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

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Abstract

Leaf blight of gladiolus caused by *Botrytis gladiolorum* is widely distributed as a major disease in gladiolus growing districts of Bangladesh where farmers usually used fungicides to manage diseases. Nowadays, it is very important to find out the more effective fungicides against *Botrytis gladiolorum* (Bg). 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 ten fungicides against *Botrytis gladiolorum* (Bg) causing gladiolus leaf blight. The aim of the study was to find out the more effective fungicides against *Botrytis gladiolorum* (Bg) in laboratory. Infected leaves samples were collected from gladiolus farmers' field and brought to the laboratory for study. Ten selected fungicides were used against the colony growth of Bg. The fungicides were Tilt 250 EC, Score 250 EC, Folicure 250 EC, Amistar top 325 SC, Nativo 75 WG, Trooper 75 WP, Autostin 50 WDG, Differ 300 EC, Indofil M-45 80 WP and Contaf 5 EC. The fungicides were applied at the rate of 100 ppm, 200 ppm and 300 ppm. Among ten fungicides, Contaf 5 EC gave the best results in arresting radial mycelia growth and it was nil (00.00 mm) after 5 DAI in 100 ppm which was statistically similar with Indofil 80 WP treated (00.00 mm) plate and the inhibition of growth was 100%. At 15 DAI the growth inhibition (87.34%) was highest in Contaf 5 EC treated plate followed by Score 250 EC (78.02%) and Autostin 50 WDG (72.34%) respectively. In 200 ppm Contaf 5 EC showed the best performance against mycelial growth at 15 DAI and gave 100% growth inhibition which was statistically similar to Score 250 EC (100% inhibition) followed by Autos-

tin 50 WDG (83.04) and Folicure 250 EC (82.90%). In 300 ppm Contaf 5 EC showed the best performance at 15 DAI against mycelial growth and showed 100% inhibition which was statistically similar to Score 250 EC (100% inhibition), Autostin 50 WDG, Tilt 250 EC and Folicure 250 EC.

Subject Areas

Plant Science

Keywords

Leaf Blight, Gladiolus, *Botrytis gladilorum*, Fungicides, Management

1. Introduction

Gladiolus (*Gladiolus grandiflorus* L) is very important and popular cut flowers in Bangladesh. The climatic conditions of Bangladesh are very much suitable for gladiolus cultivation. The cultivation process of gladiolus is relatively easy. Gladiolus flower is cultivating commercially in many areas of Bangladesh. It has more economic value and income from gladiolus flower production is six times higher than that of rice in Bangladesh (Momin 2006) [1].

Gladiolus flowers are preferable due to their beauty and aesthetic values (Bose *et al.* 1989) [2]. Its native is South Africa and has been cultivated globally. It was introduced in Bangladesh around 1992 from India (Mollah *et al.* 2002) [3]. Its demand has been increasing day by day with the advancement of aristocracy and modernization of Bangladesh. Disease is one of the most important limiting factors for commercial cultivation of gladiolus in Bangladesh.

Gladiolus plant is attacked by a number of diseases throughout the world and production strongly hampered due to diseases intensity. Gladiolus plants are affected by fungal pathogen along with bacteria, virus and nematodes such as Botrytis leaf blight (*Botrytis gladiolorum*), Corm rot, or Fusarium rot (*Fusarium oxysproum* f. sp. *gladioli*), Curvularia leaf spot (*Curvularia trifolli* f. sp. *gladioli*), Nematodes (*Meloidognye*, *Pratylenchus*, *Trichodorus*, *Belonolaimus*, *Ditylenchus*, *Hemicyliophora*, *Rotylenchus*), Scab (*Pseudomonas marginata*), Stemphylium leaf spot (*Stemphylium botryosum*), Stromatinia dry Rot (*Stromatinia gladioli*), Viruses (Bean yellow mosaic, Cucumber mosaic, Tomato ring spot, Tobacco ring spot) etc. (Elmer and Kamo 2018) [4].

Now a days, Botrytis blight which is caused by *Botrytis gladiolorum* become severe in the farmers' field of different cultivated regions in Bangladesh. The disease is manifested by spots on leaf, flower bud, inflorescence, stem and corm. Drayton (1928) [5] reported *Botrytis* disease of gladiolus from Canada in 1928. The disease has also been reported from Holland (Drayton 1929) [5], England (Moore 1939) [6], New York (Dodge and Laskaris 1941) [7], Australia (Wade 1945) [8], India (Sohi 1992 [9], Singh *et al.* 2005 [10]), Pakistan (Mirza and Sha-

kir 1991) [11] and Iran (Mirzaei *et al.* 2008) [12]. Sohi (1992) [9] worked on diseases of ornamental plants and reported *B. gladiolorum* from corms and leaves of gladiolus in India. Blight caused by *B. gladiolorum* is noted as the major threat for gladiolus production in India (Singh *et al.* 2005) [10]. Mirza and Shakir (1991) [11] reported *B. gladiolorum* from corm and leaves of gladiolus in Pakistan.

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 (Siddique *et al.* 2013) [13].

No attention has been given on Botrytis blight of gladiolus and its control earlier in Bangladesh. Application of fungicides is the most convenient and predominant way for disease control. Farmers depend on chemical pesticides for control of gladiolus leaf blight because the use of synthetic pesticides has become an integral part of agriculture and chemical practices are highly effective and low-cost management.

Researches on gladiolus disease management are very limited in Bangladesh. So, research on disease 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 fungicides against (Bg) causing gladiolus leaf blight.

2. Materials and Method

2.1. Experimental Site and Duration

The experiment was carried out at Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka during May, 2018 to October, 2018. Ten fungicides were tested *in-vitro* to evaluate their efficacy on reduction of colony growth of the fungal isolate (BGMS01). Evaluation was done the poison food techniques (Hawamdeh & Ahmad 2001) [14].

2.2. The Fungicides Used against *Botrytis gladiolorum*

Ten fungicides were used at 100 ppm, 200 ppm and 300 ppm concentration, respectively. The fungicides were Tilt 250 EC, Score 250 EC, Folicure 250 EC, Amister top 325 SC, Nativo 75 WG, Trooper 75 WP, Autostin 50 WDG, Differ 300 EC, Indofil M-45 80 WP and Contaf 5 EC in CRD design with 5 replications (**Table 1**).

2.3. Bioassay of Fungicides

The concentration of fungicides was used at 100 ppm, 200 ppm and 300 ppm. The fungicides were mixed with 100 ml PDA media in different quantities to make the desired concentration (**Table 1**). The fungicides added in autoclaved PDA Media which was distributed in ten conical flasks (Shovan *et al.* 2008) [15]. The conical flasks without fungicide served as control media. Sterilized media

Table 1. Fungicides with mode of action and doses used against colony growth of *Botrytis gladiolorum in vitro*.

Trade name	Mode of action	Active Ingredient	Doses		
			100 ppm	200 ppm	300 ppm
Tilt 250 EC	Systemic	Propiconazole	40 µl/100ml	80 µl/100ml	120 µl/100ml
Score 250 EC	Systemic	Difenoconazole	40 µl/100ml	80 µl/100ml	120 µl/100ml
Folicure 250 EC	Systemic	Tebuconazole	40 µl/100ml	80 µl/100ml	120 µl/100ml
Amistar Top 325 SC	Systemic	Azoxystrobin + Difenoconazole	30.76 µl/100ml	61.52 µl/100ml	92.28 µl/100ml
Nativo 75 WG	Systemic	Trifloxystrobin + Tebuconazole	13.33 mg/100ml	26.66 mg/100ml	39.99 mg/100ml
Trooper 75 WP	Systemic and Contact	Tricyclazol	13.33 mg/100ml	26.33 mg/100ml	39.99 mg/100ml
Autostin 50 WDG	Systemic	Carbendazim	20 mg/100ml	40 mg/100ml	60 mg/100ml
Difar 300 EC	Systemic	Difenoconazole + Propiconazole	33.33 µl/100ml	66.66 µl/100ml	99.99 µl/100ml
Indofil 80 WP	Contact	Mancozeb	12.5 mg/100ml	25 mg/100ml	37.5 mg/100ml
Contaf 5 EC	Systemic	Hexaconazole	200 µl/100ml	400 µl/100ml	600 µl/100ml

were poured@20 ml in each 9 cm petri plate. After solidification, the plates were inoculated with a 5 mm disk of 16 days old cultures of *Botrytis gladiolorum*. Five replicate plates were used for each concentration of fungicide. Radial colony diameter was measured after 5 days, 10 days and 15 days of incubation (Figure 1 and Figure 2). 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 (Sundar *et al.* 1995) [16] and data were analyzed using MSTAT-C program.

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

where,

X = Growth of fungus on control plate.

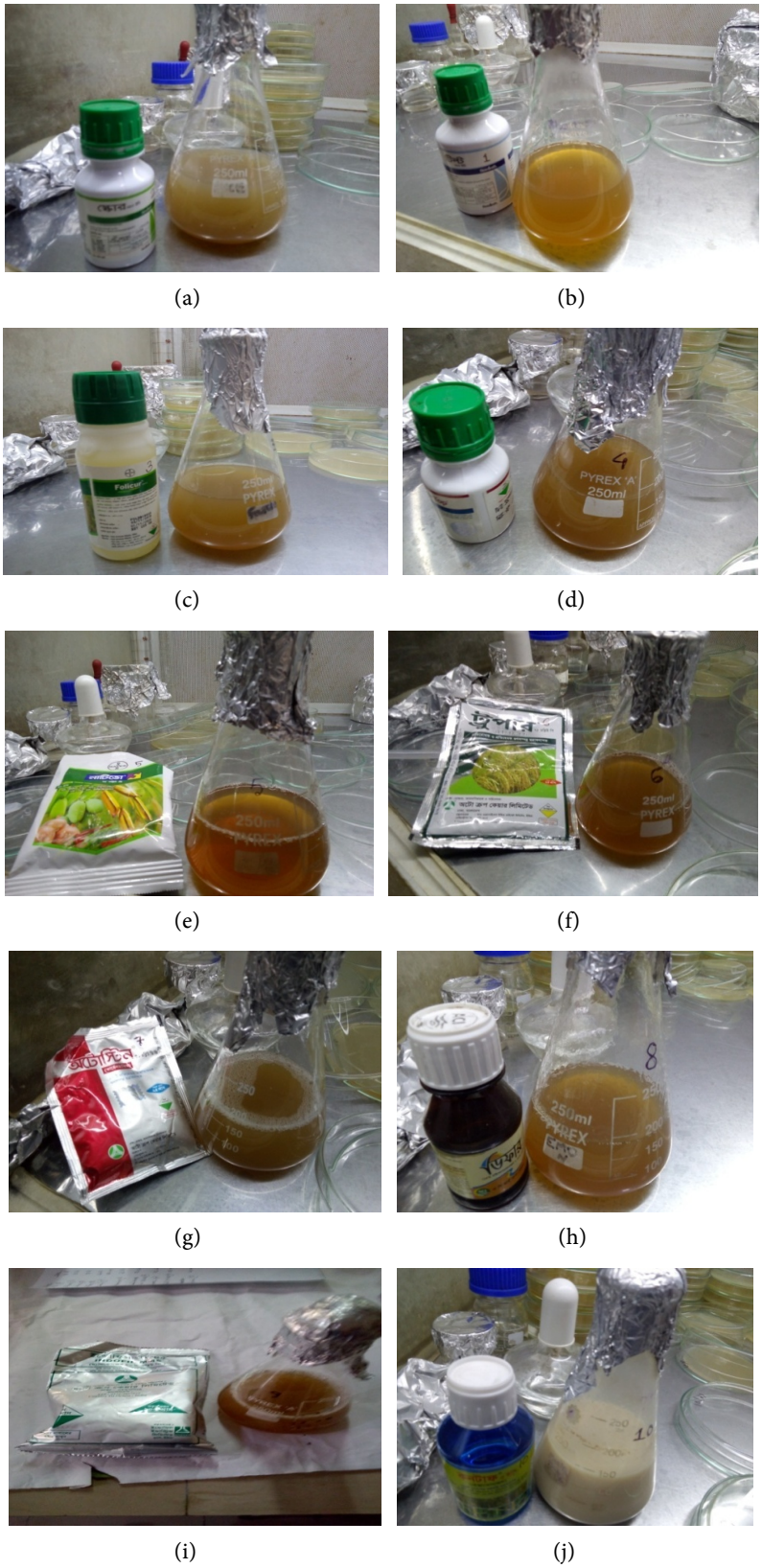
Y = Growth of fungus fungicide treated plate.

3. Result

Ten fungicides were used at 100 ppm, 200 ppm and 300 ppm, respectively. The fungicides were Tilt 250 EC, Score 250 EC, Folicure 250 EC, Amister top 325 SC, Nativo 75 WG, Trooper 75 WP, Autostin 50 WDG, Differ 300 EC, Indofil M-45 80 WP and Contaf 5 EC.

3.1. Efficacy of Fungicides at the Rate of 100 Ppm against *Botrytis gladiolorum* in the Laboratory

Ten selected fungicides viz. Tilt 250 EC, Score 250 EC, Folicure 250 EC, Amister Top 325 SC, Nativo 75 WG, Trooper 75 WP, Autostin 50 WDG, Difar 300 EC,



(a) (b)

(c) (d)

(e) (f)

(g) (h)

(i) (j)

Figure 1. Tested fungicides mixed with PDA in conical flask at 50˚C, (a) Score, (b) Tilt, (c) Folicure, (d) Amister Top, (e) Nativo, (f) Trooper, (g) Autostin, (h) Difar, (i) Indofil, (j) Contaf.

(a) (b) (c) (d) (e) (f)

Figure 2. (a) PDA media in conical flask before autoclave, (b) PDA Media in conical flask after autoclave, (c) Fungicides used against *Botrytis gladiolorum*, (d) Fungicides mixed with PDA in Petridishes, (e) and (f) Poisoned PDA media inoculated by *Botrytis gladiolorum* isolates.

Indofil 80 WP and Contaf 5 EC were tested at the rate of 100 ppm against *Botrytis gladiolorum* and radial mycelia growth of *B. gladiolorum* was measured at 5, 10 and 15 DAI (Days after inoculation). All fungicides gave significantly different results over control and found effective in reducing the mycelial growth of the isolated fungus. At 5 DAI, no the radial mycelia growth was found in Contaf 5 EC treated plate which was statistically similar with Score 250 EC treated (00.00 mm) plate, that reveals the inhibition of growth was 100%. At 10 DAI, Contaf 5 EC showed the best results to suppress the growth of mycelia (00.00 mm) followed by Score 250 EC (7.20 mm) and folicure 250 EC (13.60 mm). At 15 DAI, the growth inhibition (87.34%) was the highest in Contaf 5 EC treated plate followed by Score 250 EC (78.02%) and Autostin 50 WDG (72.34%), respectively (**Table 2** and **Figure 3**).

Table 2. Efficacy of fungicides at 100 ppm against *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 = Tilt 250 EC	00.00g	100.00	18.40de	60.77	28.70de	58.22
T_2 = Score 250 EC	00.00g	100.00	7.20h	84.65	15.10g	78.02
T_3 = Folicure 250 EC	01.40f	93.86	13.60g	71.00	20.10f	70.74
T_4 = Amistar Top 325 SC	16.50c	27.63	28.00c	40.30	37.30c	45.71
T_5 = Nativo 75 WG	20.20b	11.40	37.50b	20.04	53.20b	22.56
T_6 = Trooper 75 WP	19.50b	14.47	28.20c	39.87	31.40d	54.29
T_7 = Autostin 50 WDG	6.90e	69.74	14.50fg	69.08	19.00f	72.34
T_8 = Difar 300 EC	10.40d	54.39	20.20d	56.93	28.40de	58.66
T_9 = Indofil 80 WP	0.00g	100.00	16.30ef	65.25	26.40e	61.57
T_{10} = Contaf 5 EC	0.00g	100.00	0.00i	100.00	8.70h	87.34
T_{11} = Control (Untreated)	22.80a	-	46.90a	-	68.70a	-
LSD (P = 0.01)	0.88	-	2.34	-	2.96	-

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

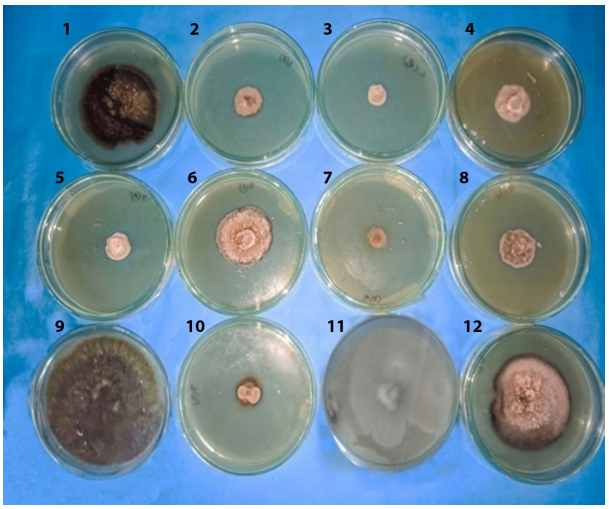


Figure 3. Mycelial growth of *B. gladiolorum* against selected fungicides (100 ppm) at 15 days after inoculation; 1 = Nativo 75 WG, 2 = Folicure 250 EC, 3 = Contaf 5 EC, 4 = Differ 300 EC, 5 = Autostin 50 WDG, 6 = Amistar Top 325 SC, 7 = Score 250 EC, 8 = Trooper 75 WP, 9 = Control, 10 = Tilt 250 EC, 11 = Indofil 80 WP, 12 = Nativo 75 WG.

3.2. Efficacy of Fungicides at 200 Ppm against *Botrytis gladiolorum* in the Laboratory

Ten selected fungicides were tested at the rate of 200 ppm against *Botrytis gladiolorum* and radial mycelia growth was measured at 5, 10 and 15 DAI (Days after inoculation). All fungicides gave significantly different results over control and found effective in reducing the mycelial growth. At 5 DAI, all the fungicides

showed 100% inhibition of growth except Amistar Top 325 SC (11.80 mm), Nativo 75 WG (13.10 mm) and Difar 300 EC (7.10 mm). At 10 DAI, Contaf 5 EC gave the best result against mycelia growth inhibition (100%) which was statistically similar with Score 250 EC (100%) and Folicure 250 EC (100%). At 15 DAI, Contaf 5 EC gave the best performance against mycelial growth and showed 100% inhibition, which was statistically similar to Score 250 EC (100% inhibition) followed by Autostin 50 WDG (83.04%) (Table 3 and Figure 4).

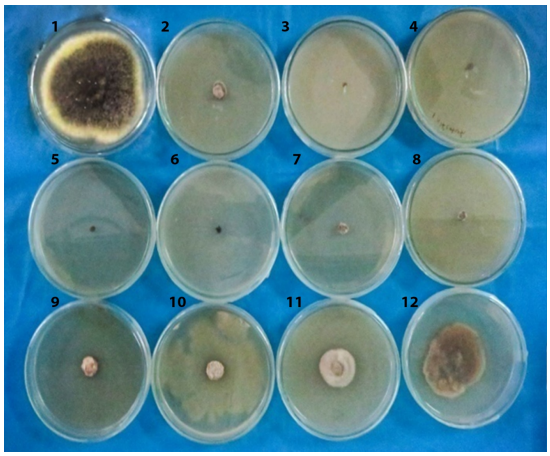


Figure 4. Mycelial growth of *B. gladiolorum* against selected fungicides (200 ppm) at 15 days after inoculation; 1 = Control, 2 = Trooper 75 WP, 3 = Autostin 50 WDG, 4 = Tilt 250 EC, 5 = Score 250 EC, 6 = Contaf 5 EC, 7 = Folicure 250 EC, 8 = Autostin 50 WDG, 9 = Differ 300 EC, 10 = Indofil 80 WP, 11 = Amistar Top 325 SC, 12 = Nativo 75 WG.

Table 3. Efficacy of fungicides at 200 ppm against *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 = Tilt 250 EC	0.00e	100.00	4.20g	91.75	15.60g	79.00
T_2 = Score 250 EC	0.00e	100.00	0.00h	100.00	0.00i	100.00
T_3 = Folicure 250 EC	0.00e	100.00	0.00h	100.00	12.60h	82.90
T_4 = Amistar Top 325 SC	11.80c	60.40	23.50b	53.83	32.30c	56.53
T_5 = Nativo 75 WG	13.10b	56.04	19.80c	61.10	44.40b	40.24
T_6 = Trooper 75 WP	0.00e	100.00	14.40d	71.71	18.50f	75.10
T_7 = Autostin 50 WDG	0.00e	100.00	8.10f	84.09	12.70h	83.04
T_8 = Difar 300 EC	7.10d	76.17	13.00e	74.46	19.70e	73.49
T_9 = Indofil 80 WP	0.00e	100.00	12.80e	74.85	21.90d	70.52
T_{10} = Contaf 5 EC	0.00e	100.00	0.00h	100.00	0.00i	100.00
T_{11} = Control (Untreated)	29.80a	-	50.90a	-	74.30a	-
LSD (P = 0.01)	0.51	-	1.10	-	1.16	-

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

3.3. Efficacy of Fungicides at 300 Ppm against *Botrytis gladiolorum* in the Laboratory

Ten selected fungicides were tested at the rate of 300 ppm against *Botrytis gladiolorum* and radial mycelia growth of fungus was measured after 5, 10 and 15 DAI. All fungicides showed significantly different results over control and found effective in reducing the mycelial growth of the pathogen. At 5 DAI, all the fungicides showed 100% inhibition of growth. At 10 DAI all the fungicides showed 100% inhibition of mycelia growth except Amistar Top 325 SC (78.45%) and Nativo 75 WG (77.72%). At 15 DAI, Contaf 5 EC showed the best performance against mycelial growth and showed 100% inhibition, which was statistically similar with Tilt, Score 250 EC (100% inhibition), Autostin 50 WDG and Folicure 250 EC (Table 4 and Figure 5).

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

The growth inhibition was found highest at the doses of 300 ppm in case of all the fungicides at 15 DAI. Among them 100% growth inhibition was recorded in Score 250 EC that showed the best performance against mycelial growth which was statistically similar with Contaf 5 EC Autostin 50 WDG and Folicure 250 EC (Figure 6). This dose can be used for further study in field condition.

Table 4. Efficacy of fungicides at 300 ppm against *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 = Tilt 250 EC	0.00b	100.00	0.00c	100.00	00.00f	100.00
T_2 = Score 250 EC	0.00b	100.00	0.00c	100.00	00.00f	100.00
T_3 = Folicure 250 EC	0.00b	100.00	0.00c	100.00	00.00f	100.00
T_4 = Amistar Top 325 SC	0.00b	100.00	11.70b	78.45	20.00b	72.49
T_5 = Nativo 75 WG	0.00b	100.00	12.10b	77.72	18.80c	74.14
T_6 = Trooper 75 WP	0.00b	100.00	0.00c	100.00	11.60e	84.04
T_7 = Autostin 50 WDG	0.00b	100.00	0.00c	100.00	00.00f	100.00
T_8 = Difar 300 EC	0.00b	100.00	0.00c	100.00	13.20d	81.84
T_9 = Indofil 80 WP	0.00b	100.00	0.00c	100.00	12.90d	82.26
T_{10} = Contaf 5 EC	0.00b	100.00	0.00c	100.00	00.00f	100.00
T_{11} = Control (Untreated)	28.60a	-	54.30a	-	72.70a	-
LSD (P = 0.01)	0.28	-	0.86	-	1.04	-

In a column mean values having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly at 0.01 level of probability.

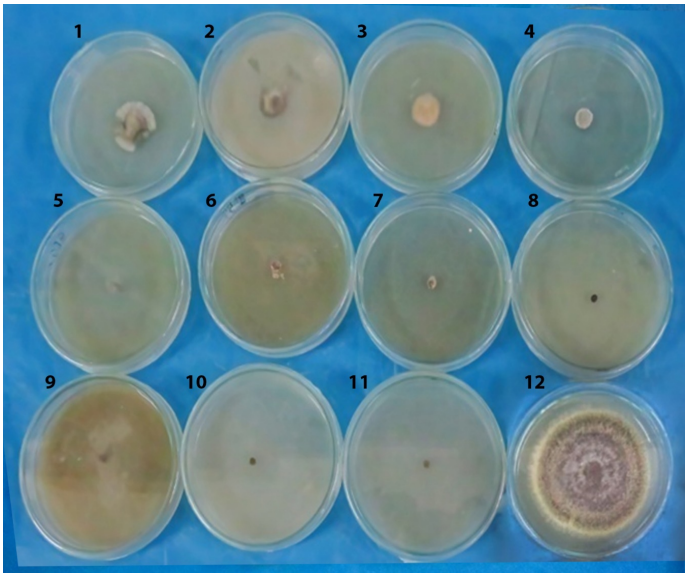


Figure 5. Mycelial growth of *B. gladiolorum* against selected fungicides (300 ppm) at 15 days after inoculation; 1 = Amistar top 325 SC, 2 = Indofil 80 WP, 3 = Nativo 75 WG, 4 = Differ 300 EC, 5 = Autostin 50 WDG, 6 = Trooper 75 WP, 7 = Trooper 75 WP, 8 = Contaf 5 EC, 9 = Folicure 250 EC, 10 = Score 250 EC, 11 = Tilt 250 EC, 12 = Control.

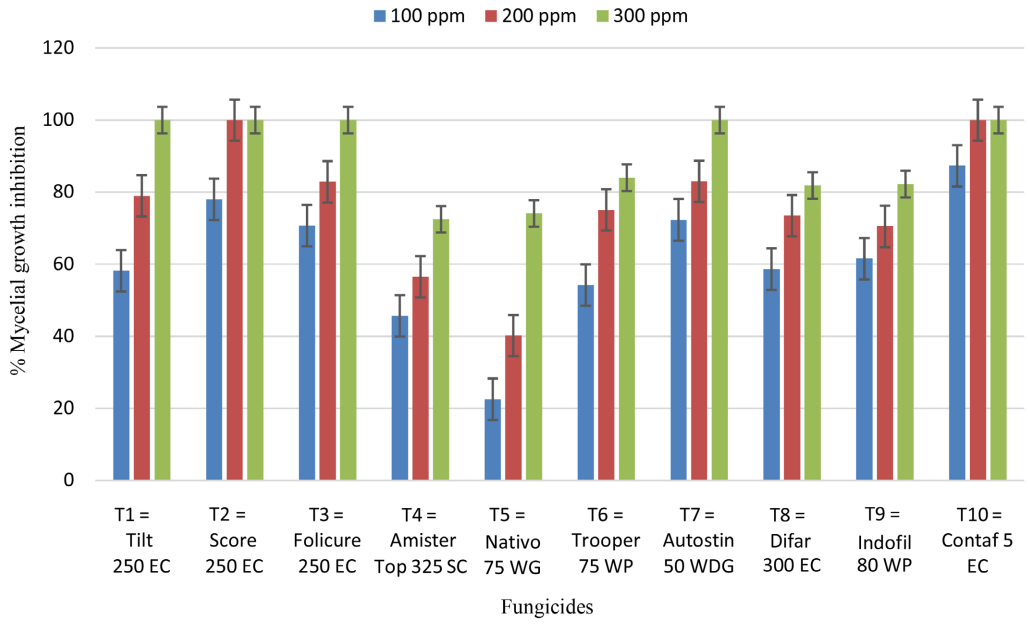


Figure 6. Comparative efficacy of different fungicides on mycelial growth inhibition of *Botrytis gladiolorum in vitro*.

4. Discussion

Gladiolus leaf blight is a destructive disease through the world but no extensive study has been done in Bangladesh. Among ten fungicides Contaf 5 EC gave the best results and the radial mycelia growth was found minimum (00.00 mm) after 5 DAI in 100 ppm which was statistically similar with Score 250 EC treated (00.00 mm) plate and the inhibition of growth was 100%. Autostin 50 WDG, Tilt

250 EC also recorded as a good efficacious fungicide against *B. gladiolorum in vitro*. Hosen *et al.* (2010) [17] found that that Bavistin 50 WP (Carbendazim), CP-Zim 50 WP (Carbendazim), Sulphonate 70 WP (Thiophanate methyl) and Rovral 50 WP (Iprodione) were the most effective fungicides to inhibit the mycelial radial growth of *B. cinerea* at 500 mg/L concentration. The findings of the present study were supported by Rony *et al.* (2021) [18] where they found that Score 250 showed the complete growth inhibition of *Colletotrichum dematium*, whereas Contaf 5 EC showed the complete growth inhibition of *Colletotrichum gloeosporioides* at 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm concentrations. Siddique (2016) [19] found that the lowest percentage (75.68) of disease control and the lowest yield (15.67 ton/ha) were recorded on fungicides containing 2.0 mg/L Ridomil MZ 72 (Metalaxyl 8% + Mancozeb 64%) with 1.0 ml/L Autostin 50 WDG (Carbendazim 50%) during 2014-2015 against blight of potato. In another study, Singh *et al.* (2008) [20] evaluated eight fungicides, both systemic and non-systemic, against the pathogen under laboratory condition. All the tested fungicides, except carbendazim and benomyl, showed good efficacy. Efficacy of three commercially available brands of mancozeb, viz. Dithane M-45, Indofil M-45 and Zebtane M-45, was also tested against the disease, but differences were non-significant. Out of the five fungicides tested under field conditions, three fungicides, namely mancozeb (Dithane M-45, 0.2%), chlorothalonil (Kavach, 0.2%) and iprodione (Rovral, 0.2%) performed very good control of the disease. These fungicides reduced foliar infection and enhanced cormel yield significantly over the control. The cost-benefit ratio was the highest with mancozeb followed by chlorothalonil. Studies on persistence of two fungicides, mancozeb and chlorothalonil, showed that mancozeb (Dithane M-45) provided protective cover for 10 days, whereas chlorothalonil (Kavach) for 15 days. Sultana *et al.* (2017) [21] found that *Trichoderma harzianum* (2%) significantly reduced the growth of *B. gladiolorum*. Maximum plant height, total number of leaves, number of spikes, rachis length, number of florets, floret diameter and yield (flower stalk/ha) were obtained with the application of 2.0% *Trichoderma harzianum* followed by Bavistin (0.2%) in the Siddique *et al.* (2013) [13] also reported that Botrytis blight caused by *B. gladiolorum* regularly attacked the gladiolus plants in Jessore regions of Bangladesh. However, this results regarding isolations, pathogenicity was in confirmity with those of Mirza and Shakir (1991) [11] and Sohi (1992) [9]. *In vitro* bioassay of *B. gladiolorum* against chemicals and bioagent showed that the highest growth was inhibited by Bavistin and *T. harzianum* than nontreated treatment. These results are in conformity with those of Singh and Arora (1994) [22] and Singh *et al.* (2005) [10] who observed that Bavistin proved its performance against *Botrytis* and *Fusarium oxysporum*.

5. Conclusion

Gladiolus leaf blight caused by *Botrytis gladiolorum* is a destructive disease in gladiolus growing district of Bangladesh. Based on findings of *in vitro* evaluation

of fungicides against Bg, Score 250 EC, Contaf 5 EC and Autostin 50 WDG were found to be most effective against *Botrytis gladiolorum* at the rate of 300 ppm and the Score 250 EC, Contaf 5 EC gave 100% inhibition of fungal growth.

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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 contribute 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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