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In Vitro Efficacy Assessment of Botanical Extracts against *Botrytis gladiolorum* Causing Gladiolus Leaf Blight

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Abstract

An experiment was conducted at the Mycology laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to find out the efficacy of selected botanical extracts against *Botrytis gladiolorum* (Bg) causing gladiolus leaf blight. Infected leaves samples were collected from gladiolus farmers' field and brought to the laboratory for study. Ten selected botanicals were used against the colony growth of (Bg). The botanicals were Mehendi, Chrysanthemum, Basil (Tulsi), Onion, Neem, Bael, Arjuna, Garlic, Aloevera (Ghritkumary) and Turmeric. Botanical extracts were applied at the rate of 5%, 10% and 20%. The radial mycelia growth was found minimum (11.60 mm) in garlic extract treated plate at the dose of 5% at 5 DAI, which was statistically similar with turmeric extract treated (14.00 mm) plate and the inhibition of growth was 60% and 51.72%, respectively. Similar trend was found at 10 DAI and 15 DAI. At 15 DAI, garlic and turmeric extract gave the best result against Bg, which was statistically similar with onion (50.07% inhibition) and mehendi (49.93%). All botanicals showed significantly different results over control and found effective in reducing the mycelial growth at the dose of 10%. At 5 DAI, no radial mycelia growth was found in garlic treated plate, which was statistically similar with onion treated plate, means that the inhibition of growth was 100%. Similar trend was also found at 10 DAI, and 15 DAI but at 15 DAI, onion (30.20 mm) gave the statistically similar results with Garlic (30.10 mm) and the mycelia growth inhibition was (57.70%) and (57.84%), respectively. In case of 20% dose, garlic extract showed the best result at 5 DAI and the radial mycelia growth was

found minimum (00.00 mm) which was statistically similar with onion extract treated (00.00 mm) plate and turmeric extract treated plate. The inhibition of growth was 100%. At 15 DAI, the inhibition of fungal growth was found (73.74%), (71.23%) and (66.90%), respectively with treated by turmeric (18.80 mm), garlic (20.60 mm) and onion (23.70 mm).

Keywords

Gladiolus, Leaf Blight, *Botrytis gladiolorum*, Botanical Extracts, Ecofriendly Management

1. Introduction

Gladiolus (*Gladiolus grandiflorus* L.), is an herbaceous annual flower that belongs to the family Iridaceae. It is one of the most important cut flowers in Bangladesh. Gladiolus is a very popular cut flower and occupying fourth place in international cut flower trade. Gladiolus has gained popularity in many parts of the world owing to its unsurpassed beauty and economic value [1]. Popularity of this crop as a cut flower is increasing day by day because of its attractive flower spikes and availability in wide range of colors of the florets, varying number of florets and their size, wide range of keeping quality and adaptability to different seasons. These characters have made it very attractive for use as a cut flower, vase and bouquet preparation, growing in herbaceous borders, beddings, rockeries and pot cultivation. At present, there are about 255 species [2] in gladiolus. The modern cultivars of *G. grandiflora* are believed to be originated from a number of wild species viz., *G. cruentus*, *G. natalensis*, *G. oppositiflorus*, *G. papilio* and *G. saundersii* [3] [4]. It ranks fifth next to tulip, lily (*Lilium* spp), freesia (*Freesia* spp) and hippeastrum (*Hippeastrum* spp) among the geophytes in international florist trade. The major gladiolus cut flower producing countries are USA, Holland, Italy, France, Poland, Bulgaria, Brazil, Australia and Israel. In Europe, gladiolus has been popular for over 500 years, whereas in Bangladesh, it is comparatively recent introduction and gained importance as a modern cut flower only in the recent past. Cultivation season of gladiolus is generally October-January but in Bangladesh gladiolus is cultivated around the year. The agro-ecological conditions of Bangladesh are very much conducive for gladiolus cultivation.

Income from gladiolus flower production is six times higher than that of rice in Bangladesh [5]. The production was around 208,000 flower stalk/hac of land in 2016-2017 in Bangladesh. The major production area of this flower is covered by Jashore district. Whereas other gladiolus growing districts are Dhaka, Manikganj, Narayanganj, Chattogram, Cox's Bazar, Bogura, Rangpur, Gaibandha, and Faridpur. Gladiolus has recently been become popular in Bangladesh. It was introduced in Bangladesh around 1992 from India [6].

Disease is one of the most important limiting factors for commercial cultiva-

tion of gladiolus in Bangladesh. Now Botrytis blight is caused by *Botrytis gladiolorum* (Bg) becoming severe in the farmers' field of different cultivated regions in Bangladesh. In recent years, disease problems appeared in Bangladesh as one of the major limiting factors for cultivation of gladiolus. In 2013-2014 crop seasons, botrytis leaf blight of gladiolus appeared as a new disease in farmers' fields in Jashore regions [7]. The disease was manifested by characteristic symptoms of Botrytis blight as spots on leaf, flower bud, flower, stem and corm. The disease incidence and severity were found very high and caused leaf and inflorescence blight. Almost all plants in a field were found to be infected by the disease. Moreover, the market price of flower sticks was reduced.

Farmers depend on chemical pesticides for control of gladiolus leaf blight. Chemical practices are harmful for environment [8]. Fungicides, and other pesticides, have recently been linked to cancer, respiratory and hormone imbalance diseases [9] [10] [11]. Use of chemicals resulted in environmental pollution and ill health to the biotic community as a whole, and this necessitates developing the natural product as an alternative to synthetic fungicides to control the disease [12]. On the other hand, botanicals are environment friendly and make it available to the farmers. The use of bio-degradable plant products especially from medicinal plants is gaining importance in plant disease management. Plants contain a wide range of secondary metabolites such as phenols, alkaloids, flavonoids, tannins, anthocyanins and saponins which are antimicrobial in nature. The inhibitory effects of some plant extracts, like neem, garlic, tulsi, ginger, lantana etc., have encouraged exploring the potential of antifungal and antibacterial compounds harbored by the plants [13]. The plant metabolites and plant base pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact in contrast to synthetic pesticides. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory. Plant extracts show antifungal activity against a wide range of fungi. At present, serious attention is drawn to extracts from higher plants known to contain antifungal substances in the form of alkaloids or prohibitins, which help in resisting the pathogens [14].

Researches on gladiolus disease management are very limited in Bangladesh. So, research on diseases management is required for better production of gladiolus in Bangladesh, which will help to manage diseases of gladiolus effectively in the field. Therefore, the present work was conducted to determine the efficacy of botanicals against Bg causing gladiolus leaf blight.

2. Materials and Methods

2.1. Experimental Site and Duration

The experiment was conducted in the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during July, 2018 to December, 2018. Ten selected botanicals were tested *in vitro* to evaluate their efficacy on colony growth of Bg. Evaluation was done through the poison food techniques [15].

2.2. Preparation of Potato Dextrose Agar (PDA)

PDA is used in the experiment with botanical extracts to inhibit the mycelial growth of the test fungus. The composition of PDA media included 20 g Dextrose and 20 g Agar mixed with potato juice obtained from 200 g peeled, sliced and boiled Potato and the volume of the whole content was up to one liter. The media was sterilized in an autoclave with 121˚C for 15 minutes at 15 PSI pressure and poured in Petri-dishes. After solidification the PDA media was used for culture of Bg in the laboratory.

2.3. Isolation of *Botrytis gladiolorum*

Blighted leaf samples of Gladiolus were collected from farmer's field (Plate 1) at Katlapur village of Singair upazila under Manikganj district, Bangladesh. Infected plant leaf samples were placed in brown paper packets and brought to laboratory for isolation of pathogens following tissue planting methods on potato dextrose agar (PDA). Infected parts of the leaves showing blight symptoms were cut into 4 - 5 mm pieces with the help of scissors and surface sterilized with 1.0% chlorox (NaOCl) solution for 1 minute subsequently rinsed in distilled water for three times. The surface sterilized pieces of leaves were placed separately in petri-dishes (9 cm) containing Potato Dextrose Agar (PDA) and incubated at 25˚C ± 1˚C. After 4 - 5 days mycelial tips were recultured in fresh PDA plate and incubated at 25˚C ± 1˚C for pure culture (Figure 1).

2.4. The Botanicals Used against *Botrytis gladiolorum*

Ten botanicals were used against the colony growth of *Botrytis gladiolorum.* The botanicals were Mehendi, Chrysanthemum, Basil, Onion, Neem, Bael, Arjuna, Garlic, Aloevera (Ghritkumary), Turmeric (Table 1 and Figure 2) in CRD design with five replications.

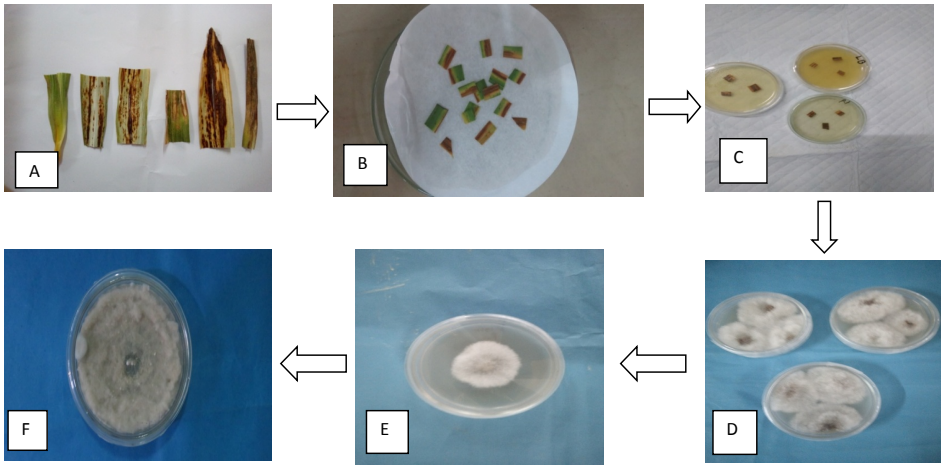
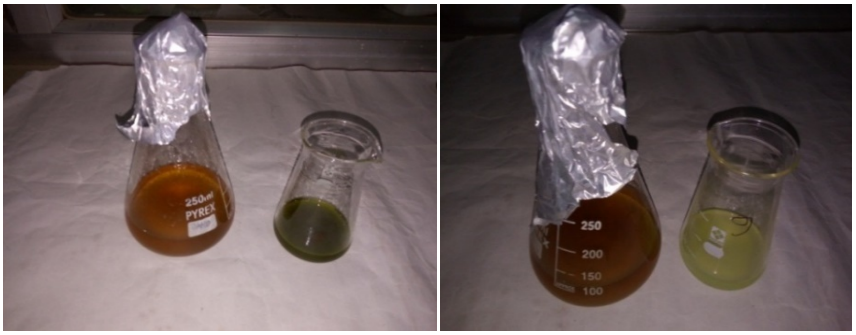


Figure 1. Flow chart of isolation of *Botrytis gladiolorum*: (A) Infected leaf sample, (B) Small pieces of leaves transferred on blotter after surface sterilization and subsequent rinsing with sterile distilled water, (C) Diseased sample in solidified PDA, (D) Mycelia growth in PDA media, (E) Reculture and (F) Pure culture.

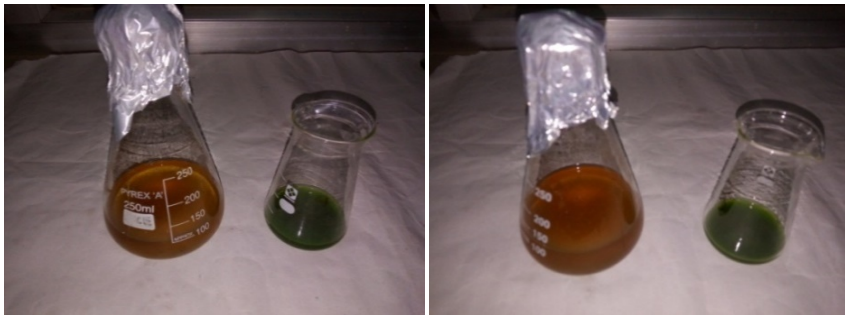
Table 1. List of botanicals used against *Botrytis gladiolorum* causing leaf blight of gladiolus *in vitro*.

Local name	English name	Scientific Name	Plant parts
Mehendi	Henna	*Lawsoniintermis*	Leaf
Chandramallika	Chrysanthemum	*Chrysanthemum morifolium*	Leaf
Tulsi	Basil	*Ocimumsactum*	Leaf
Piaj	Onion	*Allium cepa*	Bulb
Neem	Neem	*Azadirachta indica*	Leaf
Bael	Stone apple	*Ipomeapestigridis*	Leaf
Arjun	Arjuna	*Tuminalia arjuna*	Leaf
Rosun	Garlic	*Allium sativum*	Bulb
Ghritkumari	Aloe vera	*Aloe vera*	Leaf
Holud	Turmeric	*Curmuma longa*	Rhizome

(A) (B)



(C) (D)



(E) (F)

(G) (H)

(I) (J)

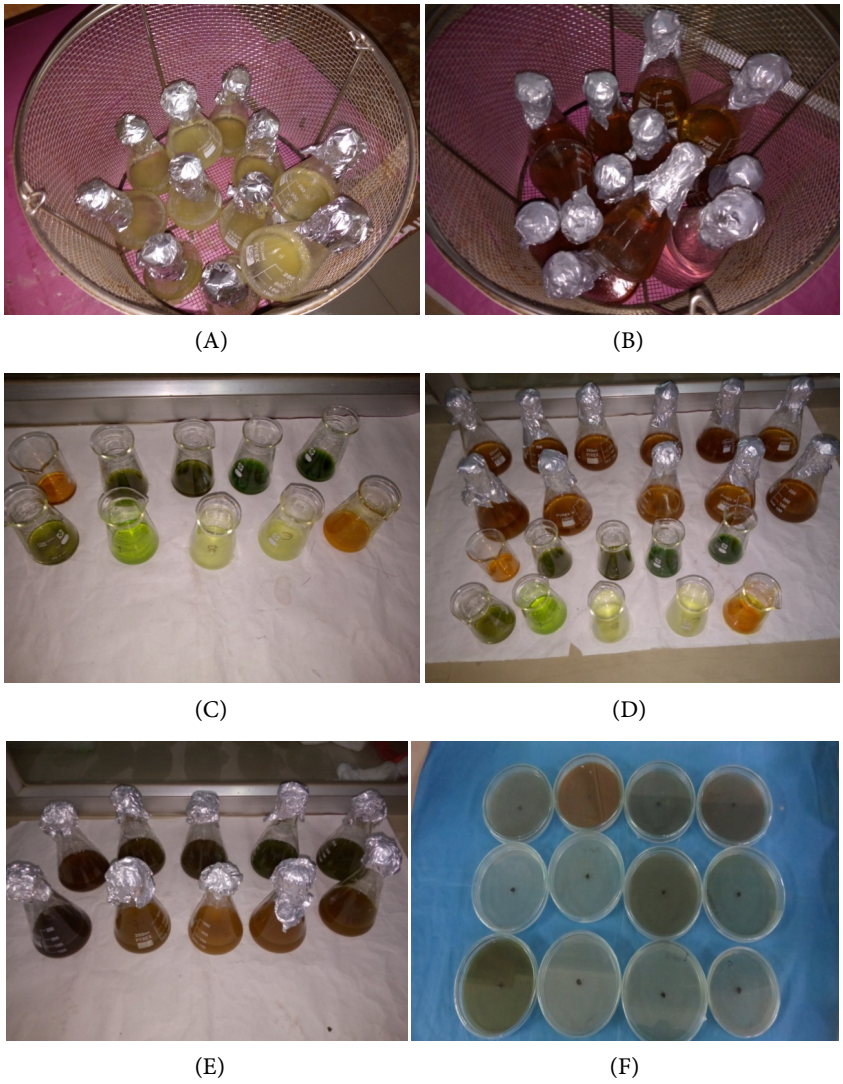
Figure 2. Botanical extracts with autoclaved PDA media in conical flask, (A) Mehendi, (B) Chrysanthemum (C) Tulsi, (D) Onion, (E) Neem, (F) Bael, (G) Arjun, (H) Garlic, (I) Aloevera and (J) Turmeric.

2.4.1. Preparation of Botanicals Leaf Extract

Fresh leaves of Mehendi, Chrysanthemum, Basil, Neem, Bael, Arjuna and, Aloevera were collected from Sher-e-Bangla Agricultural University campus, Dhaka, Bangladesh. Collected leaves were washed thoroughly with running tap water and chopped with a knife, and air dried. Five grams (5 g) leaves mixed with 20 ml distilled water and ground well by a mortar pestle and were prepared solution with leaf extract and distilled water ratio 1:4. This solution was filtered through double layered muslin cloth. The supernatant was filtered through Whatman Filter Paper and made the stock solution. Twenty mililitre (1:4) prepared stock solution was mixed with 80 ml autoclaved PDA media to make a dose 5% which was tested to determine its antifungal activity against (Bg). To follow the same procedure for making another two concentrations, ten grams leaves mixed with 20 ml distilled water for 1:2 solutions. Here 10% prepared solution mixed with 80 ml autoclaved PDA media to make a 10% dose and 20 grams leaves mixed with 20 ml distilled water for 1:1 solution. 20 ml prepared solution mixed with 80 ml autoclaved PDA that were made a 20% dose, respectively (**Figure 3**).

2.4.2. Preparation of Bulb and Rhizome Extract

Fresh bulb of onion and garlic, and rhizome of turmeric were collected from Krishi market, Mohammedpur, Dhaka, Bangladesh. Collected botanicals were washed thoroughly with running tap water and chopped with a knife, and air dried. Five grams (5 g) bulb and rhizome mixed with 20 ml distilled water separately and ground well by a mortar pestle to make the ratio (1:4). The solution



(A) (B)

(C) (D)

(E) (F)

Figure 3. (A) PDA media in conical flask before autoclave, (B) PDA media in conical flask after autoclave, (C) Ten botanical extracts, (D) Ten botanical extracts and autoclaved PDA in conical flask, (E) Plant extract mixed with 50˚C warm PDA media in conical flask, (F) Poisoned PDAmedia inoculated with *B. gladiolorum*.

was filtered through double layered muslin cloth. The supernatant was filtered through Whatman Filter Paper and made the stock solution. Twenty milliliter botanical extracts stock solution were mixed with 80 ml autoclaved PDA media for made the dose 5% which was tested to determine its antifungal activity against (Bg). To follow the same procedure for making another two concentrations, ten grams leaves mixed with 20 ml water for 1:2 solutions mixed with 80 ml autoclaved PDA media for prepared 10% dose and 20 grams leaves mixed with 20 ml distilled water for 1:1 solution that prepared 20% dose, respectively.

2.5. Bioassay of Botanicals

Twenty milliliter of each botanical extract were mixed with melt potato Dextose agar media (80 ml at 50˚C). The botanical extracts (Mehendi, Chrysanthemum,

Basil, Onion, Neem, Bael, Arjuna, Garlic, Aloevera, Turmeric) mixed with autoclaved [16] PDA Media in ten conical flasks. The conical flasks without botanical extract (100 ml) served as control media. Botanical extract treated medium @ 20 ml were poured in each 9 cm petri plate. After solidification, the plates were inoculated with a 5 mm disk of 16-days-old cultures of (Bg). Five replicate plates were used for each concentration of botanicals. Radial colony diameter measured after 5 days, 10 days and 15 days of incubation. Colony growth were measured in two directions from the underneath side, perpendicular to each other and took the growth as the mean of the two measures. Percent inhibition of radial growth was computed based on colony diameter on control plate using the following formula [17].

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

where,

X = Growth of fungus on control plate;

Y = Growth of fungus on botanicals treated plate.

2.6. Data Analysis

Data were analyzed using MSTAT-C program and mean value were separated by Duncan's Multiple Range Test (DMRT).

3. Result and Discussion

3.1. Efficacy of Botanicals (5%) against *Botrytis gladiolorum* in Laboratory

Ten selected botanicals Mehendi, Chrysanthemum, Tulsi, Onion, Neem, Bael, Arjun, Garlic, Aloevera and Turmeric applied at the rate of 5%, 10% and 20%. In case of 5% concentration the radial mycelia growth of the fungus was measured at 5, 10 and 15 DAI. All botanicals were found significantly effective in reducing the mycelial growth of the fungus compared to control. At 5 DAI, the radial mycelia growth was found minimum (11.60 mm) in garlic treated plate, which was statistically similar with turmeric treated (14.00 mm) (Plate 15) and the inhibition of growth was 60% and 51.72%, respectively. Similar trend was found at 10 DAI and 15 DAI. Garlic and turmeric gave the best result against (Bg), which was statistically similar with onion (50.7% inhibition) and mehendi (49.93%) (**Table 2** and **Figure 4**).

3.2. Efficacy of Botanicals (10%) in Controlling *Botrytis gladiolorum* in the Laboratory

Ten selected botanicals were tested with the concentration 10% against *Botrytis gladiolorum* and radial mycelia growth of fungus were measured at 5, 10 and 15 DAI. All botanicals showed significantly different results over control and found effective in reducing the mycelial growth. At 5 DAI, no radial mycelia growth was found in garlic treated plate, which was statistically similar with onion treated

Table 2. Efficacy of botanicals (5%) in controlling *Botrytis gladiolorum* in the laboratory.

Ttreatments	Radial mycelial growth (mm)					
	5 DAI	Growth inhibition (%) over control	10 DAI	Growth inhibition (%) over control	15 DAI	Growth inhibition (%) over control
T_1 = Mehendi	23.80 bc	17.93	29.00 c	38.95	36.90 cd	49.93
T_2 = Chrysanthemum	19.80 c	31.73	27.70 b	41.68	42.30 c	42.60
T_3 = Tulsi	22.80 bc	21.38	38.90 b	18.10	59.10 b	19.81
T_4 = Onion	22.70 bc	21.72	32.20 c	32.20	36.80 cd	50.07
T_5 = Neem	20.20 c	30.28	39.90 b	16.00	58.00 b	21.30
T_6 = Bael	25.60 ab	11.73	41.40 b	12.84	68.30 a	7.32
T_7 = Arjun	21.70 bc	25.17	31.10 c	34.53	43.50 c	40.98
T_8 = Garlic	11.60 d	60.00	19.60 e	58.73	35.90 cd	51.29
T_9 = Aloevera	21.80 bc	24.83	30.30 c	36.21	38.00 cd	48.44
T_{10} = Turmeric	14.00 d	51.72	23.00 de	51.59	32.20 d	56.30
T_{11} = Control (Untreated)	29.00 a	-	47.50 a	-	73.70 a	-
LSD (P = 0.01)	3.93	-	5.13	-	8.69	-

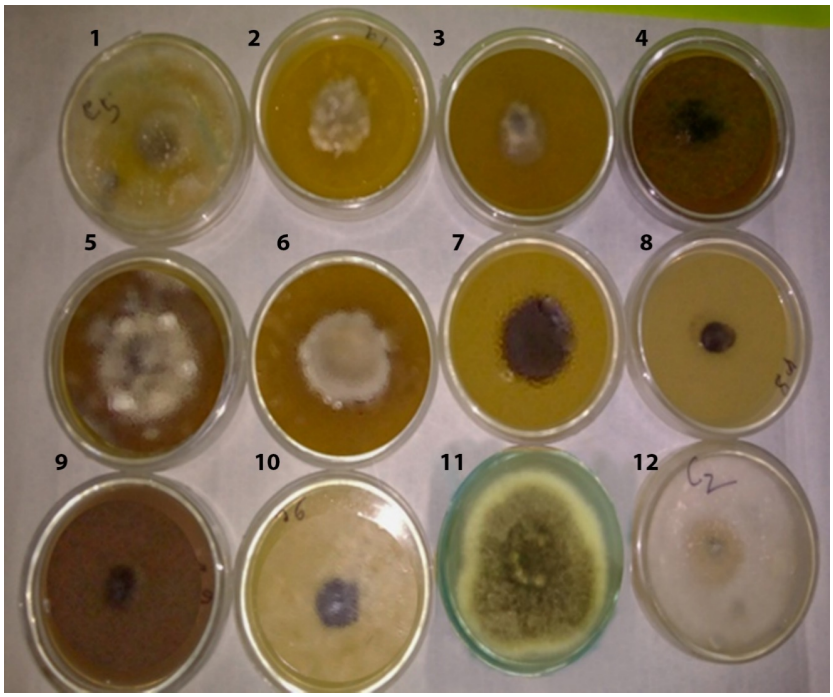


Figure 4. Mycelial growth of *B. gladiolorum* against selected botanicals extracts (5%) at 15 days after inoculation. (1 = Aloevera, 2 = Mehendi, 3 = Turmeric, 4 = Neem, 5 = Bael, 6 = Tulsi, 7 = Chrysanthemum, 8 = Garlic, 9 = Aurjun, 10 = Onion, 11 = Control, 12 = Onion)

plate, means that the inhibition of growth was 100%. Similar trend was also found at 10 DAI, and 15 DAI but at 15 DAI, onion (30.20 mm) gave the statistically similar results with Garlic (30.10 mm) and the mycelia growth inhibition was (57.70%) and (57.84%), respectively (Table 3 and Figure 5).

Table 3. Efficacy of botanicals (10%) in controlling *Botrytis gladiolorum* in the laboratory.

Treatments	Radial mycelial growth (mm)					
	5 DAI	Growth inhibition (%) over control	10 DAI	Growth inhibition (%) over control	15 DAI	Growth inhibition (%) over control
T_1 = Mehendi	17.80 e	39.86	24.70 d	46.06	32.50 de	54.48
T_2 = Chrysanthemum	18.60 de	37.16	24.50 d	46.50	33.70 de	52.80
T_3 = Tulsi	20.90 cd	29.39	28.90 cd	36.90	43.00 bc	38.93
T_4 = Onion	0.0000 h	100	17.50 e	61.79	30.20 de	57.70
T_5 = Neem	21.40 c	27.70	34.50 bc	24.01	43.60 bc	38.94
T_6 = Bael	26.80 b	9.45	38.60 b	15.72	48.60 b	31.93
T_7 = Arjun	16.90 ef	42.90	29.60 cd	35.37	38.10 cd	46.63
T_8 = Garlic	0.0000 h	100	14.60 e	68.12	30.10 de	57.84
T_9 = Aloevera	15.20 f	48.65	24.80 d	45.85	33.40 de	53.22
T_{10} = Turmeric	9.000 g	69.59	16.00 e	65.05	26.00 e	63.59
T_{11} = Control (Untreated)	29.60 a	-	45.80 a	-	71.40 a	-
LSD (P = 0.01)	2.49	-	5.45	-	7.76	-

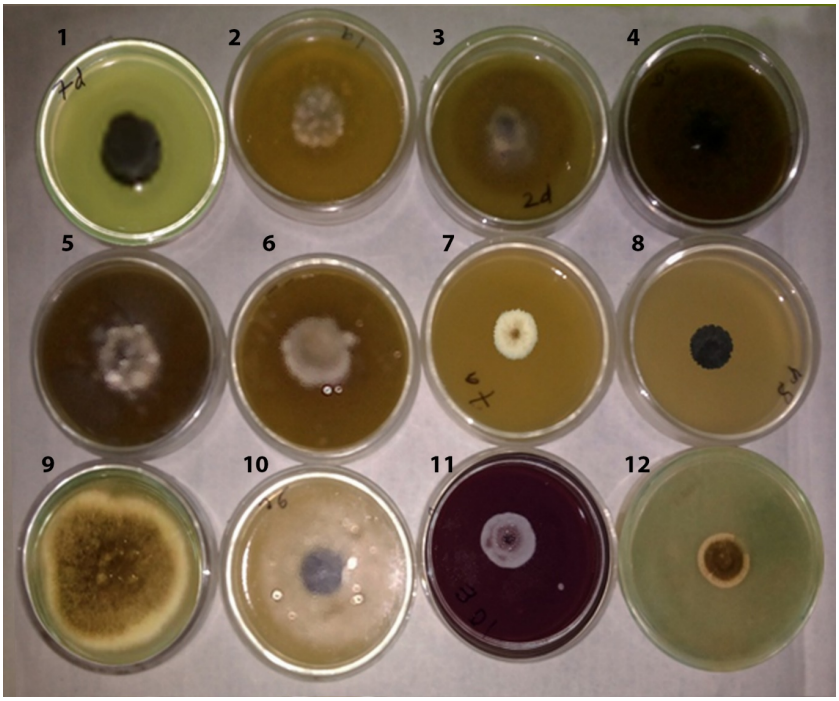


Figure 5. Mycelial growth of *B. gladiolorum* against selected botanicals extracts (10%) at 15 days after inoculation. (1 = Aloevera, 2 = Mehendi, 3 = Bael, 4 = Neem, 5 = Aurjun, 6 = Chrysanthemum, 7 = Tulsi, 8 = Garlic, 9 = Control, 10 = Onion, 11 = Turmeric, 12 = Aloevera)

3.3. Efficacy of Botanicals (20%) in Controlling *Botrytis gladiolorum* in the Laboratory

Ten selected botanicals were tested with the concentration 20% against (Bg) and

observed the radial mycelia growth of fungus at 5, 10 and 15 DAI. All botanicals significantly affected the mycelial growth of fungus and reduced the mycelial growth. At 5 DAI, no the radial mycelia growth was found in garlic treated plate which was statistically similar with onion treated plate and turmeric treated plate that means that inhibition of growth was 100%. Similar trend was also found at 10 DAI, and 15 DAI, but at 15 DAI the inhibition mycelial growth was found 73.74%, 71.23% and 66.90% treated by turmeric (18.80 mm), garlic (20.60 mm) and onion (23.70 mm), respectively (Table 4 and Figure 6).

3.4. Comparative Efficacy of Different Botanicals on Mycelial Growth Inhibition of *Botrytis gladiolorum* at 15 Days after Inoculation

The highest growth inhibition was found at the doses of 20% in case of all the botanicals at 15 DAI. At 15 DAI, the inhibition of mycelial growth was found 73.74%, 71.23% and 66.90% when treated by turmeric (18.80 mm), garlic (20.60 mm) and onion (23.70 mm), respectively (Figure 7). These doses can be used for further study in field condition.

4. Discussion

Despite the importance of Botrytis leaf blight through the world but no extensive study has been done in Bangladesh. Among ten botanicals at the rate of 20% garlic gave the best result at 5 DAI the radial mycelia growth was found minimum (00.00 mm) which was statistically similar with onion treated (00.00 mm) plate and turmeric treated plate and the inhibition of growth was 100%. At 15 DAI the

Table 4. Efficacy of botanicals (20%) in controlling *Botrytis gladiolorum* in the laboratory.

Treatments	Radial mycelial growth (mm)					
	5 DAI	Growth inhibition (%) over control	10 DAI	Growth inhibition (%) over control	15 DAI	Growth inhibition (%) over control
T_1 = Mehendi	10.00 d	65.75	16.00 e	64.60	25.00 def	65.08
T_2 = Chrysanthemum	11.00 d	62.33	18.00 e	60.18	27.50 de	61.59
T_3 = Tulsi	15.80 c	45.89	23.20 cd	48.67	35.60 bc	50.28
T_4 = Onion	00.00 e	100.00	11.40 f	74.78	23.70 def	66.90
T_5 = Neem	16.10 c	44.86	27.40 bc	39.38	35.60 bc	50.28
T_6 = Bael	20.20 b	30.82	29.90 b	33.85	41.00 b	42.74
T_7 = Arjun	11.30 d	61.30	24.50 c	45.80	30.00 cd	58.10
T_8 = Garlic	00.00 e	100.00	08.90 f	80.31	20.60 ef	71.23
T_9 = Aloevera	10.00 d	65.75	19.30 de	57.30	27.00 de	62.29
T_{10} = Turmeric	00.00 e	100.00	10.00 f	77.88	18.80 f	73.74
T_{11} = Control (Untreated)	29.20 a	-	45.20 a	-	71.60 a	-
LSD (P = 0.01)	2.50	-	4.47	-	6.46	-

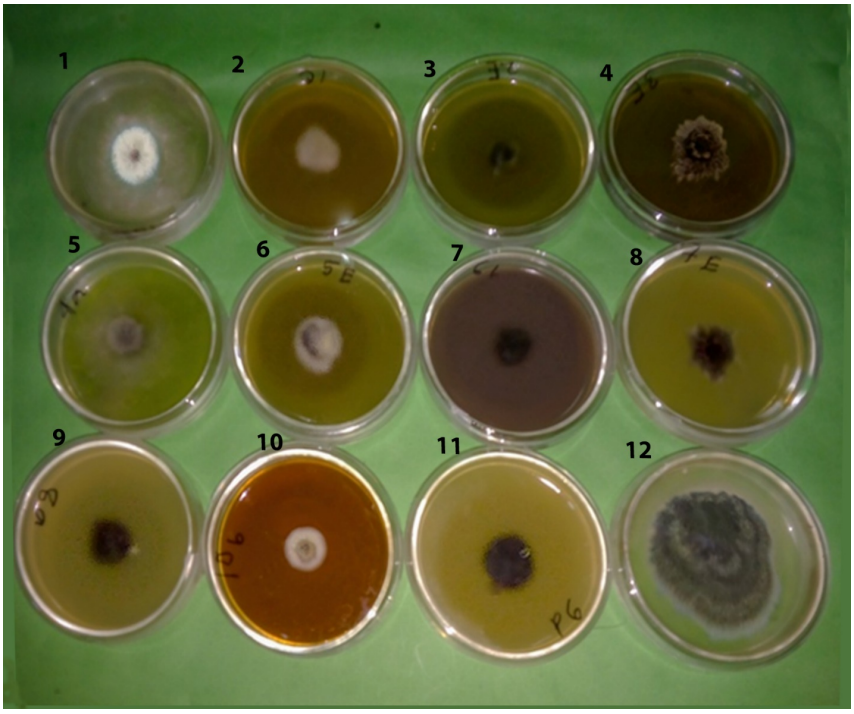


Figure 6. Mycelial growth of *B. gladiolorum* against selected botanicals extracts (20%) at 15 days after inoculation. (1 = Aloevera, 2 = Mehendi, 3 = Bael, 4 = Aurjun, 5 = Onion, 6 = Garlic, 7 = Neem, 8 = Chrysanthemum, 9 = Chrysanthemum, 10 = Turmeric, 11 = Tulsi, 12 = Control)

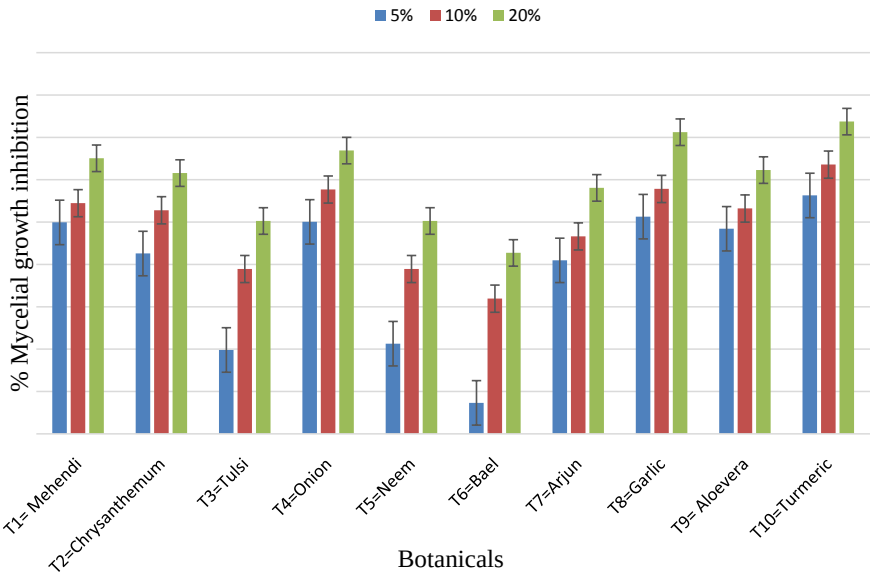


Figure 7. Comparative efficacy of different botanicals (20%) on mycelial growth inhibition of *Botrytis gladiolorum in vitro.*

inhibition of fungal growth was found (73.74%), (71.23%) and (66.90%), respectively with treated by turmeric (18.80 mm), garlic (20.60 mm) and onion (23.70 mm). Like this study, Yashoda *et al.* [18] evaluated fungitoxicity of different plant extracts, namely *Allium sativum*, *A. indica*, *Ocimum sanctum* and *Vinca*

rosea against *Cercospora beticola* under *in vitro* conditions. Among these, *A. sativum* was the best treatment for inhibiting mycelia growth (30.66%) followed by *A. indica* (24.52%). Least inhibition was observed with *V. rosea* (19.04%) followed by *O. sanctum* (19.67%). Kaur [19] tested the efficacy of *A. sativum*, *A. indica*, *Melia azedarach*, *Vitex nigundo*, *Eucalyptus globules* against *Botrytis cinerea* and found that *A. sativum* was the most inhibitory and gave the highest mean growth inhibition (57.39%) followed by *Azadirachta indica* (45.47%). Gholve *et al.* [20] evaluated *A. sativum*, *A. cepa* and *Ocimum sanctum* against *Alternaria macrospora* and found that *A. sativum* was found most inhibitory and gave the highest mean growth inhibition (37.47%) followed by *A. cepa* (34.97%) and *o. sanctum* (32.86%). Bhardwaj and Sahu [21] evaluated different botanicals *in vitro* against *Colletotrichum falcatum*. Amongst botanicals evaluated, it was found that, maximum mycelial growth inhibition was recorded in *Ocimum* (92.59%) followed by *A. cepa* (72.41%), while minimum inhibition in mycelial growth was recorded in *A. sativum* (64.44%). The results of the present investigation show that *A. sativum* and *A. cepa* gave the best control, against *Botrytis*. Avasthi *et al.* [22] reported that *A. sativum* and *A. cepa* were effective against *Aspergillus niger* with *A. sativum* showing 100 percent inhibition of mycelial growth at 20 percent concentration. In our study *A. sativum* and *A. cepa* were found effective at 5 percent concentration. Riaz *et al.* [23] evaluated effect of leaf extract of *A. cepa* and *Tagetes erecta in vitro* against *Fusarium oxysporum* f. sp. *gladioli*. Extract of *A. cepa* at 8 percent concentration significantly suppressed fungal biomass by 73 percent, while *Tagetes erecta* was not found to be effective. In present studies, *A. cepa* at 5 percent concentration inhibited 100 percent germination of spores, but marigold was not effective against *Botrytis.* Taskeen-Un-Nisa *et al.* [24] reported that different concentrations of plant extracts caused significant inhibition in the spore germination of *F. oxysporum*. The extract of *A. sativum* at the highest concentration, *i.e.* 10 percent, was the most effective in reducing the spore germination followed by *A. cepa.* In the present studies, *A. sativum* and *A. cepa* were found the most effective botanicals in reducing spore germination of *Botrytis.* Chanel *et al.* [25] showed the antifungal activity of garlic extracts applied directly and through volatile release was tested against the growth of postharvest pathogens eg, *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea alba.* Mycelial growth of *B. cinerea* and *P. expansum* was inhibited by aqueous and ethanol dilutions on garlic extract amended media (direct method) in a dose response manner.

Abd-Alla *et al.* [26] reported that hydro alcoholic extract of fresh leaves of *Aloe vera* showed significant reduction of linear growth of *Botrytis gladiolorum*, *Fusarium oxysporum* f. sp. *gladioli*, *Heterosporium prunei* and *Penicillium gladioli* at a concentration of 1.0 and 2.0%. Ogbebor *et al.* [27] reported that extracts of *Ocimum basilicum* and *Allium sativum* exhibited total inhibitory effects on the mycelial growth of *Colletotrichum gloeosporioides*. It was also observed that although not promising but still the fungitoxic effect of these plant extracts persisted even at 5% concentration. These observations suggested that fungitox-

icity of the plant extracts was found to be promising against plant pathogens like *Fusarium* sp. and *Alternaria* sp. and can be increased further by using these plant extracts at higher concentrations. Kamdi *et al.* [28] reported that aqueous leaf extracts *Azadirachta indica* was found effective followed by *Lantana camara* at 5 percent concentration reducing chickpea wilt incidence caused by *Fusarium oxysporum* f. sp*. cicer.*

5. Conclusion

Gladiolus leaf blight caused by *Botrytis gladiolorum* is a destructive disease in gladiolus growing district of Bangladesh. Botanical extracts can be possible to use gladiolus farmers' field, and it will be eco-friendly and less hazardous. Based on results of the *in vitro* evaluation three botanicals—Turmeric (*Curmuma longa*), Onion (*Allium cepa*) and Garlic (*Allium sativum*), extracts at the concentrations of 20% found to be most effective against *Botrytis gladiolorum*.

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Authors' Contributions

This work was carried out in collaboration among all authors. Author MAR conducted the research work. Author MSMC designed the study. Author FMA designed and supervised the study, managed the literature searches and edited the manuscript. All authors contributed to literature search, edited the manuscript, read and approved the final manuscript.

Conflicts of Interest

The authors have declared that no competing interests exist.

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