathogen growth in Bangladesh.

This disease damages Lilium flower quality, and productivity in greenhouses and open field [22]. Hence, varietal resistance or tolerance could be an effective BGM management strategy for Lilium. Recently, seven germplasm accessions were reported as resistant against the mentioned disease after a field screening in India [3]. Similarly, the present investigation recorded three resistant and three moderately resistant Lilium germplasms in Bangladesh. These resistant to moderately resistant germplasms could be further evaluated for high-quality Lilium flowers in the country. Interestingly, Oriental genotypes had lower disease reaction than Asiatic genotypes. Therefore, more Oriental genotypes should be evaluated for resistant varieties in Bangladesh.

BGM disease is mostly managed with chemical fungicides. Several active ingredients with distinct modes of action have been registered for BGM control on diverse crops, such as Methyl-benzimidazole carbamates (MBCs), Dicarboximides (DCF), Anilinopyrimidines, Phenylpyrroles (PPs), KetoReductase inhibitors, QoIs, SDHIs, and Multisite toxicants [23–25].

This investigation demonstrated that Iprodione reduced *in planta* disease incidence and severity. Besides, Carboxin + Thiaram, Carbendazim, and Azoxystrobin were also effective. To control Lilium BGM, this study recommends spraying Iprodione fungicide (1 mlL^{-1}) three times at weekly intervals starting from the heading stage. Alternative use of the other three fungicides with Iprodione should be tested against the disease. Several US universities have recommended Polyoxin D zinc salt, Isofetamid, Fluopyram/trifloxystrobin, Chlorothalonil, Fenhexamid, Fludioxonil, Copper Fungicides, and Thiophanate Methyl to control Lilium BGM [26,27]. The genetic diversity, intrinsic pathogen characteristics, and overuse of fungicides may cause fungicide resistance in *B. cinerea* [12,28]. Thus, further study on optimising fungicide rotation or mixing techniques for disease control and fungicide resistance management is needed.

Additionally, concerns have been raised over the residual effect of current synthetic fungicides used for this disease control [29]. A promising *B. cinerea* treatment option is microorganisms as biocontrol agents (BCAs) [30]. *Trichoderma* spp., *Ulocladium* spp., *Bacillus subtilis* have been the focus of biocontrol study with some commercial products available on the market [29]. This study presented four *Trichoderma* isolates that effectively inhibited *in vitro* growth and *in planta* disease development. A. Balode [31] reported that BioMikss, a *Trichoderma*-based biocontrol formulation, reduced *Botrytis cinerea* in Lilium ('Lolly', 'Meteorits', and 'Sonora') in greenhouses. This BioMikss-treated Lilium varieties were gray mold resistant and increased lily production considerably.

Besides another fungal agent, *E. nigram* EJS002, was also suppressed *in vitro* and *in planta* fungal growth in the present investigation. Common Ascomycetes *Epicoccum* has five effective species (*E. nigrum*, *E. layuense*, *E. dendrobii*, *E, mezzettii* and *E. minitans*). *E. nigrum* is the most promising biocontrol agent lowering a wide range of plant diseases incidence and severity (caused by *Sclerotinia sclerotiorum*, *Pythium irregulare*, *Phytophthora infestans*, *Monilinia laxa*, and a wide range of *Fusarium* species) [reviewed by 33]. The involved modes of action could be competition for nutrients and space, antibiosis, parasitism, and induced host plant resistance [30]. Besides two bacterial BCAs, *Bacillus subtilis* (PTB001) and *Pseudomonas fluorescens* (PPR001) were also effective against Lilium gray mold. Recently, *B. amyloliquefaciens* (VB7) showed *in vitro* (46 %) antagonism against Lilium *B. cinerea* [4]. Similar *in vitro* antagonism was observed by

Bacillus subtilis (PTB001) against the same pathogen in this investigation. Additionally, bulb dipping, soil drenching, and foliar application of *B. amyloliquefaciens* successfully reduced the mentioned disease symptom earlier [4,32]. However, the biocontrol agents were not evaluated in the nethouse due to insufficiency of funds and time in the present investigation.

5. Conclusion

This investigation identifies BGM as a significant disease concern for Lilium in Bangladesh, and the growth season's natural environment may favours the disease infection. Moreover, this investigation suggests three spray of Ipridione fungicide at weekly intervals from the heading stage could be used to control this disease. To prevent disease resistance, future study should investigate alternative sprays or rotation and mixing of other effective fungicides such as Carboxin + Thiaram, Carbendazim, and Azoxystrobin. This investigation also presented four *Trichoderma*, one *Epicoccum*, one *Bacillus*, and one *Pseudomonas* isolates as promising isolate for biocontrol research. Biofungicides should be tested with chemical fungicides and other cultural practices to combat Lilium gray mold. In addition, the seasonal effect of Lilium cultivation, flower quality, and fungicide resistance of *B. cinerea* could also be interesting subjects of future research.

Ethics statement

Since neither humans nor animals were involved in this investigation, informed consent was not necessary.

Data availability statement

Data associated with this study has been deposited at NCBI accession No. MN756674 and MN757648.

CRediT authorship contribution statement

Md Mahfuz Alam: Project administration, Methodology, Investigation, Data curation, Conceptualization. **Khondoker Mohammad Alam:** Supervision, Investigation, Formal analysis, Data curation. **Rumana Momotaz:** Visualization, Investigation, Data curation. **Most Arifunnahar:** Investigation, Data curation. **Md Mosiur Rahman Bhuyin Apu:** Resources, Investigation. **Shaikh Sharmin Siddique:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank Plant Pathology Division, BARI, for technical assistance and acknowledged BARI for financial support. Authors are also thankful to Professor Dr. Md. Zinnatul Alam (Retd.), Department of Entomology, BSMRAU for proof reading the manuscript.

References

[1] R. Daneshvar, Efficient approaches to in vitro multiplication of *Lilium candidum* L. with consistent and safe access throughout year and acclimatization of plant under hot-summer Mediterranean (Csa Type) climate, Not. Bot. Horti Agrobot. Cluj-Napoca 47 (3) (2019) 734–742, https://doi.org/10.15835/nbha47311486.

[2] Y.K. Lapiz-Culqui, J.B. Meléndez-Mori, G. Mállap-Detquizán, J.J. Tejada-Alvarado, N.C. Vilca-Valqui, E. Huaman-Human, M. Oliva, M. Goñas, *In vitro* bulbification of five lily varieties: an effective method to produce quality seeds and flowers, Int J Agron 2022 (2022) 8775989, https://doi.org/10.1155/2022/8775989.

[3] R. Latif, K. Ahmad, F. Mohiddin, Z.A. Bhat, Z. Badri, T. Bashir, I. Hamid, M. Hassan, Screening of *Lilium* germplasm against botrytis blight (*Botrytis cinerea* Pers, Fr.), J. Pharm. Innov. 11 (11) (2022) 418–421.

[4] S. Nakkeeran, R. Priyanka, S. Rajamanickam, U. Sivakumar, *Bacillus amyloliquefaciens* alters the diversity of volatile and non-volatile metabolites and induces the expression of defence genes for the management of Botrytis leaf blight of Lilium under protected conditions, J. Plant Pathol. 102 (4) (2020) 1179–1189, https://doi.org/10.1007/s42161-020-00602-6.

[5] A. Grassotti, F. Gimelli, Bulb and cut flower production in the genus *Lilium*: current status and the future, II International Symposium on the Genus Lilium 900 (2010) 21–35, https://doi.org/10.17660/ActaHortic.12011.17900.17661.

[6] K. Ambia, F. Khan, A. Naznin, M. Bhuiyin, K. Ara, Characterization and evaluation of Lilium in Bangladesh, Bangladesh, J. Agric. Res. 47 (1) (2022) 39–50.

[7] J.-Y. Jang, S. Subburaj, G.-J. Lee, H.-S. Kim, *In vitro* screening for *Botrytis* leaf blight resistance in *Lilium* species, Sci. Hortic. 239 (2018) 133–140, https://doi.org/10.1016/j.scienta.2018.05.009.

[8] X. Cao, S. Shi, Z. Zhang, First report of Botrytis leaf blight on lily (*Lilium longiflorum*) caused by *Botrytis cinerea* in Beijing, China, Plant Dis. 102 (5) (2018) 1033, https://doi.org/10.1094/PDIS-10-17-1583-PDN.

[9] J.W. Pscheidt, C.M. Ocamb, Lily (*Lilium* spp.) botrytis blight (fire). Pacific Northwest Plant Disease Management Handbook, Oregon State University, 2023.

[10] K. Alam, M. Alam, M.M. Islam, R. Momotaz, M. Arifunnahar, N. Sultana, H. Raihan, T. Mujahidi, F. Khatun, S. Banu, First report on *Fusarium oxysporum* f. sp. *niveum* causing watermelon Fusarium wilt in Bangladesh, Plant Dis. 104 (6) (2020) 1859, https://doi.org/10.1094/PDIS-11-19-2466-PDN.

[11] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, X. Mega, Molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547, https://doi.org/10.1093/molbev/msy096.

[12] S. Sundravadana, D. Alice, S. Kuttalam, R. Samiyappan, Efficacy of azoxystrobin on *Colletotrichum gloeosporiodes* Penz growth and on controlling Mango anthracnose, J Agric Biol Sci 2 (3) (2007) 10–15.

[13] R. Prasad, B. Kumaraswamy, Screening of different genotypes against castor gray mold disease, Int J Pure Appl Biosci 5 (4) (2017) 1641–1644, https://doi.org/10.18782/2320-7051.5719.
[14] M.M. Alam, T. Tanaka, H. Nakamura, H. Ichikawa, K. Kobayashi, T. Yaeno, N. Yamaoka, K. Shimomoto, K. Takayama, H. Nishina, et al., Overexpression of a rice heme activator protein gene (OsHAP2E) confers resistance to pathogens, salinity and drought, and increases photosynthesis and tiller number, Plant Biotechnol. J. 13 (1) (2015) 85–96, https://doi.org/10.1111/pbi.12239.
[15] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, PCR protocols: a guide to methods and applications 18 (1990) 315–322.
[16] K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol. 10 (3) (1993) 512–526, https://doi.org/10.1093/oxfordjournals.molbev.a040023.
[17] M.I. Hosen, Cultural, physiological comparison and fungicidal sensitivity between two isolates of *Botrytis cinerea* and *Stemphylium botryosum*, Emir. J. Food Agric. (2011) 120–129, https://doi.org/10.9755/ejfa.v23i2.6346.
[18] S. Ma, Y. Hu, S. Liu, J. Sun, M. Irfan, L.-J. Chen, L. Zhang, Isolation, identification and the biological characterization of *Botrytis cinerea*, Int. J. Agric. Biol. 20 (2018) 1033–1040, https://doi.org/10.17957/IJAB/15.0600.
[19] H.-C. Wang, L.-C. Li, B. Cai, L.-T. Cai, X.-J. Chen, Z.-H. Yu, C.-Q. Zhang, Metabolic phenotype characterization of *Botrytis cinerea*, the causal agent of gray mold, Front. Microbiol. 9 (2018) 470, https://doi.org/10.3389/fmicb.2018.00470.
[20] N. Ciliberti, M. Fermaud, J. Roudet, V. Rossi, Environmental conditions affect *Botrytis cinerea* infection of mature grape berries more than the strain or transposon genotype, Phytopathology 105 (8) (2015) 1090–1096, https://doi.org/10.1094/PHYTO-10-14-0264-R.
[21] B. Bai, H. Yang, S. He, A. Yu, Studies on morphology and biological characteristics of *Botrytis elliptica* (Berk.) Cooke causing foliage blight of *Lilium davidii* Duch. var. unicolor (Hoog) Cotton, Chin Veg 16 (2013) 78–84.
[22] S. Lebiush-Mordechai, O. Erlich, M. Maymon, S. Freeman, T. Ben-David, T. Ofek, E. Palevsky, L.T. Lahkin, Bulb and root rot in lily (*Lilium longiflorum*) and onion (*Allium cepa*) in Israel, J Phytopatho 162 (7–8) (2014) 466–471, https://doi.org/10.1111/jph.12214.
[23] FRAC, FRAC (Fungicide Resistance Action Committee), Code List ©2020: fungal control agents sorted by cross resistance pattern and mode of action (including FRAC Code numbering) Fungicide Resistance Action Committee, Brussels, https://www.frac.info, 2020.
[24] S. Fillinger, A.-S. Walker, Chemical control and resistance management of Botrytis diseases, in: Botrytis–the Fungus, the Pathogen and its Management in Agricultural Systems, 2016, pp. 189–216, 110.1007/1978-1003-1319-23371-23370.
[25] J.K. Richards, C.-L. Xiao, W.M. Jurick, *Botrytis* spp.: a contemporary perspective and synthesis of recent scientific developments of a widespread genus that threatens global food security, Phytopathology 111 (3) (2021) 432–436, https://doi.org/10.1094/PHYTO-10-20-0475-IA.
[26] N. Doubrava, J.H. Blake, J. Williamson, Gray mold (botrytis blight), in: College of Agriculture, Forestry and Life Sciences, Clemson University, 2021.
[27] M. Hausbeck, B. Harlan, Recommendations for botrytis fungicides for 2020, in: Michigan State University Extension, Department of Plant, Soil & Microbial Sciences, Michigan State University: Michigan State University, 2020.
[28] A. Dhyani, B. Nautiyal, M. Nautiyal, M.C. Rivera, D. Prasad, K. Singh, First report of *Botrytis cinerea* on *Lilium polyphyllum*, a critically endangered herb in Uttarakhand, India, Phyton 81 (2012) 157.
[29] J.A. Abbey, D. Percival, L. Abbey, S.K. Asiedu, B. Prithiviraj, A. Schilder, Biofungicides as alternative to synthetic fungicide control of grey mould (*Botrytis cinerea*) – prospects and challenges, Biocontrol Sci. Technol. 29 (3) (2019) 207–228, https://doi.org/10.1080/09583157.2018.1548574.
[30] G. Fedele, C. Brischetto, V. Rossi, Biocontrol of *Botrytis cinerea* on grape berries as influenced by temperature and humidity, Front. Plant Sci. 11 (2020) 1232, https://doi.org/10.3389/fpls.2020.01232.
[31] A. Balode, Biocontrol of grey mould in lilies spp. by *Trichoderma* spp. and *Bacillus* spp, XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 937 (2010) 583–587, 510.17660/ActaHortic.12012.17937.17670.
[32] A.L. Chiou, W.S. Wu, Formulation of *Bacillus amyloliquefaciens* B190 for control of lily grey mould (*Botrytis elliptica*), J Phytopatho 151 (1) (2003) 13–18, https://doi.org/10.1046/j.1439-0434.2003.00669.x.