STUDY ON LEAF BLIGHT OF GLADIOLUS (*BOTRYTIS GLADIOLORUM*) IN BANGLADESH AND ITS MANAGEMENT

BY

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CERTIFICATE

This is to certify that the Dissertation entitled **'STUDY ON LEAF BLIGHT OF GLADIOLUS (*BOTRYTIS GLADIOLORUM*) IN BANGLADESH AND ITS MANAGEMENT'** has been submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in PLANT PATHOLOGY**, embodies the result of a piece of *bonafide* research work carried out by **Md. Abdur Rahaman**, Registration No.: **15-06997** under my supervision and guidance. No part of the dissertation has been submitted for any other degree or diploma.

I further certify that any help or source of information received during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

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DEDICATED

TO

MY BELOVED PARENTS AND
Family Members

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The Author

STUDY ON LEAF BLIGHT OF GLADIOLUS (*BOTRYTIS GLADIOLORUM*) IN BANGLADESH AND ITS MANAGEMENT

ABSTRACT

By

Md. Abdur Rahaman

A set of investigations comprising six experiments were conducted in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, during the consecutive years 2016 to 2020 in Bangladesh. The investigation was aimed to find out the disease incidence and severity of gladiolus leaf blight disease in major gladiolus growing districts in Bangladesh and its management *in vitro* and *in vivo*. A survey was conducted in selected gladiolus growing districts in Bangladesh and the data on the disease incidence and disease severity were collected. Leaf blight infected leaves, stems and flowers samples were collected and studied. Survey data revealed that leaf blight incidence in all the districts showed statistically similar result except Bogura and Dhaka. Disease incidence was varied from 18.89% to 32.22%. On the other hand, the highest severity (17.22%) was found in Manikganj district which was statistically similar with Cox's Bazar (15.56%), Faridpur (13%) and Jashore district (12.33%). The lowest disease severity (5.56%) was found in Gaibandha district. Forty-four (44) isolates of *Botrytis gladiolorum* were isolated and identified from the sample collected from survey areas. The highest mycelial radial growth of *Botrytis gladiolorum* (72.00 mm) was recorded from BGCCO3 whereas the lowest (33.00 mm) growth was recorded from BGCCO5 in PDA media at 16 DAI. The radial mycelia average growth rate /day ranged from 2.06 mm to 4.5 mm. Fourteen (14) cultural groups of *B. gladiolorum* were determined based on cultural characteristics. 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 Autostin 50 WDG (83.04) and Folicure 250 EC (82.90%). In 300 ppm Contaf 5 EC showed the best performance at 15 DAI against mycelia 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. Among ten botanicals studied 20% garlic extract showed the best result at 5 DAI and the radial mycelia growth was nil (00.00 mm) which was statistically similar with onion extract and turmeric extract treated plate. At 15 DAI the inhibition of fungal growth was found (73.74%), (71.23%) and (66.90%), respectively with treated by turmeric extract (18.80 mm), garlic extract (20.60 mm) and onion extract (23.70 mm). Among nine organic acids tested at the rate of 1000 ppm Acetic acid gave the best performance and showed (57.02%) inhibition of mycelia growth at 15 DAI followed by Benzoic acid (48.04%) and oxalic acid (41.97%). Three fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field against gladiolus leaf blight. At the flowering stage lowest incidence (14.81%) was found in Score 250 EC treated plot which was statistically similar with Contaf 5 EC (14.81%), Autostin 50 WDG (18.51%) and Turmeric extract (20.37%). Disease severity was lowest in Score 250 EC treated plot (8.33%) which was statistically similar with Contaf 5 EC (9.00%), Autostin 50 WDG (10.00%) and Turmeric extract (10.67%). The growth parameters and yield were also found highest in score 250 EC treated plot, which was statistically similar with Contaf 5 EC, Autostin 50 WDG and Turmeric extract treated plot.

CONTENTS

ACRONYM

ABBREVIATE FORM	ELABORATION
AEZ	Agro-Ecological Zone
FAO	Food and Agriculture Organization
et al.	And others / Co-workers
BBS	Bangladesh Bureau of Statistics
BARI	Bangladesh Agricultural Research Institute
CRD	Complete Randomized Design
DAI	Days after inoculation
No.	Number
%	Percentage
PDA	Potato Dextrose Agar
DAS	Days After Sowing
DAA	Days after application
LSD	Least Significant Difference
^{0}C	Degree Centigrade
NS	Not significant
LSD	Least Significant Difference
CV	Coefficient of variance
ha	Hectare
Hr	Hour
mm	Millimeter
cm	Centimeter

ml	Milliliter
w/v	Weight/Volume
Min	Minimum
SAU	Sher-e-Bangla Agricultural University

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CHAPTER 1

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L) is one of the most popular commercial flowers in Bangladesh. The agro-ecological conditions of Bangladesh are very much conducive for gladiolus cultivation. The major production area of this flower is Jashore, Dhaka, Manikganj, Narayanganj, Chattogram, Cox's Bazar, Bogura, Rangpur, Gaibandha, Faridpur. It has great economic value as a cut-flower and its cultivation is relatively easy. Income from gladiolus flower production is six times higher than that of rice in Bangladesh (Momin 2006).

Gladiolus flower are the preferable as cut flowers due to their different sizes, shapes, and excellent vase life (Bose *et. al.* 1989). Its native is South Africa and has been cultivated globally. It was introduced in Bangladesh around 1992 from India (Mollah *et. al*. 2002). It has recently been become popular in Bangladesh. 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).

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) reported *Botrytis* disease of gladiolus from Canada in 1928. The disease has also been reported from Holland (Drayton 1929), England (Moore 1939), New York (Dodge and Laskaris 1941), Australia (Wade 1945), India (Sohi 1992, Singh *et. al.* 2005), Pakistan (Mirza and Shakir 1991) and Iran (Mirzaei *et. al*. 2008). Sohi (1992) 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). Mirza and Shakir (1991) reported *B. gladiolorum* from corm and leaves of gladiolus in Pakistan.

Cucumber mosaic virus (CMV) and bean yellow mosaic virus (BYMV) are the most prevalent in commercial gladiolus (Loebenstein, 1995). Corm rot and stem rot are the most serious diseases of gladiolus, affecting plants in the field and corms in storage. Corm rot is also called "yellows" on infected plants in the field. The causal organism is *Fusarium oxysporum*. f. sp. *gladioli* (Armitage 1993; Remotti *et. al*. 1997; Chandel and Bhardwaj 1999). The pathogen is affecting plants in the field, causing corms to rot before digging, in storage, or after planting (Jones and Jenkins, 1975). Symptoms of the disease include root, crown, stem rot, vascular wilts, foliage chlorosis, yellowing and necrosis, and discoloured and misshapen flowers (Heimann and Worf 1997). The disease is also known as fusarium yellows where leaves tend to turn downward, yellow progressively, and die prematurely. Brown rot of corms begins in basal plate and core, and extends upward into the leaf bases via vascular strands. Corms may rot in ground or while in storage. The fungus survives in infected corms and in the soil as mycelium, chlamydospores, macro conidia and micro conidia. The infected corms show brownish to black dry rot symptoms. Foliage of affected plants first turns yellow and then brown. Infected roots remain small and are gradually killed. Despite of many attempts to control this disease, the problem is still widespread (Roebroeck and Mes 1992).

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). 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.

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. Chemical practices are highly

effective and low-cost management but harm the environment (Slusarenko *et. al*. 2008). It was observed that most farmers used locally available fungicides, but often the diseases were not well managed. Fungicides, and other types of pesticides, have recently been linked to cancer, respiratory and hormone imbalance diseases (Piel 2019; Hoppin 2017; Juntarawijit 2018).

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 (Hubert *et. al.* 2015). 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 harboured by the plants (Mishra *et. al.* 2003). The plant metabolites and plant base pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers 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. Plants in their natural state possess a relative stable biological balance with microbes on their surface. 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 (Kithan and Daiho 2014).

Researches on detection, identification and management of diseases of gladiolus are very limited in Bangladesh. Thus, there is utmost need to conduct the systematic research on survey, isolation and identification, measurement of disease incidence and disease severity of gladiolus leaf blight in Bangladesh in order to manage diseases of gladiolus effectively in the field. Considering the above facts and points this research work is designed to achieve the following objectives.

OBJECTIVES

1. To estimate the disease incidence and severity of Botrytis leaf blight of gladiolus in Bangladesh.

2. To determine the morphological variation of *Botrytis gladiolorum* causing gladiolus leaf blight in different growing regions.
3. To evaluate the selected fungicides, botanicals and organic acids against *Botrytis gladiolorum in vitro* and *in vivo*.

CHAPTER II

REVIEW OF LITERATURE

Gladiolus **(***Gladiolus grandiflorus* L) is a popular cut flower, which easily gladden our hearts by 'their majestic flower spike'. Besides having aesthetic value, it represents and reflects the glory of collectivism, a 'symbol of beauty, love and tranquility in its arrangement as inflorescence on the spike, resulting and opening an avenue of immense commercial value in the present era of globalized economy. Many diseases attack in this flower. But very few researches work has been carried out in this arena in Bangladesh. There is also very limited significant research works on diseases of gladiolus in the South Asia. However, research works are found regarding diseases of gladiolus in the world. The literatures on diseases of gladiolus and their pathogens are accumulated in this section. This chapter is to review the previous studies that are related to the present study. The review of some related studies is described below:

2.1. Gladiolus (*Gladiolus grandiflorus* L.)

Mollah *et al*. (2002) stated that, gladiolus was introduced in Bangladesh around 1992 from India. It has recently been become popular in Bangladesh. Its demand has been increasing day by day with the advancement of aristocracy and modernization of Bangladesh.

Parthasarathy and Nagaraju (1999) said that, the magnificent long-lasting spike of gladiolus come in a variety of colours and forms which makes it more attractive for use in herbaceous borders, bedding, rockeries, pots, as well as cut flowers.

Noor-un-Nisa *et al*. (2009) conducted a field experiment in Pakistan to investigate the effect of corm size on the vegetative, floral attributes, corm and cormel production in gladiolus. Corm and cormel production of gladiolus has a major role in the growth and development of the gladiolus. Large sized corms significantly increased the leaf breadth, length of flowering spike, and number of florets per spike. Regarding corm production, large sized corms produced significantly higher weight of corms per plant, cormels per plant and combined total weight of corms and cormels per plant.

McKay *et al*. (1986) conducted two experiments in South-East Queensland of Australia to investigate the effects of size and division of the mother corm on yield of gladiolus inflorescences, corms and cormels and inflorescence quality and noticed that, different factors such as corm size influence the production of corms.

2.1.1. Cultivation seasons of gladiolus

Momin (2006) stated that, gladiolus (*Gladiolus communis*) is very popular flower and grown throughout the world in a wide range of climatic conditions. Its magnificent inflorescence with various colour have made it attractive in Bangladesh also. Income from gladiolus flower production is six times higher than from that of rice.

Lu *et al.* (1996) stated that, the gladiolus plants thrive in warm weather conditions, good availability of water throughout the cycle, soil with good drainage, fertile and rich in organic matter, and soil pH in the range of 5.5 to 6.5.

2.2. Diseases of Gladiolus

Gladiolus plants are affected by fungal diseases along with bacteria, virus and nematodes such as Botrytis leaf blight (*Botrytis gladiolorum*) Corm rot, or Fusarium rot (*Fusarium oxysporum* 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) (Singh 1994)

Gladiolus plant is attacked by a number of diseases throughout the world. Of them Botrytis blight caused by B. gladiolorum is very destructive one. The disease is manifested by spots on leaf, flower bud, inflorescence and stem, and corm rot. Sohi (1992) 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).

Cucumber mosaic virus (CMV) and bean yellow mosaic virus (BYMV) are the most prevalent in commercial gladiolus (Stein 1995). Corm rot and stem rot are the most serious diseases of gladiolus, affecting plants in the field and corms in storage. Corm rot is also called "yellows" on infected plants in the field. The causal organism is *Fusarium oxysporum*. f. sp. *gladioli* (Armitage 1993; Remotti *et al*. 1997; Chandel and Bhardwaj 2000). The pathogen is affecting plants in the field, causing corms to rot before digging, in storage, or after planting (Jones and Jenkins 1975). Symptoms of the disease include root, crown, stem rot, vascular wilts, foliage chlorosis, yellowing and necrosis, and discoloured and misshapen flowers (Heimann and Worf 1997). The disease is also known as Fusarium yellows where leaves tend to turn downward, yellow progressively, and die prematurely. Brown rot of corms begins in basal plate and core, and extends upward into the leaf bases via vascular strands. Corms may rot in ground or while in storage. The fungus survives in infected corms and in the soil as mycelium, chlamydospores, macro conidia and micro conidia. The infected corms show brownish to black dry rot symptoms. Foliage of affected plants first turns yellow and then brown. Infected roots remain small and are gradually killed. Despite of many attempts to control this disease, the problem is still widespread (Roebroeck 1992).

Cantor (2006) conducted *Gladiolus* breeding programs attempt to improve gladiolus features such as the color, number and shape of flowers, flowering capacity in winter, multiplication and resistance to foliar and corm diseases.
In Brazil, in addition to agronomic characteristics such as productivity and adaptability, breeding programs are focused on the search for genotypes that are resistant or tolerant to rust (*U. transversalis*), which is the most significant disease of gladiolus in this country (Magie 1960).

Mollah *et al*. (2002) stated that, gladiolus suffers from many diseases such as corm rot, leaf spot and leaf blight. Now days, leaf blight which is caused by *B. gladiolorum* become severe in the farmers' field of Jashore region that thrives in high humidity and cool weather. No attention has been given on the diagnosis of botrytis blight and its control in hazardous to our environment. Tomar (1997) reported that, in Himachal Pradesh of India, the disease incidence of gladiolus ranged between 7.12- 64.23%. The disease incidence is comparatively more in sub-mountainous regions than in temperate ones.

Roy *et al*. (1995) noticed that, gladiolus plant is affected by a number of diseases, out of which gladiolus corm rots. Fusarium wilt and leaf spots are most destructive diseases.

Sohi (1992) worked on diseases of ornamental plants and reported *Fusarium oxysporum* f. sp. *gladioli* from corms and roots and *Botrytis gladiolorum*, *curvularia* sp. and *Stemphylium sp.* from corms and leaves of gladiolus from India.

Chandel and Bhardwaj (2000) stated that, gladiolus is susceptible to a number of diseases incited by fungal, bacterial and viral pathogens such as Fusarium wilt, core or spongy rot, dry or neck rot, Curvularia blight, bacterial scab, grey mould, storage rot etc. Pathological problems, particularly diseases caused by fungal pathogens, take a heavy toll in terms of plant stand, quality and yield.

Buxton and Robertson (1953) found that, the four species of Fusarium namely, *F. oxysporum* f. sp. *gladioli, F. solani, F. moniliforme* and *F. roseum* have been reported to cause wilt or yellows in gladiolus. *F. oxysporum* f. sp. *gladioli* has the widestworld distribution and it can survive in infected corms and soil as mycelium, clamydospores, microconidia and macroconidia.

Magie (1953), Baiswar *et al*. (2007) and Tombolato *et al*. (2010) reported on several pathogens of gladiolus, such as *B. gladiolorum, Curvularia* spp., *F. oxysporum* f. sp. *gladioli, F. solani, Geotrichum candidum*, *Septoria gladioli*, *Phyllosticta gladioloides*, *Stemphylium* sp. and *Uromyces transversalis* infect gladiolus and cause reduction in the yield and quality of the flowers and corms, as well as increasing the cost of production.

2.2.1. Botrytis leaf blight of gladiolus

Classification of *Botrytis* is largely based on morphological and cultural characteristics. Species of *Botrytis* have been named based on host association (Jarvis 1977). Features such as sclerotial size and form and conidium size are useful in delimiting some species, but many species are morphologically similar and growing conditions significantly influence these characters.

Sultana *et. al.* (2017) found significantly highest incidence (100%) of Botrytis blight at 75 days after sowing on 21 January, 2015 and lower incidence (6%) was found at the younger plants of 45 days on December, 2014 in Mymensingh, Bangladesh. The similar trend was observed in case of disease severity that ranged from 8-60%. Highest incidence (100%) and severity (60%) of Botrytis blight was observed at older plants than the younger ones after the 3rd week of January 2015. Sung *et al*. (2003) also found that the Botrytis gray mold *(B. gladiolorum*) reached up to 50% in damaged fields in Korea and *B. gladiolorum* spores produced gray mold on older plants drifted onto the flowers before harvest. However, severe outbreaks of Botrytis blight in mature stage were induced may be due to low temperature 17.9 ^{0}C, high humidity (89%), Rainfal (15 mm) with wind speed (3.06 kmph) and no sunshine at that time. This is supported by Sehajpal *et al*. (2015) who revealed that the progression of botrytis blight disease was more in cool weather and towards the winds and wind direction during January-February.

Sehajpal *et al*. (2015) reported that, the progression of botrytis blight disease of gladiolus was more in cool weather and towards the winds and wind direction during January-February. The severely infected leaves become reddish-brown with grayish conidial masses and dried from the tips. As the disease progressed, the lesions developed and blighted completely the spike, petal, flower bud with grey rot of flowers.

Tesfaye and Kapoor (2010) reported that, *B. gladiolorum* can infect the corm, leaves and flowers. Sclerotia can form on all parts of the plant, including the corm. Under damp conditions, this fungus produces masses of spores above ground that are distributed by the wind. The sclerotia formed underground are large, black, and flat and range in size from 1-9 mm. Under excessively damp conditions, corms and harvested products in cold stores can also be infected. The infection of leaves and stem occurs at cool temperatures (approximately 10°C) and under damp conditions. If the plant remains wet for too long, the infection spreads to other leaves. This disease occurs very often in a crop in which corms have been planted too closely together and also in unventilated greenhouses where the RH reaches excessively high levels.

Sung *et al*. (2003) also found Botrytis gray mold disease of gladiolus (*B. gladiolorum*) reached up to 50% in damaged fields in Korea and *B. gladiolorum* spores produced gray mold on older plants drifted onto the flowers before harvest. However, severe outbreaks of Botrytis blight in

mature stage were induced may be due to low temperature 17.9 °C, high humidity (89%), Rainfall (15 mm) with wind speed (3.06 kmph) and no sunshine at that time.

Sultana *et al.* (2017) noticed Botrytis blight symptoms appeared on Gladiolus grown in Mymensingh regions of Bangladesh during 2014-2015. The disease caused spots on leaves, stems/spikes, buds and flowers. In severe infection, the disease caused both flower and leaf blight. In cool and moist weather Botrytis blight incidence was recorded up to 100% in some fields. The causal pathogen identified as *B. gladiolorum*. The effect of temperature on mycelial growth, sporulation and sclerotial production of *B. gladiolorum* was investigated in different temperatures. The maximum radial was found 20 $\pm 1^0$C. An excellent degree of conidial and sclerotial production also took place at 20 and 25 ± 1^0C. The optimum spore concentration for disease development on the leaf tissue was at $4x10^4$ conidia/ml of water that was identical as recorded from the field. *Trichoderma harzianum* (2%) significantly reduced the growth of *B. gladiolorum*. Maximum plant height, total number of leaves, number of spikes, rachis length, and 10 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 field experiment.

Hosen *et al.* (2010) found the optimum temperature and pH for the best mycelial radial growth of *B. cinerea* was 20°C. The mycelial radial growth increased with the temperature up to 20°C thereafter it decreased gradually up to 30°C and no growth was observed at 35°C.

2.2.2. Taxonomical classification of *Botrytis gladiolorum*

Kingdom: Fungi

Phylum: Ascomycota

Class: Leotiomycetes

Family: Sclerotiniaceae

Genus: *Botrytis*

Species: *Botrytis gladiolorum*

2.2.3. Distribution of Botrytis leaf blight of gladiolus

Drayton (1928) reported *Botrytis* disease of gladiolus from Canada in 1928. The disease has also been reported from Holland (Drayton 1929), England (Moore 1939), New York (Dodge and Laskaris 1941), Australia (Wade 1945), India (Sohi (1992, Singh *et al.* 2005), Pakistan (Mirza and Shakir 1991) and Iran (Mirzaei *et al*. 2008). Sohi (1992) 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). Mirza and Shakir (1991) reported *B. gladiolorum* from corm and leaves of gladiolus in Pakistan.

2.2.4. Leaf Spot Diseases of Gladiolus

Torres *et al.* (2013a) stated that, curvulara leaf spot disease affects leaves, stems, and petals of gladiolus. Symptoms usually begin on leaves first as light to dark brown, oval spots. The symptomatic tissues show leaf spots that are oval to circular, brown with dark edges, and surrounded by a yellow halo. Often the lesions become necrotic and the leaves acquire a dry and wilted appearance.

2.3. Management of Botrytis leaf blight

Botrytis spp. is among the most problematic fungal pathogens in agricultural and horticultural crops worldwide. Botrytis blight of gladiolus is a serious disease that plays havoc under cool and wet weather conditions (McClellen *et al* 1949). It is very difficult to control the disease once it appears in the field. Several approaches have been adopted to control *Botrytis* including botanicals, organic acids and fungicides.

2.3.1. Efficacy of fungicides for management of Botrytis leaf blight

Application of fungicides is the most convenient and predominant way for disease control. Farmers depend on chemical pesticides for control leaf blight of gladiolus because use of synthetic pesticides has become an integral part of agriculture. Their extensive use has encountered two main challenges. First, concerns have been raised over the residual effects and toxicity that affect the environment and human health. For example, fungicides, and other types of pesticides, have recently been linked to cancer and respiratory and hormone imbalance

diseases, thereby depending on the level of exposure (Piel 2019; Hoppin 2017; Juntarawijit 2018). Chemical practices are highly effective and low-cost management but harm the environment (Slusarenko *et al*. 2008).

Sharma *et al.* 2005 stated that Fusarium wilt of gladiolus may be minimized by the integrated management approach under pot culture and polyhouse conditions. The integrated approach using pots treated with neem cake, carbendazim and *Trichoderma harzianum* revealed the highest disease control and enhanced plant health and corm yield. Application of carbendazim (200 ppm), *T. harzianum*(P=0.001) and *Pseudomonas fluorescence* (P= 0.05) decreased corm rot, yellows and the pathogen population in soil resulting increased plant growth and flowering (Khan and Mustafa 2005). Singh and Arora (1994) reported that Bavistin-HCl and Emisan as better fungicides in reducing disease severity (%) and enhancing corm and cormel yield.

Singh *et al.* (2008) evaluated Eight fungicides, both systemic and non-systemic, against the pathogen under laboratory conditions. All the test 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%) provided 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.

Nikam *et al.* (2007) reported that chickpea wilt due to *Fusarium oxysporum*f. sp. *Ciceri* being soil borne disease could be managed by integrating varoius practices like using resistant varieties, seed treatment with chemicals, seed and soil application of bio-agents and amendments of soils with oils seeds cake.This report is supported by Sharma *et al.* (2005) who studied integrated approach using pots treated with neem cake, carbendazim and *Trichoderma harzianum* revealed the highest disease control (Fusarium yellows), enhanced corm yield and improved plant health of gladiolus.

Khan and Mustafa (2005) reported application of carbendazim, *T. harzianum* and *Pseudomonas fluorescence* decreased the corm rot and yellows scores and the soil population of the pathogen and increased plant growth and flowering. Mishra *et al*. (2004) studied the effect of integration of chemicals and biological control agents against gladiolus corm rot. Mirsha *et al.* (2000) used *Trichoderma virens,* carboxin and a combination of both and found good for the control of gladiolus corm rot and wilt caused by *Fusarium oxysporum*f. sp. *gladioli* in glasshouse and field experiment.

Sultana *et al*. 2017 found that *Trichoderma harzianum* (2%) significantly reduced the growth of *B. gladiolorum.* Maximum plant height, total number of leaves, number of spikes, rachis length, and 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 field experiment.

Alemu and Kapoor (2010) and Kapoor (2010) reported that *T. harzianum* could effectively control *Botrytis gladiolorum.* Hermosa *et al*. (2000) also reported that *Trichoderma harzianum* reduces mycelial growth of plant pathogens. Alemu and Kapoor (2007) indicated that *In vitro* treatment of *Trichoderma harzianum, T. viride,* and *Gliocladium* species reduce mycelial growth of Botrytis corm rot (*Botrytis gladiolorum).*

Kapoor (2010) have shown that i*n vivo* evaluation of *Trichoderma* species against *Botrytis corm* rot (*Botrytis gladiolorum*) drastically reduced the disease incidence and severity and simultaneously obtained maximum yield of Gladiolus. Spraying Bavistin or chemical was impractical because concentrated or frequent sprays injured and stained the petals (Mirzaei *et al*. 2008) but *Trichoderma* was not only effective in controlling the *B. gladiolorum* infection, but also increased the yield of flowers as well. Jegathambigati *et al*. (2009) also reported that the *Trichoderma* treatment enhenced plant growth, leading to a significant increase in plant height and weight in relation to untreated control.

2.3.2. Botanical management of Botrytis leaf blight

Botanicals that are target-specific, biodegradable, and relatively safe to non-target organisms would be the best alternative (Pandey 2018). Natural compounds as economically accessible disease control methods are receiving increased attention due to their nontoxicity and biodegradability (Zarandi *et al*. 2009, Sukanya *et al*. 2011, Hajano *et al*. 2012, Bhattacharji and Ali and Nadarajah 2014). Plant extracts have been known for their medicinal and antimicrobial properties since ancient times (Lalitha *et al*. 2010). They offer a greater scope than synthetic chemicals as they are relatively safe, easily biodegradable, and ecofriendly (Sukanya *et al*. 2011, Gurjar 2012).

Ark and Thompson (1959) showed that garlic extracts contain potent fungicides which effectively protect peaches against brown rot (*Monilinia fructicola*). Ethanol extracts of garlic followed by those of *Ocimum santum* and *Datura alba* were found to be most inhibitory to growth of the fungus. Garlic extracts have shown to be inhibitory to a number of fungi like *Fusarium, Alternaria* (Tansey and Appleton 1975).

Based on results obtained in *in vitro* tests, six plant extracts were tested and screened *in vivo*, under field conditions. *Satureja hortensis*, *Allium sativum*, *Hyssopus officinalis*, *Mentha* and *Tagetes patula* extracts have been efficient in limiting grey mould severity in blackcurrant applied at 10 percent compared to untreated control. No *in vivo* activity was registered for *Valeriana officinalis* extract. Plant extracts with highly efficiency can be recommended as a non-polluting and environmentally friendly alternative (organic horticulture) in the protection of blackcurrant as medicinal crop against grey mould (Sesan *et. al.* 2015).

Bhowmick and Vardhan (1982) evaluated the antimycotic activity of leaf extracts of some medicinal plants on *Dreschslera turcica* and observed that extracts from *Vitex negundo* and *Catharanthus roseus* hold the potential to completely inhibit the growth of the *Botrytis* fungus under *in vitro* conditions.

Asthana *et al.* (1986) found the leaf extract of *Ocimum adscendens* was fungitoxic against *Aspergillus flavus*. Five antifungal substances that showed activity against *Alternaria alternata*

in vitro were isolated from extract of *Portulaca oleracea* (Park *et al.* 1986). *In vivo* spraying of rice leaves with extracts from *Lawsonia inermis* was reported to give better control of *Dreschslera oryzae* than seed treatment (Natarajan and Lalitha 1987).

Bandara *et al.* (1988) reported *Acorus calamus, Zingiber zerumbet* and *Curcuma longa* to possess several important antifungal activities. Crude extracts of *Curcuma longa, Zingiber officinale, Allium sativum,* tested *in vitro* showed significant antifungal activity against *Curvularis* sp. Significant inhibition of *Dreschlera graminae, Curvularia lunata, Aspergillus fumigatus. Phytophthora infestans, Pythium, Pyricularia oryzae* and *Candida albicans* were observed with petroleum ether and benzene fractions of the leaf of *Lawsonia inermis.* Their findings revealed that extracts of *A. calamus* and *Z. Zerumbet* had profound effect on growth of all fungi tested. Sporulation of *B. theobromae, F. solani, P. oryzae*was also inhibited.

Manoharachary and Gourinath (1988) determined the efficacy of *Calatropis, Datura, Ocimum, Ricinus and Thidax* against *Curvularia lunata, Cylindrocarpon lichenicola, Fusarium solani* and *Myrothecium leucotrichum*. *Alternaria alternata* and *Fusarium oxysporum* were reported to be inhibited by extracts leaves of *Codieum variegatum* (Naidu 1988). Tiliacorine was reported to be translocated symplastically in plant tissue and thus worked as a systemic fungicide (Singh and Pandey 1988).

Bandara *et al.* (1989) screened plant species for their activity against *Cladosporium cladosporioides*. Plant species whose extracts displayed significant activity were, *Butea monosperma* (stem bark), *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Eupatorium riparium* (whole plant, root), *Pleisospermium alatum* (stembark, rootbark) and Z. *zerumbet* (tuber). The activity of the compounds against *Penicillium*, *Aspergillus niger* and *Curvularia* spp. were evaluated and its activity was found to be comparable to that of the standard fungicide Benlate.

Mishra *et al.* (1989) reported the leaves of *Chenopodium ambrosioides*, *Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. grattissium* showed fungitoxicity against *Aspergillus flavus* at 200, 300, 400 and 500 ppm. Alternaria leaf blight is one of the major diseases of pigeon pea. From *Tiliacora racemosa,* two alkaloids were isolated

and evaluated for their antifungal activity against *Alternaria termissina*. Tiliacorine reduced the germination of the fungus at concentrations greater than 100 ppm (Tripathi and Dwivedi 1989). Extracts of *Portulaca oleracea* have been found to possess protective and therapeutic activities against *Helminthosporium maydis* (Noriel and Robles 1990).

Upadhyaya and Gupta (1990) demonstrated the antifungal activities of *A. sativum, O. sanctum* and *D. alba* on mycelial growth and spore germination of *M. Phaseolina* against *Curvularia lunata* (*Cochliobolus lunatus*). From methanol extracts of twigs of *Oxymitra velutina*, which was active against *Bacillus subtilis, Botrytis cinerea, Saprolegnia asterophora* and *Rhizoctonia solani* (Achenbach and Hemrich 1991).

Dubey and Dwivedi (1991) reported that Onion bulb and *Acacia arabica* leaf extracts completely checked the mycelial growth of *Macrophomina phaseolina*. Jinatko and Vesela (1992) reported that extract from *Chelidonium majus* was highly active *in vitro* against *Botrytis* in reducing pathogen growth by 90 percent.

Yegen *et al.* (1992) studied the fungitoxic effect of extracts of six selected plants and showed that that aqueous extracts of *Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia futicasa* and *Inula viscosa* were fungitoxic to *Fusarium moniliforme*, *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Phytophthoracapsici.* Favaron *et al.* (1993) conducted *in vitro* experiment on extracts of *Allium cepa* and *A. porrum* and found inhibition of some rot fungi including *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium moniliforme* etc.

Meena and Mariappan (1993) studied antifungal effect of the complete ethanolic fraction of three native Chilean plants *Ephedra breana*, *Fabiana imbricate* and *Nolana sedifolia* and reported these to be effective against *B. cinerea* under *in vitro* conditions. The results of this study showed that the ethanolic fractions of *E. breana* and *N. sedifolia* have a fungistatic effect for 14 days, while the fungus is exposed to the media with extracts. The complete methanolic fractions of the three studied plant species and the ethanolic fraction of *F. imbricada* did not show any fungicidal effect. Studies also indicated that foliar application of *Allium sativum* bulb extracts at 1 percent concentration was significantly superior in controlling the disease caused by *Fusarium* spp. and

azadirachtin also reduced the disease intensity and increased the yield over control (Srinivas *et al.* 1997).

Wilson *et al.* (1997) evaluated 345 plants against *B. cinerea*. Among the plant extracts 13 botanicals, *viz*. *Adenocalyma alleaceum*, *Allium ampeloprasum*, *A. ramosum*, *A. sativum*, *Tulbaghia violacea*, *Capsicum annuum*, *C. chinense* and *C. frutescens* showed highest antifungal activities, especially *Allium* and *Capsicum* spp proved to be the most antifungal against *B. cinerea*.

Thiribhuvanmala and Narasinmhan (1998) showed that the leaf extracts from *Delonix regia*, *Pongamia glabra* and *Acacia nilotica* significantly inhibited the mycelial growth of *M. phaseolina*.

Parvu (1998) reported that plant extracts of *Berberis vulgaris* and *Chelidonium majus* effectively inhibited fungus *Botrytis gladiolorum* with increasing alkaloid concentration. The extracts from *Berberis vulgaris* containing 1 percent alkaloids and *C. majus* containing 0.25 percent alkaloids were added to PDA after autoclaving to give alkaloid concentrations ranging from 25 to 250µg/ml. The extract from both plants had increasing inhibitory activity against *Botrytis gladiolorum* with increasing alkaloid concentration reported. *Allium obliquum, A. fistulosum, A. ursinum, Aloe vera, Berberis vulgaris* and *Chelidonium majus* plant extracts were obtained and the antifungal activity was determined against *Apergillus niger*, *Botrytis cinerea*, *B. paeoniae*, *Fusarium oxysporum* f. sp. *gladioli*, *Fusarium oxysporum* f.sp. *tulipae, Heterosporium pruneti*, *Penicillium gladioli*, *Penicillium expansum* and *Sclerotinia sclerotiorum*. Nine botanicals, i.e. *Bhang*, *Bael*, *Drek*, *Eucalptus*, *Congress grass*, *Curry leaves*, *Tulsi*, *Paanch Phooli* and *Onion*, each at 5, 10, 15, 20 percent were evaluated *in vitro* against *Rhizoctonia solani.* All the plant leaf extracts significantly inhibited mycelial growth of test pathogen over untreated control. Among botanicals *Drek* extract was effective inhibiting 46.5 percent of mycelial growth of *Rhizoctonia solani* followed by Bhang (29.7%), Onion (25.4%), Tulsi (23.9%), Bael (20.6%), Panch phooli (17.9%), Curry leaves (14.1%), Congress grass (13.4%) and Eucalyptus (10.4%) (Sharma *et al.* 1999).

Singh *et al.* (1999) reported that aqueous extract of fifty-two plants from different families were tested for their antifungal potential against *Aspergillus* spp. such as *A. candidus*, *A. columnaris*, *A. flavipes*, *A. flavus*, *A. fumigatus, A. niger, A. ochraceus* and *A. tamarii.* Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Sygigium cumini* recorded significant antifungal activity against one or the other *Aspergillus* species tested. *A. flavus* recorded high susceptibility. The percentage of inhibition of aqueous extract of the twelve plants was more than 50 percent against all the test fungi, except *Manilkara zapota, Polyanthia longifolia* and *Eucalyptus globules* against *A. ochraceus* and *A. tamarii*.

Sindhan *et al.* (1999) reported that leaf extract of neem, mint, eucalyptus, tulsi, datura, bougainvillea, ginger, garlic and onion inhibited the mycelial growth of *tcrophomina phaseolina.* Iacomi *et al.* (2000) recorded the highest antibotrytis *in vitro* activity (efficiency between 80 and 100%) was obtained using the following extracts: *Hyssopus officinalis* (at 20, 10 and 5%), *Satureja hortensis*, *Allium sativum, Tagetes patula* (at 20 and 10%) and Mentha (at 20%) against *Botrytis cinerea* in blackcurrant crop. A moderate antibotrytis activity (efficiency between 35.7 and 65.7%) has been noticed for Mentha (at 10 and 5%), Satureja *hortensis*, *Allium sativum* and *Tagetes patula* (at 5%) extracts. Further plant extracts were tested and screened *in vivo*, under field and reported that the extracts of *Satureja hortensis*, *Allium sativum*, *Hyssopus officinalis*, *Mentha* and *Tagetes patula* extracts have been efficient in limiting gray mold severity in blackcurrant applied at 10 per cent compared to untreated control.

Fairs *et al.* (2002) had reported zone of inhibition of aqueous extract of *Curcuma longa* against *Aspergillus spp.* with recorded inhibition zones.

Ichikawa and Yoshida (2002) reported that plant extract of *A. sativum* completely inhibited the growth of *Botrytis* due to sulphur containing compounds such as allicin, diallylsulfides, dithiins, alliin and S-allylcysteine.

Choi *et al.* (2004) reported that the extracts of *A. sativum*, *Azadirchta indica*, *Lawsonia inermis* and *Azadirchta indica* were also effective to some extent against *Curvularia* sp. There are reports that phytochemicals of *Melia azedarach*, *Euclyptus citriodora* and *Alstonia scholaris* showed fungicidal activity against *Botrytis* and *Fusarium* (Charmaine *et al.* (2005).

Fatehi *et. al.* (2005) reported that hydroalcoholic of *B. vulgaris* have stronger antifungal effect against *Sclerotinia sclerotiorum*. Bhosale *et al* (2008) tested the botanicals under field conditions against leaf blight of onion caused by *Alternaria porr*i. The botanicals, *i.e., Lantana camara* and *Pongamia pinnata* were found effective and gave 27.08 and 22.44 percent disease control, respectively.

Singh *et al.* (2008) found that use of plant products was tested against Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense*. Of the 22 plant species tested, the leaf extract of *Datura metel* (10%) showed complete inhibition of the mycelial growth of *Fusarium oxysporum*. Phytochemical screening was performed for phenolic compounds, allicin and allin in *Allium* species, for aloine in *Aloe vera*, and for berberine in *Berberis vulgaris*. These different antifungal compounds present in botanicals help in inhibiting disease. All botanicals were effective and gave good control against fungal pathogens. The antifungal effects of the studied plant extracts recommend them as candidates for *in vivo* biological control (Parvu *et al.* 2008).

Webster *et al.* (2008) reported that the extracts of *Salvia sclarea, S. officinalis* and *Ricinus officinalis* could be considered as potential sources of antifungal compounds for treating diseases in plants. Antifungal activity of *Aloe vera* plant extract against the mycelial growth of *Botrytis* has been reported (Casian *et al.* 2009). Mahmoudabadi and Nasery (2009) evaluated *A. ver*a against the mycelial growth of *B. gladiolorum*, *Fusarium. oxysporum* f.sp. *gladioli*, *Heterosporium pruneti* and *Penicillium gladioli*.

Sehajpal *et al.* (2009) reported the antifungal effect of 44 plant extracts against the pathogen *Rhizoctonia solani*. Out of 44 plants tested, 36 plant extracts showed varied degree of antimicrobial effect at different concentrations against the pathogen whereas 8 plants extracts, *viz. Abrus precatorious, Acacia auriculiformis, Bougainvillea glabra, Convolvulus arvensis,*

Hibiscus rosa-sinensis, *Morus alba*, *Thevatia peruviana,* and *Withania somnifera* did not exert any effect. Among all the plant extracts, *A. sativum* exhibited strong fungitoxicity even at the lowest concentration, i.e. 100 ppm, with relative magnitude of inhibition 2.0 mm against the pathogen *R. solani*.

Soylu *et al.* (2010) reported the antifungal activity of *Alliumm* spp against *B. cinerea.* Efforts have been made to study the effects of some botanicals and chemicals on the management of *Alternaria alternata* and *A. helianthi* on sunflower. Results indicated that *Allium cepa* accelerated the growth. On the other hand, addition of dextrose to the medium, carbendazim and *A. sativum* were inhibitory to *A. helianthi*. Akila *et al.* (2011) evaluated plant products along with biocontrol agents against Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense*. Of the 22 plant species tested, the leaf extract of *Datura metel* (10%) showed complete inhibition of the mycelial growth of *F. oxysporum.*

Ling *et al.* (2011) reported the antifungal activity of the extracts from *Atractylodes macracepha* and *Pulsatilla chinensis* that inhibited the mycelial growth of *Botrytis cinerea* up to 80.25 percent. The antifungal activities of the extracts from *Atractylodes macracephal Koidz, Pulsatilla chinensis* and *Bunge Regel* against *Botrytis cinerea* and *Alternaria solani* were studied under *in vitro*conditions. The extracts of all the two plants showed strong antifungal activity against the target pathogenic fungi, especially the antifungal activity of the extract from *Pulsatilla chinensis*; *Bunge regel* was stronger and more stable. The inhibition rate of the mycelium growth of *B. cinerea* was 80.25 percent. At the same concentration the extract from *Atractylodes macracephal Koidz* showed little inhibition of *B. cinerea* and *Alternaria solani. Jatropha curcas,* a tropical perennial plant, is known to contain a wide range of phytochemicals to which its antimicrobial effect is attributable (Namuli *et al.* 2011).

Pathak and Yadav (2011) tested botanicals, namely Garlic, Neem, Datura, Tulsi and Onion leaf extracts at different concentrations against early blight of Potato caused by *Alternaria solani*. All botanicals were found effective. However, among botanicals Garlic bulb extract used at 10 percent concentration was found most efficacious followed by Neem, Datura leaf extarcts.

Patil *et al.* (2011) reported that *Allium sativum* was found to be most effective in inhibiting the growth of *Alternaria dianthicola* causing leaf blight of Gladiolus followed by *Azadirachta indica* and *Ocimum sanctum.*

Sharma and Sharma (2011) reported that acetone extract of *Lawsonia inermis* leaves and Petroleum ether extract of *Eucalyptus citriodora* leaves showed highest activity against *Alterneria solani, Drechslera halodes, Rhizoctonia solani, Fusarium solani, Curvularia lunata, Dreschlera graminae, Fusarium moniliformae, Aspergillus flavus, A. parasiticus* var. *globosus, Trichophyton rubrum, Aspergillus fumigatus* and *Candida albicans.*

Chethana *et al.* (2012) studied the bioefficacy of six plant products (Clerodendron, cinnamon, garlic, neem oil, pongamia oil and turmeric) against *Alternaria porri, Verticillium lecanii* and *Metarhizium anisopliae*) under *in vitro* conditions. Among plant products evaluated, fresh aqueous extract of garlic (20%) was effective in causing 100 percent inhibition of mycelial growth of *Alternaria porri, Verticillium lecanii* and *Metarhizium anisopliae*. Gurjar *et al.* (2012) evaluated botanicals namely; Neem, Garlic and Eucalyptus against *Alternaria* spp. Garlic was found to be highly effective against *Alternaria* spp.

Jhalegar *et al.* (2012) tested botanicals namely *Aloe vera, Eucalyptus* and *Ocimum* against *Penicillium digitatum* and *P. italicum* and results indicated that all botanicals inhibited the growth (colony diameter) of both pathogens over untreated PDA plates, but the inhibition was the strongest by *Aloe vera* extracts. Similarly, under *in vivo* conditions, all botanicals influenced the decay incidence, decay loss, but *Aloe vera* was the most effective.

Panwar *et al.* (2012) evaluated various plant extracts like Neem, Harda, Periwinkle, Nirgudi, and Garlic etc. Garlic (*Allium sativum*) completely inhibited the mycelial growth of *urvularia. lunata* and *C. pallescen* followed by *Azadirachta indica*.

Rongai *et al.* (2012) evaluated four plant extracts (*Adhatoda vasica, Jatropha curcas, Sapindus emarginatus* and *Vitex negundo*) were able to control wilt disease of *Solanum melogena* caused by *Fusarium oxysporum.* Six plant extracts were tested and screened, *in vitro* and *in vivo*, against *Botrytis cinerea*. The highest antibotrytis *in vitro* activity (efficiency between 80 and 100%) was

obtained using the following extracts: *Hyssopus officinalis* (at 20, 10 and 5%), *Satureja hortensis*, *Allium sativum*, *Tagetes patula* (at 20 and 10%) and *Mentha* (at 20%). A moderate antibotrytis activity (efficiency between 35.7 and 65.7%) has been noticed for *Mentha* (at 10 and 5%), *Satureja hortensis*, *Allium sativum* and *Tagetes patula* (at 5%) extracts. The lowest antibotrytis activity or no efficiency was noticed using extracts obtained from *Achillea millefolium*, *Artemisia dracunculus 'sativa'*, *Rosmarinus officinalis* and *Valeriana officinalis* even applied at 20 percent.

Rose *et al.* (2012) conducted a field experiment to evaluate the efficacious nature of some botanicals such as *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum* and *Eichhornia echinulata* against *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phyllosticta phaseolina*, and *Sclerotium rolfsii* by the application of botanicals to soil. In this study, soil amendments with botanicals such as *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum* and *Eichhornia echinulata* significantly reduced the population of plant parasitic nematodes and soil-inhabiting fungi.

Babaei *et al.* (2013) evaluated the antifungal activity of different extracts of *Aloe vera* plant on the growth of *Aspergillus flavus*. The antifungal activity of different extracts of *Aloe vera* on radial growth of *A. flavus* fungus in 0, 2, 20, 200 and 2000 µL in 20 µL assessed. Results showed that acetone extract of *Aloe vera* leaves in concentration of 2000 µL could significantly inhibited (100%) the growth of *A. flavus* and at the lowest concentration of this extract (2µL), the growth inhibition was found to be 51.72 percent.

Chavan and Suryawanshi (2014) evaluated bio efficacy of two effective botanicals, *viz.* garlic (*Allium sativum*) and onion (*Allium cepa*) as foliar sprays against *Colletotrichum truncatum*. In experiments these botanicals were highly effective with highest average reduction in the disease intensity and pod infection to the tune of 51.37 and 76.89 percent and 47.92 and 72.05 percent, respectively and significantly highest yield of 2525 kg/ha and 2513 kg/ha.

Gholve *et al.* (2014) reported that botanicals were evaluated at different concentrations 5, 10, 15 percent, under *in vitro* conditions against *Alternaria macrospora* causing leaf blight of cotton. All the treatments significantly inhibited mycelial growth of the test fungus over untreated

control. Among the botanicals tested, *Allium sativum* was found to be most inhibitory and recorded highest mean growth inhibition (37.47%) followed by *Allium cepa* (34.97%) and *Oscimum sanctum* (32.86%). The present study was conducted with the objective of screening the potential antifungal activities of nine plant extracts *in vitro* against leaf blight of *E. linguiformis* caused by *C. lunata.* Among the plant extracts, *Millettia pachycarpa* root extracts at 10 percent was superior (55.78%), followed by *Acorus calamus* with (53.40%) inhibition (Kithan and Daiho 2014).

Masih *et al.* (2014) showed that the leaf extract of *Pongamia pinnata, Calotropis procera, Nerium indicum* and *Curcuma longa* were taken in aqueous solution, tested against plant pathogenic fungi namely; *Aspergillus fumigatus, Alternaria solani, Helminthosporium* spp. and *Fusarium solani*. The antifungal activity of aqueous extract of *Curcuma longa* was determined against *Aspergillus fumigatus, Alternaria solani, Fusarium solani* and *Helminthosporium* spp. The aqueous extract of *Curcuma longa* showed better activity against *Aspergillus, Fusarium solani, Alternaria solani* and *Helminthosporium* spp.

Mokhtar *et al.* (2014) showed that the antifungal effect of three botanical powdered plants and their extracts against root rot disease incidence of bean caused by *Fusarium solani* and *Rhizoctonia solani* was evaluated under laboratory and field conditions. Powder of chilli pods (*Capsicum annuum*), Cabbage leaves (*Brassica oleracea*) and Eucalyptus leaves (*Eucalyptus obliqua*) were used in the present work. The botanicals tested at different concentrations of 2, 4 and 8 per cent. All applied treatments reduced root rot incidence comparing with untreated control. Higher significant reduction in disease incidence was observed for combined treatment than that of individuals. It is interesting to note that botanical plants powder gave a similar effect to the fungicide Rhizolex-T in reducing root rot incidence either at pre- or post-emergence stages of bean growth.

Regmi *et al.* (2014) evaluated extracts of six plants *viz*; *Jatropa curcas, Datura strumarium, Azadirachata indica, Moringa oleifera, Calotropis gigantean* and *Morus Alba* used concentration of 50 percent were evaluated *in vitro* by poisoned food techniques against the fungus. The results revealed that all plant extracts at 50 percent significantly inhibited the mycelial growth of

pathogen. However, leaf extract of *J. curcas* demonstrated maximum mycelial growth inhibition of *Alternaria alternata (*62.9%) followed by *D. strumarium leaf* extract (55.6%) and was significantly superior to all other tested extracts. *A. indica* extract (51.9%) also inhibited its mycelial growth followed by *M. oleifera (*46.9%), *C. gigantea* extract (23.45%) and *M. Alba* (13.6%) as compared to control.

Four aromatic and medicinal plants were tested for their efficiency in reducing postharvest gray mould of tomato fruits caused by *Botrytis cinerea in vitro* and *in vivo* using two types of extracts: organic plant extract and aqueous extracts. When they were used at 1000 ppm, the four organic plant extracts of *Asteriscus imbricatus* inhibited completely the growth of *B. cinerea*. However complete inhibition of the mycelia growth of the pathogen was observed at 2000 ppm concentration by ether and chloroform extracts of *Pulicaria mauritanica*. Moreover, the organic extracts of *Lavandula dentata* showed a moderate antifungal effect, while the four organic extracts of *Globularia alpym* had no effect on the studied fungus. The aqueous extract of *Asteriscus imbricatus* inhibited completely the growth of *B. cinerea* at 2000 ppm. The aqueous extract of *P. mauritanica* showed a moderate antifungal effect, while the aqueous extracts *L. dentata* and the aqueous extracts of *G. alpym* were ineffective against *B. cinerea*. The *in vivo* test showed that disease incidence decreased as the concentration of *A. imbricatus* and *P. mauritanica* extracts increased. This study has demonstrated that organics and aqueous extracts of these two plants are promising antifungal agents which could be used as biofungicide in tomato crops protection against *B. cinerea* (Senhaji *et al.* 2014).

2.3.3. Effect of botanicals and fungicides on suppression of Botrytis leaf blight of gladiolus

Laurian *et al.* (2006) reported that the hydroalcoholic plant extract obtained from *Aloe vera* fresh leaves had antifungal activity against mycelial growth of *Botrytis*. This study investigated the antimicrobial potential and minimum inhibitory concentrations (MICs) of aqueous, chloroform and ethanol extracts of *Jatropha curcas* and *Calotropis procera* leaves against *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus niger, Penicillum fellutanum* and *Candida* sp.

Parvu *et al.* (2011) reported that aqueous extracts of *A. cepa* and *A. sativum* had antifungal action against *C. albicans* and *Malassezia furfur* isolates. *A. cepa* plant extract had antifungal action

against *Tricophyton rubrum* and *T. mentagrophytes* species too. Another species with antimicrobial activity was *A. ascalonicum*. It had antifungal action against *C. albicans*, dermatophytes (*Microsporum gypseum, Trichophyton mentagrophytes, Epidermophyton floccosum*), *Syncephalastrum* spp., *A. niger, Penicillium* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Cladosporium* spp., *Alternaria* spp and *Drechslera* spp. at MIC of 0.25 percent. MICs of aqueous extracts of both the plants were between 12.5 and 50 mg/ml of extract in all susceptible isolates, while MICs of ethanol extracts were between 12.5 and 100mg/ml. The MICs of chloroform extracts were between 50 and 100 mg/ml for test isolates, while failing to inhibit *S. aureus* and *E. coli* at the highest concentration tested. Leonard *et al.* (2013) Investigated the antimicrobial potential and minimum inhibitory concentrations (MICs) of aqueous, chloroform and ethanol extracts *of Jatropha curcas* and *Calotropis procera* leaves against *Aspergillus niger* and *Penicillum.* MICs of aqueous extracts of both plants was between 12.5 and 50 mg/ml of extract in all susceptible isolates, while MICs of ethanol extracts was between 12.5 and 100 mg/ml. The MICs of chloroform extracts was between 50 and 100 mg/ml for most test isolates.

Kohinoor *et al.* (2003) compared two botanicals, namely leaf extract of neem (*A. indica*) and bishkatali (*Polygonum hydropiper*) with 2 fungicides namely, Rovral 50 WP (0.20% and 0.30%) and Dithane M-45 (0.25%), to find out their efficacy to control Alternaria blight (*A. brassicicola* and *A. brassicae*) of cauliflower seed crop. Leaf extracts of neem and bishkatali were effective in controlling the disease and increasing the yield whereas, in the present study, *A. sativum* and *A. cepa* showed good results as compared to Dithane M-45 fungicide against B. *gladiolorum*.

Kamdi *et al.* (2012) reported that carbendazim and Thiram fungicides were found significantly superior for increasing seed germination, reducing chickpea wilt incidence caused by *Fusarium oxysporum* f. sp. *ciceri* and increase yield. Amongst aqueous leaf extracts *A. indica* was found effective followed by *L. camara* at 5 percent concentration whereas, in present study *A. indica* was found effective against *B. gladiolorum* but *L. camara* was not found to be effective against this fungus.

Rose *et al.* (2012) studied the efficacious nature of some botanicals such as *Argemone mexicana, Calotropis procera, Solanum xanthocarpum* and *Eichhornia echinulata* in combination with normal as well as deep 15 ploughing against soil-inhabiting fungi infesting chickpea such as

Macrophomina phaseolina, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phyllosticta phaseolina* and *Sclerotium rolfsii*.

2.3.4. Organic acids for management of Botrytis blight

Angell *et al.* (1930) demonstrated that one of the toxic substances present in pigmented scales was protocatechuic acid which showed inhibitory effect to *Botrytis* fungi. It must also be noted that the toxicity of the pure protocatechuic acid isolated from onion scales is identical with the toxicity of the pure acid from other sources. Some of the organic acids that have antimicrobial activities are chlorogenic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, vanillic acid, caffeic acid and 3, 4-hydroxybenzoic acid (Clarke 1972).
The organic acids have been reported to be primarily responsible for their antimicrobial activity (Banwart 1981).

Morsy *et al.* (1999) also reported that tuber crops treated with safety chemical like acetic acid showed promising control of Botrytis blight. They found that acetic acid at 7.5 percent completely prevented the infection caused by *Botrytis*. Lang *et al.* (2000) showed that acetic acid and lactic acid at higher concentrations was comparable to fungicidal control of *A. zinniae* and *A. alternata*.

Abd-El-Kareem (2001) reported that acetic acid vapours caused complete inhibition of linear growth of *Botrytis cinerea* and reduced grey mould incidence of table grapes by more than 84.6 percent compared with control berries.

Capdeville *et al.* (2003) studied the effect of pulsing rose cv. Kiss with solutions of citric acid, salicylic acid against *Botrytis cinerea.* Van der Wolf *et al.* (2008) reported that antimicrobial effects 14 with organic acids were variable.

Lagopodi *et al.* (2009) studied the effects of acetic acid fumigation, ethanol fumigation, and steam heat treatment on growth of *Botrytis cinerea in vitro*. Fumigation with 4 or 6 ⁄ µl acetic acid for 6 min, and 8 µl acetic acid for 3 or 6 min resulted in complete inhibition of fungal growth of *Botrytis cinerea*.

Shekhar *et al.* (2009) reported that Acetic acid and Sodium propionate, screened in different concentration (5 mM, 10 mM, 20 mM, 30 mM, 4 mM and 50 mM) for inhibition of radial growth of toxic isolates of *Aspergillus flavus in vitro*. Out of them Ammonium carbonate, Potassium carbonate and Sodium carbonate were found very effective in reducing the radial growth of of *A. flavus* at 20 mM concentration and fungitoxic at 30 mM concentration. Kim *et al.* (2010) showed that a number of benzoic acid analogues showed antifungal activity against strains of *Aspergillus flavus.*

Kumar and Jain *et al.* (2010) studied antifungal activity of chemical food preservatives against food associated fungi isolated from bakery product and pickles. Acetic acid showed maximum antifungal activity against two isolates each of *Aspergillusn luchuensis, A. flavus, Rhizopus stolonifer, Mucor* sp. (100%) followed by one isolate of *Penicillium. oxalicum* (66.6%) and minimum in *Scopulariopsis* sp. (60%). Lactic acid showed antifungal activity against two isolates each of *A. luchuensis* and *A. flavus* (50%), but it did not show any antifungal activity against other food-associated fungi. Benzoic acid produced 75 to 100 percent mycelial growth inhibition against all the selected food-associated fungi except one isolate each of *A. flavus* and *P. oxalicum.* Citric acid and sodium acetate were found to be inhibitory only against *Scopulariopsis* sp. with mycelial growth inhibition of 12.5 percent. Hence, acetic acid is the most active chemical food preservative as compared with other test chemical preservatives in inhibiting the growth of food-associated fungi. No antifungal activity was observed in control. These findings indicated that acetic acid could be used to inhibit the growth of fungal food spoilage and food-borne pathogens and can be used to improve the safety of food products.

Shokri (2011) evaluated inhibitory effects of citric and tartaric acids and their combination on the growth of *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida albicans*, and *Malassezia furfur*. The results showed that citric acid had more fungistatic and fungicidal activities than those of tartaric acid against all pathogenic fungi tested, and its effect on filamentous fungi was higher than that on the yeasts. Antifungal activity of the citric acid alone was higher than tartaric acid alone.

Gutierrez *et al.* (2012) showed that some members of a series of cinnamic acid derivatives possess promising inhibitory activities in cellular assays against fungi of the *Aspergillus* genus.

Montenegro *et al.* (2012) analyzed the extracts and fractions by high-performance liquid chromatography and the assayed compounds were: chlorogenic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, vanillin, vanillic acid, rutin, caffeic acid, 3, 4-hydroxibenzoic acid (veratric acid), 3, 4-dimethoxycinnamic acid (caffeic acid dimethyl ester) and protocatechuic acid. Korosec *et. al.* (2013) showed high antifungal activity of seven cinnamic acid derivatives against *C. lunatus* and two other fungi, *Aspergillus niger* and *Pleurotus ostreatus*.

Kamel *et al.* (2014) conducted a greenhouse experiment on cucumber cultivar to evaluate the efficacy of three concentrations of fulvic acid to control downy and powdery mildew diseases compared with the recommended fungicides and their effects on plant growth. Results revealed that all fulvic acid concentrations significantly reduced disease severity of both the diseases. The highest reduction in disease severity of downy mildew was recorded using 75 ppm of fulvic acid, which was more effective than the recommended fungicide. The significant effect of inhibition of powdery mildew diseases increased gradually with increased fulvic acid concentration. At the same time, the reduction of diseases severity was greater than or equal to the recommended fungicides.

Mohamed *et al.* (2015) tested five organic acids, *i.e.* ascorbic, citric, boric, salicylic and acetic for controlling fungal diseases attacking snap bean pods caused by *P. aphanidermatum* and *B. cinerea.* Pre-harvest spray of the tested acids inhibited completely the decay development of naturally infected pods of both snap bean varieties during storage at 7±1°C and 90-95 percent RH for 18 days, expect acetic acid on cv. Xera at the lower concentration, 0.1 percent, which controlled both post-harvest diseases with efficacy about 70 percent. Boric, acetic and ascorbic acids showed minimum decay caused by *B. cinerea* at the high concentration (0.2, 1 and 2%, respectively) compared with those treated with citric and salicylic acids. As for cv. Valentino, citric acid at 2 percent was the most effective treatment against fungal decay of artificially inoculated snap bean pods with *B. cinerea* as well as all tested concentrations inhibited the cottony rot on snap bean pods artificially inoculated with *P. aphanidermatum*. Pre-harvest

spraying of snap bean with organic acids incited anatomical changes in cuticle and epidermis of pods.

Zhang *et al.* (2015) reported that cinnamic is widely used in food in suppressing plant diseases. In present study showed that application of cinnamic acid was significantly effective on controlling the gray mould of table grape caused by *Botrytis cinerea*. Cinnamic acid can directly inhibit the mycelial growth of *B. cinerea* on potato dextrose agar plates.

CHAPTER III

MATERIALS AND METHODS

Consequently, six experiments were conducted during the study to achieve the objectives. The experiments were as follows

1. Survey of major gladiolus growing districts and determination of disease incidence and disease severity of gladiolus leaf blight
2. Study on morphological variation of *Botrytis gladiolorum*
3. Evaluation of selected fungicides against *Botrytis gladiolorum in vitro*
4. Efficacy of selected botanicals against *Botrytis gladiolorum in vitro*
5. Efficacy of selected organic acids against *Botrytis gladiolorum in vitro*
6. Evaluation of selected fungicides, botanicals and organic acids for management of gladiolus leaf blight in field conditions.

3.1. Experiment 1. Survey of major gladiolus growing districts and determination of disease incidence and severity of gladiolus leaf blight

3.1.1. Survey Area

Gladiolus flower is cultivated twenty-eight (28) districts in Bangladesh (Appendix 1) but some districts cultivated gladiolus in very small scale and some districts cultivated in commercially (Appendix 2). The survey was conducted in the major ten gladiolus growing districts of Bangladesh which were Bogura, Chattogram, Cox's Bazar, Dhaka, Faridpur, Gaibandha, Jashore, Manikganj, Narayanganj and Rangpur. Farmers' fields with standing gladiolus plant that selected for survey and investigation. Three villages were selected in one upazila under a district and three farmers were selected in each village except Jhikargacha, Jashore. Thirteen (13) villages were selected from Jhikargacha, Jashore and also three farmers were selected in each village for collecting required information. Altogether 40 villages along with 120 gladiolus farmers under 10 upazilla that were Sonatala in Bogura, Satkania in Chattogram, Chakaria in Cox's Bazar, Savar in Dhaka, Sadar in Faridpur, Sadullapur in Gaibandha, Jhikargacha in Jashore, Singair in Manikganj, Bandor in Narayanganj and Sadar in Rangpur those were

intensively surveyed and data were collected on disease incidence and severity from selected gladiolus field (Figure 1). Present and previous gladiolus cultivation with different problems and constraints and other socio-economic condition of farmers' data were recorded. The data collected from farmers that have been focused on gladiolus production of Bangladesh.

3.1.2. Methods of survey

Farmers' field were investigated to find out the occurrence and distribution of leaf blight disease caused by *B. gladiolorum* in selected gladiolus fields. The survey was conducted using structural questionnaire among the 120 farmers' from 40 villages under 10 districts. The farmer answered the questions about their socio-economic condition and the problems and constraints of gladiolus cultivation. The interview arranged with farmers at the farmer's field in every villages (Plate 1). The data were collected on present and previous gladiolus cultivation and the data were recorded. Gladiolus Plant parts showed typical disease symptoms that were collected in separate bags and brought to the laboratory. All samples collected from different locations were used to isolate the causal organisms of gladiolus leaf blight.

3.1.3. Experimental period

The survey study was carried out during the period from November, 2016 to February, 2018.

3.1.4. Soil characteristics of surveyed districts

The Savar regions belong to Shallow Red Brown Terrace Soils under Tejgaon Series under the agro-ecological region of "Madhupur Tract" (AEZ No. 28). The Jashore region occupies extensive low-lying areas between the Gangesriver floodplain and the Ganges tidal floodplain under the agro-ecological region of "Gopalganj-Khulna Beels" (AEZ NO. 14). Active Tista flood plain (AEZ No. 02) covered some area of Rangpur and Gaibandha and Karatoya bangali flood (AEZ. 04) plain in Bogura. Young Bramaputra and Jamuna flood plain (AEZ No. 08) covered some districts along with Manikganj, Faridpur in lower Ganges River flood plain (AEZ No. 12) and Narayanganj in Old Bramaputra flood plain (AEZ No. 09). Finlly Chattogram and Cox's Bazar are situated in Chattogram Coastal plain (AEZ No. 23).

3.1.5. Collection of diseased samples

Infected leaves, stem and flowers of gladiolus were collected from farmers' field in surveyed districts of Bangladesh. Sixty-two (62) deseased samples were collected. The diseased samples of gladiolus brought to the laboratory for further examined in laboratory for external symptoms and preserved for isolation of diseased causing pathogen.

3.1.6. Determination of the disease incidence and severity

Disease incidence was calculated on the basis of number of infected plant and it was expressed in percentage. Disease severity was recorded based on the symptom as shown on the surface of the gladiolus leaves. Disease severity was also expressed in percentages to consider the infected area of gladiolus leaves and calculated the percentage from the total area of the leaf. One hundred plants were selected randomly from a field and measured their infected area calculation for severity. The incidence and severity were calculated by following formulas (Agrios 2005)

$$\%\ \text{Disease incidence (DI)} = \frac{\text{No. of diseased plant}}{\text{No. of total plant}} \times 100$$

$$\%\ \text{Disease Severity (DS)} = \frac{\text{Infected area of leaf}}{\text{Total leaf area}} \times\ 100$$

Figure 1. A. Survey and sample collection sites (star marked) under ten selected districts of Bangladesh.

Plate1. Data collection on socio-economic condition of farmer's and gladiolus cultivation, (A. and B. data collection, C. Investigation of infected field to calculate disease incidence and severity).

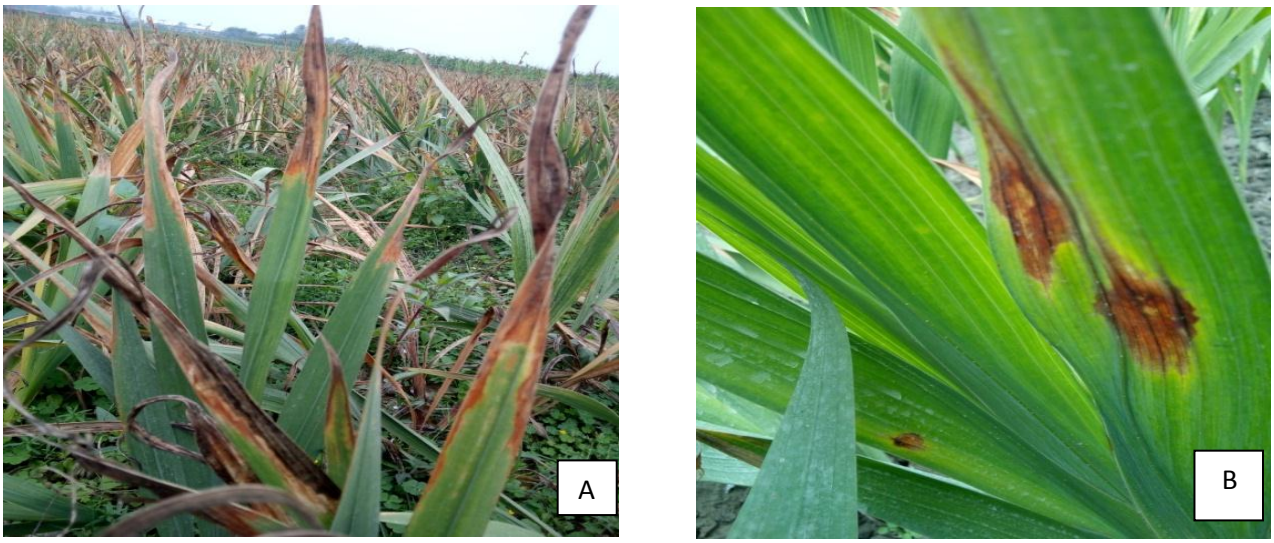


Plate 2. A & B. Infected plant in field.

3. 2. Experiment 2. Study on morphological variation of *Botrytis gladiolorum*

The present investigation was carried out during November, 2017 to April, 2018 at Mycology Laboratory, Department of Plant Pathology, Sher-e- Bangla Agricultural University to examine morphological variations and pathogenicity of *Botrytis gladiolorum* the causal organism of gladiolus leaf blight.

3.2.1. Isolation and Identification of Casual organism

Diseased samples of Gladiolus leaf blight were collected from different farmer's field (Plate 2) under ten selected districts of Bangladesh. Infected plant leaf samples were placed in brown paper packets and brought to laboratory for isolation and identification of pathogens. 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 min sub-sequently rinsed in sterile distilled water for three times. The surface sterilized pieces of leaves were placed separately in petridishes (9 cm) containing Potato Dextrose Agar (PDA) and incubated at 25±1°C. After 4-5 days mycelial tips were recultured in fresh PDA plate and incubated at 25±1^{o}C. To identify the fungus, morphological characters such as colony color, colony growth, and the associated fungus was identified based on the morphology (Mirzaei *et al*. 2008, Barnet and Hunter 1972).

3.2.2. Morphological variability

The identified fungus from four districts were re-cultured and finally 44 isolates prepared for radial growth data collection. The data of the radial mycelial growth were recorded at 2, 4, 6, 8, 10, 12, 14 and 16 days after inoculation.

3.2.2.1. Isolates designation, growth and morphology study

Isolates of *B. gladiolorum* collected from different locations were designated following Aminuzzaman *et. al.* (2010). Growth study was carried out using the method of Hossain and Azad (1992). The PDA plates were inoculated with 5 mm mycelium block at the center of the plate maintaining five replications. After 16 days of incubation at 25±1^{o}C the radial growth was determined and the growth rate was calculated.

3.2.3. Pathogenicity study

Pathogenicity of the isolated fungus was performed under control conditions by inoculating healthy gladiolus leaf with spore suspension of *Botrytis gladiolorum* isolates. Gladiolus plants were grown in earthen pots (20 cm height and 20 cm diameter). The isolate BGMS01 collected from Manikganj, Singair was multiplied on PDA media. Sixteen days after incubation, conidia were harvested from the culture by flooding the plates with sterilized distilled water and scraping with sterilized glass slides. The conidial suspension was filtered through muslin cloth to remove mycelium fragments. The suspension was adjusted to 6×10^4 conidia ml^{-1} using distilled water. One drop of tween-20 was added into the spore suspension. At three leaf stage, apparently healthy gladiolus leaves were inoculated with the conidial suspension. For inoculation, the inoculums suspension was sprayed over the tested plants and plants under control were sprayed with distilled water. Both inoculated and control plants were covered with polythene sheet to keep the plants humid for 48 hours. The pots with plants were placed in a glass house having ambient temperature of 20-22^oC until development of disease symptoms. Characteristic symptoms of the disease appeared within 12 days of inoculation. The inoculated fungus was re-isolated from the inoculated plant parts showing characteristic symptoms following the procedures as mentioned earlier. Pieces of leaf specimens were also plated on moist blotting paper in Petri dishes and incubated at 25±1^oC. The fungus grew on the leaf samples were isolated, purified and morphological characteristics of the fungus were recorded.

3.3. Experiment 3. Evaluation of selected fungicides against *Botrytis gladiolorum in vitro*

3.3.1. 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 September, 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).

3.3.2. 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).

3.3.3. 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). The conical flasks without fungicide served as control media. Sterilized media 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 were measured after 5 days, 10 days and 15 days of incubation (Plate 3 and Plate 4). 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) and data were analyzed using MSTAT-C program (Khan *et al*. 2007).

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

Where,

X= Growth of fungus on control plate

Y= Growth of fungus fungicide treated plate

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

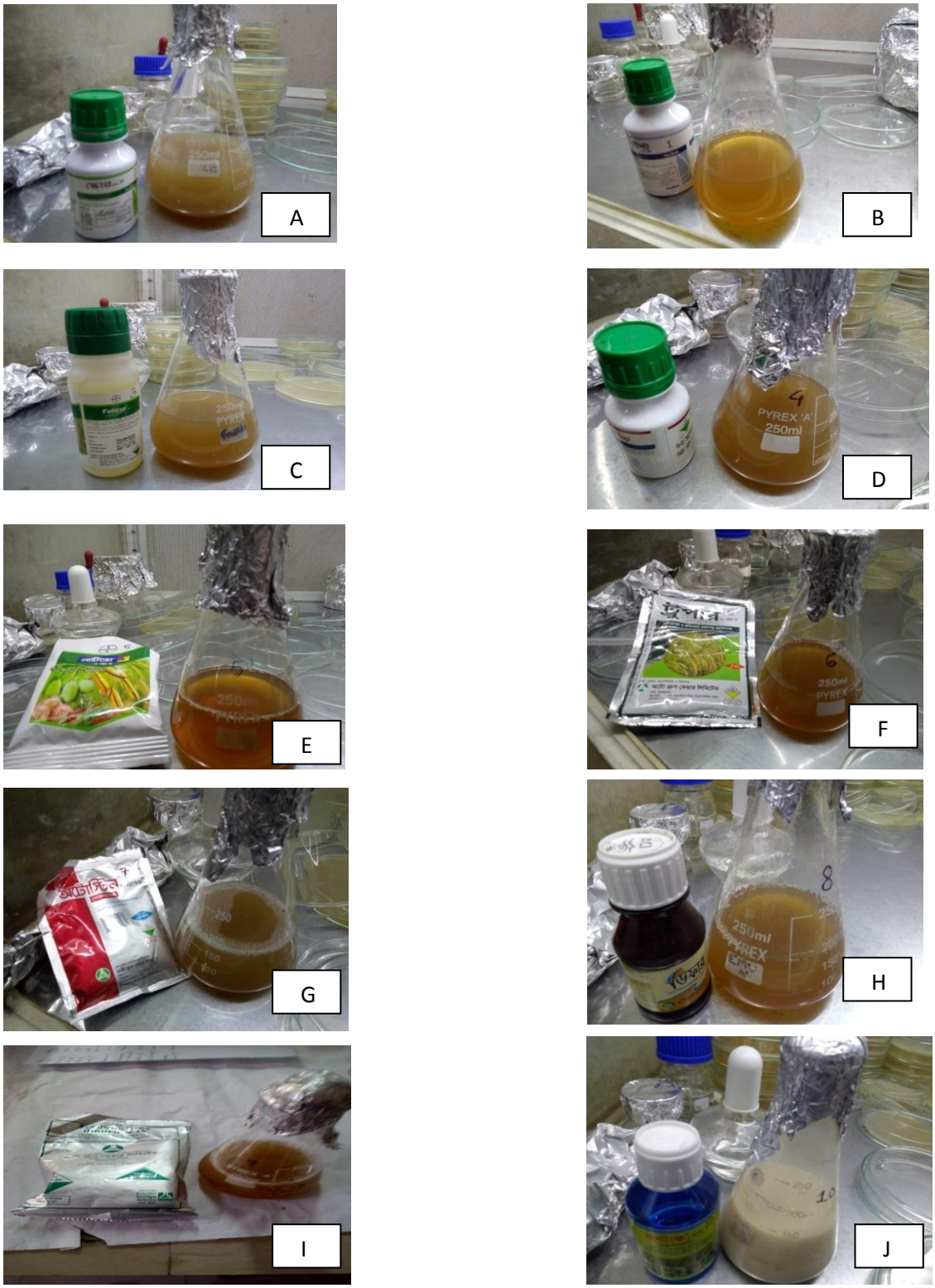
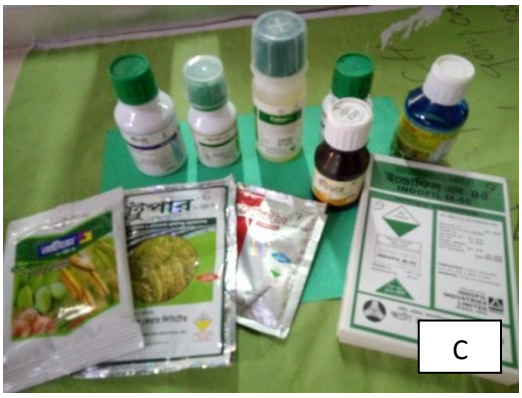


Plate 3. Tested fungicides mixed with PDA in conical flask at 50^0C, A. Score, B. Tilt, C. Folicure, D. Amister Top, E. Nativo, F. Trooper G. Autostin, H. Difar I. Indofil, J. Contaf.



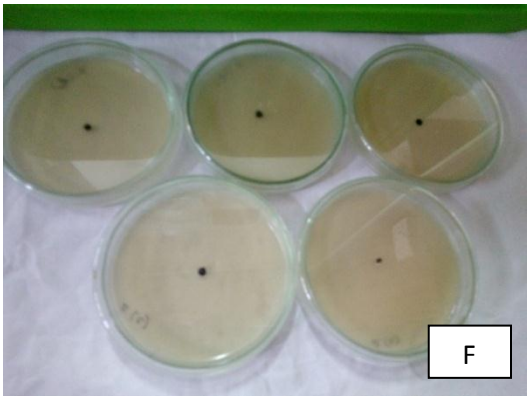


Plate 4. A. PDA media in conical flask before autoclave, B. PDA Media in conical flask after autoclave, C. Fungicides used against *Botrytis gladiolorum*, D. Fungicides mixed with PDA in Petridishes, E. & F. Poisoned PDA media inoculated by *Botrytis gladiolorum* isolates.

3. 4. Experiment 4. Efficacy of selected botanicals against *Botrytis gladiolorum in vitro*

3.4.1. Experimental site and duration

The experiment was conducted in the Mycology Laboratory, Department of Plant Pathology, Shere-e-Bangla Agricultural University, Dhaka during July, 2018 to August, 2018. Ten selected botanicals were tested *in vitro* to evaluate their efficacy on colony growth of the fungal isolate. Evaluation was done through the poison food techniques (Hawamdeh and Ahmad 2001.

3.4.2. The Botanicals used against *Botrytis gladiolorum*

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

3.4.3. Preparation of botanicals leaf extract

Fresh leaves of Mehendi, Chrysanthemum, Basil, Neem, Bael, Arjuna and, Aloevera were collected from SAU campus 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 to make the 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. This stock solution was mixed with 80 ml autoclaved PDA media for made the dose 5% which was tested to determine its antifungal activity against *B. gladiolorum.* 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 (Table 3).

3.4.4. Preparation of bulb and rhizome extract

Fresh bulb of onion and garlic and rhizome of turmeric were collected from Krishi market, Mohammedpur, Dhaka and 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). This solution was filtered through double

layered muslin cloth. The supernatant was filtered through Whatman Filter Paper and made the stock solution. This stock solution was mixed with 80 ml autoclaved PDA media for made the dose 5% which was tested to determine its antifungal activity against *B. gladiolorum.* 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.

3.4.5. Bioassay of botanicals

The botanicals were mixed with melt potato Dextose agar media (80 ml at 50ºC). The botanicals (Mehendi, Chrysanthemum, Basil, Onion, Neem, Bael, Arjuna, Garlic, Aloevera, Turmeric) added in autoclaved (Shovan *et al.* 2008) PDA Media which was distributed in ten conical flasks. The conical flasks without botanicals served as control media. Sterilized 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 *B. gladiolorum*. Five replicate plates were used for each concentration of botanicals (Plate 5 and Plate 6). 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 (Sundar *et al*. 1995) and data were analyzed using MSTAT-C program (Khan *et al*. 2007).

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

Where,

X= Growth of fungus on control plate

Y= Growth of fungus on botanicals treated plate

Table 2. List of botanicals used against *Botrytis gladiolorum* causing leaf blight of gladiolus *in vitro*

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

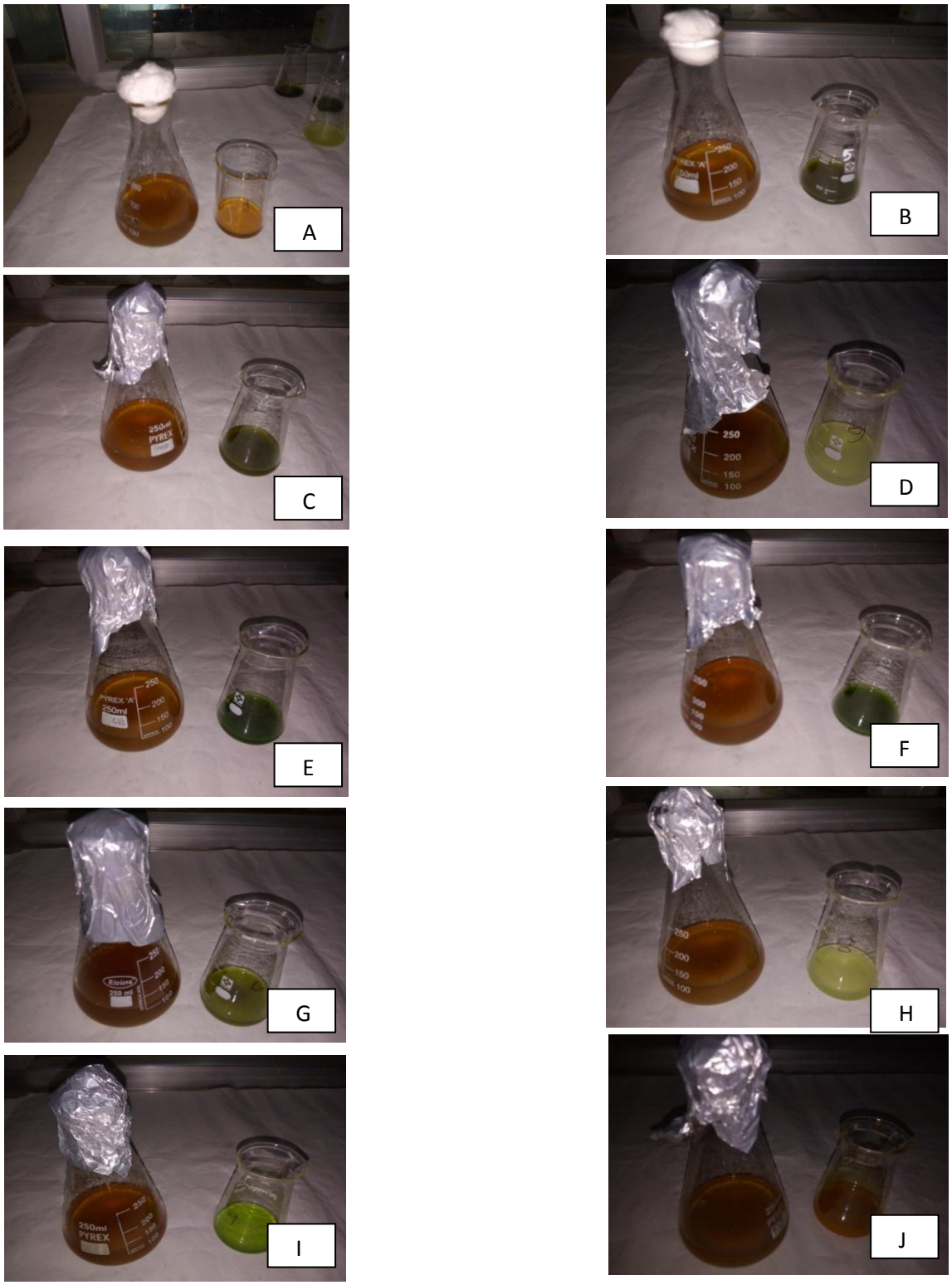


Plate 5. Botanical extracts with autoclaved PDA media in conical flask, A. Mehendi, B. Chrysanthemum C. Tulsi, D. Onion, E. Neem, F. Bael, G. Arjun, H. Garlic, I. Aloevera and J. Turmeric.

Plate 6. 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^{o}C warm PDA media in conical flask, F. Poisoned PDA media inoculated with *B. gladiolorum*.

3. 5. Experiment 5. Efficacy of selected organic acids against *Botrytis gladiolorum in vitro*

3.5.1. Experimental site and duration

The experiment was conducted in the Mycology Laboratory, Department of Plant Pathology, Shere-e-Bangla Agricultural University, Dhaka during September, 2018 to Octoberber, 2018. Nine selected organic acids were tested *in vitro* to evaluate their efficacy on colony growth of the fungal isolate. Evaluation was done through the poison food techniques (Hawamdeh and Ahmad 2001).

3.5.2. The acids used against *Botrytis gladiolorum*

Nine organic acids were evaluated against the colony growth of *Botrytis gladiolorum.* The acids were Tartaric acid, Oxalic acid, Citric acid, Ascorbic acid, Acetic acid, Benzoic acid, Galic acid, Glutamic acid and ortho phosphoric acid at the laboratory in CRD with five replications.

3.5.3. Preparation of the concentration of organic acids

The concentrations of the organic acids were 1000 ppm, 2000 ppm and 3000 ppm. The organic acids were mixed with PDA media at 50^0C in different quantities to make the desired doses in ppm (Table 3).

3.5.4. Bioassay of organic acids

Organic acids added in autoclaved (Shovan *et al.* 2008) PDA Media which was distributed in ten conical flasks. The conical flasks without organic acid served as control media. 20 ml of sterilized PDA media were poured in each 9 cm petridishes. After solidification, the plates were inoculated with a 5 mm disk of 16-days-old cultures. Five replications were used for each concentration of organic acids in CRD design. Radial colony growth diameter was measured after 5 days, 10 days and 15 days of incubation (Plate 7 and plate 8). Colony growth were measured in two directions from the underneath side, perpendicular to each other, taking growth as the mean of the two measures. Percent inhibition of radial growth were computed based on colony diameter on control plate using the following formula (Sundar *et al.* 1995) and data were analyzed using MSTAT-C program (Khan *et al.* 2007).

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

Where,

X= Growth of control plate

Y= Growth of organic acids treated plate

Table 3. Selected organic acids and their doses for the suppression of *Botrytis gladiolorum in vitro*

Name of organic acids	Concentrations		
	1000 ppm	2000 ppm	3000 ppm
Tartaric acid	100 mg/100 ml	200 mg/100 ml	300 mg/100 ml
Oxalic acid	100 mg/100 ml	200 mg/100 ml	300 mg/100 ml
Citric acid	101 mg/100 ml	202 mg/100 ml	303 mg/100 ml
Ortho phosphoric acid	118 µl/100 ml	236 µl/100 ml	354 µl/100 ml
Ascorbic acid	100 mg/100 ml	200 mg/100 ml	300 mg/100 ml
Acetic acid	100 µl/100 ml	200 µl/100 ml	300 µl/100 ml
Benzoic acid	102 mg/100 ml	202 mg/100 ml	306 mg/100 ml
Galic acid	101 mg/100 ml	202 mg/100 ml	303 mg/100 ml
Glutamic acid	100 mg/100 ml	200 mg/100 ml	300 mg/100 ml

Plate 7. A. PDA media in conical flask after autoclaved, B. Organic acids and *Botrytis gladiolorum* isolates. C. PDA mixed with organic acids in conical flask, D. PDA media mixed with organic acids poured in petridishes.

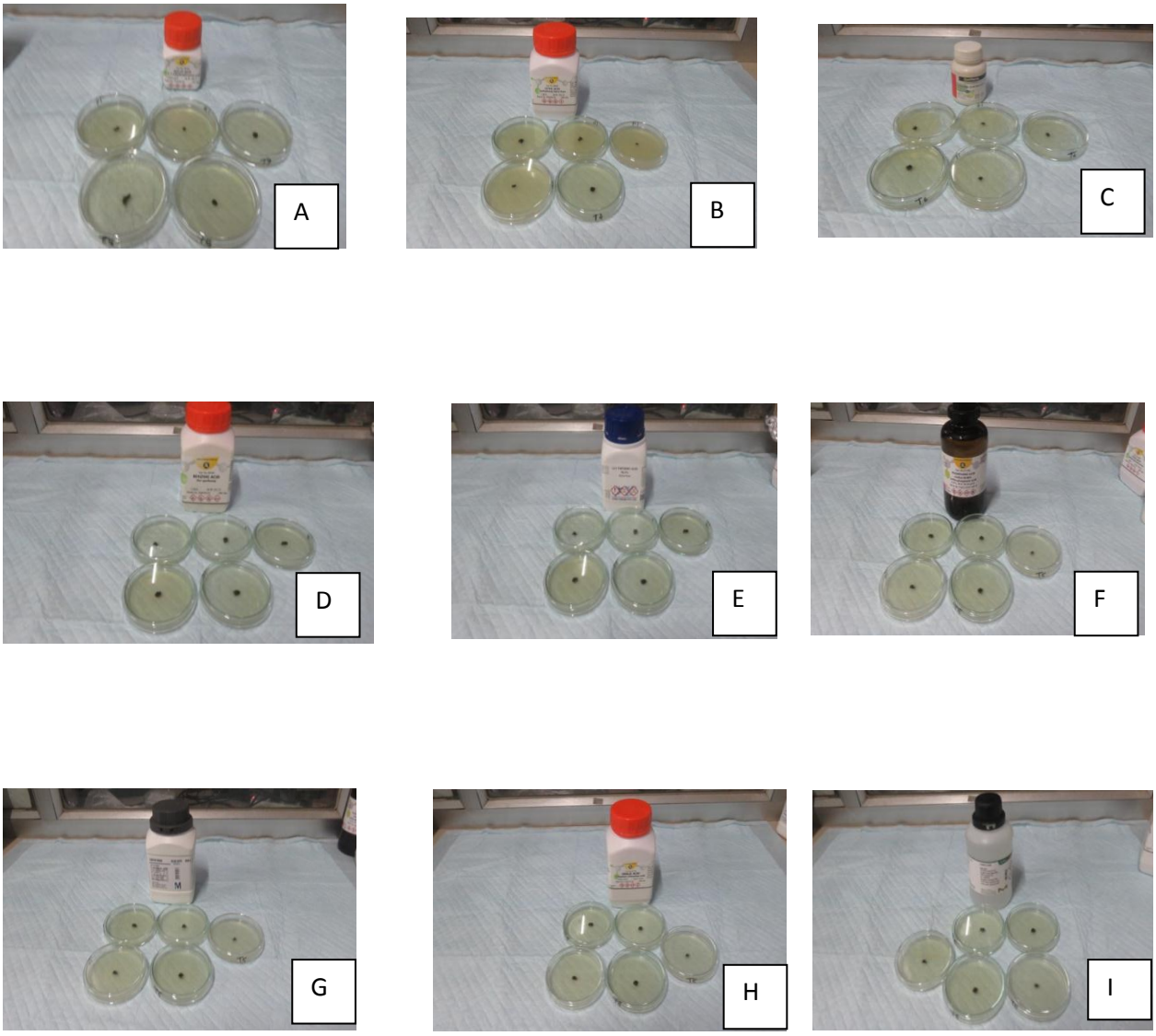


Plate 8. Organic acid amended PDA plates inoculated with *B. gladiolorum*. A. Tartaric acid, B. oxalic acid, C. Citric acid, D. Orthophosphoric acid, E. Ascorbic acid, F. Acetic acid, G. Benzoic acid, H. Galic acid and I. Glutamic acid.

3.6. Experiment 6. Evaluation of selected fungicides, botanicals and organic acids for management of gladiolus leaf blight in field conditions

3.6.1. The experimental site and duration: The experiment was conducted in farmers' field at Katlapur, Singair, Manikganj during October 2019 to January 2020. The experiment was situated in the latitude 23.827553 and longitude 90.243138.

3.6.2. Soil: The experimental site was situated in the sub-tropical zone. The soil of the experimental site lies in Agro-ecological region of Low Ganges River floodplain (AEZ no. 12). The experimental site was medium high land and organic matter content is low (1.5). The soil is slightly alkaline reaction and pH is 7.4. The soil was silt clay loams in texture (Appendix 3).

3.6.3. Climate: The geographical situation of the experimental site was under the subtropical climate, characterized by heavy precipitation during the month of May to August and scanty precipitation during the period from October to March. The mean temperature during the research period was 20.38^0C with average maximum and minimum being 27.25^0C and 13.5^0C, respectively. The average relative humidity and rainfall was 86.41% and 131mm, respectively (Appendix 4).

3.6.4. Planting Materials: Gladiolus corms were used as planting materials in this experiment which was collected from local farmers in Katlapur, Singair, Manikganj.

3.6.5. Land preparation: The experimental plot was opened in the first week of October 2019 with a power tiller and was exposed to the sun for a weak. After a week the land was prepared by several ploughing and cross ploughing followed by laddering and harrowing with power tiller and country plough to bring about good tilth. Weeds and other stubbles were removed carefully from the experimental plot and leveled properly. The final land preparation was done on 13^{th} October 2019.

3.6.6. Manure and fertilizer Application: The manures and fertilizers were applied with the following doses recommended by gladiolus booklet (HRC, 2000) which is presented in the

Table 4. Manure (well decomposed cow dung), MP and TSP were applied as basal dose during the final land preparation and incorporated into the soil, but Urea was applied two installments as top dressing. HaIf of the Urea were applied after emerging 4 leaves and the rest half of the Urea also be applied after emerging 6-7 leaves (before flowering) of the plants.

Table 4. Doses of Manure and Fertilizers applied in the field

Sl no.	Manure/ Fertilizers	Doses/ha	System of application
1	Cow dung	10 MT	Basal application
2	Urea	200 kg	Top dressing
3	TSP	225 kg	Basal application
4	MP	190 kg	Basal application

3.6.7. Seed sowing/planting of corms: The experimental area prepared with thirty individual plots where corms have planted at a depth of 5 cm adopting with ridges and furrows system. Individual plot size was 1 m^2, plot to plot distance 40 cm, row to row distance 30 cm and plant to plant distance 15 cm. The individual plot having three rows and six corms were planted. Thirty individual plots were divided in three blocks which considered as three replications. Medium sized corms were planted in furrows on 15th October 2020.

3.6.8. Intercultural Operations: Intercultural operations viz, weeding, mulching, irrigation, earthing up, stacking were done as and when necessary.

3.6.9. Irrigation: The experimental plots were irrigated as when required during the crop period. The optimum irrigation was given 10-15 days interval after sowing the corms.

3.6.10. Earthing up: Earthing up was done twice during growing period. The first earthing up was done at 25 days after sowing (DAS). The second earthing up was done after 40 DAS.

3.6.11. Fungicides, botanicals and organic acids application

The best three fungicides (Contaf 5 EC, Score 250 EC, Autostin 50 WDG) was selected the evaluation in the laboratory at the rate of 300 ppm, three botanicals (Turmeric, Garlic, Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid, Oxalic acid) at the rate of

3000 ppm were used in this experiment (Table 5). Selected fungicides, botanicals and organic acids were sprayed three times in experimental plot at 20 DAS, 40 DAS and 60 DAS (Plate 9).

Table 5. Selected fungicides, botanicals and organic acids and their doses used in controlling gladiolus leaf blight in field.

Treatments	Doses
T_1 = Contaf 5 EC	0.6 %
T_2=Score 250 EC	0.12 %
T_3=Autostin 50 WDG	0.06 %
T_4=Turmeric	20.00 %
T_5=Garlic	20.00 %
T_6=Onion	20.00 %
T_7=Acetic acid	0.3 %
T_8=Benzoic acid	0.3 %
T_9=Oxalic acid	0.3 %
T_{10}=Spraying water (Control)	100 ml/plot

3. 6. 12. **Design**: The design followed in this experiment was Randomized complete block design (RCBD) with three replications.

3.6.13. Data collection: Data were collected from 18 plants at vegetative (45 DAS) and flowering stage (70 DAS) after treatment application in respect of the following parameters.

3.6.13.1. Average plant height (cm): Plant height refers to the length of the plant from ground level up to shoot apex of the plant. The plant height measured from each treatment used against *Botrytis gladiolorum* and from control at vegetative (45 DAS) and flowering stage (70 DAS) the mean was calculated.

3.6.13.2. Percent Plant infection (disease incidence): The percent plant infection data were collected from experimental field at vegetative (45 DAS) and flowering stage (70 DAS) and the mean was calculated by using the following formula

$$\%\ \text{of Disease incidence (PDI)} = \frac{\text{No. of diseased plant}}{\text{No. of total plant}} \times 100$$

3.6.13.3. Disease severity: Leaf blight severity was determined by the following formula

$$\%\ \text{of Disease Severity (PDS)} = \frac{\text{Infected area of leaf}}{\text{Total leaf area}} \times\ 100$$

3.6.13.4. Total number of leaves/plant: All the leaves of selected plants were counted at vegetative (45 DAS) and flowering stage. Number of leaves per plant was recorded by counting all the leaves from the selected plants of each plot.

3.6.13.5. Total number of healthy leaves/plant: Total healthy leaves of selected plants were counted at vegetative (45 DAS) and flowering stage (70 DAS). Number of healthy leaves per plant was recorded by counting all healthy leaves from the selected plants of each plot and the mean was calculated.

3.6.13.6. Total number of infected leaves/plant: Total infected leaves of selected plants were counted at vegetative (45 DAS) and flowering (70 DAS) stage. Number of infected leaves per plant was recorded by counting all infected leaves from the selected plants of each plot and the mean was calculated.

3.6.13.7. Width of leaves/Breadth of leaves (cm): Width of leaves of selected plants of each plot was measured at vegetative (45 DAS) and flowering stage (70 DAS).

3.6.13.8. Rachis length (cm): Length of the rachis refers to the length from the axial of first floret tip to the tip of the inflorescence. Rachis length was measured by a measuring scale from spike base to the tip. Data were collected at flowering stage (70 DAS).

3.6.13.9. Number of floret per spike: All the florets of the spike were counted from the selected plants of each plot the mean value was calculated.

3.6.13.10. Floret diameter (cm): The floret diameters were measured (cm) from selected plants of each plot and the mean was calculated.

3.6.13.11. Weight of a single spike (g): Ten spikes were cut from selected plants of each plot and the weights of spike (g) were recorded and calculated.

3.6.13.12. Yield (flower stalk/ha): The yields were counted from individual plot and then it was converted into yield flower stalk/ ha (number). The mean value was calculated.

3.6.14. Statistical analysis: The recorded data on different parameters were statistically (MSTAT-C program) analyzed. The mean value was compared by Duncan's multiple range Test (DMRT). The mean of collected data for the treatments was calculated and Correlation and regression analysis were performed to determine the treatments efficacy.

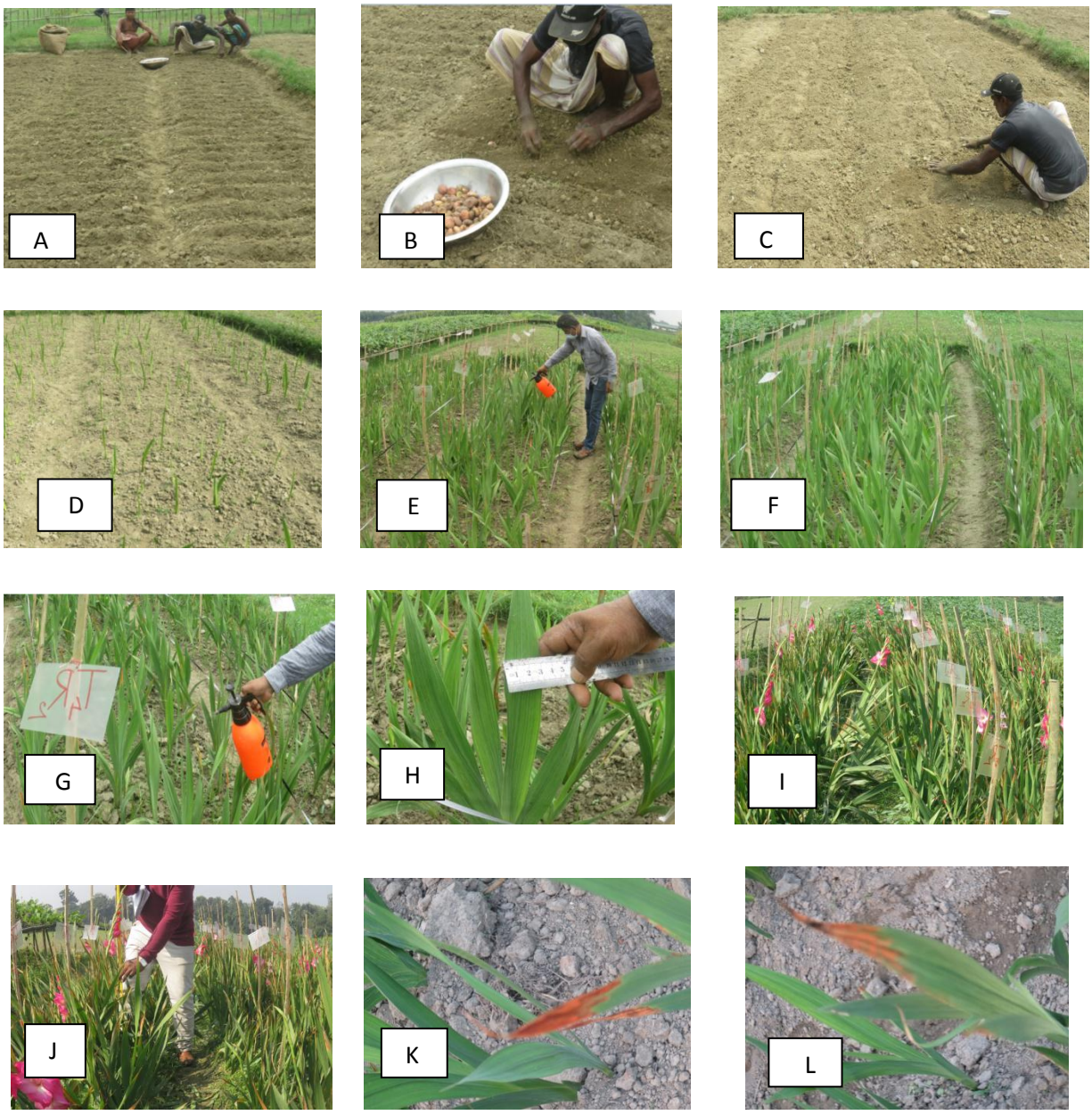


Plate 9. A. Experimental plot preparation, B. Corm transplanting, C. Level after transplanting, D. Seedling, E. Fungicide spray, F. Experimental plot at vegetative stage, G. Fungicide spray, H. Data collection at vegetative stage, I. Experimental plot at flowering stage, J. Data collection at flowering stage, K. Infected leaf, L. Infected leaf.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Experiment 1. Survey of major gladiolus growing districts and determination of disease incidence and severity of gladiolus leaf blight

The study was done by data collection and gladiolus field visit to make interview with farmers through a questionnaire for searching many aspects regarding leaf blight diseases in ten major gladiolus growing districts of Bangladesh. Farmers provided information about their socio-economic condition and the problems and constraints of gladiolus cultivation as well as major diseases that can hampered the normal production of gladiolus in the surveyed area. The aim of the survey was to assess the socio-economic condition of farmers' and to know the knowledge level of farmers about gladiolus cultivation and find out different diseases especially leaf blight of gladiolus which is a major growth limiting factor of gladiolus production. The data were collected from the field and entry in the computer for analyzing the comparisons. The results obtained from the studies conducted in the surveyed areas are presented below sequentially and discussed as to extract the findings systematically in line with the objective of the research work.

4.1. 1. Farmers information on different aspects of gladiolus cultivation

The results of the farmers' knowledge on gladiolus cultivation and diseases have been discussed under the following sub-headings:

4.1.1.1. Gender of the gladiolus farmers

There were 120 gladiolus farmers were participated in the field survey, among them the most (95%) farmers were male (Table 6).

Table 6. Gender of the gladiolus farmers

Gender	No. of the respondent [N=120]	Percentage (%) response
Male	114	95
Female	06	05
Total	120	100

4.1.1.2. Age of the gladiolus farmers

The farmers of different age group were found to engaged in the gladiolus culvation. Among 120 farmers of different age group, 36 to 45 years old ranked first (30.83%) (Table 7).

Table 7. Age of the farmers engaged in gladiolus cultivation

Ages	No. of respondent [N=120]	Percentage (%) response
15-25	15	12.5
26-35	23	19.17
36-45	37	30.83
46-55	24	20.00
56-65	13	10.83
Above 65 years	08	6.67
Total	120	100

4.1.1.3. Education of the gladiolus farmers

The farmers participated in the survey of gladiolus diseases were illiterate to SSC that were 87.50%. Among them Class VI to SSC were ranked first that was 33.33% followed Class I to V (29.17%). About one fourth of the total farmers were illiterate (Table 8).

Table 8. Education level of the gladiolus farmers

Education Level	No. of respondent [N=120]	Percentage (%) response
Illiterate	30	25
Class 1-5	35	29.17
Class VI -SSC	40	33.33
HSC	10	8.33
Degree	05	4.17
Total	120	100

4.1.1.4. Land utilization pattern of the farmers for gladiolus cultivation

According to the farmers opinion, on an average total land area owned of each farmer was 1.25 hactare of which cultivable land under total owned was .80 hactare. The land under gladiolus cultivation was 0.40 hactare. From these findings it was revealed that large portion (50%) of the cultivable land of the gladiolus farmers were engaged under gladiolus cultivation. The Average lease land area was 0.05 hactare that was the (6.25%) of gladiolus cultivable land (Table 9).

Table 9. Farmers opinion on the land utilization pattern for gladiolus cultivation

Land utilization pattern	Land size (Trimmed mean)	
	Decimal	Hectare
1. Total land area owned	307.42	1.25
2. Cultivable land under total land owned	196.75	0.80
3. Land area under gladiolus cultivation	98.37	0.40
4. Gladiolus cultivation under lease area	12.30	0.05

4.1.1.5. Selection of the season for gladiolus cultivation

About 100% of farmers (120) Participated in the survey program were engaged in Rabi season for gladiolus cultivation. From total farmers 70% (84) were engaged in Kharif season and 30% (36) were engaged for gladiolus cultivation in around the year (Table 10). Farmers have been cultivated gladiolus around the year in Dhaka, Manikganj, Narayanganj and Jashore districts.

Table 10. Farmers opinion of the season of gladiolus cultivation

Season	Farmers engaged in gladiolus cultivation	Percentage (%) response
Rabi	120	100
Kharif	84	70
Around the year	36	30

4.1.1.6. Farmers' opinion on the source of gladiolus seeds (corm) used for cultivation

Gladiolus farmers collected gladiolus seeds (corm) from different sources for cultivation. Among those 33.33% of the farmers used gladiolus seeds (corm) from their own source and 20.83% of farmers collected corms from neighbour farmers. Other sources were from flower traders 16.67%, NGO 12.5% and others 8.33% (Table 11).

Table 11. Farmers opinion on the source of gladiolus seeds (corm) used for cultivation

Source of Seeds (corm)	No. of respondents [N=120]	Percentage (%) response
Own seeds (corm)	50	33.33
Neighbour farmers	35	20.83
Seed company	00	00.00
Local Nursery	00	00.00
Importer	00	00.00
Research institutions	00	00.00
NGO	10	12.50
Flower Traders	15	16.67
others	10	8.33
Total	120	100.00

4.1.1.7. Farmers opinion about the variety of gladiolus used by the farmers

Farmers used the different varieties for gladiolus cultivation. Among them, 30% farmers used Mount Everest variety, 15% farmers used BARI-3 (white colour), 15% farmers used BARI-4 (pink colour), 10% farmers used BARI-5 (yellow colour) and 30% farmers used other varieties (Table 12).

Table 12. Variety of gladiolus used by the farmers for cultivation

Variety	No. of respondents [N=120]	Percentage (%) response
Mount Everest	36	30
White (BARI-3)	18	15
Pink (BARI-4)	18	15
Yellow (BARI-5)	12	10
Others	36	30
Total	120	100

4.1.1.8. Farmers response on the major diseases of gladiolus

According to the farmers response, the major diseases were leaf blight, leaf spot, leaf rot, corm rot, stem rot, dry rot, flower rot, scab, mosaic viruses, and aster yellow. The farmers expressed the rank first to eleventh by 54.17%, 30%, 25%, 16.66%, 12.50%, 11.67%, 10%, 9.17%, 6.67%, 5.83% and 5.83%, respectively the diseases were leaf blight, leaf spot, leaf rot, corm rot, stem rot, flower rot, wilt, mosaic viruses, aster yellow, dry rot and scab (Table 13).

Table 13. Farmers response on the major diseases of gladiolus

Name of diseases of Gladiolus	Farmers response on the disease's incidence	
	No of respondent [N=120]	Percentage (%) response
Leaf blight	65	54.17
Leaf spot	36	30.00
Leaf rot	30	25.00
Corm rot	20	16.66
Stem rot	15	12.5
Dry rot	7	5.83
Flower blight	14	11.67
Wilt	12	10.00
Scab	7	5.83
Mosaic viruses	11	9.17
Aster yellow	8	6.67
Total	120	100.00

4.1.1.9. Disease infection conditions

The highest response was 45.83% for leaf blight and the lowest response of farmers 5.83% for scab can infested in flowering stages and also in vegetative stages the highest responsed of farmers 29.16% for leaf blight and the farmers had no response for aster yellow. In the seedling stages the highest response of farmers 16.67% for corm rot and the farmers had no response for aster yellow (Table 14).

Table 14. Farmers response on diseases severity of gladiolus in different growth stages

Name of gladiolus diseases	Diseases severity in different growth stages						
	No of respon-dent [N=120] answer about disease infestation to the seedlings stage	%Res-ponses	No of respondent [N=120] answer about disease infestation to the vegetative stage	%Responses	No of respondent[N =120] answer about disease infestation to the flowering stage	% Respons-es	Total Respon-dents
Leaf blight	15	12.5	35	29.16	55	45.83	120
Leaf spot	12	10.00	20	16.67	26	21.66	
Leaf rot	10	8.33	18	15.00	25	20.83	
Corm rot	20	16.67	15	12.50	20	16.67	
Stem rot	15	12.50	12	10.0	15	12.50	
Dry rot	05	4.67	10	8.33	15	12.50	
Flower blight	00	00.00	00	00.00	18	15.0	
Wilt	12	10.00	15	12.50	20	16.67	
Scab	10	8.33	12	10.00	7	5.83	
Mosaic viruses	12	10.00	18	15.00	15	12.50	
Aster yellow	00	00.00	00	00.00	08	6.67	

4.1. 1.10. Farmers response on the fungicides uses

According to pesticides use, 25% farmers were responsed for Score 250 EC, 20.83% for Tilt 250 EC, 8.33% for Folicure 250 EC, 6.33% for Amister Top 325 SC, 15% for Nativo 75 WG, 10% for Trooper 75 WP, 05% for Autostin 50 WDG, 4.17% for Differ 300 EC, 11.67% for Indofil M-45 80 WP, 10% for Contaf 5 EC and 16.66% for other pesticides used for protection of gladiolus disease (Table 15).

Table 15. Farmers responsed about fungicides use for combating gladiolus diseases

Name of fungicides	Farmers response about fungicides use	
	No. of respondent [N=120]	Percentage % response
Score 250 EC	30	25.00
Tilt 250 EC	25	20.83
Folicure 250 EC	10	8.33
Amister Top 325 SC	08	6.33
Nativo 75WG	18	15.00
Trooper 75 WP	12	10.00
Autostin 50 WDG	06	05.00
Differ 300 EC	05	4.17
Indofil M-45 80 WP	14	11.67
Contaf 5 EC	12	10.00
Others	20	16.66

4.1.2. Disease incidence and severity of gladiolus leaf blight in different villages of different districts

In case of Bogura (Sonatala) district the highest (28.3%) incidence of gladiolus leaf blight was found at Taluknagor village and the severity was also observed the highest (10%) in field of the same village. The lowest incidence (8.33%) and severity (3.33%) was found at Morichbari village. The incidence and severity were 20% and 8.33%, respectively at Bosurhat village (Table 16). Whereas the highest incidence (35%) and severity (11.67%) were found at Fakirhat village of Sathkania upazila under Chattogram district. The lowest incidence (16.67%) and severity (5%) were found at Char Ghagria and the incidence and severity of Rosulpur village was found 28.33% and 10.0%, respectively (Table 17).

The incidence and severity of villages of Chakaria upazila under Cox's Bazar district were also measured. The highest (31.67%) incidence was found at Maizepara village but the highest (21.66%) severity was found at Alamnogor village. The lowest incidence and severity were

found at Purbo Korratipara, 28.33% and 8.33%, respectively (Table 18). In case of Savar Upazila under Dhaka district with considering three villages the highest incidence and severity were found at Birulia village 21.66% and 9%, respectively. The lowest incidence and severity were found at Shaduulah village 16.66% and 5%, respectively (Table 19). In case of Faridpur district, the highest incidence (26.55%) was found at Shiberumpur village of Faridpur Sadar upazila and the highest (15%) severity was found at Kanaipur village. The lowest incidence and severity were observed at Pyarpur village 20% and 11%, respectively (Table 20).

In case of Gaibandha distric the highest incidence (30%) and severity (6.66%) were found at Alipur village. The lowest incidence was found at Tajnagor village (20%) and the lowest severity (5%) was found at Choknodi village (Table 21).

Incase of Jhikargacha upazila under Jashore district the highest incidence (45%) was found at Mothuapara village but the highest severity (25%) was found at Haria Nimtola village. The lowest incidence was found (18.33%) at Taora village. The lowest severity (7.33%) was found at Dhaliapara village (Table 22). Highest incidence (40%) was found at Katlapur village under Singair Upazila, Manikganj district and the lowest incidence (20%) was found at Kashempur village. The highest severity (21.66%) was found at Footnogor village and the lowest severity (10%) was found at Kashempur village (Table 23).

In case of Bandor upazila of Narayanganj district, the highest incidence (38.33%) was found at Digolthi village and the lowest incidence (21.6%) was found at Harjathi village. The highest severity (10%) was found at Digolthi village and the lowest severity (7.33%) was found at Harjathi village (Table 24). Highest incidence (30%) was found at Kamarpara village and the lowest incidence (23.33%) was found at Goalu village. The highest severity (13.33%) was found at Hajirhat village and the lowest severity (8.33%) was found at Goalu village (Table 25).

Table 16. Incidence and severity of gladiolus leaf blight in Sonatala Upazila under Bogura district

Name of District	Name of Upazila	Name of Villages	Disease Incidence% (Mean±SE)	Diseases Severity% (Mean±SE)
Bogura	Sonatala	Morichbari	8.33±7.64	3.33±2.89
		Bosurhat	20±18.03	8.33±7.63
		Taluknagor	28.33±17.56	10±5.00

Table 17. Incidence and severity of gladiolus leaf blight in Satkania Upazila under Chattogram district

Name of District	Name of Upazila	Name of Villages	Disease Incidence% (Mean±SE)	Diseases Severity% (Mean±SE)
Chattogram	Satkania	Char Ghagria	16.67±15.27	5.0±5.0
		Rosulpur	28.33±7.64	10.0±5.0
		Fakirhat	35.0±5.00	11.67±2.88

Table 18. Incidence and severity of gladiolus leaf blight in Chakaria Upazila under Cox's Bazar district

Name of District	Name of Upazila	Name of Villages	Disease Incidence% (Mean±SE)	Diseases Severity% (Mean±SE)
Coxsbazar	Chakaria	Purbo Korratipara	28.33±7.64	8.33±2.89
		Maigepara	31.67±2.88	16.66±5.77
		Alamnogor	30±10.00	21.66±2.89

Table 19. Incidence and severity of gladiolus leaf blight in Savar Upazila under Dhaka district

Name of District	Name of Upazila	Name of Villages	Disease Incidence% (Mean±SE)	Diseases Severity% (Mean±SE)
Dhaka	Savar	Birulia	21.66±12.58	09±3.61
		Shaduulah	16.66±15.27	5.0±5.0
		Bonogram	20.0±20.0	6.66±7.64

Table 20. Incidence and severity of gladiolus leaf blight in Faridpur Sadar Upazila under Faridpur district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Faridpur	Faridpur Sadar	Shiberumpur	26.55±2.89	12.33±2.52
		Pyarpur	20.0±5.00	11.66±2.89
		Kanaipur	25.0±10.0	15.0±5.00

Table 21. Incidence and severity of gladiolus leaf blight in Sadullapur Upazila under Gaibandha district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Gaibandha	Sadullapur	Choknodi	30±5.00	5.00±00
		Alipur	30±10.0	6.66±2.89
		Tajnagor	20±18.08	5.00±5.00

Table 22. Incidence and severity of gladiolus leaf blight in Jhikargacha upazila under Jashore district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Jashore	Jhikargacha	Godkhali	40.0±5.00	16.66±2.89
		Haria Nimtola	26.66±7.64	25±13.23
		Taora	18.33±7.64	10.0±5.00
		Narangali	23.33±7.64	13.33±10.41
		Dhaliapara	18.33±16.07	7.33±6.43
		Kulia	21.66±18.93	8.33±7.64
		Sharifpur	38.33±12.58	10.0±8.66
		Chandropur	35.0±7.64	15.0±5.00
		Baisha	43.33±15.28	13.33±2.89
		Mothuapara	45.0±13.23	13.33±7.64
		Namapara	43.33±7.64	11.66±2.89
		Patuapara	23.0±7.64	10.0±5.00
		Panisara	18.33±16.07	10±05

Table 23. Incidence and severity of gladiolus leaf blight in Singair Upazila under Manikganj district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Manikganj	Singair	Footnogor	36.66±27.54	21.66±7.64
		Katlapur	40.0±5.00	20.0±5.00
		Kashempur	20.0±17.32	10.0±10.0

Table 24. Incidence and severity of gladiolus leaf blight in Bandor Upazila under Narayanganj district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Narayanganj	Bandar	Digolthi	38.33±20.21	10.0±5.0
		Harjathi	21.66±20.21	7.33±6.43
		Madhobpasha	25.0±22.91	8.33±7.64

Table 25. Incidence and severity of gladiolus leaf blight in Rangpur Sadar upazila under Rangpur district

Name of District	Name of Upazila	Name of Villages	Disease Incidence (%) (Mean±SE)	Diseases Severity (%) (Mean±SE)
Rangpur	Rangpur Sadar	Kamarpara	30±26.46	11.66±10.41
		Hajirhat	28.33±7.64	13.33±2.89
		Goalu	23.33±20.82	8.33±7.64

4.1.3. Disease incidence and severity of gladiolus leaf blight in different districts of Bangladesh

Survey was done in 10 selected districts to measure the incidence (%) and severity (%) of gladiolus leaf blight. All the districts showed statistically similar incidence (%) except Bogura and Dhaka. Percent incidence was 32.32, 31.44, 30, 28.33 and 27.22 found in Manikganj, Jashore, Cox's Bazar, Narayanganj, Rangpur district, respectively. Incidence was found 18.89% in Bogura district. Incase of severity, the highest severity was found in manikganj district

(17.22%) which was statistically similar with Cox's Bazar (15.56%), Faridpur (13%) and Jashore district (12.33%). The lowest severity was found in Gaibandha district (5.56%) (Table 26).

Table 26. Incidence and severity of gladiolus leaf blight in different locations of Bangladesh

District	Name of upazila	Disease incidence (%)	Disease severity (%)
Bogura	Sonatala	18.89 c	7.22 cde
Chottagram	Satkania	26.67 abc	8.89 cde
Cox's Bazar	Chakaria	30.00 a	15.56 ab
Dhaka	Savar	19.45 bc	6.89 de
Faridpur	Faridpur Sadar	23.89 abc	13.00 abc
Gaibandha	Sadullapur	26.67 abc	5.56 e
Jashore	Jhikargacha	31.44 a	12.33 abcd
Manikganj	Singair	32.22 a	17.22 a
Narayanganj	Bandor	28.33 ab	8.55 cde
Rangpur	Rangpur Sadar	27.22 abc	11.11 bcde
LSD=(P=0.05)	-	8.04	5.40

4.2. Experiment 2. Study on morphological variation of *Botrytis gladiolorum*

4.2.1. Isolation and identification of *Botrytis gladiolorum*

The isolates of *B. gladiolorum* were identified based on morphological and cultural characteristics. After confirming microscope examination, each isolate was prepared from polyconidial culture and maintained on Potato Dextrose Agar (PDA) for further study (Plate 10).

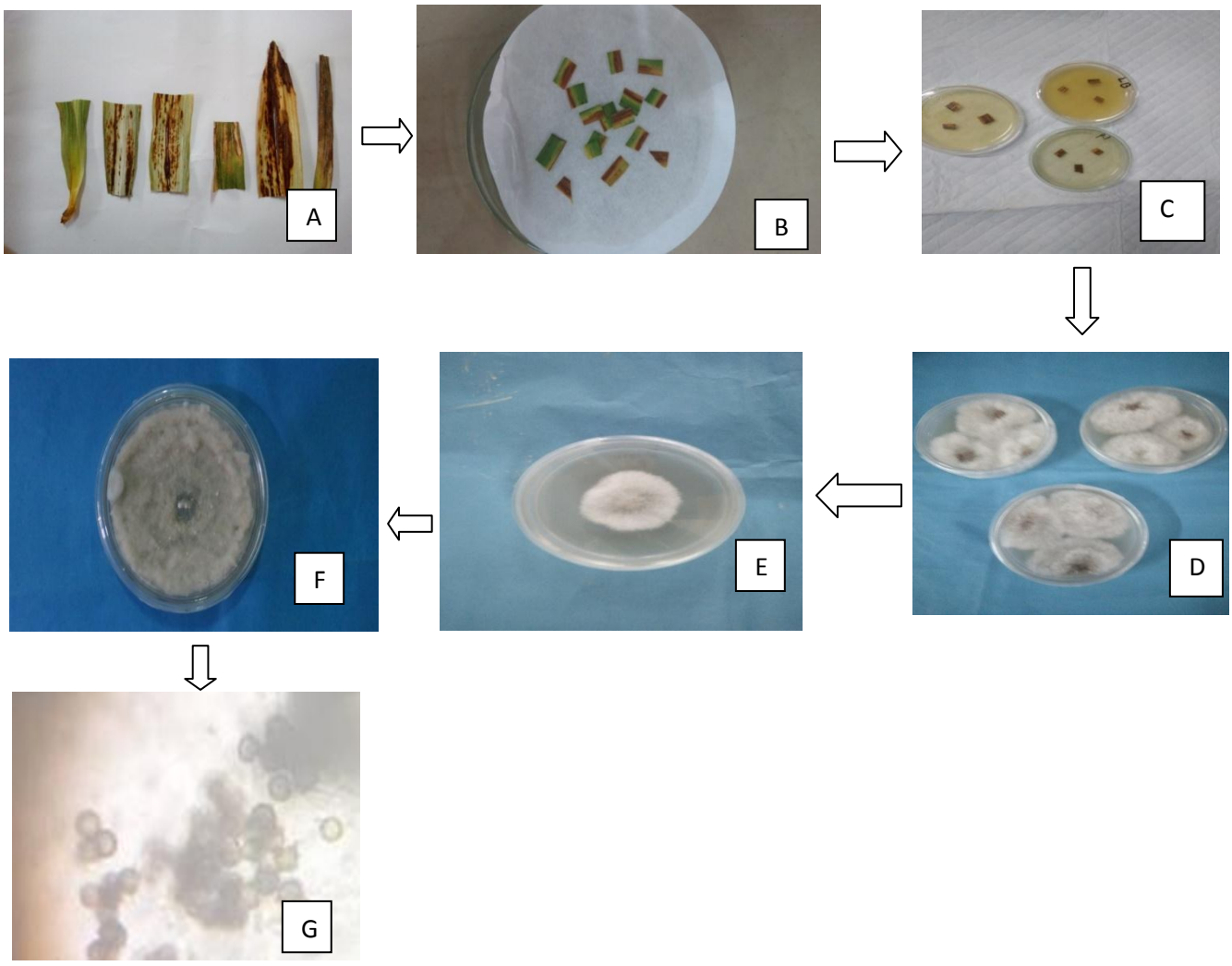


Plate 10. Flow chart of isolation and identification of *Botrytis gladiolorum* A. Infected leaf sample, B. Leaf cutting transfered on blotter, C. Diseased sample in solidified PDA, D. Mycelia growth in PDA media, E. Reculture and F. Pure culture. G. Conidia (×100).

4.2.2. Confirmation of *Botrytis gladiolorum*

The conidia were found obovoid, unicellular, pale brown, smooth. And the sclerotia were abundant, single or aggregated in large masses (Plate 11).

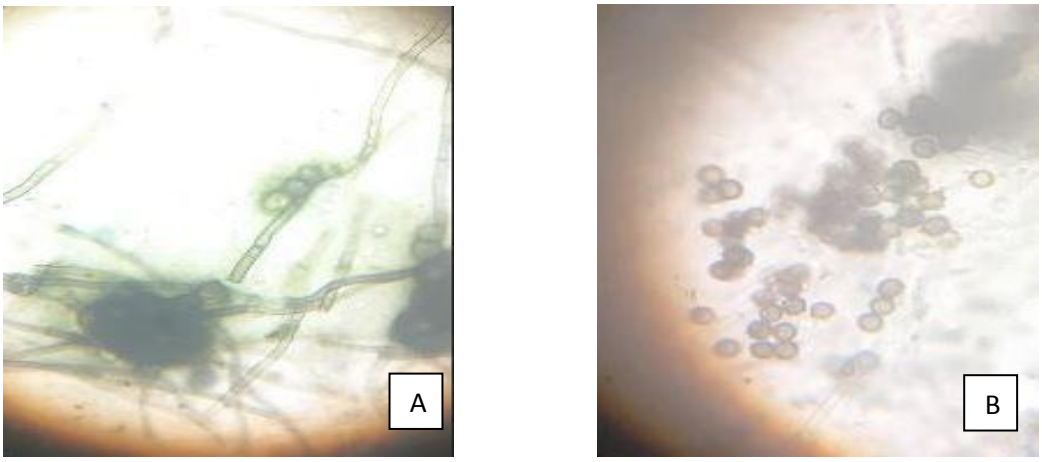


Plate 11. A. Conidiophore with conidia, B. Conidia (×100).

4.2.3. *In vitro* mycelial growth of *Botrytis gladiolorum* on culture media

Mycelial radial growth of *Botrytis gladiolorum* was observed in Potato Dextrose Agar (PDA), variation was recorded (Table 27 and Table 28) among 44 collected isolates. Furthermore, it was revealed that the redial mycelial growth of *B. gladiolorum* varied from 33 mm to 72 mm and average myceial growth rate /day varied from 2.06 mm to 4.5 mm.

4.2.4. Cultural and morphological characterization of *Botrytis gladiolorum* isolates

Forty-four (44) isolates were isolated from leaf blight samples of gladiolus collected from different districts. Isolate colony characteristics was recorded in culture plates in the laboratory. Mycelial growth rate of forty-four isolates were also recorded. All the 44 isolates grown on PDA media were observed the mycelial color, surface texture, shape, and growth (Table 27). The isolates BGCC01 and BGCC03 were gray color on the other hand the isolates BGCC02, BGCC04, BGCC05, BGCC06, and BGCC08 were dark gray colors; the isolate BGCC07 and BGCC10 were light gray color. Grayish ash color was found in the isolates of BGCC09, BGJJ01, BGJJ02, BGJJ03, BGJJ04, BGJJ05, BGJJ06, BGJJ07, BGJJ08, BGJJ09, BGJJ10, BGJJ11, BGJJ12 and BGJJ13. White color was found in the isolates of BGDS01, BGDS02, BGDS04, BGDS05, BGDS06, BGDS10, BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS06, BGMS07, BGMS08, BGMS09, BGMS10 and BGMS11. On the other hand, milky white color was found in BGDS03 and BGDS07 isolate. The isolates BGDS08 and BGDS09 showed Buff color (Plate 12).

The smooth, velvety surface texture and the regular shape were found in the isolates BGCC01, BGCC02, BGCC03, BGCC04 BGCC08, BGCC09 and BGCC10. On the other hand, smooth, velvety surface texture and the irregular shape were found in the isolates BGCC05 and BGCC07. The isolates BGDS01, BGDS03, BGDS04, BGDS05, BGDS07 and BGDS10 have a smooth, cottony surface texture and regular shape. On the other hand, the isolates BGDS02, BGDS05 and BGDS06 showed smooth, cottony surface texture and irregular shape. Rough velvety surface texture and regular shape found in BGJJ01, BGJJ02, BGJJ03, BGJJ04, BGJJ05, BGJJ07, BGJJ08, BGJJ09, BGJJ10, BGJJ11 and BGJJ13. On the other hand, BGJJ06 and BGJJ12 showed rough velvety surface texture and irregular shape. Rough cottony surface texture and irregular shape found in all isolates isolated from samples collected from Manikganj district, these were BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS06, BGMS07, BGMS08, BGMS09, BGMS10 and BGMS11 isolates (Plate 12).

Good mycelial growth appeared in the isolates BGCC02, BGCC03, BGCC08, BGCC10, BGDS03, BGDS06, BGDS07, BGDS08, BGDS09, BGDS10, BGJJ07, BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS09 and BGMS10. On the other hand, medium mycelial

growth showed in the isolates BGCC01, BGCC04, BGCC06, BGCC07, BGCC09, BGDS01, BGDS02, BGDS04, BGDS05, BGJJ01, BGJJ02, BGJJ04, BGJJ06, BGJJ08, BGJJ09, BGJJ10, BGJJ11, BGJJ12, BGJJ13, BGMS06, BGMS07, BGMS08, BGMS09 and BGMS11. Poor mycelial growth was recorded in the isolates BGCC05, BGJJ03 and BGJJ05.

Table 27. Cultural characteristics of different isolates of *Botrytis gladiolorum* collected from farmers field in four major gladiolus growing districts of Bangladesh

Isolates code	Source area of Isolates			Colony Characteristics			Mycelial growth
	District	Upazila	Village/union	Color	Surface Texture	Shape	
BGCC01	Cox's Bazar	Chakaria	Purbo khorrati para	Gray Color	Smooth Velvety	Regular	Medium growth
BGCC02			Purbo khorrati para	Dark Gray Color	Smooth Velvety	Regular	Good growth
BGCC03			Purbo khorrati para	Gray Color	Smooth Velvety	Irregular	Good growth
BGCC04			Purbo khorrati para	Dark Gray Color	Smooth Velvety	Regular	Medium growth
BGCC05			Maizepara	Light Gray Color	Smooth Velvety	Irregular	Poor growth
BGCC06			Maizepara	Dark Gray Color	Smooth Velvety	Regular	Medium growth
BGCC07			Maizepara	Light gray Color	Smooth Velvety	Irregular	Medium growth
BGCC08			Alam nagor	Dark Gray Color	Smooth Velvety	Regular	Good growth
BGCC09			Alam nagor	Grayish Ash Color	Smooth Velvety	Regular	Medium growth
BGCC10			Alam nagor	Light Gray Color	Smooth Velvety	Regular	Good growth
BGDS01	Dhaka	Savar	Birulia	White Color	Smooth Cottony	Regular	Medium growth
BGDS02			Birulia	White Color	Smooth Cottony	Irregular	Medium growth
BGDS03			Birulia	Milky White Color	Smooth Cottony	Regular	Good growth
BGDS04			Birulia	White Color	Smooth Cottony	Regular	Medium growth
BGDS05			Shadullapur	White color	Smooth Cottony	Irregular	Medium growth

Isolates code	Source area of isolates			Colony Characteristics			Mycelial growth
	Distric	Upazilz	Village	Color	Surface Texture	Shape	
BGDS06	Dhaka	Savar	Shadullahpur	White Color	Smooth Cottony	Irregular	Good growth
BGDS07			Shadullapur	Milky White Color	Smooth Cottony	Regular	Good growth
BGDS08			Bonogram	Buff White Color	Rough Cottony	Regular	Good growth
BGDS09			Bonogram	Buff White Color	Rough Cottony	Regular	Good growth
BGDS10			Bonogram	White Color	Smooth Cottony	Regular	Good growth
BGJJ01	Jashore	Jhikargacha	Godkhali	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ02			Haria Nimtola	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ03			Taora	Grayish Ash Color	Rough Velvety	Irregular	Poor growth
BGJJ04			Narangali	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ05			Dhaliapara	Grayish Ash Color	Rough Velvety	Regular	Poor growth
BGJJ06			Kulia	Grayish Ash Color	Mixed Sandy	Irregular	Medium growth
BGJJ07			Sharifpur	Grayish Ash Color	Rough Velvety	Regular	Good growth
BGJJ08			Chandropur	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ09			Baisha	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ10			Mothuapara	Grayish Ash Color	Rough Velvety	Regular	Medium growth

Isolates code	Source area of isolates			Colony Characteristics			Mycelial growth
	Distric	Upazila	Village	Color	Surface Texture	Shape	
BGJJ11	Jashore	Jhikargacha	Namapara	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGJJ12			Patuapara	Grayish Ash Color	Rough Velvety	Irregular	Medium growth
BGJJ13			Panisara	Grayish Ash Color	Rough Velvety	Regular	Medium growth
BGMS01	Manikganj	Singair	Katlapur	White Color	Rough Cottony	Regular	Good growth
BGMS02			Katlapur	White Color	Rough Cottony	Regular	Good growth
BGMS03			Katlapur	White Color	Rough Cottony	Regular	Good groth
BGMS04			Katlapur	White Color	Smooth Cottony	Regular	Good growth
BGMS05			Footnagor	White Color	Rough Cottony	Regular	Good growth
BGMS06			Footnagor	White Color	Rough Cottony	Regular	Medium growth
BGMS07			Footnagor	White Color	Rough Cottony	Regular	Medium growth
BGMS08			Footnagor	White Color	Rough Cottony	Regular	Medium growth
BGMS09			Kashempur	White Color	Rough Cottony	Regular	Good growth
BGMS10			Kashempur	White Color	Rough Cottony	Regular	Medium growth
BGMS11			Kashempur	White Color	Rough Cottony	Regular	Good growth

BGCC (*Botrytis gladiolorum* Cox's Bazar Chakaria), BGDS (*Botrytis gladiolorum* Dkaka Savar) BGJJ (*Botrytis gladiolorum* Jashore Jhikargacha), BGMS (*Botrytis gladiolorum* Manikganj Sigair)

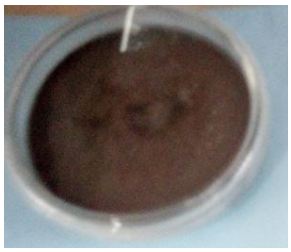
4.2.5. Grouping of isolates of *B. gladiolorum* based on cultural characteristics

Fourteen (14) cultural groups were found on the basis of cultural characteristics. Highest (25%) isolates were found in the cultural group CG-8 (Rough cottony white regular). Lowest (2.27%) isolates were found in CG-3 (Smooth velvety light gray regular). Similar results were found in

CG-4 (Smooth velvety grayish ash regular), CG-10 (Smooth velvety gray irregular) and CG-14 (Mixed sandy grayish ash irregular) (Table 28).

Table 28. Grouping of different isolates of *B. gladiolorum* based on cultural characteristics

Cultural group (CG)	Cultural characteristics	Number of isolates	Designation of isolates	% isolates under each group
CG-1	Smooth velvety gray regular	1	BGCC01	2.27
CG-2	Smooth velvety dark gray regular	4	BGCC02, BGCC04, BGCC06 and BGCC08	9.09
CG-3	Smooth velvety light gray regular	1	BGCC10	2.27
CG-4	Smooth velvety grayish ash regular	1	BGCC09	2.27
CG-5	Smooth cottony white regular	3	BGDS01, BGDS04 and BGDS10	6.82
CG-6	Smooth cottony milky white regular	2	BGDS03 and BGDS07	4.54
CG-7	Rough cottony buff white regular	2	BGDS08 and BGDS09	4.55
CG-8	Rough cottony white regular	11	BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS06, BGMS07, BGMS08, BGMS09, BGMS10 and BGMS11	25
CG-9	Rough velvety grayish ash	10	BGJJ01, BGJJ02, BGJJ04, BGJJ05, BGJJ07, BGJJ08, BGJJ09, BGJJ10, BGJJ11 and BGJJ13	22.73
CG-10	Smooth velvety gray irregular	1	BGCC03	2.27
CG-11	Smooth velvety light gray irregular	2	BCC05 and BGCC07	4.55
CG-12	Smooth cottony white irregular	3	BGDS02, BGDS05 and BGDS06	6.82
CG-13	Rough velvety grayish ash irregular	2	BGJJ03 and BGJJ12	4.55
CG-14	Mixed sandy grayish ash irregular	1	BGJJ06	2.27



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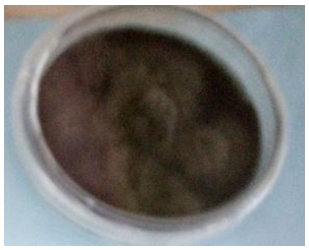

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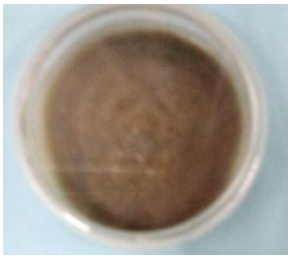

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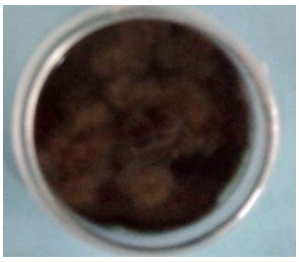

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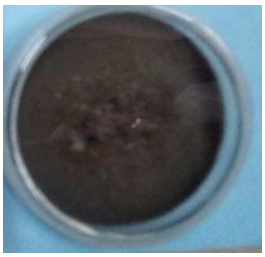

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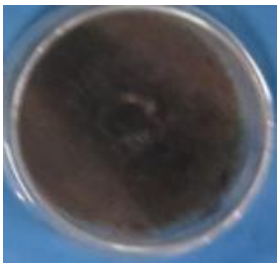

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BGDS02

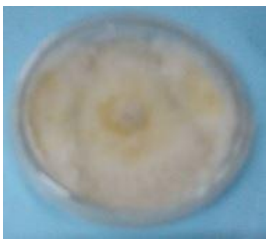
BGDS03

BGDS04

BGDS05

BGDS06

BGDS07



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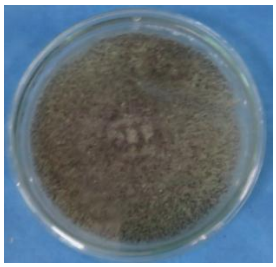

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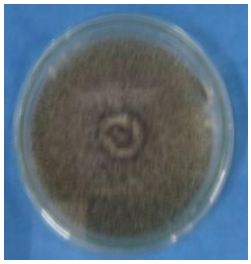

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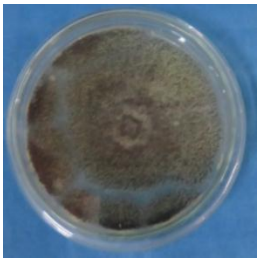

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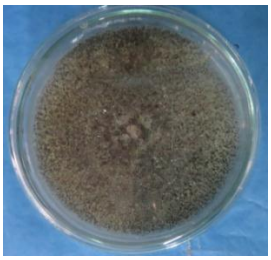

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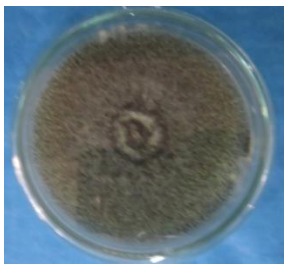

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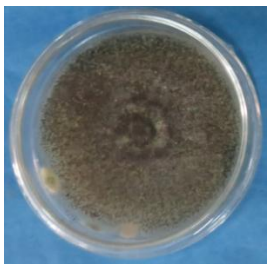

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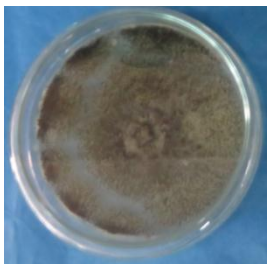

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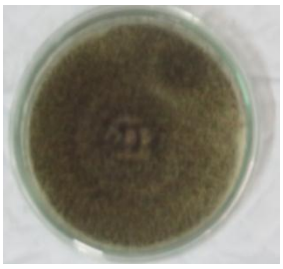

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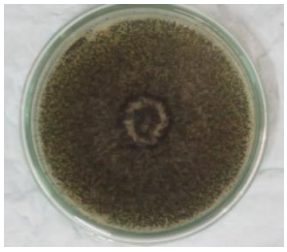

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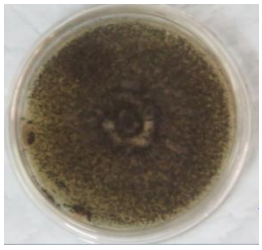

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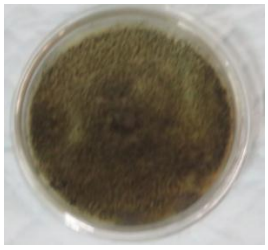

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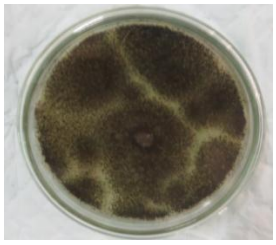

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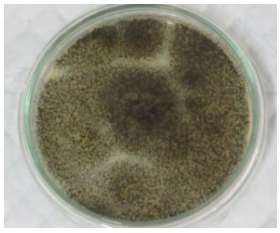
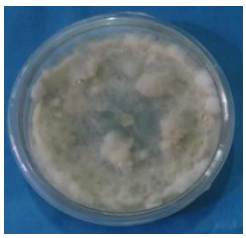


BGJJ13

BGMS01



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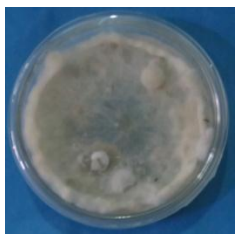

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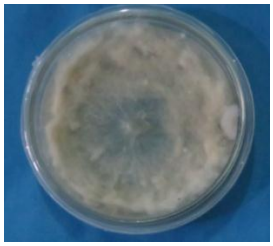

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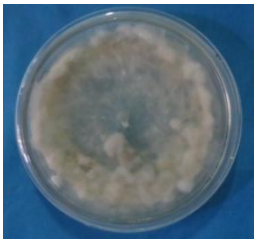

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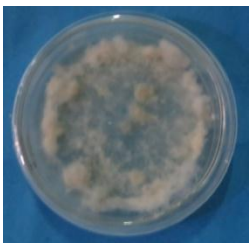

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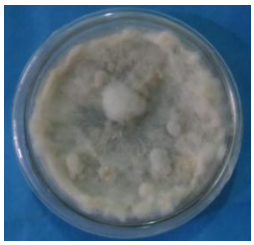

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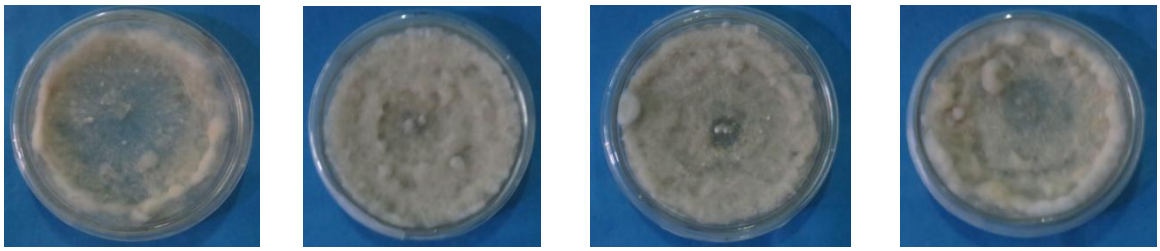

BGMS08 BGMS09 BGMS10 BGMS11

Plate 12. Pure culture of 44 isolates of *Botrytis gladiolorum* on PDA media at 16 days after inoculation

4.2.6. Radial mycelial growth of *Botrytis gladiolorum*

A total of 44 isolates of *Botrytis gladiolorum* were isolated from leaves of Gladiolus from major Gladiolus growing districs such as Joshore, Mnikgonj, Dhaka and Cox's Bazar of Bangladesh. Where 10 isolates were isolated from samples collected from Cox's Bazar, 10 isolates were isolated from Dhaka distric, 13 isolates from Joshore and 11 isolates from Manikgonj district. The maximum number of isolates (13) was collected from Jassore. The isolates were cultured on PDA and average radial growth rate/date was recorded. In the present study, the average radial mycelia growth rate /day ranged from 2.06 mm to 4.5 mm were collected from Chakoria, Cox's Bazar. Similarly, the average radial mycelia growth rate /day ranged from 2.75 mm to 4.12 mm and the isolates were collected from Savar, Dhaka. The average radial mycelia growth rate /day ranged from 2.18 mm to 3.59 mm collected from Jhikargacha, Jashore. In Singair, Manikganj the average radial mycelia growth rate/day was found 3.18 mm to 4.06 mm. In this study, the highest average mycelia growth rate/day was found in Singair, Manikganj and the lowest average mycelia growth rate/date was found in Jhikargacha, Joshore. The growth rate recorded in this study was slower than previous recorded growth rate of other pathogens (Table 29).

Table 29. Radial mycelial growth of *Botrytis gladiolorum* isolated from leaf blight samples collected from different locations of Bangladesh

Isolates code	Radial mycelia growth (mm)								Growth rate/day (mm)
	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI	16 DAI	
BGCC01	8.5	11.5	20.5	23.5	25.5	29.5	33.5	36.5	2.28
BGCC02	10	13	23	30	45.5	52.5	63	67	4.19
BGCC03	16	28	37.5	44.5	55	63	68	72	4.50
BGCC04	10.5	13.5	19.5	26.5	31.5	35.5	38.5	41.5	2.59
BGCC05	8.0	12.5	18.5	22	26	29	31	33	2.06
BGCC06	12.5	16	18	20	23.5	29.5	35.5	41.5	2.59
BGCC07	10.5	14.5	16.5	19,5	24.5	31.5	36.5	42.5	2.65
BGCC08	9.5	17	27.5	32.5	36.5	43	59+55	59.5	3.72
BGCC09	10.5	16	18	24	31	36	42	47	2.93
BGCC10	11.5	15.5	20.5	24.5	29.5	43.5	55.5	64	4.00
BGDS01	13.5	27	34	40	45	48	52	56	3.50
BGDS02	7.5	19	27.5	31.5	36.5	41	45	48.5	3.03
BGDS03	12.5	17	37.5	21	45.5	49	52,5	56.5	3.53
BGDS04	6.5	15	37.5	26	31	35	40	44	2.75
BGDS05	11.5	24.5	32.5	37.5	41.5	45.5	48,5	53.5	3.34
BGDS06	14.5	22	32.5	37.5	43.5	50.5	55.5	60.5	3.78
BGDS06	12.5	23	31	41	46	53	58	63	3.93
BGDS07	11.5	21.5	31	38	45	51	56	62	3.87

Isolates code	Radial mycelial growth (mm)								Growth rate/date (mm)
	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI	16 DAI	
BGDS08	9.0	20	30	40	47	55	60	66	4.12
BGDS10	13	23	32	39	45	50	55	61	3.81
BGJJ01	8.5	13.5	22.5	24	30	37	47.5	52	3.25
BGJJ02	12.5	18.5	25.5	30.5	38	43	44.5	47.5	2.96
BGJJ03	11	17	20.5	21	24.4	30	32	35	2.18
BGJJ04	14.5	19.5	28.5	30.5	33.5	38.5	40.5	42	2.62
BGJJ05	13	17	24	25	28	31	33	37.5	2.34
BGJJ06	9.5	20	25	29	34	37	41	45	2.81
BGJJ07	10	23	33.5	37.5	45.5	49.5	53.5	57.5	3.59
BGJJ08	9.0	17	26	32	37.5	43	47	51	3.18
BGJJ09	9.0	18	22.5	28.5	34.5	37.5	41	44	2.75
BGJJ10	9.0	17	23.5	27.5	32.5	36	41	44	2.75
BGJJ11	10	17	23.5	30	36.7	40.5	44.5	50	3.12
BGJJ12	10.5	19.5	27.5	30	35	39	43	49	3.06
BGJJ13	9.5	20	26	32	36.5	40.5	44.5	48	3.0
BGMS01	10.5	23	35	40	46	49	53	57	3.56
BGMS02	13.5	27	45.5	49.5	55.5	58.5	61	65	4.06
BGMS03	15.5	25	35.5	41.5	48.5	52.5	56.5	60.5	3.78

BGMS04	12.5	27.5	45	49	54.5	58.5	61.5	62.5	3.90
BGMS05	13	26	36.5	43	47	50	54	57	3.56
BGMS06	14.5	23	35.5	39.5	43.5	46.5	50.5	53.5	3.34
Isolates code	Radial mycelial growth (mm)								Growth rate/date (mm)
	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	12 DAI	14 DAI	16 DAI	
BGMS07	8.0	23.5	28	33.5	39.5	43.5	47.5	51	3.18
BGMS08	7.5	16	30	36	42	46	50	54	3.37
BGMS09	8.5	25	35	40	45	49	55	59	3.68
BGMS10	8.5	19	29	35	39	44	49	53	3.31
BGMS11	10.5	21.5	32.5	37.5	43.5	46.5	51.5	56.5	3.53

DAI = Days after inoculation

4.2.7. Pathogenicity study of *Botrytis gladiolorum*

Pathogenicity assays confirmed that *Botrytis gladiolorum* was the causal pathogen of leaf blight of gladiolus. Due to profuse growth and high sporulation ability, the isolate BGMS01 was selected for pathogenicity test. The conidial spore suspension was sprayed on gladiolus plant in pots at 3-4 leaf stage. Apparently healthy gladiolus leaves were inoculated with the conidial suspension with a concentration of 6×10^4 conidia/ml. For inoculation, the inoculum suspension was sprayed over the plants. Plants under control were sprayed with distilled water. Both inoculated and control plants were covered for 48 hours with polythene sheet to keep the plants humid. The pots with plants were placed in a glass house having ambient temperature of 20-22^{o}C until development of symptoms. Characteristic symptoms of the disease appeared within 12 days of inoculation. The inoculated fungus was re-isolated from the inoculated plant parts showing characteristic symptoms following the procedures as

mentioned earlier. Pieces of leaf specimens were also plated on moist blotting paper in Petri dishes and incubated at 21^{o}C. The fungi grew on the leaf samples were isolated, purified and morphological characteristics of the fungus were recorded (Plate 13.) The observed conidia were found obovoid, unicellular, pale brown; these characterics symptom confirm that the pathogen was *Botrytis gladiolorum*. Thus, the isolate BGMS01 collected from Manikganj, Singair was pathogenic.

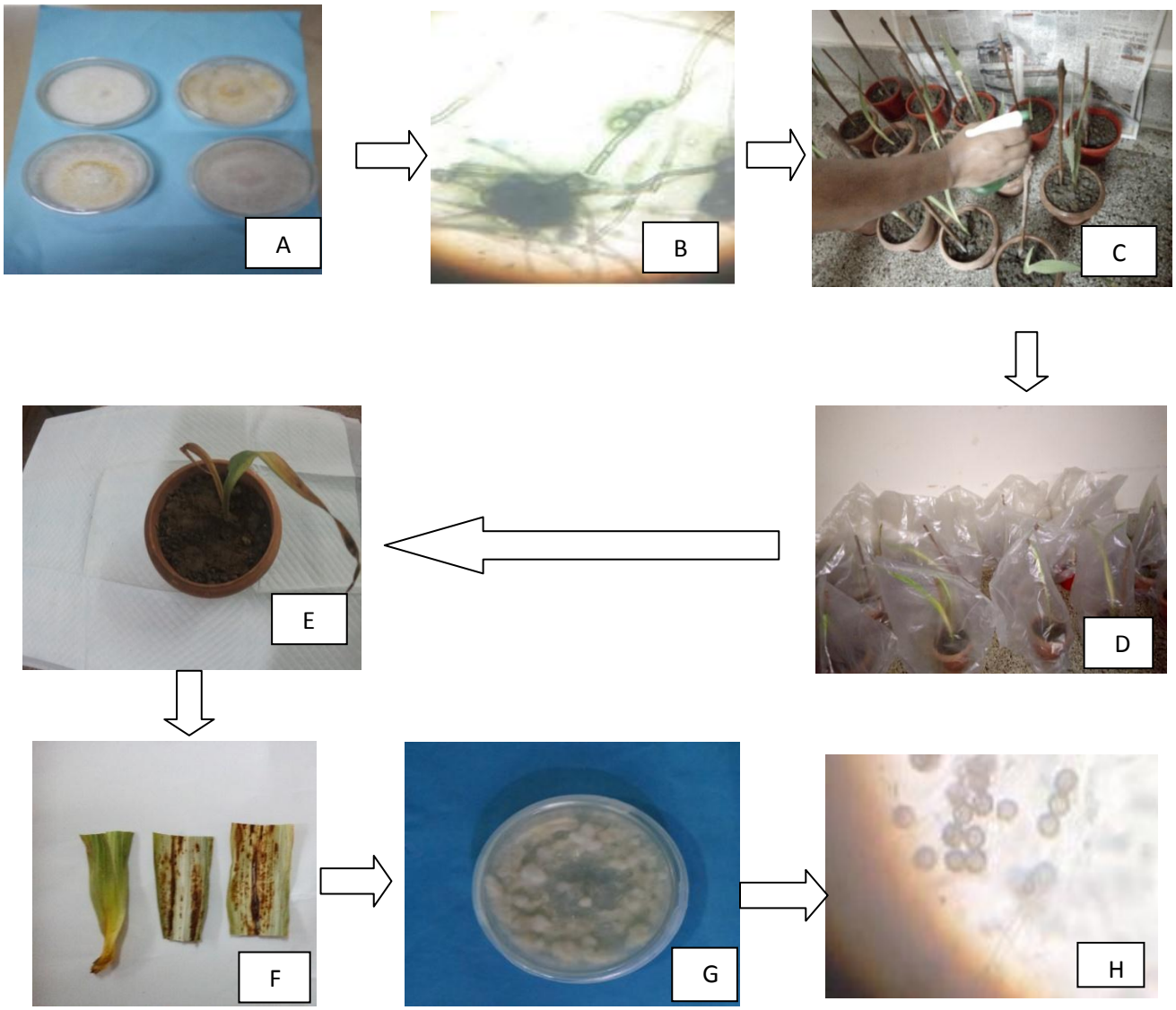

Plate 13. Flow chart of Pathogenicity study of *Botrytis gladiolorum* A. Pure culture, B. Mycelia and conidia of *Botrytis gladiolorum*, C. Inoculation, D. Incubation, E. Infected plant. F. Infected leaf, G. Pure culture and H. Conidia of *Botrytis gladiolorum* (×100).

4.3. Experiment 3. Evaluation of selected fungicides against *Botrytis gladiolorum in vitro*

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.

4.3.1. Efficacy of fungicides at the rate of 100 ppm gainst *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, 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 30 and Plate 14).

Table 30. Efficacy of fungicides at 100 ppm against *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= Tilt 250 EC	00.00 g	100.00	18.40 de	60.77	28.70 de	58.22
T_2= Score 250 EC	00.00 g	100.00	7.20 h	84.65	15.10 g	78.02
T_3= Folicure 250 EC	01.40 f	93.86	13.60 g	71.00	20.10 f	70.74
T_4= Amister Top 325 SC	16.50 c	27.63	28.00 c	40.30	37.30 c	45.71
T_5= Nativo 75 WG	20.20 b	11.40	37.50 b	20.04	53.20 b	22.56
T_6= Trooper 75 WP	19.50 b	14.47	28.20 c	39.87	31.40 d	54.29
T_7= Autostin 50 WDG	6.90 e	69.74	14.50 fg	69.08	19.00 f	72.34
T_8= Difar 300 EC	10.40 d	54.39	20.20 d	56.93	28.40 de	58.66
T_9= Indofil 80 WP	0.00 g	100.00	16.30 ef	65.25	26.40 e	61.57
T_{10}= Contaf 5 EC	0.00 g	100.00	0.00 i	100.00	8.70 h	87.34
$T_{11=}$Control (Untreated)	22.80 a	-	46.90 a	-	68.70 a	-
LSD (P=0.01)	0.88	-	2.34	-	2.96	-

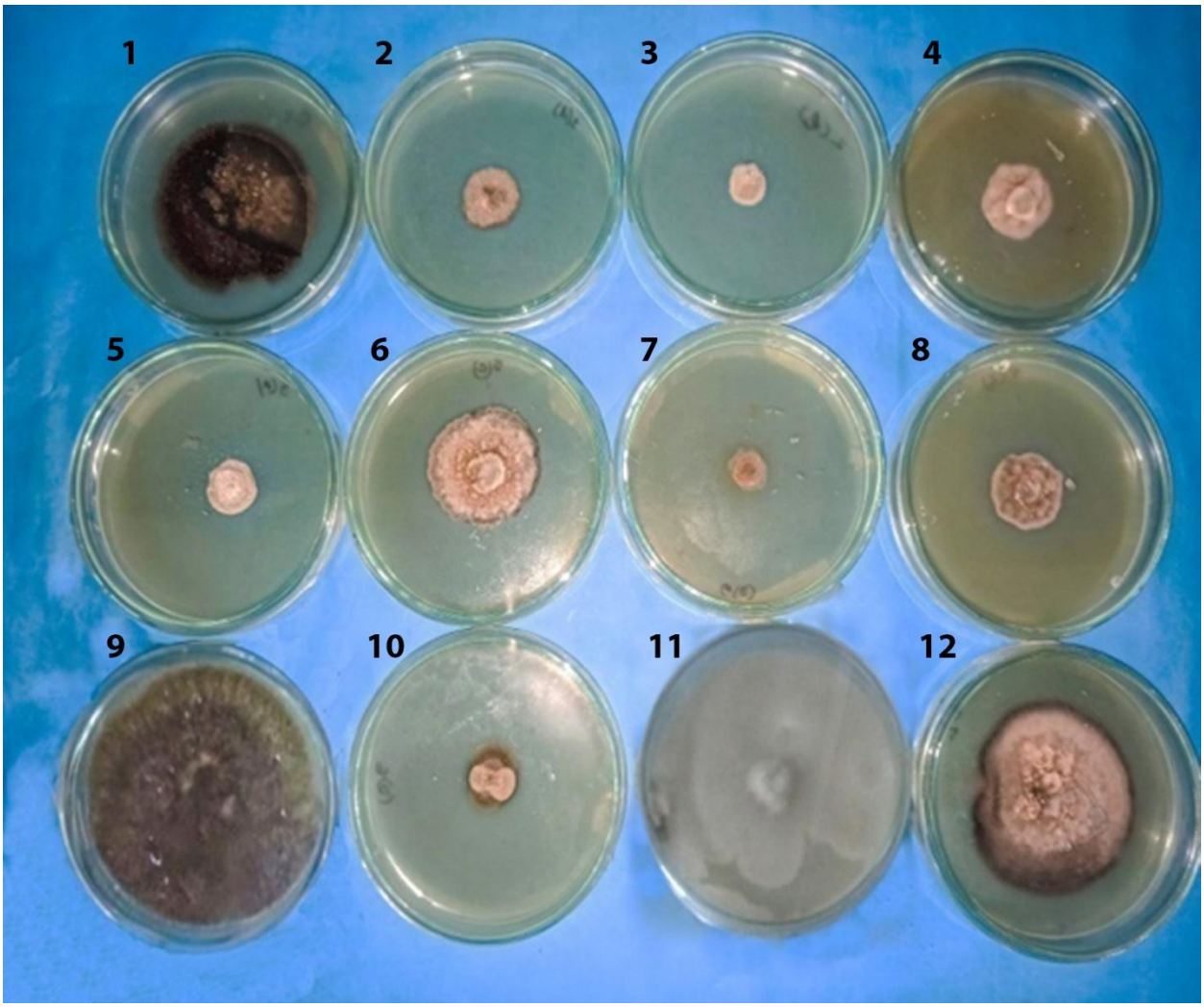

Plate 14. Mycelial growth of *B. gladiolorum* against selected fungicides (100 ppm) at 15 days after inoculation (1= Nativo 75 WG, 2= Folicure 250 EC, 3= Contaf 5 EC, 4= Differ 300 EC, 5= Autostin 50 WDG, 6= Amistar Top 325 SC, 7= Score 250 EC, 8= Trooper 75 WP, 9= Control, 10= Tilt 250 EC, 11= Indofil 80 WP, 12= Nativo 75 WG).

4.3.2. 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 Amister 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 31 and Plate 15).

Table 31. Efficacy of fungicides at 200 ppm against *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= Tilt 250 EC	0.00 e	100.00	4.20 g	91.75	15.60g	79.00
T_2= Score 250 EC	0.00 e	100.00	0.00 h	100.00	0.00 i	100.00
T_3= Folicure 250 EC	0.00 e	100.00	0.00 h	100.00	12.60 h	82.90
T_4= Amister Top 325 SC	11.80 c	60.40	23.50 b	53.83	32.30 c	56.53
T_5= Nativo 75 WG	13.10 b	56.04	19.80 c	61.10	44.40 b	40.24
T_6= Trooper 75 WP	0.00 e	100.00	14.40 d	71.71	18.50 f	75.10
T_7= Autostin 50 WDG	0.00 e	100.00	8.10 f	84.09	12.70 h	83.04
T_8= Difar 300 EC	7.10 d	76.17	13.00 e	74.46	19.70 e	73.49
T_9= Indofil 80 WP	0.00 e	100.00	12.80 e	74.85	21.90 d	70.52
T_{10}= Contaf 5 EC	0.00e	100.00	0.00 h	100.00	0.00 i	100.00
$T_{11=}$Control (Untreated)	29.80a	-	50.90 a	-	74.30 a	-
LSD (P=0.01)	0.51	-	1.10	-	1.16	-

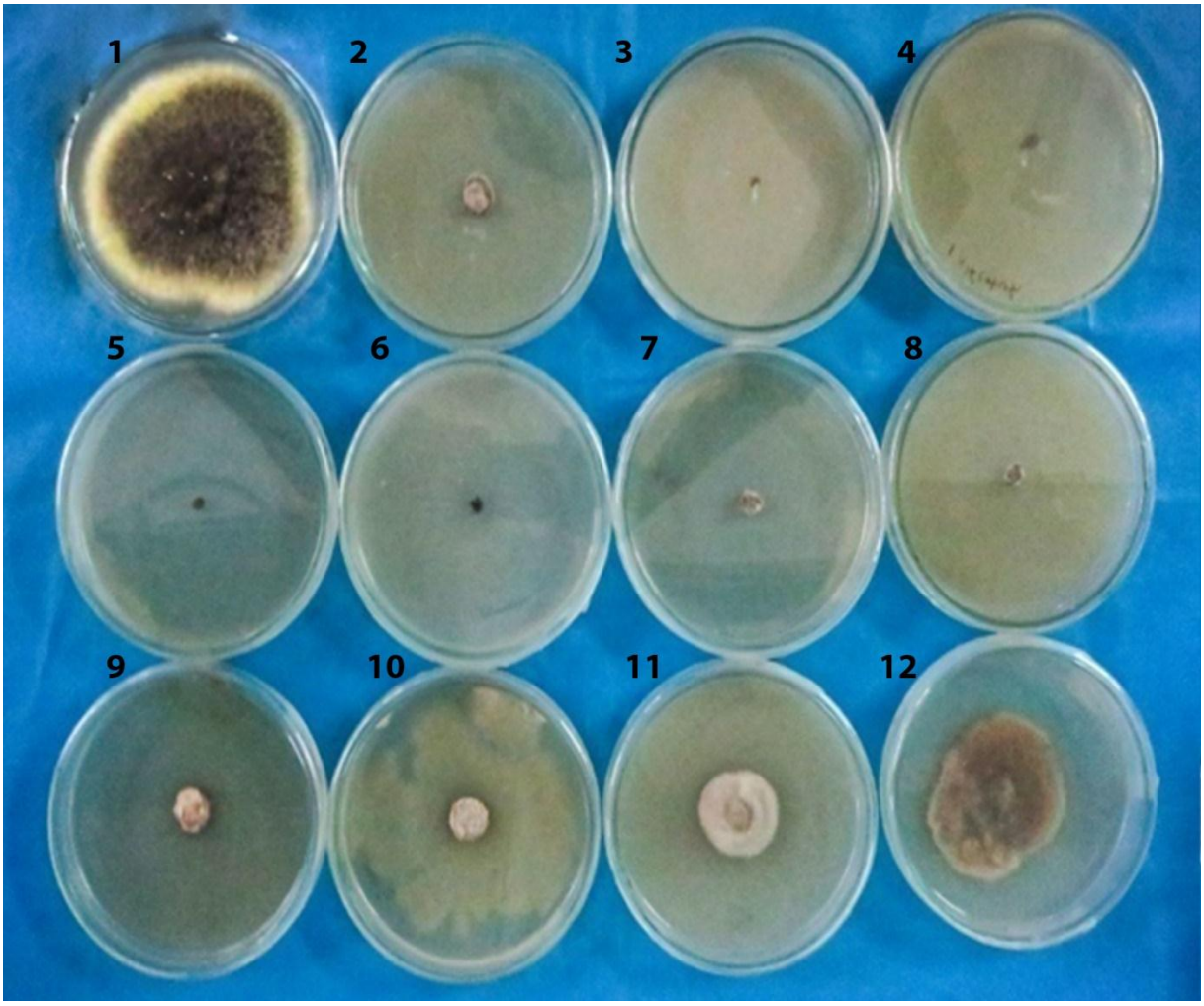

Plate 15. Mycelial growth of *B. gladiolorum* against selected fungicides (200 ppm) at 15 days after inoculation (1= Control, 2= Trooper 75 WP, 3= Autostin 50 WDG, 4= Tilt 250 EC, 5= Score 250 EC, 6= Contaf 5 EC, 7= Folicure 250 EC, 8= Autostin 50 WDG, 9= Differ 300 EC, 10= Indofil 80 WP, 11= Amistar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

4.3.3. 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 Amister 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 32 and Plate 16).

Table 32. Efficacy of fungicides at 300 ppm against *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= Tilt 250 EC	0.00 b	100.00	0.00 c	100.00	00.00 f	100.00
T_2= Score 250 EC	0.00 b	100.00	0.00 c	100.00	00.00 f	100.00
T_3= Folicure 250 EC	0.00 b	100.00	0.00 c	100.00	00.00 f	100.00
T_4= Amister Top 325 SC	0.00 b	100.00	11.70 b	78.45	20.00 b	72.49
T_5= Nativo 75 WG	0.00 b	100.00	12.10 b	77.72	18.80 c	74.14
T_6= Trooper 75 WP	0.00 b	100.00	0.00 c	100.00	11.60 e	84.04
T_7= Autostin 50 WDG	0.00 b	100.00	0.00 c	100.00	00.00 f	100.00
T_8= Difar 300 EC	0.00 b	100.00	0.00 c	100.00	13.20 d	81.84
T_9= Indofil 80 WP	0.00 b	100.00	0.00 c	100.00	12.90 d	82.26
T_{10}= Contaf 5 EC	0.00 b	100.00	0.00 c	100.00	00.00 f	100.00
$T_{11=}$Control (Untreated)	28.60 a	-	54.30 a	-	72.70 a	-
LSD (P=0.01)	0.28	-	0.86	-	1.04	-

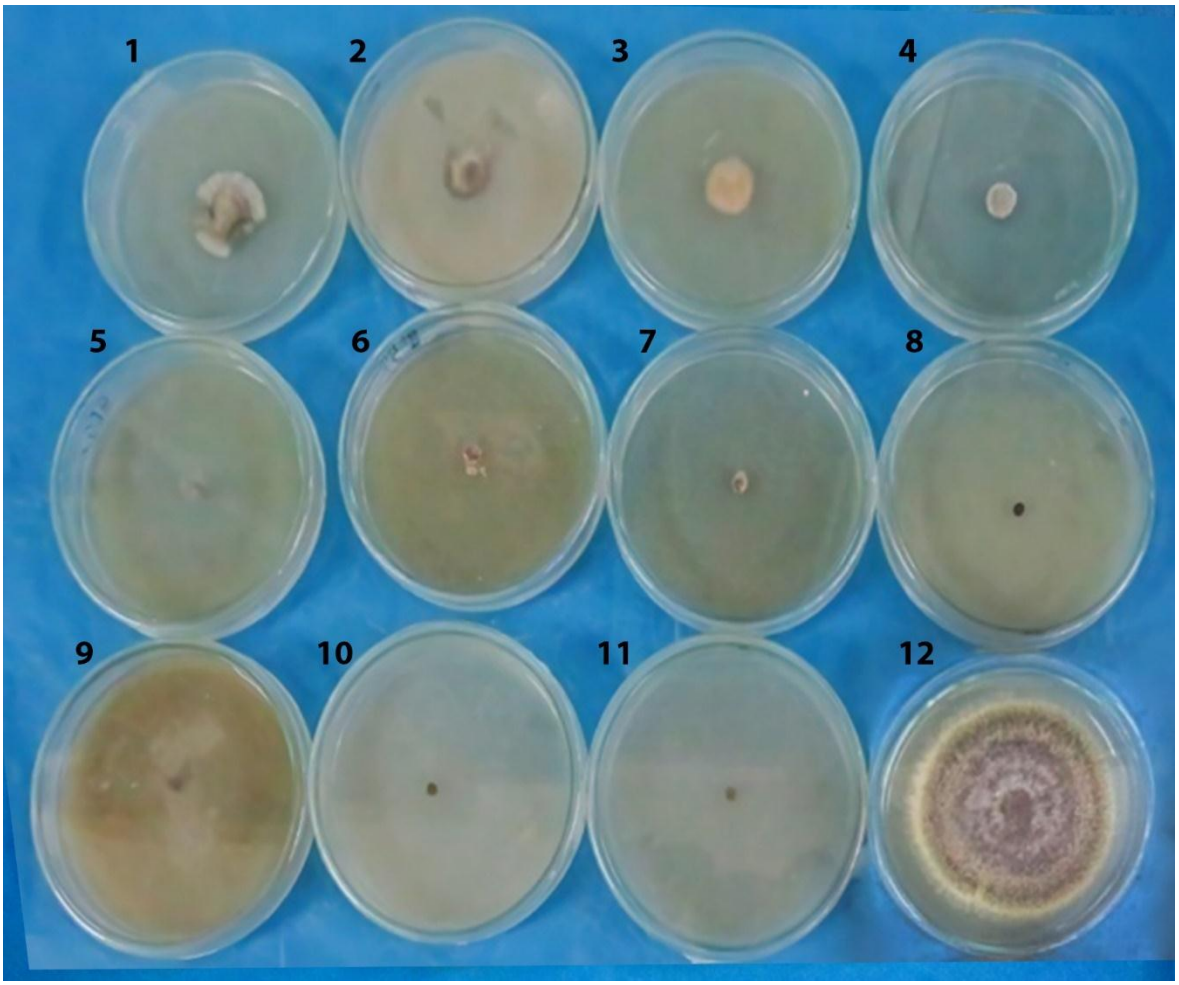

Plate 16. Mycelial growth of *B. gladiolorum* against selected fungicides (300 ppm) at 15 days after inoculation (1= Amistar top 325 SC, 2= Indofil 80 WP, 3= Nativo 75 WG, 4= Differ 300 EC, 5= Autostin 50 WDG, 6= Trooper 75 WP, 7= Trooper 75 WP, 8= Contaf 5 EC, 9= Folicure 250 EC, 10= Score 250 EC, 11= Tilt 250 EC, 12= Control).

4.3.4. 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 2). This dose can be used for further study in field condition.

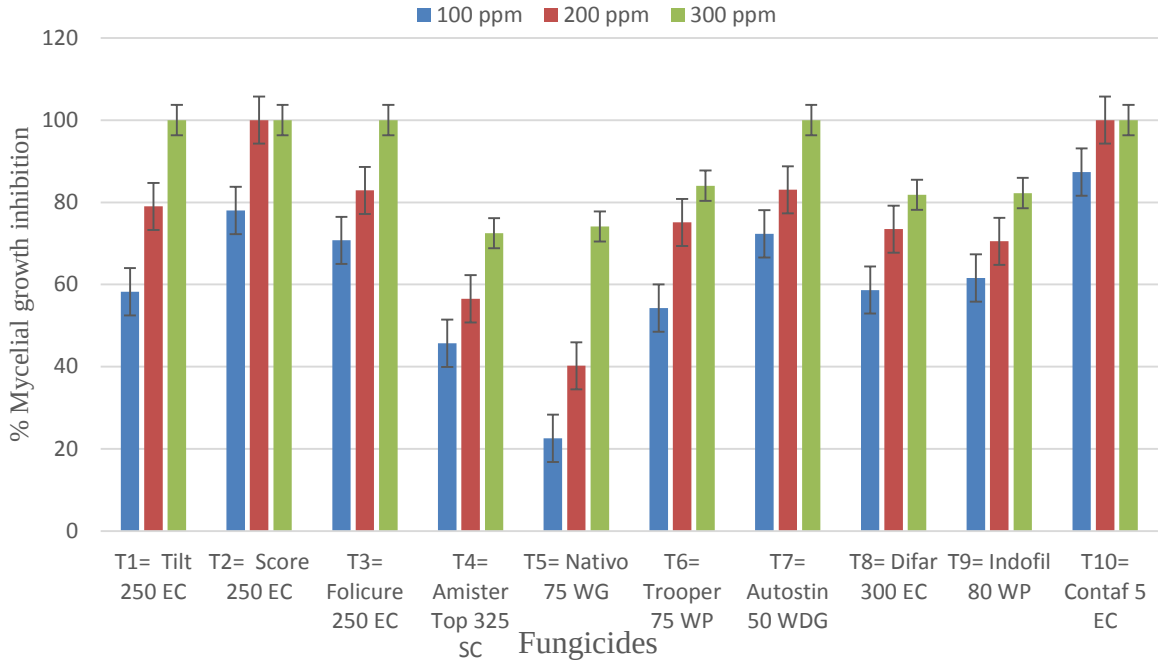

Figure 2. Comparative efficacy of different fungicides on mycelial growth inhibition of *Botrytis gladiolorum in vitro.*

4.4. Experiment 4. Efficacy of selected botanicals against *Botrytis gladiolorum in vitro*

Ten selected botanicals were evaluated against the mycelial growth of *Botrytis gladiolorum* the selected botanicals were Mehendi, Chrysanthemum, Tulsi, Onion, Neem, Bael, Arjun, Garlic, Aloevera (Ghritkumary), Turmeric applied at the rate of 5%, 10% and 20%.

4.4.1. Efficacy of botanicals (5%) against *Botrytis gladiolorum* in laboratory

Ten selected botanicals were tested with 5% concentration against *Botrytis gladiolorum* and 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 *Botrytis gladiolorum*, which was statistically similar with onion (50.07% inhibition) and mehendi (49.93%) (Table 33 and Plate 17).

Table 33. 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	-

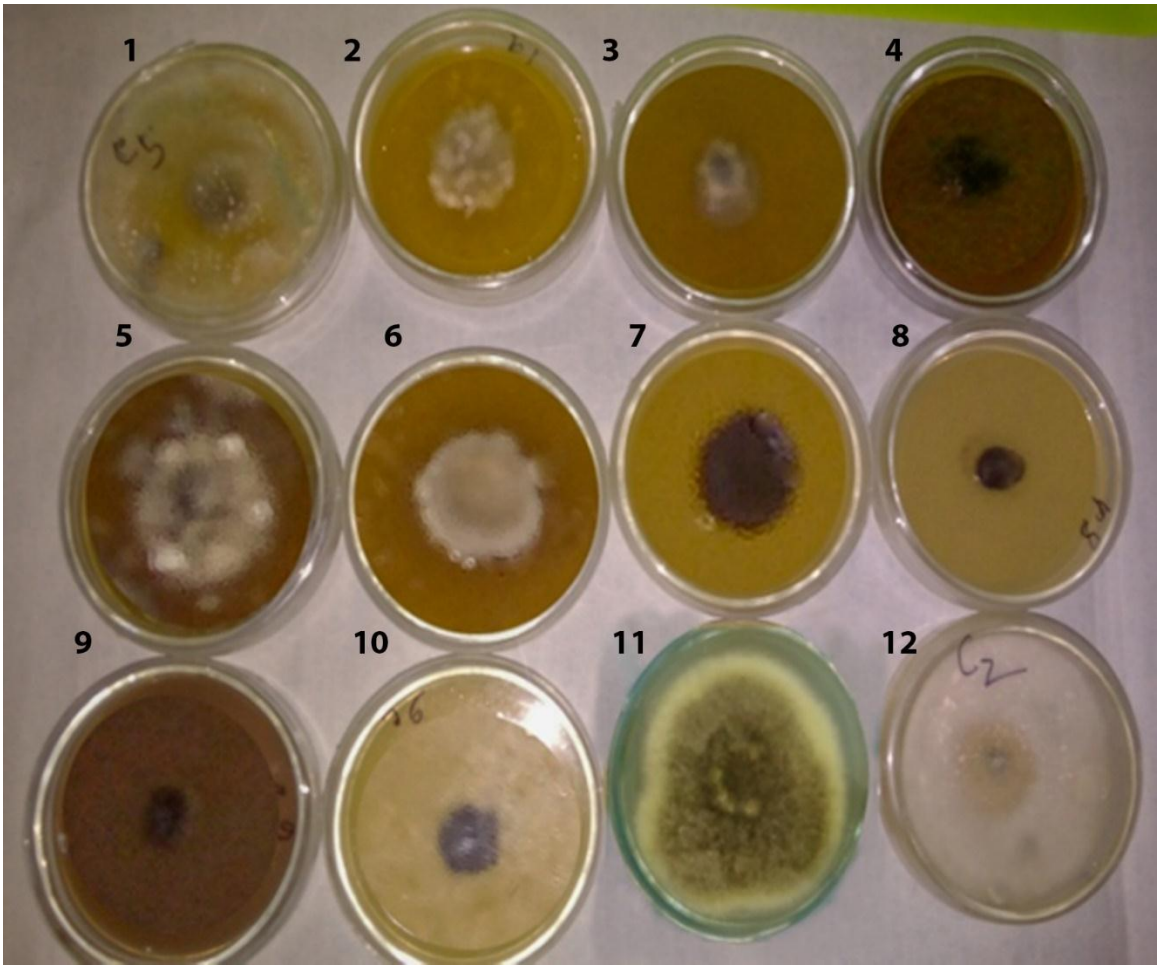

Plate 17. Mycelial growth of *B. gladiolorum* against selected botanical extracts (5%) at 15 days after inoculation (1= Aloevera, 2= Mehendi, 3= Turmeric, 4= Neem, 5= Bael, 6= Tulsi, 7= Chrysanthemum, 8= Garlic, 9= Aurjun, 10= Onion, 11= Control, 12= Onion).

4.4.2. 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 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 34 and Plate 18).

Table 34. Efficacy of botanicals (10%) 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	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	-

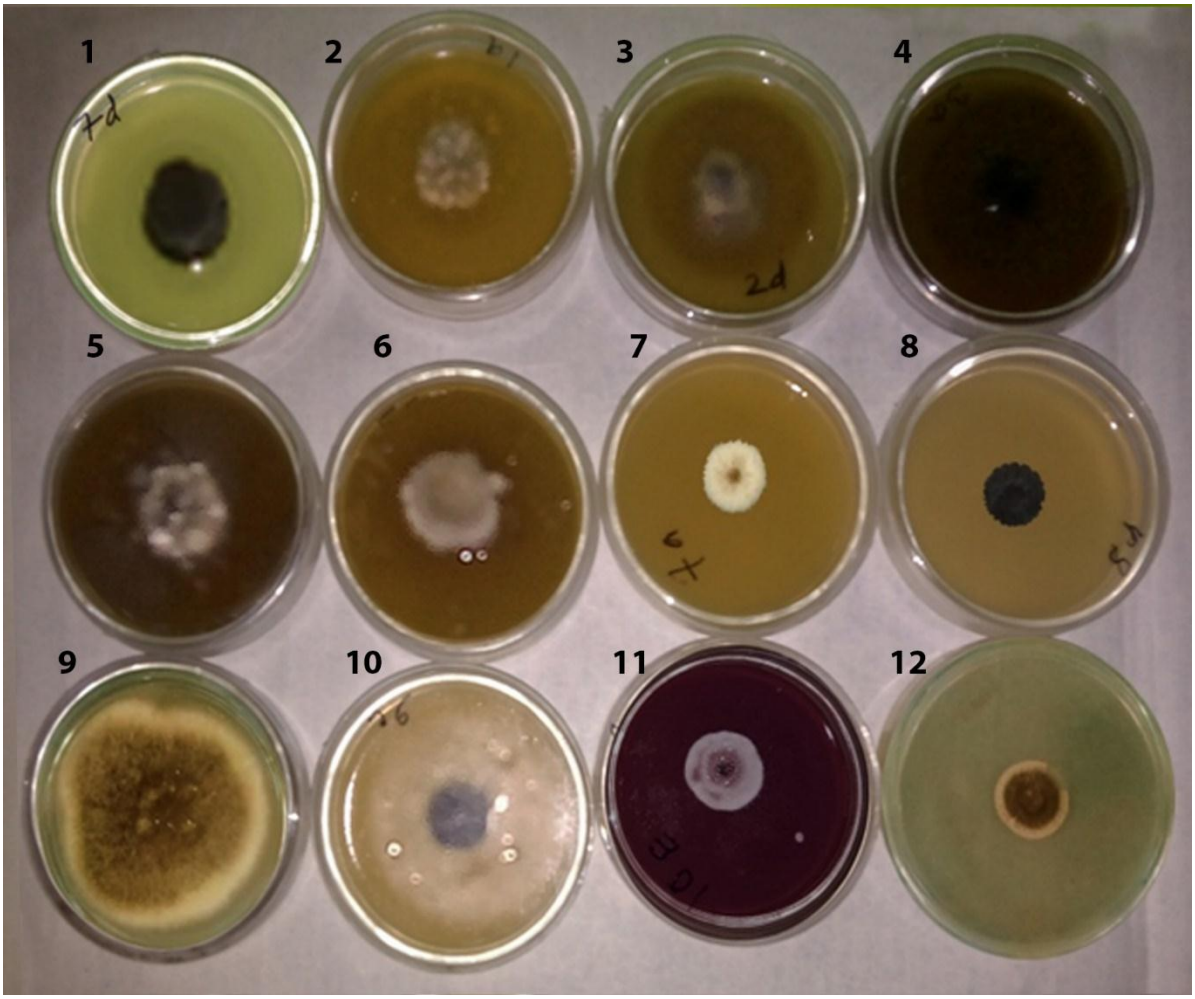

Plate 18. Mycelial growth of *B. gladiolorum* against selected botanical extracts (10%) at 15 days after inoculation (1= Aloevera, 2= Mehendi, 3= Bael, 4= Neem, 5= Aurjun, 6= Chrysanthemum, 7= Tulsi, 8= Garlic, 9= Control, 10= Onion, 11= Turmeric, 12= Aloevera).

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

Ten selected botanicals were tested with the concentration 20% against *Botrytis gladiolorum* 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), espectively (Table 35 and Plate 19).

Table 35. Efficacy of botanicals (20%) 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	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	-

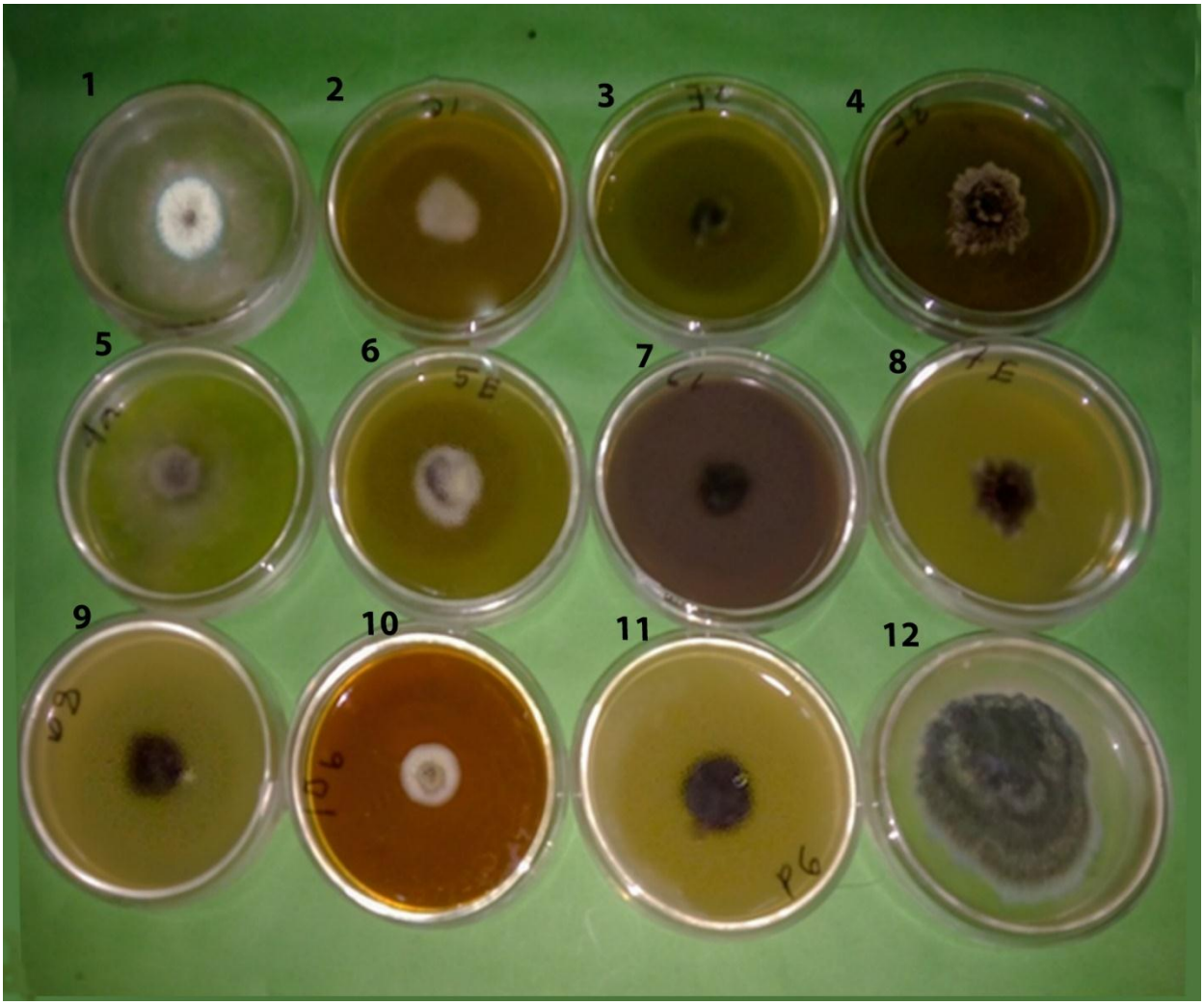

Plate 19. Mycelial growth of *B. gladiolorum* against selected botanical extracts (20%) at 15 days after inoculation (1= Aloevera, 2= Mehendi, 3= Bael, 4= Aurjun, 5= Onion, 6= Garlic, 7= Neem, 8= Chrysanthemum, 9= Chrysanthemum, 10= Turmeric, 11= Tulsi, 12= Control).

4.4.4. 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 3). This dose can be used for further study in field condition.

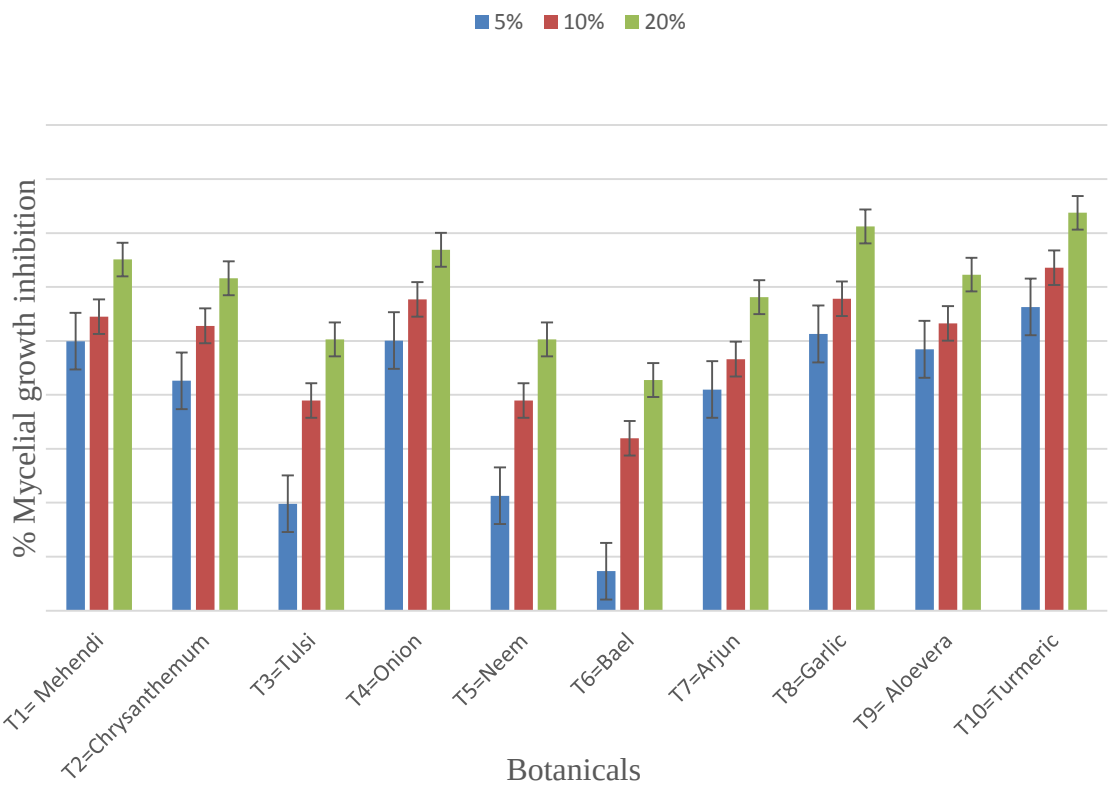

Figure 3. Comparative efficacy of different botanicals on mycelial growth inhibition of *Botrytis gladiolorum in vitro.*

3.5. Experiment 5. Efficacy of selected organic acids against *Botrytis gladiolorum in vitro*

Nine selected organic acids were evaluated against mycelial growth of *Botrytis gladiolorum.* The organic acids tested were Tartaric acid, oxalic acid, Citric acid, Ascorbic acid, Acetic acid, Benzoic acid, Galic acid, Glutamic acid and Ortho phosphoric acid at laboratory.

4.5.1. Efficacy of organic acids at 1000 ppm dose in controlling *Botrytis gladiolorum* in the laboratory

Nine selected organic acids were tested with at the rate of 1000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids showed significantly different results as compared to control. At 5 DAI, the radial mycelia growth was found minimum (26.10 mm) in oxalic acid which was statistically similar with acetic acid (26.30 mm) followed by benzoic acid (28.80 mm) and the inhibition of growth was 44.47%, 40.04% and 38.72%, respectively. At 10 DAI, the highest inhibition of growth was found in acetic acid (46.23%), which was statistically similar with oxalic acid (42.92%) and benzoic acid (41.87%). At 15 DAI, the growth inhibition (43.22%) was highest in acetic acid followed by benzoic acid (38.11%) and oxalic acid (33.50%), respectively (Table 36 and Plate 20).

Table 36. Efficacy of organic acids at 1000 ppm dose 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=Tartaric acid	38.70 b	17.66	55.00 b	17.17	69.40 b	11.25
T_2=Oxalic acid	26.10 f	44.47	37.90 d	42.92	52.00 e	33.50
T_3=Citric acid	40.30 b	14.26	51.40 c	22.59	62.20 d	20.46
T_4=Ortho phosphoric acid	33.20 d	29.36	51.70 c	22.14	65.80 c	15.86
T_5=Ascorbic acid	35.50 c	24.47	54.70 b	17.62	65.30 c	16.50
T_6=Acetic acid	26.30 f	40.04	35.70 d	46.23	44.40 g	43.22
T_7=Benzoic acid	28.80 e	38.72	38.60 d	41.87	48.40 f	38.11
T_8=Gallic acid	39.50 b	15.96	51.40 c	22.59	69.50 b	11.13
T_9=Glutamic acid	35.00 cd	25.53	51.40 c	22.59	72.30 b	7.54
$T_{10=}$Control (Untreated)	47.00 a	-	66.90 a	-	78.20a	-
LSD (P=0.01)	2.15	-	2.81	-	2.978	-

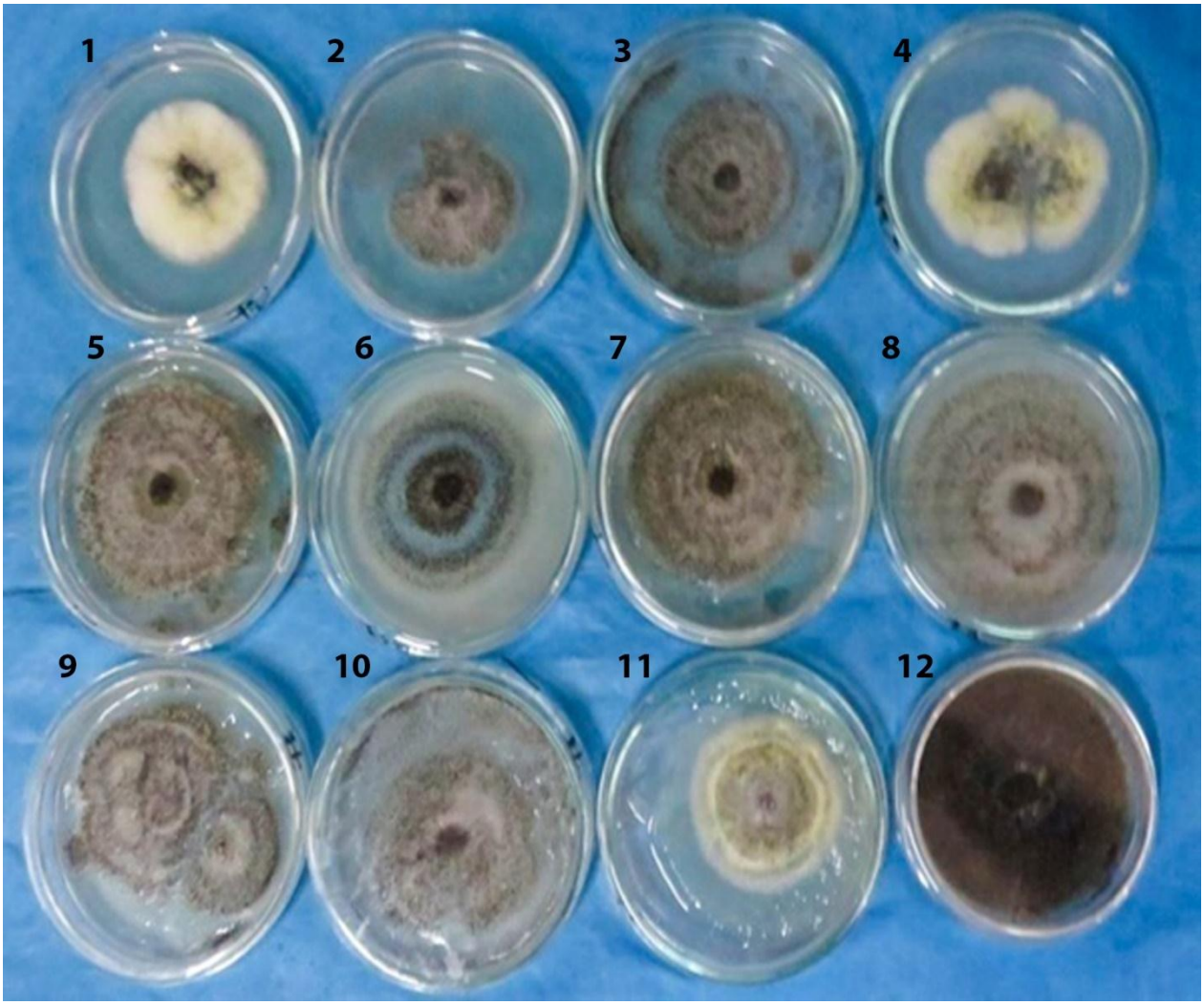

Plate 20. Mycelial growth of *B. gladiolorum* against selected organic acids (1000 ppm) at 15 days after inoculation (1= Oxalic acid, 2= Acetic acid, 3= Benzoic acid, 4= Oxalic acid, 5= Galic acid, 6= Ascorbic acid, 7= Tartaric acid, 8= Glutamic acid, 9= Orthophosphoric acid, 10= Ascorbic acid, 11= Citric acid, 12= Control).

4.5.2. Efficacy of organic acids at 2000 ppm dose in controlling *Botrytis gladiolorum* in the laboratory

Nine selected organic acids were tested with at the rate of 2000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids performed significantly different results as compared to control. At 5 DAI, the radial mycelia growth was found minimum in acetic acid (10.30 mm) followed by oxalic acid (21.40 mm). benzoic acid showed (20.50 mm) growth inhibition were 75.65%, 49.41% and 51,53%. At 10 DAI, the highest growth inhibition was found in acetic acid (71.11%) followed by benzoic acid (54.69%). At 15 DAI, highest inhibition of growth found in acetic acid (57.02%) followed by benzoic acid (48.04%) and oxalic acid (41.97%) (Table 37 and Plate 21).

Table 37. Efficacy of organic acids at 2000 dose ppm 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=Tartaric acid	31.80 b	24.82	48.70 b	28.29	64.40 b	18.58
T_2=Oxalic acid	21.40 e	49.41	31.90 e	53.22	45.90 f	41.97
T_3=Citric acid	32.40 b	23.40	44.80 c	34.31	56.80 d	28.19
T_4=Ortho phosphoric acid	28.10 d	33.57	38.00 d	44.28	51.90 e	34.39
T_5=Ascorbic acid	30.50 bc	27.90	47.60 b	30.20	61.00 c	22.88
T_6=Acetic acid	10.30 f	75.65	19.70 f	71.11	34.00 h	57.02
T_7=Benzoic acid	20.50 e	51.53	30.90 e	54.69	41.10 g	48.04
T_8=Gallic acid	32.90 b	22.22	44.60 c	34.60	61.50 bc	22.25
T_9=Glutamic acid	29.20 cd	30.97	43.00 c	36.95	59.70 cd	24.53
T_{10}=Control (Untreated)	42.30 a	-	68.20 a	-	79.10 a	-
LSD (P=0.01)	2.25	-	2.64	-	3.20	-

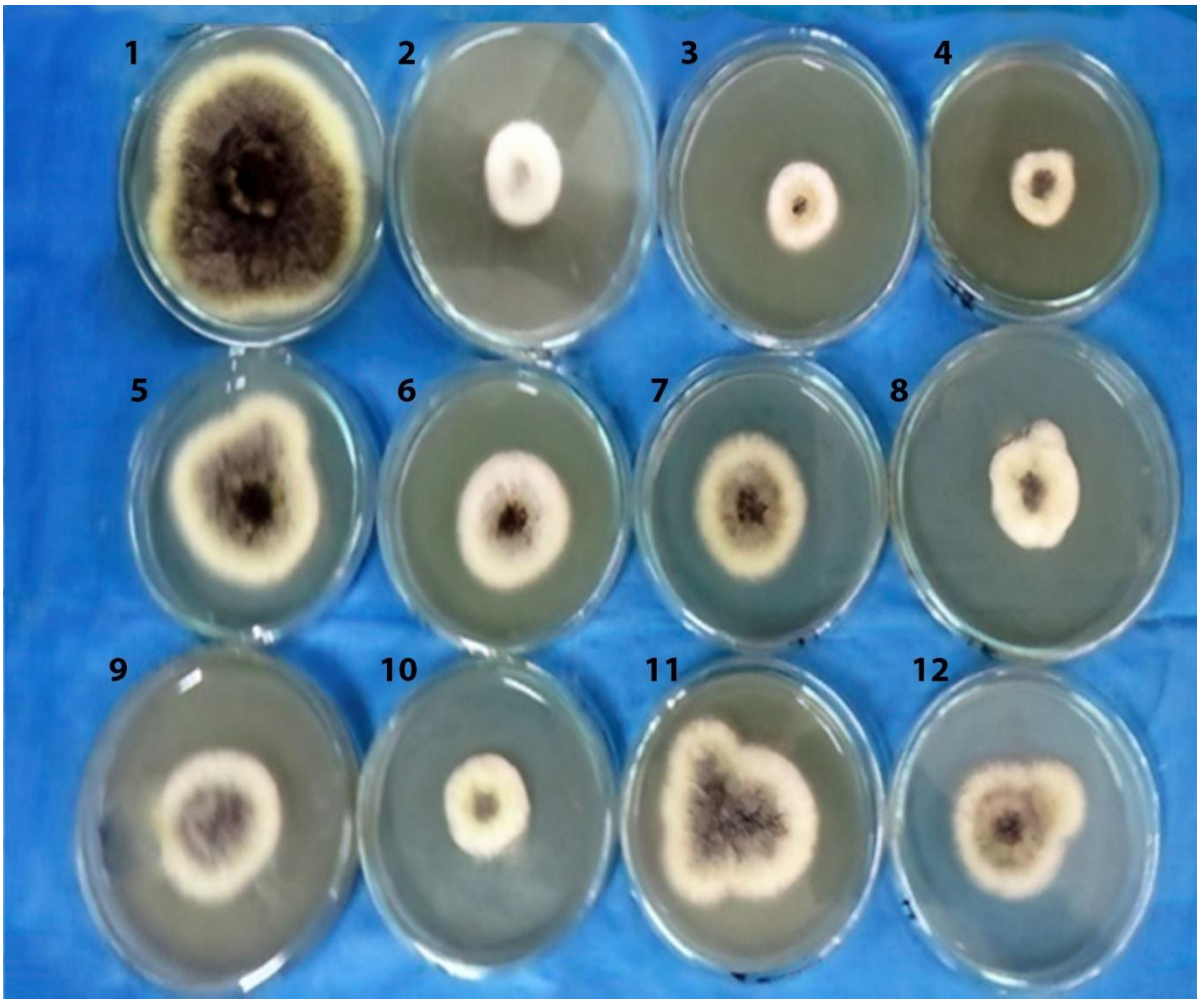

Plate 21. Mycelial growth of *B. gladiolorum* against selected organic acids (2000 ppm) at 15 days after inoculation (1= Control, 2= Benzoic acid, 3= Oxalic acid, 4= Acetic acid, 5= Tartaric acid, 6= Citric acid, 7= Ascorbic acid, 8= Orthophosphoric acid, 9= Ascorbic acid, 10= Benzoic acid, 11= Glutamic acid, 12= Galic acid).

4.5.3. Efficacy of organic acids at 3000 ppm dose in controlling *Botrytis gladiolorum* in the laboratory

Nine selected organic acids were tested with at the rate of 3000 ppm against *Botrytis gladiolorum* and the radial mycelia growth of fungus was measured at 5, 10 and 15 DAI. All organic acids showed significantly different results as compared to control. At 5 DAI, no radial mycelial growth was found in acetic acid (00) which was followed by benzoic acid (8.60 mm) and oxalic acid (16.40 mm) and the inhibition of growth of these organic acids were 100%, 80.54%, 62.90%, respectively. At 10 DAI, the highest growth inhibition found in acetic acid (100%), which was statistically followed by benzoic acid (64.69%) and oxalic acid (54.29%). At 15 DAI, the growth inhibition (86.75%) was highest in acetic acid followed by benzoic acid (58.54%) and oxalic acid (46.36%), respectively (Table 38 and Plate 22).

Table 38. Efficacy of organic acid at 3000 ppm 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=Tartaric acid	26.50 b	40.05	43.60 b	28.05	58.30 b	22.78
T_2=Oxalic acid	16.40 d	62.90	27.70 f	54.29	40.50 e	46.36
T_3=Citric acid	22.40 c	49.32	35.50 e	41.42	47.00 d	37.75
T_4=Ortho phosphoric acid	24.50 bc	44.57	35.70 e	41.09	47.10 d	37.62
T_5=Ascorbic acid	26.80 b	39.37	40.30 cd	33.50	52.80 c	30.07
T_6=Acetic acid	0.00 f	100	0.00 h	100	10.00 g	86.75
T_7=Benzoic acid	8.60 e	80.54	21.40 g	64.69	31.30 f	58.54
T_8=Gallic acid	27.40 b	38.00	41.90 bc	30.86	55.80 b	26.09
T_9=Glutamic acid	26.00 b	41.18	38.50 d	36.47	50.30 c	32.72
T_{10}=Control (Untreated)	44.20 a	-	60.60 a	-	75.50 a	-
LSD (P=0.01)	2.72	-	2.34	-	2.59	-

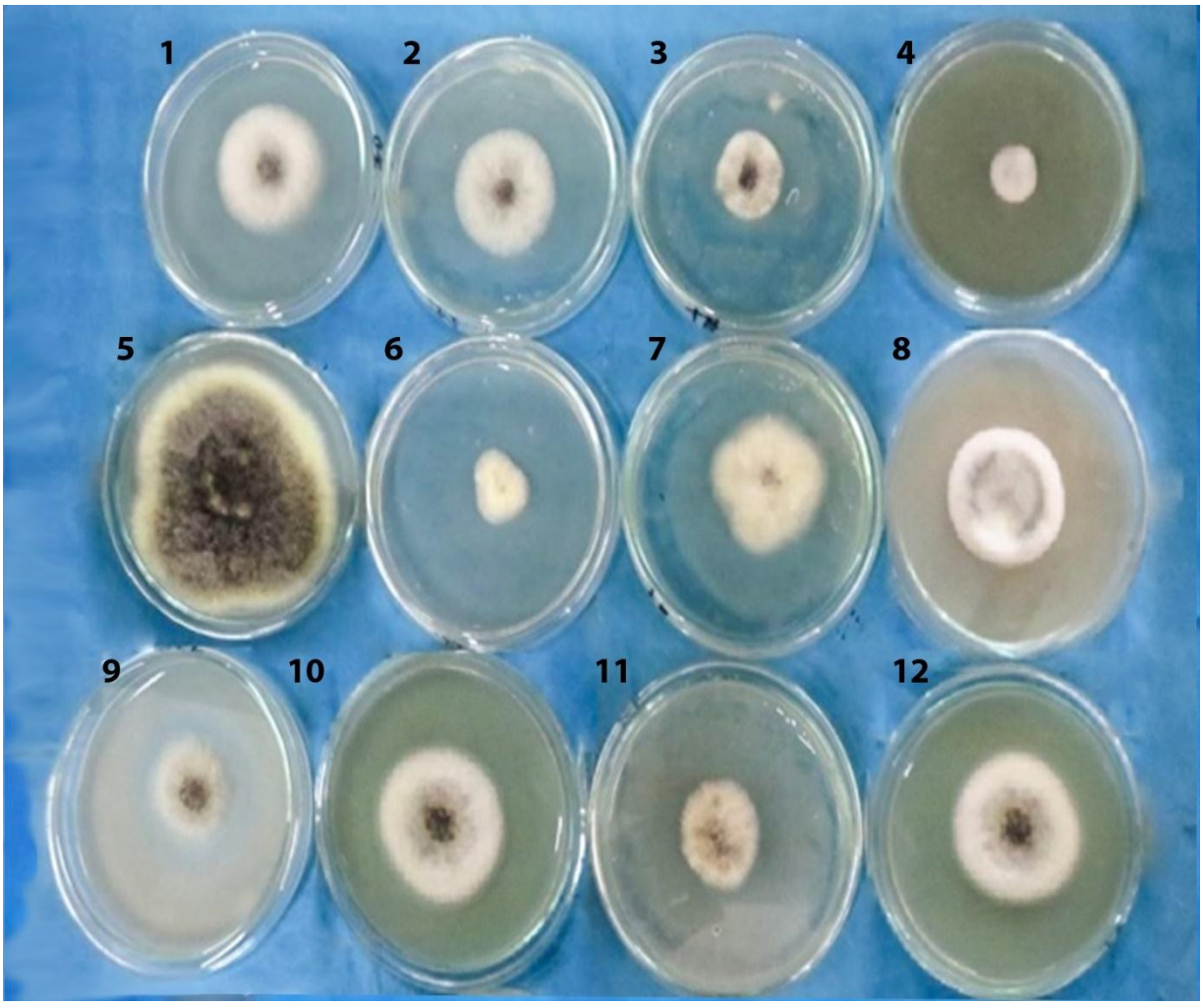

Plate 22. Mycelial growth of *B. gladiolorum* against selected organic acids (3000 ppm) at 15 days after inoculation (1= Ascorbic acid, 2= Citric acid, 3= Benzoic acid, 4= Acetic acid, 5= control, 6= Acetic acid, 7= Citric acid, 8= Tartaric acid, 9= Orthophosphoric acid, 10= Glutamic acid, 11= Oxalic acid, 12= Galic acid).

4.5.4. Comparative efficacy of different organic acids on mycelial growth inhibition of *Botrytis gladiolorum* at 15 days after inoculation

The highest growth inhibition was found at the doses of 3000 ppm in case of all organic acids at 15 DAI. At 15 DAI, the growth inhibition (86.75%) was highest in acetic acid followed by benzoic acid (58.54%) and oxalic acid (46.36%), respectively (Figure 4). This dose can be used for further study in field condition.

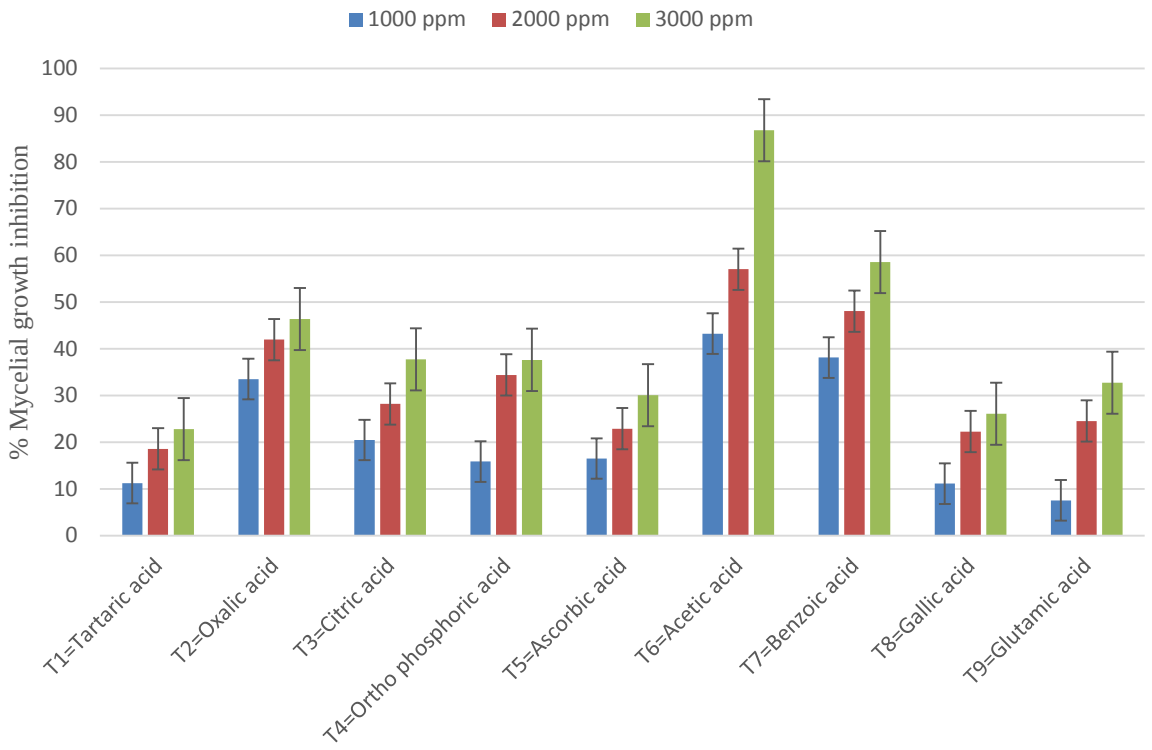

Figure 4. Comperative Efficacy of different organic acids on mycelial growth inhibition of *Botrytis gladiolorum in vitro*.

3.6. Experiment 6: Evaluation of fungicides, botanicals and organic acids for management of gladiolus leaf blight in field conditions

The most effective fungicides (Contaf 5 EC, Score 250 EC, Autostin 50 WDG) those were evaluated in the previous experiment conducted in the laboratory at the rate of 300 ppm, three botanicals (Turmeric, Garlic, Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid, Oxalic acid) at the rate 3000 ppm were used in this experiment. Selected fungicides, botanicals and organic acids were sprayed three times in experimental plot at 20 DAS, 40 DAS and 60 DAS.

3.6.1. Evaluation of fungicides, botanicals and organic acids in controlling leaf blight and on growth parameters of gladiolus in the field at vegetative stage (45 DAS)

Three fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field for controlling leaf blight of gladiolus. At the vegetative stage, the lowest disease incidence (1.85%) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (3.70%), Autostin 50 WDG (7.40%) and Turmeric (9.25%). Similar results were also found in disease severity. Where the disease severity was lowest in Score 250 EC treated plot (1.33%), which was statistically similar with Contaf 5 EC (3.33%), Autostin 50 WDG (5.00%) and Turmeric (5.67%). In case of plant height, the highest plant height was in Score 250 EC treated plot which was statically similar with Contaf 5 EC, Autostin 50 WDG and Turmeric treated plot. In case of leaf/plant, healthy leaf/plant and leaf length/plant was found the highest in Score 250 EC treated plot which was statically similar with Contaf 5 EC and Autostin 50 WDG. The highest infected Leaf/plant was found in control plot which was statistically different than all other treatments. The lowest infected leaf/plant was found in Score 250 EC, Contaf 5 EC, Autostin 50 WDG and Turmeric treated plot. Statically similar width/plant was observed in Score 250 EC, Contaf 5 EC, Autostin 50 WDG, Turmeric, Garlic, Onion and Acetic acid treated plot (Table 39).

3.6.2. Evaluation of fungicides, botanicals and organic acids in controlling leaf blight of gladiolus and on growth parameters in the field at flowering stage (70 DAS)

Three fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field for controlling leaf blight of gladiolus. At the flowering stage (70 DAS), the lowest disease incidence (14.81%) was found in Score 250 EC and contaf 5 EC treated plot, which was statistically similar with Autostin 50 WDG (18.51%) and Turmeric (20.37%). Lowest disease severity was found in score 250 EC (8.33%) which was statistically similar with contaf 5 Ec (9.00%). Highest plant height (89.33 cm) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (85.00 cm), Autostin 50 WDG (82.67 cm), Turmeric (81.00 cm), Garlic (79.00 cm), Onion (76.00 cm) and Acetic acid (74.00 cm) than others. Similar results were found in case of leaf/plant. Highest healthy leaf/plant (9.25) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (9.06), Autostin 50 WDG (8.88), Turmeric (8.64) and Garlic. Similar trend was found in leaf length and leaf width. Highest infected leaves (1.43) were found in control which was statistically different than other treatments. Lowest infected leaves were found Score 250 EC treated plot (0.28), which was statistically similar with Contaf 5 EC (0.33) and Autostin 50 WDG (0.38) (Table 40).

Table 39. Evaluation of fungicides, botanicals and organic acids in controlling leaf blight and on growth parameters of gladiolus in the field at vegetative stage (45 DAS)

Treatments	Disease Incidence (%)	Disease Severity (%)	Plant height (cm)	Number of leaf/plant (no.)	Number of healthy leaf/plant (no.)	Number of infected Leaf/Plant (no.)	Leaf length (cm)	Leaf width (cm)
T_1= Contaf 5 EC	3.70 de	3.33 ef	51.00 ab	5.64 a	5.31 ab	0.33 f	30.00 ab	2.85 ab
T_2=Score 250 EC	1.85 e	1.33 f	52.00 a	5.77 a	5.50 a	0.28 f	32.00 a	2.92 a
T_3=Autostin 50 WDG	7.40 cde	5.00 def	49.67 abc	5.51 ab	5.13 ab	0.38 ef	28.67 abc	2.78 abc
T_4=Turmeric	9.25 cde	5.67 cde	48.67 abcd	5.33 bc	4.89 bc	0.44 def	27.33 bcd	2.66 abc
T_5=Garlic	11.11 bcd	5.67 cde	46.33 bcde	5.11 cd	4.53 cd	0.58 cde	26.00 cde	2.59 abc
T_6=Onion	12.96 abc	7.33 bcd	45.33 cdef	4.98 de	4.37 de	0.61 cd	25.33 cde	2.54 abc
T_7=Acetic acid	14.81 abc	8.67 bcd	44.33 cdef	4.74 ef	3.96 ef	0.77 bc	24.00 def	2.48 abc
T_8=Benzoic acid	12.96 abc	9.33 bc	43.67 def	4.51 fg	3.74 fg	0.78 bc	23.67 ef	2.44 bcd
T_9=Oxalic acid	18.51 ab	10.67 ab	42.33 ef	4.37 gh	3.47 g	0.90 b	21.67 f	2.33 cd
$T_{10=}$Control (Water spray)	20.37 a	14.00 a	40.00 f	4.12 h	2.70 h	1.43 a	18.00 g	2.01 d
LSD (P=0.01)	7.50	3.57	5.08	0.28	0.44	0.21	3.27	0.41

3.6.3. Evaluation of fungicides, botanicals and organic acids on flower production of gladiolus in the field (70 DAS)

Three fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field for controlling leaf blight of gladiolus. At the reproductive stage, the highest spike length (71.67 cm) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (68.33 cm), Autostin 50 WDG (65.67 cm), Turmeric (63.33 cm), Garlic (62.33 cm), Onion (59.67 cm) and Acetic acid (58.00 cm). Similar trend of growth was found in floret/spike. Highest rachis length was found in Score 250 EC (31.33 cm) treated plot which was statically similar with Contaf 5 EC, Autostin 50 WDG and turmeric extract. Floret diameter and yield was statistically similar in Score 250 EC, Contaf 5 EC, Autostin 50 WDG, Turmeric, Garlic and onion treated plot (Table 41)

Table 40. Evaluation of fungicides, botanicals and organic acids and on growth parameters of gldiolus in the field at flowering stage (70 DAS)

Treatments	Disease incidence (%)	Disease severity (%)	Plant height (cm)	Number of leaf/plant (no.)	Number of healthy leaf/plant (no.)	Number of infected Leaf/Plant (no.)	Leaf length (cm)	Leaf width (cm)
T_1= Contaf 5 EC	14.81 d	9.00 e	85.00 ab	9.39 a	9.06 ab	0.33 f	35.67 ab	3.15 ab
T_2=Score 250 EC	14.81 d	8.33 e	89.33 a	9.52 a	9.25 a	0.28 f	38.00 a	3.22 a
T_3=Autostin 50 WDG	18.51 cd	10.00 de	82.67 ab	9.26 ab	8.88 ab	0.38 ef	35.33 ab	3.08 abc
T_4=Turmeric	20.37 bcd	10.67 de	81.00 ab	9.08 abc	8.64 abc	0.44 e	34.67 ab	2.96 abcd
T_5=Garlic	22.22 bc	10.67 de	79.00 abc	8.86 abcd	8.28 abcd	0.58 d	32.00 abc	2.89 a-d
T_6=Onion	24.07 bc	12.00 cd	76.00 abc	8.73 abcd	8.12 bcde	0.61 d	31.33 bc	2.84 bcd
T_7=Acetic acid	25.92 abc	13.67 bc	74.00 abc	8.49 abcd	7.71 cde	0.77 c	30.00 bcd	2.78 cd
T_8=Benzoic acid	24.07 bc	14.33 bc	70.67 bc	8.28 bcd	7.50 de	0.78 c	28.33 cd	2.74 cd
T_9=Oxalic acid	27.77 ab	15.67 b	70.00 bc	8.12 cd	7.22 ef	0.90 b	28.00 cd	2.63 d
T_{10}=Control (Water spray)	31.48 a	19.00 a	64.00 c	7.89 d	6.46 f	1.43 a	24.67 d	2.31 e
LSD (P= 0.01)	6.79	2.49	13.93	0.97	0.97	0.11	5.74	0.32

Table 41. Evaluation of fungicides, botanicals and organic acids on gladiolus flower production in the field (70 DAS)

Treatments	Spike length (cm)	Rachis length (cm)	Number of floret/spike	Floret diameter (cm)	Weight/spike (g)	Yield (flower stalk/ ha)
T_1= Contaf 5 EC	68.33 ab	29.33 ab	9.33 ab	10.20 ab	68.00 ab	165000.00 ab
T_2=Score 250 EC	71.67 a	31.33 a	10.00 a	10.57 a	72.33 a	170000.00 a
T_3=Autostin 50 WDG	65.67 ab	28.00 abc	9.00 ab	10.10 ab	66.00 abc	161666.67 ab
T_4=Turmeric	63.33 ab	27.33 abcd	8.67 ab	10.07 ab	63.33 bcd	157666.67 abc
T_5=Garlic	62.33 ab	26.33 bcd	8.33 ab	9.93 ab	60.67 cde	155000.00 abc
T_6=Onion	59.67 abc	24.33 cde	8.00 ab	9.83 abc	60.00 cde	153000.00 abc
T_7=Acetic acid	58.00 abc	23.33 de	8,00 ab	9.70 bc	58.00 def	150000.00 bcd
T_8=Benzoic acid	53.33 bc	21.33 ef	7.33 bc	9.57 bc	55.33 ef	146333.33 bcd
T_9=Oxalic acid	52.67 bc	21.00 ef	7.33 bc	9.47 bc	53.00 fg	141000.00 cd
T_{10}=Control (Water spray)	45.33 c	17.33 f	5.67 c	9.03 c	48.67 g	135666.67 d
LSD (P= 0.01)	13.92	4.08	1.89	0.74	6.1	17120

DISCUSSION

Botrytis leaf blight of gladiolus has been studied in the growing districts of Bangladesh. Data were collected on disease incidence and severity from the ten selected districts. The highest severity (17.22%) was found in Manikganj district which was statistically similar with Coxsbazar (15.56%), Faridpur (13%) and Jashore district (12.33%). The lowest disease severity (5.56%) was found in Gaibandha district. In a previous study, Botrytis blight was recorded in five surveyed fields of Sutiakhali and Babukhali of Mymensingh region and significantly the highest incidence (100%) was recorded at 75 days after sowing and lower incidence (6%) was found at the younger plants of 45 days (Sultana *et al.*, 2017). The similar trend was also observed in disease severity. The findings of the present work were supported by many scientists of the world. Sung *et al.* (2003) found that the Botrytis gray mold caused by *B. gladiolorum* reached up to 50% in damaged fields in Korea and *B. gladiolorum* spores produced gray mold on older plants drifted onto the flowers before harvest. However, severe outbreaks of Botrytis blight in mature stage were induced may be due to low temperature (17.9^0C), high humidity (89%), rainfal (15 mm) with wind speed (3.06 kmph) and no sunshine at that time. The variation in diseseae incidence and disease severity in different districts of Bangladesh was due to difference in weather parameters that was supported by Sehajpal *et al*. (2015) who revealed that the progression of botrytis blight disease was more in cool weather and towards the winds and wind direction. This is the first record on the occurrence of *Botrytis* blight and its causal pathogen, *B. gladiolorum* in ten districts of Bangladesh though the first report on the same disease was recorded by Siddique *et al.* (2013) in Jashore and Sultana *et al.* (2017) in Mymensingh. The symptoms appeared in the field was recorded and compared with the symptoms reported by other workers (Mirza and Shakir 1991, Sohi 1992, Singh *et al.* 2005 and Mirzaei *et al*. 2008). In the present study, artificially inoculated and severely infected leaves become reddish-brown with grayish conidial masses and dried from the tips. As the disease progressed, the lesions developed and blighted completely the spike, petal, flower bud with grey rot of flowers. The present findings are supported with the findings of Sung *et al*. (2013) and Siddique *et al.* (2013).

Forty-four (44) isolates of *B. gladiolorum* was isolated and identified on the basis of their morphological and cultural characters and according to the key literature and were recorded. Botrytis species have been named based on host association (Jarvis, 1977). For confirmation of the causal agent, Kock's postulate was performed through artificial inoculation as healthy leaves of gladiolus grown in pots in a glass house. Conidial suspension of *B. gladiolorum* isolated from naturally infected plants were used as inocula for inoculation. Characteristic symptoms of Botrytis blight developed on inoculated gladiolus plants were identical as recorded from the field. Based on pathogenicity test it was confirmed that the disease was Botrytis blight of gladiolus and the causal fungus was *B. gladiolorum*.

The radial mycelia growth rate /day ranged from 2.06 mm to 4.5 mm among 44 isolates. Colony color of the collected isolates were whitish, gray, milky white or ash color, regular or irregular having both of good, medium and poor growth. This finding supported the previous study (Siddique *et al.,* 2013; Sultana *et al.,* 2017). Siddique *et al.* (2013) recorded whitish mycelial growth that appeared on the infected leaf pieces of gladiolus on moist blotting paper. Like this study they also reported brown colony of *B. gladiolorum* having initiation of black sclerotia after 14-16 days of incubation. In this study the conidiophores were dark brown and twisted with ellipsoid and ovoid or oval in shape, pale brown conidia were in color those were similar as described by Siddique *et al.* (2013). The morphological characteristics of mycelia, conidiophores, conidia and sclerotia of *B. gladiolorum* recorded in the present investigation are almost similar to the descriptions of Sung *et al.* (2003), Mirzaei *et al.* (2008) and Sultana *et al*. (2017). The collected isolates were grouped in to 14 cultural group based on cultural characteristics (Aminuzzaman *et al*., 2010; Sharmin *et al*., 2022).

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) found that that Bavistin 50 WP (Carbendazim), CP-Zim 50WP (Carbendazim), Sunphanate 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) 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 (2019) 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) 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.* (2019) 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) 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) and Sohi (1992). *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 Shakir *et al*. (1998), Singh and Arora (1994) and Singh *et al.* (2005) who observed that Bavistin proved its performance against *Botrytis* and *Fusarium oxysporum*.

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 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.* (2011) evaluated fungitoxicity of different plant extracts, namely *A. 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%). In another study, Ashraf *et al.* (2014) reported that *A. sativum* and *Eucalptus* spp. at 1000 ppm concentration showed maximum inhibition of spore germination of the different *Fusarium* spp, whereas, in present study *A. sativum* at 5 per cent showed maximum inhibition of spore germination of *Botrytis gladiolorum,* while *Eucalptus* spp. were not found to be effective as *A. sativum* but showed statistically significant results. Kaur (2014) tested the efficacy of *A. sativum, A. indica, Melia azedarach, Vitex nigundo, Eucalyptus globules* against *B. 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.* (2014) 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 (2014) 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.* (2010) 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.* (2010) evaluated effect of leaf extract of *A. cepa* and *Tagetes erecta in vitro* against *F. 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 per cent concentration inhibited 100 percent

germination of spores, but marigold was not effective against *Botrytis*. Taskeen-Un-Nisa *et al.* (2011) 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.* (2015) showed the antifungal activity of garlic extracts applied directly and through volatile release was tested against the growth of postharvest pathogens *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 doseresponse manner.

Abd-Alla *et al.* (2013) reported that hydroalcoholic 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* (2007) 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 fungitoxicity 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.* (2012) 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*. The findings of the present studies were also supported by previous works (Rehena *et. al*., 2022; Nazifa *et. al*., 2021).

Among nine organic acids at the rate of 2000 ppm Acetic acid gave the best performance and showed (57.02%) inhibition of mycelia growth at 15 DAI followed by Benzoic acid (48.04%) and oxalic acid (41.97%). Abd-El-Kareem (2001) reported that acetic acid vapours caused complete inhibition of linear growth of *Botrytis cinerea* and reduced grey mould incidence of table grapes by more than 84.6% compared with control berries. In another

study, Lagopodi *et al.* (2009) recorded the effects of acetic acid fumigation, ethanol fumigation, and steam heat treatment on growth of *Botrytis cinerea in vitro*. Fumigation with 4 or 6 ⁄ µl acetic acid for 6 min, and 8 µl acetic acid for 3 or 6 min resulted in complete inhibition of fungal growth of *Botrytis cinerea*. Shokri (2011) evaluated inhibitory effects of citric and tartaric acids and their combination on the growth of *Trichophyton mentagrophytes, Aspergillus fumigatus*, *Candida albicans*, and *Malassezia furfur*. The results showed that citric acid had more fungistatic and fungicidal activities than those of tartaric acid against all pathogenic.

Three selected fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three selected botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three selected organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field experiment. At the flowering stage the lowest disease incidence (14.81%) was found in Score 250 EC treated plot which was statistically similar with Contaf 5 EC (14.81%), Autostin 50 WDG (18.51%) and Turmeric (20.37%). Similar results were found in disease severity. Disease severity was recorded the lowest in Score 250 EC treated plot (8.33%) which was statistically similar with Contaf 5 EC (9.00%), Autostin 50 WDG (10.00%) and Turmeric (10.67%). The highest spike length (71.67 cm) was found in Score 250 EC treated plot which was statistically similar with Contaf 5 EC (68.33 cm), Autostin 50 WDG (65.67 cm) and Turmeric (63.33 cm). Similar trend was found in rachis length, floret/spike, floret diameter, weight/ spike and flower stalk. Sultana *et al.* (2019) reported that Bavistin @ 0.2% and Tricho-suspension @ 2.0% significantly reduced the blight disease (14.2 and 12.5%) where control yielded 42.8% disease incidence. The height of plants, number of leaves/plants, rachis length, no. of floret/spike, floret length and diameter of florets significantly increased with the application of *Trichoderma harzianum* @ 2% followed by Bavistin. *Trichoderma harzianum* was found superior in terms of yield/ha (2.42 lac flower stalk) followed by Bavistin (1.90 lac flower stalk/ha) where control yielded 1.78 lac flower stalk/ha.

CHAPTER VI

SUMMARY AND CONCLUSION

Six experiments were conducted including survey on socio-economic conditions of gladiolus farmers, incidence and severity of gladiolus leaf blight (GLB) and management of *Botrytis gladiolorum* in the laboratory and in the field. Survey was conducted in ten major gladiolus growing districts of Bangladesh to determine the disease incidence and severity of gladiolus leaf blight, and subsequently, infected leaves, stems and flower were collected from the farmers' field. The survey was conducted through a questionnaire among the 120 farmers in 40 villages under 10 districts. The farmers answered the questions about their socio-economic condition, present and previous gladiolus cultivation, and the problems and constraints of gladiolus production. The interview arranged with the farmers at the farmers field in every village. *In vitro* evaluation of fungicides, botanicals, and organic acids were carried out against *B. gladiolorum* at the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, from November 2016 to January 2020.

In the field survey 120 gladiolus farmers were participated, among them 95% farmers were male, and rest of them are female. From total the farmers, 70% (84) were engaged in kharif season and 30% (36) were engaged for gladiolus cultivation in around the year. About 33% farmers used gladiolus seeds (corm) from own source and 20.83% farmers collected corms from neighbour farmers. Other sources were flower traders 16.67%, NGO 12.5% and others 8.33%. About 30% farmers used Mount Everest variety, 15% farmers used BARI gladiolus-3 (white colour), 15% farmers used BARI gladiolus-4 (pink colour), 10% farmers used BARI gladiolus-5 (yellow colour), whereas 30% farmers used other unknown varieties. From the farmers opinion, leaf blight was the most important disease followed by leaf spot, leaf rot, corm rot, stem rot, flower rot, wilt, mosaic, aster yellow, dry rot and scab. During survey, disease incidence and severity of gladiolus leaf blight caused by *B. gladiolorum* were recorded in 10 districts. Incidence of GLB was statistically similar in all districts. Percent incidence was 32.32, 31.44, 30, 28.33 and 27.22 found in Manikganj, Jashore, Cox's Bazar and Rangpur district, respectively. Disease incidence was found 18.89% in Bogura district. In case of disease severity, the highest disease severity was found in Manikganj district

(17.22%), which was statistically similar with Cox's Bazar (15.56%), Faridpur (13%) and Jashore district (12.33%). The lowest disease severity was found in Gaibandha district (5.56%).

The isolates of *Botrytis gladiolorum* were identified based on morphological and cultural characteristics. Under compound microscope obovoid, unicellular, pale brown, smooth conidia were observed. Mycelial radial growth of *B. gladeolorum* was measured in Potato Dextrose Agar (PDA) and significant variation was recorded. Forty-four (44) isolates of *B. gladiolorum* were isolated from leaves of Gladiolus from major Gladiolus growing districs viz. Jashore, Mnikgonj, Dhaka and Cox's Bazar of Bangladesh. Among them 10 isolates were collected from Cox's Bazar, 10 isolates from Dhaka district, 13 isolates from Jashore and 11 isolates from Manikgonj district. The maximum number 13 of isolates was collected from Jashore. The radial mycelia growth rate /day ranged from 2.75 mm to 4.12 mm among the isolates collected from Savar Upazilla, Dhaka. On the other hand, the radial mycelia growth rate /day ranged from 2.18 mm to 3.59 mm found in isolates collected from Jhikorgacha, Jashore. But in Singair, Manikgonj the radial mycelia growth rate/day of the fungus was recorded as 3.18 mm to 4.06 mm. Among all isolates collected from four districts, the highest radial mycelia growth rate /day (4.5 mm) and the lowest growth rate /day (2.06 mm) were found in the isolates collected from Chakoria, Cox's Bazar. Pathogenicity assays on susceptible gladiolus variety BARI gladiolus-4 confirmed that *B. gladiolorum* was the causal pathogen of leaf blight of gladiolus.

Colony characteristics of isolates were recorded in culture plates in the laboratory. Mycelial growth rate of forty-four (44) isolates were recorded. All the 44 isolates grown on PDA media were observed the mycelial color, surface texture, shape, and growth. The isolates BGCC01 and BGCC03 were gray color. On the other hand, the isolates BGCC02, BGCC04, BGCC05, BGCC06, and BGCC08 were dark gray colors; the isolate BGCC07 and BGCC10 were light gray color. Grayish Ash color was found in the isolates of BGCC09, BGJJ01, BGJJ02, BGJJ03, BGJJ04, BGJJ05, BGJJ06, BGJJ07, BGJJ08, BGJJ09, BGJJ10, BGJJ11, BGJJ12 and BGJJ13. White color was found in the isolates of BGDS01, BGDS02, BGDS04, BGDS05, BGDS06, BGDS10, BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS06, BGMS07, BGMS08, BGMS09, BGMS10 and BGMS11, On the other hand milky

white were found in BGDS03 and BGDS07 isolates. The isolates BGDS08 and BGDS09 showed Buff colour.

The smooth, velvety surface texture and the regular shape were found in the isolates BGCC01, BGCC02, BGCC03, BGCC04 BGCC08, BGCC09 and BGCC10. On the other hand, smooth, velvety surface texture and the irregular shape were found in the isolates BGCC05 and BGCC07. The isolates BGDS01, BGDS03, BGDS04, BGDS05, BGDS07 and BGDS10 having a smooth, cottony surface texture and regular shape. On the other hand, the isolates BGDS02, BGDS05 and BGDS06 have a smooth, cottony surface texture and irregular shape. Rough velvety surface texture and regular shape found in BGJJ01, BGJJ02, BGJJ03, BGJJ04, BGJJ05, BGJJ07, BGJJ08, BGJJ09, BGJJ10, BGJJ11 and BGJJ13. On the other hand, BGJJ06 and BGJJ12 isolates have rough velvety surface texture and irregular shape. Rough cottony surface texture and irregular shape found in all the isolates collected from Manikganj district, the isolates were BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS06, BGMS07, BGMS08, BGMS09, BGMS10 and BGMS11.

Good mycelial growth appeared in the isolates BGCC02, BGCC03, BGCC08, BGCC10, BGDS03, BGDS06, BGDS07, BGDS08, BGDS09, BGDS10, BGJJ07, BGMS01, BGMS02, BGMS03, BGMS04, BGMS05, BGMS09 and BGMS10. On the other hand, medium mycelial growth showed in the isolates BGCC01, BGCC04, BGCC06, BGCC07, BGCC09, BGDS01, BGDS02, BGDS04, BGDS05, BGJJ01, BGJJ02, BGJJ04, BGJJ06, BGJJ08, BGJJ09, BGJJ10, BGJJ11, BGJJ12, BGJJ13, BGMS06, BGMS07, BGMS08, BGMS09 and BGMS11. Poor mycelial growth was recorded in the isolates BGCC05, BGJJ03 and BGJJ05.

Fourteen (14) cultural groups were found on the basis of cultural characteristics. Highest (25%) isolates were found in the cultural group CG-8 (Rough cottony white regular). Lowest (2.27%) isolates were found in CG-3 (Smooth velvety light gray regular). Similar results were found in CG-4 (Smooth velvety grayish ash regular), CG-10 (Smooth velvety gray irregular) and CG-14 (Mixed sandy grayish ash irregular).

Ten fungicides such as 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, Indofil 80 WP and Contaf

5 EC were tested against *Botrytis gladiolorum* at the rate of 100 ppm, 200 ppm and 300 ppm and radial mycelia growth of fungus were measured after 5, 10 and 15 DAI. In 100 ppm after 5 DAI, no radial mycelia growth was found in Contaf 5 EC treated plate which was statistically similar with Score 250 EC treated plate that indicates the inhibition of growth was 100%. At 10 DAI, Contaf 5 EC showed the best results to suppress the growth of mycelia (00.0 mm) followed by Score 250 EC (7.20 mm) and Autostin 50 WDG (14.50 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.

In 200 ppm, all fungicides gave significantly different results as compared with control and were found effective in reducing the mycelial growth. At 5 DAI, all the fungicides showed 100% inhibition of growth except Amister Top 250 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 effect against mycelial growth (00.0 mm) which is statistically similar with Score 250 EC (00.0 mm) and Folicure 250 EC (00.0 mm). At 15 DAI, Contaf 5 EC showed the best performance against mycelial growth and gave 100% inhibition, which was statistically similar to Score 250 EC (100% inhibition) followed by Autostin 50 WDG (83.04%).

In 300 ppm, all fungicides gave significantly different results as compared with control and found effective in reducing the mycelial growth. At 5 DAI, all the fungicides showed 100% inhibition of growth. At 10 DAI, all the fungicides gave 100% inhibition of mycelia growth except Amister Top 250 SC (78.45%) and Nativo 75 WG (77.72%). At 15 DAI, Contaf 5 EC gave the best performance against mycelia growth and showed 100% inhibition, which was statistically similar to Score 250 EC (100% inhibition), Autostin 50 WDG and Trooper 75 WP.

Ten botanicals such as Mehendi, Chrysanthemum, Tulsi, Onion, Neem, Bael, Arjun, Garlic, Ghritkumai and Turmeric at the rate of 5%, 10% and 20% were tested against *Botrytis gladiolorum* and radial mycelia growth of fungus were measured after 5, 10 and 15 DAI, all botanicals were found significantly effective in reducing the mycelial growth of the pathogens as compared to control. In the dose 5% 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) and the inhibition of growth was 60% and 51.72%, respectively. Similar trend was found after 10 DAI and 15 DAI. At 15 DAI, Garlic and Turmeric gave the

best performance against *B. gladiolorum,* which was statistically similar with onion (50.07%) and mehendi (49.93%) inhibition.

When at the rate of 10% botanicals mixed with water no radial mycelia growth was found in garlic treated plate at 5 DAI, which was statistically similar with onion treated (00.0 mm) plate and the growth inhibition was 100%. Similar trend was also found after 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 the dose 20%, no radial mycelia growth was found, in garlic treated plate at 5 DAI, which was statistically similar with onion treated (00.00 mm) plate and turmeric treated plate and the inhibition of growth was 100%. Similar trend was also found after 10 DAI and 15 DAI but 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).

Nine organic acids such as Tartaric acid, Oxalic acid, Citric acid, Ortho phosphoric acid, Ascorbic acid, Acetic acid, Benzoic acid, Gallic acid and Glutamic acid at the dose 1000 ppm were tested against *Botrytis gladiolorum* and radial mycelia growth of fungus were measured after 5, 10 and 15 DAI. All organic acids gave significantly different results as compared with control. At 5 DAI, the radial mycelia growth was found minimum in oxalic acid (26.10 mm) which was statistically similar with acetic acid (26.30 mm) followed by benzoic acid (39.50) and the inhibition of growth was 44.47%, 40.04% and 38.72%. At 10 DAI, the highest inhibition of growth was found in acetic acid (46.23%) which was statistically similar with oxalic acid (42.92%) benzoic acid (41.87%). At 15 DAI, the growth inhibition (43.22%) was highest in acetic acid followed by benzoic acid (38.11%) and oxalic acid (33.50%), respectively.

At the dose 2000 ppm, the radial mycelia growth was (10.30) mm found in acetic acid followed by oxalic acid (21.40 mm) at 5 DAI. Benzoic acid showed 51.53% growth inhibition, which was statistically similar with oxalic acid. At 10 DAI the highest growth inhibition was found in acetic acid (71.11%) followed by benzoic acid (54.69%). At 15 DAI, the highest inhibition of growth was found in acetic acid (57.02%) followed by benzoic acid (48.04%) and oxalic acid (41.97%). At the dose 3000 ppm, no radial mycelial growth of fungus was found in acetic acid at 5 DAI, which was followed by benzoic acid (8.6 mm) and

oxalic acid (16.40 mm) and the inhibition of growth of these organic acids were 100%, 80.54%, 62.90%, respectively. At 10 DAI the highest inhibition of growth was found in acetic acid (100%), which was statistically followed to benzoic acid (64.69%) and oxalic acid (54.29%). At 15 DAI, the growth inhibition (86.75%) was the highest in acetic acid followed by benzoic acid (58.54%) and oxalic acid (46.36%), respectively.

Three fungicides (Score 250 EC, Contaf 5 EC and Autostin 50 WDG) at the rate of 300 ppm, three botanicals (Turmeric, Garlic and Onion) at the rate of 20% and three organic acids (Acetic acid, Benzoic acid and Oxalic acid) at the rate of 3000 ppm were evaluated in the field for controlling leaf blight of gladiolus. At the vegetative stage and the lowest incidence (1.85%) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (3.70%), Autostin 50 WDG (7.40%) and Turmeric (9.25%). Similar results were also found in severity, where the severity was found the lowest in Score 250 EC treated plot (1.33%), which was statistically similar with Contaf 5 EC (3.33%), Autostin 50 WDG (5.00%) and Turmeric (5.67%). In case of plant height, these was no statistically significant effect found on number of leaves per plant, leaf length and leaf width. At the reproductive stage of the plant, the lowest disease incidence (14.81%) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (14.81%), Autostin 50 WDG (18.51%) and Turmeric (20.37%). Similar results were also found in case of disease severity, where the severity was found the lowest in Score 250 EC treated plot (8.33%), which was statistically similar with Contaf 5 EC (9.00%), Autostin 50 WDG (10.00%) and Turmeric (10.67%) treated plots. In case of plant height, there was no statistically significant effect found on number of leaves per plant, leaf length and leaf width. The highest spike length (71.67 cm) was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC (68.33 cm), Autostin 50 WDG (65.67 cm) and Turmeric (63.33 cm) treated plots. Similar trend was also found in rachis length, floret/spike, floret diameter, weight/ spike and flower stalk.

Based on the findings of the study it may be concluded that

- Leaf blight of gladiolus caused by *Botrytis gladiolorum* is widely distributed and major biotic problem in the gladiolus growing districts of Bangladesh. Among the different growing districts, the disease incidence and severity varied from 18.89 to 32.22% and 5.56 to 17.22%, respectively.
- Morphological and cultural variabilities exist among the isolates of *Botrytis gladiolorum* associated with leaf blight disease of gladiolus found in Bangladesh. The highest mycelial radial growth of *Botrytis gladiolorum* (72.00 mm) was recorded for BGCCO3 isolate, whereas the lowest (33.00 mm) was recorded in BGCCO5 isolate cultured in PDA media at 16 DAI. The average radial mycelial growth rate /day ranged from 2.06 mm to 4.5 mm.
- Based on findings of *in vitro* evaluation of fungicides, 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, which was statistically similar with Autostin 50 WDG.
- 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*.
- Among organic acid evaluated *in vitro,* Acetic acid, Benzoic acid and Oxalic acid at the dose of 3000 ppm were found the most effective against *Botrytis gladiolorum.*
- In field experiment minimum disease incidence and severity was found in Score 250 EC treated plot, which was statistically similar with Contaf 5 EC, Autostin 50 WDG and Turmeric extract treated plot. The growth parameters and yield were also found highest in score 250 EC treated plot, which was statistically similar with Contaf 5 EC, Autostin 50 WDG and Turmeric extract treated plot.

Recommendations

- Cultural Characteristics of different isolates of *Botrytis gladiolorum* collected from farmer's field of four major glaadiolus growing districts in Bangladesh showed remarkable variations. Further research should be conducted including molecular characterization to determine the variants, physiologic races and pathogenicity of this pathogen.
- Farmers should be suggested to apply Score 250 EC (0.12 % at three times) or Contaf 5 EC (0.6 % at three times), Autostin 50 WDG (0.06% at three times) to effectively control of gladiolus leaf blight.
- Spray of turmeric extract (20% at three times) showed promising in arresting the disease *in vivo* and as such this could be an effective ecofriendly alternative approach in controlling gladiolus leaf blight.
- In *in vivo* disease management, experiment was conducted in Manikganj District. Hence for further confirmation the result of present study should be tested in different AEZ region especially in commercially Gladiolus producing areas.

CHAPTER VI

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CHAPTER VII
APPENDICES

Appendix 1. Gladiolus growing disticts of Bangladesh (cultivation area and production, 2016-2017)

Sl. No.	Name of districts	Cultivation area (ha.)	Production (No. of flower)
1	Dhaka	41.00	7380000
2	Manikganj	16.55	2979000
3	Narayanganj	45.40	8172000
4	Tangail	1.05	189000
5	Kishoreganj	2.00	360000
6	Jamalpur	1.00	180000
7	Moulovibazar	0.50	90000
8	Sylhet	2.00	360000
9	Chittagong	59.00	10620000
10	Cox's Bazar	20.00	3600000
11	Rajshahi	0.65	117000
12	Natore	1.00	180000
13	Nogaon	1.00	180000
14	Bogura	3.00	540000
15	Pabna	1.00	180000
16	Faridpur	6.00	1240000
17	Rajbari	0.50	90000
18	Borishal	1.00	180000
19	Pirojpur	0.80	144000
20	Rangpur	2.75	495000
21	Kurigram	0.65	117000
22	Lalmonirhat	0.22	39600
23	Gaibantha	2.70	486000
24	Dinajpur	0.10	18000
25	Joshore	275.00	49263750
26	Magura	0.001	2580
27	Jhinaidoho	12.00	2035000
28	Khulna	0.40	72000

Source: Horticulture wing, DAE

Appendix 2. Cultivation area and production of gladiolus in ten selected districts of Bangladesh in 2016-2017.

Sl. No.	Name of districts	Cultivation area (ha.)	Production (No. of flower)
1	Bogura	3.00	540000
2	Cox's Bazar	20.00	3600000
3	Chattagram	59.00	10620000
4	Dhaka	41.00	7380000
5	Faridpur	6.00	1240000
6	Gaibandha	2.70	486000
7	Jashore	275.00	49263750
8	Manikganj	16.55	2979000
9	Narayanganj	45.40	8172000
10	Rangpur	2.75	495000

Source: Horticulture wing, DAE

Appendix 3. Soil characteristics of Singair upazila under Manikganj district

Sl. No.	Elements	% Elements	Soil Textural class
01	P^H	7.4	Silt Clay Loam
02	Organic Matter	1.5	
03	Total N	0.068	
04	Available P (mgP205/I00g soil)	6.00	
05	Exchangeble K (meq/100g soil)	0.075	

Source: Agriculture office, Upazila- Singair, Dist.-Manikganj.

Appendix 4. Monthly average temperature, relative humidity and total rainfall of field experimental site Singair, Manikganj During the Period from October, 2018 to September, 2019.

Year	Months	Air Tem. (^{0}C)		Relative humidity (%)	Rainfall (mm)
		Max.	Min.		
2018	October	30	20		
2018	November	30	15		
2018	December	27	11		
2019	January	22	09		
2019	February	27	15		
2019	March	32	20		
2019	April	33	22		
2019	May	38	25		
2019	June	37	25		
2019	July	34	24		
2019	August	35	25		
2019	September	32	22		
Average		31.42	19.42	86.41%	131 mm

Source: Agriculture office, Upazila- Singair, Dist.-Manikganj.

Appendix 5. Survey Questionare for gladiolus farmers' interview

সেট-১: গ্লাডিওলাস ফুলচাষীদের জন্য জরিপ ও প্রশ্নাবলী

কোড			

মোবাইল :

ক. গ্লাডিওলাস ফুলচাষীর ব্যাক্তিগত তথ্যাদি ঃ

ক. ১ উত্তরদাতার নাম: ---------- ক. ২ গ্রাম: ----------

ক. ৩ কৃষি ব্লক : ---------- ক. ৪ উপজেলা:---------- ক. ৫ জেলা: ----------ক.

৬ শিক্ষাগত যোগ্যতা: ---------- ক. ৭ বয়স:---------- ক. ৮ পেশা: ---------- (কোড: ১ = ক্ষুদ্র চাষী, ২ = মধ্যম চাষী, ৩ = বড় চাষী, ৪ = ফুল ব্যবসায়ী, ৫= নার্সারী মালিক, ৬= কোল্ড স্টোরেজ)

খ. ফুলের আবাদ সংক্রান্ত

খ. ১ গ্লাডিওলাস ফুল চাষে ব্যবহৃত জমির ধরন/ প্রকৃতি:

ফুল চাষে ব্যবহৃত জমির ধরন	
১. এ বছর ফুল চাষ করেছেন এমন জমির পরিমাণ বলুন	
২. অন্য ফসলের তুলনায় এ বছর ফুল চাষে নিয়োজিত জমির আনুমানিক শতকরা পরিমান (%)	
৩. কত বছর যাবত ফুল চাষ করেন ?	
৪. গত বছরের তুলনায় এ বছর ফুল চাষ বেড়েছে কি?	
৫. অর্জিত আয় হাজার/একর)	

খ. ২ চাষকৃত ফুলের জাত কি কি?

১. ---------- ২. ---------- ৩. ---------- .৪. ----------

খ. ৩ গ্লাডিওলাস ফুল চাষের জন্য সাধারনত: কোন কোন উৎস থেকে বীজ/চারা ক্রয় /সংগ্রহ করেন?

উৎস সমুহ	উত্তরের ধরন কোড= ১=হ্যা, ২=না	
১. নিজের তৈরী বীজ বা চারা		
২. প্রতিবেশী কৃষকের কাছ থেকে পাওয়া		
৩. অন্য কোন কোম্পানী বীজ/চারা/কন্দ		
৪.স্থানী বীজ বা চারা উৎপাদনকারী নার্সারী		
৫. আমদানীকারকের কাছ থেকে		
৬. গবেষণা প্রতিষ্ঠান থেকে		
৭. এনজিও এর কাছ থেকে		
৮. ফুল বীজ বা চারা ব্যবসায়ীর কাছ থেকে		
অন্যান্য		

খ. ৪ কোল্ডস্টোরেজে গ্লাডিওলাস ফুলের বীজ কন্দের রোগ হয় কি না? হ্যা=১, না =২ ----- যদি হ্যা হয় তাহলে কি কি রোগ হয়?

১.---------- ২. ---------- ৩.---------- ৪. ----------

খ. ৫ গ্লাডিওলাস ফুলের কি কি রোগ হয়?

১. ---------- ২. ---------- ৩. ---------- ৪. ----------

৫. ---------- ৬. ---------- ৭. ----------

খ. ৬ গ্লাডিওলাস ফুল চাষে রোগের কারণে কি পরিমান ফলন কমে?

খ. ৭ গ্লাডিওলাস ফুল চাষে রোগ নিয়ন্ত্রণে কোন কোন ওষুধ ব্যবহার হয়?

১. ---------- ২. ---------- ৩. ---------- ৪. ----------

৫. ---------- ৬. ----------

খ. ৮ গ্লাডিওলাস ফুলে বিভিন্ন রোগের আক্রমনের অবস্থা, গাছের ঝুকিপূর্ণ ধাপ সমূহ, রোগাক্রান্ত গাছের অংশ এবং আক্রমনের তীব্রতা কেমন?

নং	রোগ সমূহের নাম	আক্রমনের অবস্থা: (১= মূখ্য, ২= গৌণ, ৩=আক্রমণ হয় না	ফুল গাছের ঝুকিপূর্ণ ধাপ সমূহ ১= চারা, ২= বাড়ন্ত গাছ, ৩=ফুল ফোটা বা বৃদ্ধি পর্যায়	আক্রমনের তীব্রতা

গ. ফলনের পরিমান কত ? উ:-

ঘ. আর্থিক লাভের পরিমান কত ? উ:-

ঙ. রোগের ইনসিডেন্স:

চ. রোগের সিভিয়ারিটি:

ছ. গ্লাডিওলাস ফুল চাষে সমস্যা:

১. ------ ২. ---- ৩. ----- ৪. --------

তথ্য সংগ্রহকারীর নাম ও স্বাক্ষর