2013

Fungal Diseases of Strawberry in Bangladesh with Special Reference to Crown Rot Disease

Khatun, Most. Rehena

University of Rajshahi

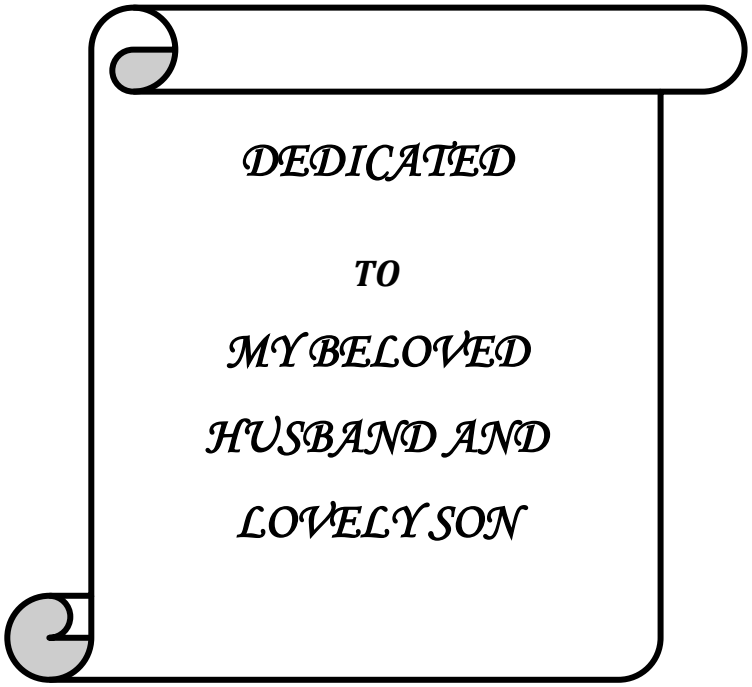
FUNGAL DISEASES OF STRAWBERRY IN BANGLADESH WITH SPECIAL REFERENCE TO CROWN ROT DISEASE

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI, BANGLADESH

BY

MOST. REHENA KHATUN
B. Sc. (Hons.), M. Sc.

PLANT PATHOLOGY, MYCOLOGY AND MICROBIOLOGY LAB
DEPARTMENT OF BOTANY
RAJSHAHI UNIVERSITY
RAJSHAHI 6205
BANGLADESH
DECEMBER, 2013


DEDICATED
TO
MY BELOVED
HUSBAND AND
LOVELY SON

DECLARATION

I hereby declare that the whole work submitted as a thesis entitled *"**FUNGAL DISEASES OF STRAWBERRY IN BANGLADESH WITH SPECIAL REFERENCE TO CROWN ROT DISEASE**"* in the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh for the degree of Doctor of Philosophy is the results of my own investigation and was carried out under the supervision of Professor Dr. Md. Shahadat Hossain, Department of Genetic Engineering and Biotechnology, Rajshahi University, Rajshahi. The thesis has not already been submitted in the substance for any degree and has not been concurrently submitted in the candidature for any other degree.

December, 2013

Most. Rehena Khatun
Candidate

CERTIFICATE

This is to certify that the thesis entitled "***FUNGAL DISEASES OF STRAWBERRY IN BANGLADESH WITH SPECIAL REFERENCE TO CROWN ROT DISEASE***" prepared by Most. Rehena Khatun for the award of degree of Doctor of Philosophy is a record of bonafide research work carried out by her under our supervision. The work is original and to the best of our knowledge and belief, no part of the thesis has been submitted before for any degree or diploma in any University.

Supervisors:

1. (Md. Shahadat Hossain, *Ph.D.*)

Professor
Department of Genetic Engineering and Biotechnology
University of Rajshahi
Rajshahi-6205
Bangladesh.

2. (Dr. Mst. Ferdowsi Mahal)

Associate Professor
Department of Botany
University of Rajshahi
Rajshahi-6205
Bangladesh.

ACKNOWLEDGEMENT

The author is grateful to Almightily Allah for her blessings, protection help and wisdom in all aspect of her life. She would like to express her indebtedness with heartiest gratitude to her supervisors Md. Shahadat Hossain, Professor, Department of Genetic Engineering and Biotechnology and Dr. Mst. Ferdowsi Mahal, Associate Professor, Department of Botany, University of Rajshahi, for their generous advice, constructive discussion and criticisms throughout this study.

She expresses her sincere gratitude to Professor, Dr. M. Shahidul Alam and Dr. M. Monzur Hossain, Department of Botany, University of Rajshahi for providing their laboratory and field facilities to conduct this research work.

Special thanks are due to Professor (Ret.) Dr. M. Shah Alam, Professor Dr. A. K. M. Rafiul Islam and Professor Dr. Most. Ferdowsi Begum, Department of Botany and to Dr. M. Khalekuzzaman, Professor, Department of Genetic Engineering and Biotechnology, University of Rajshahi, for their technical assistance, valuable comments and suggestions.

She would like to extend her gratefulness and thanks to Professor Dr. Tanzima Yeasmin, Director, Institute of Biological Sciences; Professor Dr. K. A. M. Shahadat Hossain Mondal; Professor Dr. M. A. Bari Miah; Professor Dr. Wahedul Islam and Professor Dr. Parvez Hassan, Institute of Biological Sciences, University of Rajshahi. She is also grateful to Professor Dr. M. Khalekuzzaman, ex. Director, Institute of Biological Sciences, University of Rajshahi.

The author also heartily expressed thanks to the “Ministry of Science and Technology of the People’s Republic of Bangladesh” for the funding of this research work.

Her special thanks to Mr. Montaj Ali Sarker, Kaosar Rahman and Ms. Nargis Tangila, and other researchers of Plant Pathology, Mycology and Microbiology Laboratory, Department of Botany, University of Rajshahi.

She is very much grateful to her husband’s Md. Rezaul Karim and to her son Nafis Reza Diganta and to her father, mother, brothers and well wishers for their inspiration and encouragement leading to moral support throughout the whole present research work.

December, 2013 The Author

LIST OF ABBREVIATIONS AND SYMBOLS

PDA	: Potato Dextrose Agar
NAA	: α-Naphthalene Acetic Acid
IBA	: Indole-3-butyric acid
BA	: 6-Benzyle adenine
IAA	: Indol-3-acetic acid
2,4- D	: 2,4-Dichlorophenoxyacetic acid
NaOH	: Sodium hydroxide
cm	: Centimetre (s)
cm^2	: Centimetre square
°C	: Degree Celsius
etc.	: Et cetera (= and the others)
e.g.	: Exempli gratia (= for example)
et al.	: Et alia (= and others)
Fig.	: Figure (S)
g	: Gram (S)
$HgCl_2$	: Mercuric chloride
HCl	: Hydrochloric acid
l	: Litre
ml	: Millilitre (S)
pH	: Negative logarithm of hydrogen ion (H^+) concentration
0.1N	: 0.1 Normal
sp.	: Species
sq.	: Square
viz.	: Videlicet (= namely)
mm	: Millimitre (S)
min.	: Minute (S)
i.e.	: Id est. = that is
Kg.	: Kilogram (S)
%	: Percentage

/	: Per
No.	: Number
L.	: Linnaeus (Carolus Linnaeus)
mg	: Milligram (s)
v/v	: Volume per volume
w/v	: Weight per volume
µ	: Micron
rpm	: Rotation per minute
KJ	: Kilojoules
µm	: Micrometre
LSD	: Least Significant Difference
hr	: Hour
DF	: Dry Flowable
WG	: Water Dispersible Granules
WP	: Wettable Powder
EC	: Emulsifiable Concentrate
ppm	Pers per million
&	And
ha	Hectare

ABSTRACT

The present investigation was conducted on various aspects on crown rot disease of strawberry with reference to survey and surveillance of fungal diseases. This study revealed that many fungal diseases of strawberry were noticed in Bangladesh**.** Among these diseases, crown rot is an important and very intensive disease of strawberry. The severity of crown rot was more at Rajshahi district followed by Sherpur, Dinajpur and Natore districts. The identity of the fungus was confirmed as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

Cultural studies revealed that among solid media, Richard's and PDA media were found to be good for mycelial growth and sporulation of *C. gloeosporioides* on 10th day of incubation.

Nutritional studies revealed that potassium nitrate and glucose were better for nitrogen and carbon sources respectively for growth and sporulation of the pathogen.

Physiological studies revealed that optimum pH of 6.5 was favoured for growth and sporulation of the pathogen. The highest mycelial growth and sporulation of the fungus was recorded at 30^{o}C and light duration having 12 hours light alternated with 12 hours dark.

In vitro evaluation of plant extracts mehedi and garlic were found to be most effective against *C. gloeosporioides.* Among the fungicides tested, Bavistin and Folicur were found to be effective at four concentrations (100-1000 ppm) and Hayconazole at 1000 ppm. Among two biomatters, cow urine was most effective against *C. gloeosporioides.* Out of five phytohormones, NAA was found to be effective followed by IAA, 2,4-D and IBA. Among the biocontrol agent *T. harzianum* was found to be effective against *C. gloeosporioides.*

In the field evaluation of plant extracts, garlic bulb extract (25%) was most effective on crown rot disease of strawberry and recorded higher yield (149.29 g per plant) followed by neem leaf extract (145.99 g per plant). On the other hand the antagonist *T. harzianum* was found to be most effective in the field condition recorded higher yield (151.53 g per plant).

Among the different fungicides, Bavistin at 1000 ppm was most effective and recorded higher yield (148.56 g per plant) followed by Score (143.44 g per plant) Hayconazole (139.41 g per plant) and Dithane (134.86 g per plant).

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I
INTRODUCTION

1.1. STRAWBERRY: A GENERAL ACCOUNT

Strawberry (*Fragaria* × *ananassa* Duch.) is a popular fruit all over the world where suitable soil and moisture are available. It is a fruit of winter leading country. It is one of most popular, nutritious and lovely looking fruits in the world. The production and consumption of strawberry is increasing day by day because of its food value and other importance. The winter season preferably November to March can be favorable for strawberry cultivation in Bangladesh. It is cultivated in an area of 0.2 million hectares of land producing 0.3 million metric tons. America is the first in position (27%) for the production of strawberry in the world (Hossain, 2007). It has been commercially cultivated in Canada, USA, Japan, Spain, Germany, Korea, Italy, Poland, Thailand and so many other countries in the world (Biswas *et al*., 2007).

Strawberry has traditionally been a popular delicious fruit for its flavour, taste, and fresh use, freezing and prosing. It contains relatively high quantities of ellagic acid, which has a wide range of biological activity. Strawberries are now getting popularity in Bangladesh (Sakila *et al.,* 2007). Due to its popularity and increasing demand in Bangladesh, a research study has been undertaken at Department of Botany, University of Rajshahi, Bangladesh to establish a rapid *in vitro* clonal propagation of different strawberry genotypes and evaluate their field performance in Bangladesh condition. But, the strawberry plants has been infected by many diseases, such as Colletotrichum crown rot, Phytophthora crown rot, Anthracnose fruit rot, Botrytis fruit rot (Gray mold), Alternaria rot, Rhizopus rot or leak, Black root rot, Leaf spot, Phomopsis leaf blight, Phomopsis soft rot, Verticillium wilt etc. Among the diseases crown rot is a serious disease of strawberry in Bangladesh. The time of harvest is very

important in strawberry. Berries were harvested every day to maintain the quality of the berries. When 2/3th of the berries were become red colour then berries was ready to collect. The other standard agronomic practices were followed.

1.2. IMPORTANCE OF STRAWBERRY

Strawberries are often used to flavour other foods. Importances of strawberry are described as following heads:

Nutritional importance: Strawberry fruits have potential to be used as jam, jelly, syrup, and as a foundation in beverage, in ice creams, cakes and in cosmetic industries. Strawberry shortcake, an all-time favorite America dessert is a frequent star on the sweet table when strawberries are in season from early spring thorough summer. Strawberries and cream, a tasty combination, has a rather long history. In Eastern Europe, strawberries are paired with sour cream while in France and Italy; strawberries are topped with wine and sugar. (Http//www.vegparadise. com/highest perch 45.html # uses). One cup of strawberries (149 g) constitutes approximately 45 calories (188 KJ) and is an excellent source of vitamin C and flavonoids as well as other nutrients mentioned in following chart:

Nutrient	**Amount**
Vitamin C	81.65 mg
Vitamin K	20.16 mg
Vitamin B_2	0.10 mg
Manganese	0.42 mg
Dietary fiber	3.31g
Iodine	12.96 mg
Potassium	239.04 mg
Foliate	25.49 mg

Medicinal importance: During the 13th century French cultivated strawberries to use as a medicinal herb for numerous digestive discomforts. The roots, leaves, and fruits of the Alpine strawberry, *Fragaria vesca* were used as a digestive aid and skin tonic. According to the ancients, strawberries could cure inflammations, fevers, throat infections, kidney stones, gout, fainting spells, and diseases of the blood, liver and spleen.

Economic importance: Strawberries are one of the highest values per acre crops grown in North America with annual yields ranging from 4 to 20 tons per acre and gross values ranging from 2,800 to $ 14,000 per acre. The future of strawberry production and sales is very positive. The demand of strawberry is increasing day by day all over the world.

1.3. ORIGIN AND DISTRIBUTION OF STRAWBERRY

Strawberry (*Fragaria* × *ananassa* Duch.) belongs to the group of the most popular fruit crops in the world. Strawberries were cultivated and traded in Chile and Peru of South America before the Spanish explorers arrived. *Fragaria* × *ananassa,* a relatively young fruit species in comparison to other pomological plants, came into existence as a result of random cross of two wild species *F. chiloensis* Ehourh. and *F. virginiana* Duch. Hybridization of both species resulted in new plants with valuable traits (aroma, flavour, contents of vitamin and antioxidants). Simultaneously, strawberry plants are graceful model for different biological studies like analysis of metabolic path ways, molecular assays and experimental with transgenesis recorded history of the *Fragas*, fruit of the strawberry, dates back to 23-79 A.D. in the writings of Pliny (Darrow, 1966a). The woodland strawberry of Northern Europe, *Fragaria vesca* L. was cultivated as early as 1300 in France. It was appreciated as much for its flowers as for the fruit. Cultivation also took place in England were documentation of the history of the name strawberry is found in literature from the tenth to fifteenth centuries. In the 1500s, other species and subspecies of *Fragaria* were discovered in Europe. Early colonists in North America cultivated their native

strawberry, *Fragaria virginiana.* The North American very was a hardy plant with the ability to withstand cold temperatures and drought. In the early 1600s, *Fragaria virginiana* was imported to Europe from North America.

In the 1700s, French explorers found a wild strawberry in Chile. The South American berry grew large fruit but was not well suited to a wide range of climates. The European importation of the Chilean strawberry, *Fragaria chiloensis,* in 1714 was the most important event in the history of the large fruited strawberry grown today (Darrow, 1966a). A French army officer named Amedee Francois Frezier nursed 5 *F. chiloensis* plants back to Europe from Concepcion of Chile. When he arrived he distributed his plants. The plant he gave to his superior in Brest, France was interred planted along side *F. vigrininana* (Hancock *et al.*, 1996). A natural hybrid developed and its combination of hardy plant and large fruit, made plant lovers take notice. The hybrid quickly spread throughout the Europe. Frenchman, Antoine Nicloas Duchesne is credited with identification of the natural hybrid of the *Fragaria* × *ananassa.* Duchesne was a contemporary of the famous Swedish taxonomist Carolus Linnaeus (1707-1778), also known as the Father of Taxonomy. Both were sons of avid gardeners. Duchesne's father was the chief garden at Versailles in France. His interest in horticulture began at an early age. He studied the strawberry and discovered that there were two main groups with several sub-groups. His study led him to the discovery that the large fruited and flavorful strawberry sweeping Europe at the time was a hybrid of the two American species, *F. chiloensis* and *F. virginiana.* Duchsne's discovery changed the name from *F. ananassa* to *Fragaria* × *ananassa.* The Chilean parent plant was suited to a mild climate whereas, the North American parent was more adaptive to heat, drought, and cold weather conditions. European plant breeding from the mid-1700s throughout the following one hundred years provided selections from the progeny of crosses between the Chilean and North American varieties resulting in the large fruited cultivars. The Europeans called the large fruited strawberries *Ananas* because of their resemblance to pineapple in

fragrance and shape. *Ananas* strawberries were introduced to the American colonies in the late 1700s and the hardy North American strawberries were crossed with them to produce large fruit and hardy plants. At present plant breeders have developed strawberry cultivars with more disease resistance, better testing, larger and redder berries to meet consumer demands (Darrow, 1966b).

1.4. TAXONOMIC POSITION

The cultivated strawberry (*Fragaria* × *ananassa* Duch.) is a member of the family rosaceae, subfamily Rosoideae along with blackberries and raspberries.The Rosoideae also includes roses (*Rosa* sp), *Duchesnea indica,* the mock or Indian strawberry, a naturalized weedy species of the eastern US, and *Potentilla*, the *Cinquefoils.* There are about 34 other species of *Fragaria* found in Asia, North and South America and Europe, of which two are cultivated commercially for their fruit *Eragaria moschata* and *F. vesca*. These species were cultivated for centuries, but there is very little production of them today, due of the success of *Fragaria* × *ananassa* (Guttridge, 1985).The systemic position of *Fragaria* × *ananassa* Duch. according to the classification is mentioned below:

Division- Magnoliophyta
Class- Magnoliopsida
Sub-class- Rosidae
Order- Rosales
Family- Rosaceae
Genus- *Fragaria*
Species- *F.* × *ananassa*

1.5. MORPHOLOGY

Strawberry is an herbaceous plant. It looks very beautiful. The description of different parts of strawberry plant is given below:

Plant: Strawberries are perennial, stoloniferous herbs, meaning that they spread via stolons or runners. The runners produce daughter plants at every other node particularly in the summer, which root where they touch the ground and become in dependent plants. It has a reduce stem in the centre of the plant called crown.

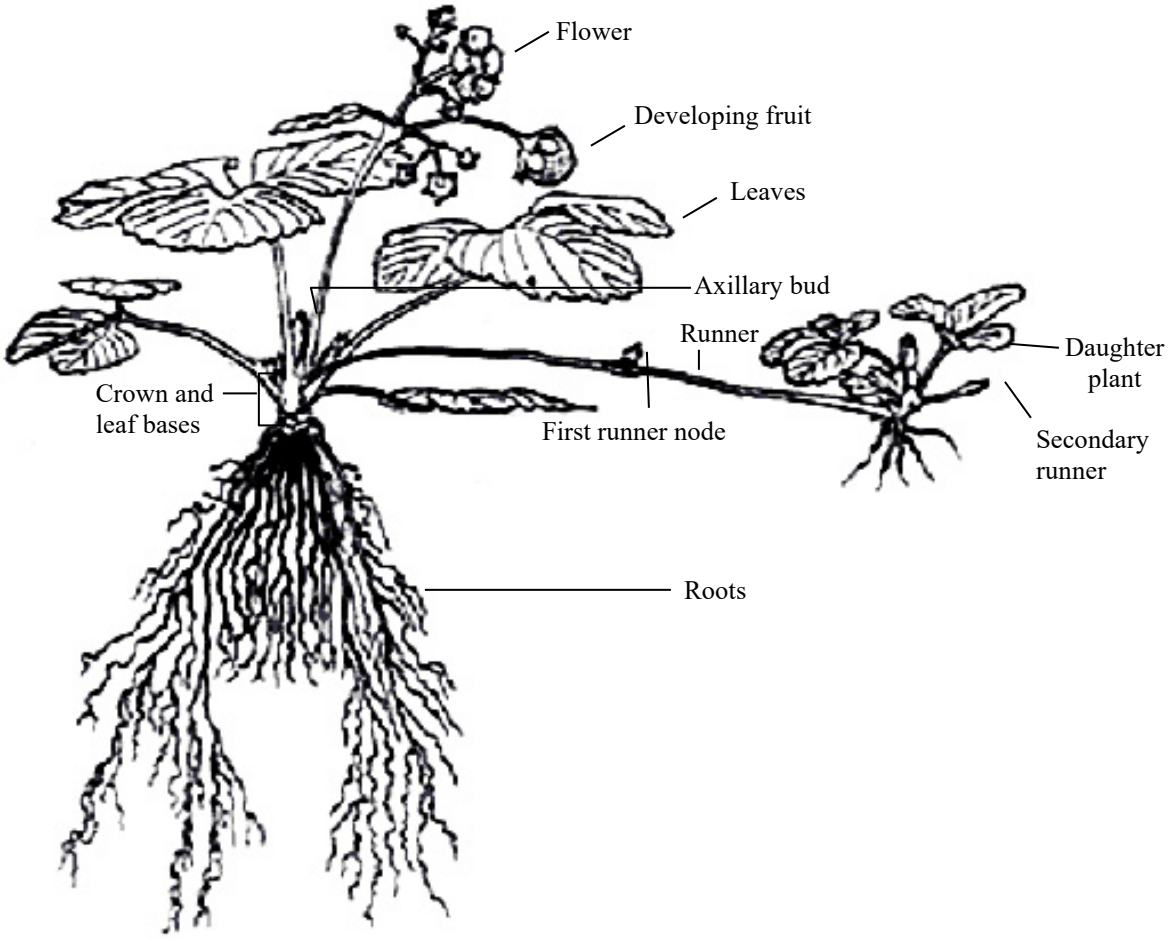


Fig. 1.1: An ideal strawberry plant

Leaves: The leaves have toured leaflets and arise from the crown (a reduced stem in the centre of the plant). Leaflets are ovate or broadly oval, obtuse, dentate or coarsely serrate.

Flowers: Strawberry flowers are white about one inch across with 25-30 yellow stamens and 50-500 pistils on a raised, yellow, conical receptacle. Borne on a dichasial cyme, the centre most terminal flower opens first and is largest, producing the largest fruit. Subordinate flowers are smaller, have fewer pistils and produce smaller fruit. Flowering occurs over several weeks, and plants may have ripe fruity, developing fruit and flowers all at once (Darrow, 1966a).

Flowers on short day cultivars are initiated when day lengths are less than 14 hours and temperature are less than 15°C. The number of photo inductive cycles varies from 7-14, but can be up to 23. But if the temperature is low, flower initiation can take place at high latitudes when day lengths of 21 hours occur.

Pollination: Most cultivars are self pollinated and therefore do not need cross pollination for fruit set. However, bee activity is beneficial in transferring pollen to stigmas in an individual flower. A few hundred pollination events must take place to produce a well formed berry.

Fruit: The strawberry is an accessory fruit; since the edible portion is non ovarian in origin (it is largely swollen receptacle tissue). The true fruits which contain the seed of the strawberry are achiness, which are similar to tiny sunflower seeds. In strict botanical sense, the strawberry fruit is not a true fruit, but is not a true fruit, but is termed a pseudo carp. The achiness are numerous, tiny, ellipsoid specks that cover the fruit surface. They are essential to fruit development because they produce growth regulators (auxin) that enhance growth of the underlying fleshy tissue. Areas on the fruit surface devoid of functional achenes do not grow, causing irregularly shaped fruits. Ultimate fruit size and shape is therefore heavily dependent on achene set. Fruiting begins in the spring after fall planting, and continuous for three years, although declining in size and quality after the first year. Fruits mature rapidly ripening accurse in 20 to 50 days after pollination.

1.6. AGRONOMICAL ASPECT

1.6.1. Site Selection and Preparation

Strawberries need full sun for maximum production and should not be planted near wooded areas that will shade the planting. A gentle south facing well drained sandy loam is ideal for growing strawberries. Sites for planting strawberries should be in areas were adequate air and water drainage occurring.

1.6.2. Soil Types and pH

Strawberries are adapted to a variety of different soil types, provided they are well drained. Plants usually bloom earlier on lighter soils and thus may increase the need for frost protection. Light or sandy soils are suitable for commercial production when irrigation is available and close attention is paid to nutritional (fertilizer) needs of the crop. Strawberries are also grown on heavier soils, such as loam, silt loam and salty clay loam. On heavy clay soils, yields may be reduced because of poor drainage, root diseases and the resulting poor root development. Soils that are very heavy or extremely sandy, as well as those that are rocky, will be more difficult to shape into uniform raised beds. Strawberry likes sandy loam slightly acidic (around pH 6.5) soil. Site selection is important for successful Strawberry production. Plants do best on well drained soils with a pH of 6.0 to 6.5.

1.6.3. Temperature and Climate Requirement

Strawberry plants need about 20-26°C (68-79°F) in day time and 12-16°C (54-60°F) in night time temperature to produce high yields of high-quality fruits. Growth rates will be lower if day time or night time temperatures go above or below this range. Strawberries grow best in a location receiving at least 8 hours plus of direct sun light per day. Short day strawberry variety initiate flowers when photoperiods are less than 14 hours. However, high or low temperatures can over the photoperiodic influence on flower bud initiation. In general, runners

and leaf formation is encouraged under warm long days (September-October in Bangladesh), while crown formation increases under cool short days (November-February in Bangladesh), regardless of genotype. In order to produce Strawberry in Bangladesh, short day variety of strawberry plants is recommended for winter cultivation. If the temperature reaches 72°C (161°F) inside the plant of cultivated varieties, injury may occur, with the killing point about 38° C (101°F)

1.7. A BRIEF REVIEW OF FUNGAL DISEASES OF STRAWBERRY

Anthracnose crown rot caused by the fungus**,** *Colletotrichum fragariae*, may infect all above ground parts of the plant (Smith, 1998). Petiole and stolon infections are characterized by dark sunken lesions and leaf infections are characterized by small black leaf spots. When the fungus infects the crown, wilt and rapid plant death often follows. *Colletotrichum gloeosporioides* and *C. acutatum* also cause antracnose diseases of strawberry (Howard *et al.,* 1992, Smith, 1998). Anthracnose crown rot and fruit rot are often severe in strawberry crop fields when the *Colletotrichum* spp. pathogens is spread rapidly during wet harvest seasons, sometimes a total crop loss. *Colletotrichum* was first reported infecting strawberry in 1931 by Brooks. He reported a disease of stolons and petioles that was caused by *Colletotrichum fragariae.* Subsequently he also reported that it caused a wilt and crown rot in 1935. In the 1960's it was found that *Colletotrichum gloeosporioides* could also cause a crown rot and wilt of strawberry and this species has since become the predominant cause of *Colletotrichum* crown rot. Occasionally a third species of *C. acutatum* has been found to cause a slow decline and ultimately a crown rot of strawberry. However, the development of symptoms with *C.acutatum* is not typical of the rapid plant wilt and death caused by *Colletotrichum gloeosporioides* and *Colletotrichum fragariae. Colletotrichum* crown rot can be a serious disease in Bangladesh. The disease cause severe losses, killing up to 80% of the plants when susceptible cultivars are used. This disease can also be important in other

subtropical production regions where warm temperatures and frequent rainfall can occur during the season. Strawberry transplants appear to be the most likely source of primary inoculums of *Colletotrichum gloeosporioides* for crown rot epidemics in Bangladesh. Because strawberries are clonally propagated, the pathogen can easily be transported from an infested nursery to a fruiting field on strawberry transplants. Often, crown rot epidemics on different farms are associated with plant material from the same nursery. Weed and other no cultivated host plants of *Colletotrichum gloeosporioides* may also provide inoculums epidemics of crown rot. Some isolates of *Colletotrichum gloeosporioides* from plants around strawberry fields are capable of causing crown rot when inoculated into strawberry crowns. Fruit rot and flower blight are the common symptoms in fruiting fields, whereas lesions on stolons, petioles and leaves are particularly damaging in nurseries (Freeman and Katan, 1997).

1.8. FUNGAL DISEASES OF STRAWBERRY IN BANGLADESH

High yields of quality strawberries require vigorous growth and healthy plants. Strawberry production is hampered by various biotic factors and abiotic factors in the country. Diseases are the most important factors that contribute to the reduction of strawberry production. Strawberries are attacked by many fungal diseases. Strawberry diseases are causing 35 fungal species, belonging to Oomycetes (11%), Zygomycetes (9%), Ascomycetes (69%) and Basidiomycetes (11%) (Sesan, 2006). The following fungal diseases are available in strawberry.

1.8.1. Leaf Diseases

Leaf spot (*Mycosphaerella fragariae*): The symptoms begin as round purple spots 1/8 to 1/4 inch in diameter on upper leaf surfaces. Later, the centre of the spot becomes tan or gray, then almost white with a purple border. The disease can also occur on immature petioles, fruit stalks, runners and caps of susceptible cultivars.

Leaf scorch (*Diplocarpon earliana*): It is the most common leaf disease in Bangladesh, appearing as small dark purple spots up to one-fourth of an inch in diameter on upper leaf surfaces. If the spots become numerous, the entire leaf dries up and dies. Similar spots may appear on leaf petioles and runners.

Leaf blotch (*Gnomonia fructicola*): It is characterized by purplish to brown blotches and in later stages by large necrotic spots. The spots often occur on the end of a leaflet and are wedge shaped. This fungus can also attack the fruit stalk, cap and fruit.

Leaf blight (*Dendrophoma obscurans*): The spots are large, 1/2-1 inch in diameter, circular to oval in shape. Young spots are reddish purple, enlarge with age and develop a brown centre bordered by a purple zone. Small black fungal fruiting structures may be observed in the centre of the spot.

Powdery mildew (*Sphaerotheca macularis*): Disease causes infected leaflets to curl upwards along the margins. The lower leaf surface may turn reddish on heavily infected leaflets. The white fluffy fungal growth is not as obvious on strawberry leaves as on many other plants infected by powdery mildews.

1.8.2. Fruit Diseases

***Alternaria* fruit rot (*Alternaria tenuissima*):** Occurs infrequently and usually not important in most strawberry growing regions. This rot affects ripened fruit in the field as well as post harvest. Lesions are irregular in shape and slightly sunken. Older lesions are circular, firm, sunken and dark green to black due to sporulation of the fungus.

Anthracnose fruit rot (*Colletotrichum acutatum*): It is an important disease for strawberry production. Symptoms of Anthracnose fruit rot appear as dark, sunken lesions on infected fruit. On green fruit, anthracnose lesions are small (1/6 to 1/8 inch across) hard, sunken, dark brown or black. Lesions on ripening fruit are large (1/8 to 1/2 inch) hard, sunken and tan to dark brown.

Gray mold (*Botrytis cinerea*): Disease develops as a cover of velvety gray mycelial and /or conidia on the fruit surface. The rot usually starts at a point of contact such as the soil or other fruits. The berry turns brown and remains firm with little leakage. Frequent rains produce maximum rot development.

Phomopsis soft rot (*Phomopsis obscurans*): Initial symptoms on fruit are round, light pink and water-soaked lesions. Frequently, two or more lesions may coalesce into large soft brown lesions with dark fruiting structures (pycnidia) on the surface.

Leather rot (*Phytophthora fragariae*): Occurs occasionally on either green or ripe fruit in Bangladesh. The rotted area is light brown in the centre and shades into purple at the edge. In the late stages of decay, the fruit becomes tough and leathery.

Leak (*Rhizopus stolonifer*): It has been a very common and destructive post-harvest fruit rot of commercially picked and shipped berries, but is of much less importance now with good refrigeration and of little importance in pick your own or home plantings. The symptoms of leak are so characteristic that they are not easily confused with those of other fruits rots. The colour of the infected ripe berry remains unchanged at first but later it changes to light brown. The berry becomes soft and watery and collapses flat with the juice. The rotted fruit and particularly packaged fruit soon become covered with white fluffy cottony fungus growth with black spore producing structures.

1.8.3. Root Diseases

Red stele (*Phytophthora fragariae*): The disease is very serious fungus disease of strawberries, attacks plants during the cool part of the year but above ground symptoms are most apparent from March to July. The fungus persists for many years in the soil, and it occurs most frequently in poorly drained areas. The causal fungus spreads from one area to another in the roots of infected plants and within an area in surface water or in soil carried on farm implements. Red stele

affected plants become stunted and wilt in dry weather. Older leaves turn yellow or red particularly along the margin. The symptom that helps to identify red stele is the brick red discolouration in the centre (stele) of live white roots. The red colour may extend the length of the root, or it may show up for only a short distance above the dead root tip. This symptom is obvious only during winter and spring. The discolouration does not extend to the crown of the plant. Infected plants usually die by June or July. To reduce fruits yield losses, strawberries should not be planted in fields where red stele has occurred. **Verticillium wilt (*Verticillium albo-atrum*):** This disease of strawberry occurs infrequently in North Carolina. The symptoms are similar to those for red stele except *Verticillium* does not cause red discolouration in the roots.

Black root rot (*Fusarium oxysporum*): It is a name for a general condition of older plantings for which several fungi and nematodes are associated. Normal strawberry roots are white, but naturally turn dark on the surface with age. The root system of a plant affected by black root rot is smaller with black lesions or with the roots completely black. Such plants become stunted and produce few berries and runners.

1.8.4. Crown Disease

Crown rot (*Colletotrichum gloeosporioides*): The crown rot disease is a serious disease of strawberry in Bangladesh. This disease are caused by several fungal pathogens such as *Colletotrichum gloeosporioides*, *Colletotrichum fragariae* and *Phytophthora* spp. These pathogens among the disease cannot be distinguished in the field by symptoms alone (Peres *et al*., 2005). *Colletotrichum fragariae* is most often associated with crown rot of strawberry grown in hot and humid areas. *Colletotrichum gloeosporioides* usually causes petiole and stolon lesions and crown rot on a strawberry but may also produce fruit symptoms (Smith, 1998). Symptoms usually begin in the upper part of the crown and spread downward. The youngest leaves wilt suddenly and often turn on bluish green. Wilting spreads quickly throughout the plant, and complete collapse

occurs within a few days. Collapse may be one sided depending on the number of crowns infected. Infected plants will often break at the upper part of the crown when lifted from the soil. When cut lengthwise, the crown appears brown or sometimes rose pink throughout. Crown tissue will disintegrate with time. Below ground symptoms do not appear until the above ground plant parts are dead. If the plants may recover from the disease, they would be are usually stunted.

Symptoms are most common between flowering and harvesting stage when the plants are under stress condition. The crown rot disease causes severe yield lose, killing up to 80% of the plants when susceptible cultivars are used (Legard, 2000a).

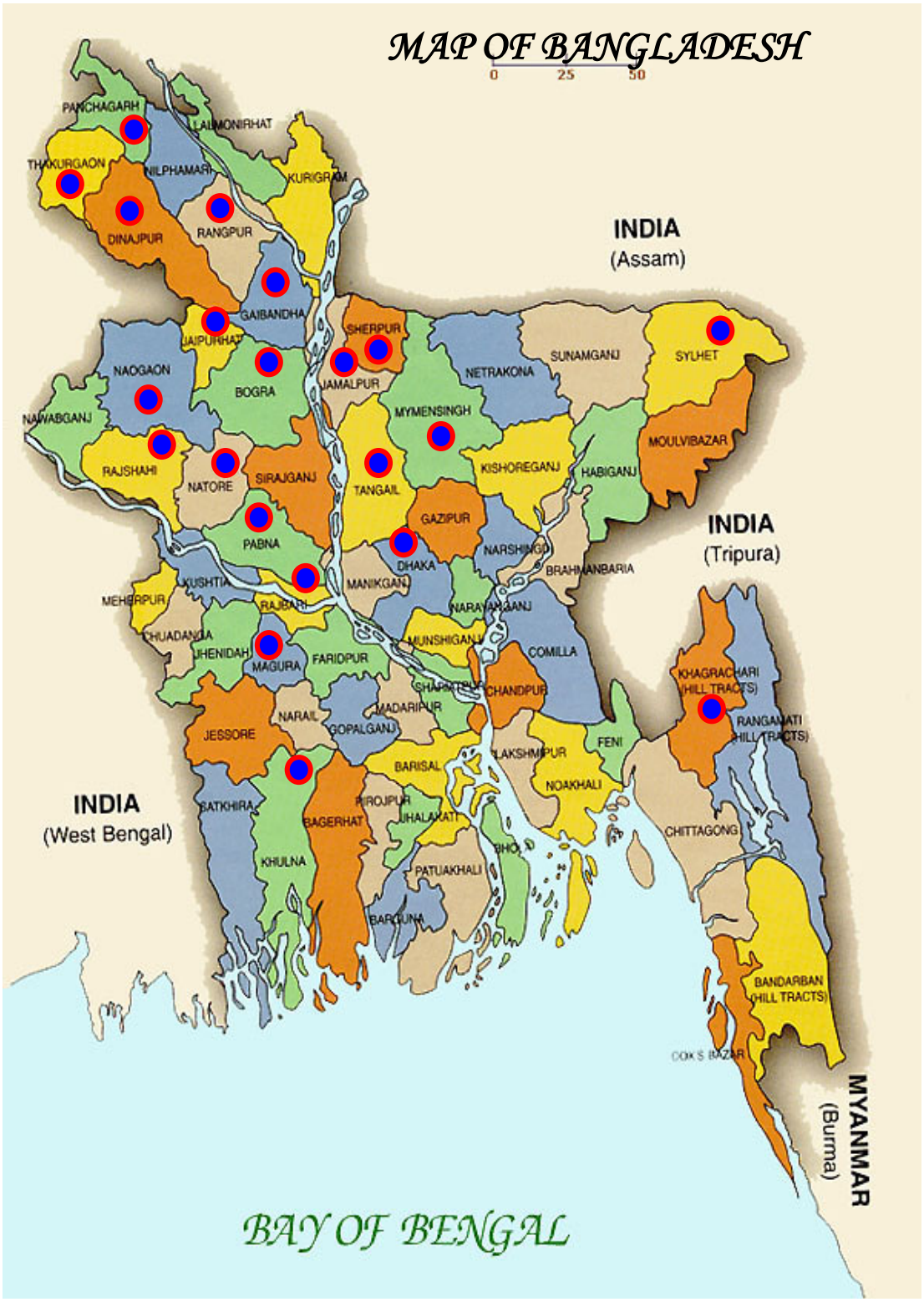


Fig . 1.2: Commercially strawberry cultivating areas (● marked) of Bangladesh

1.9. RATIONALE OF THE PRESENT STUDY

Strawberry is an important and delicious fruit. Strawberries are produced in 71 countries worldwide on 506000 areas of land. Production has increased 33% since 1992 in response to increase in yield of 45%, since acreage in stable or slightly lower than a decade ago. Strawberries are among the highest yielding of all the fruit crops. Average yields world side are just under 14000 lbs/acre, but approach 40000 lbs/acre in the USA, the most productive country. Many tree crops with much greater leaf area per unit of land cannot produce fruit yields as high as strawberry. Strawberries are not commonly found in Bangladesh. It is a new fruit plant in Bangladesh. Bangladesh is a tropical country. In winter the climatic condition is very much favorable for cultivation of strawberry as the temperature remains low. Strawberries grow well in 10°- 26°C temperature as it is a high yielding plant and might be an important new source in our cultivation. Strawberry has a great food value. Protein, carbohydrate, fat, calories, water, vitamin, mineral salt all are found in strawberry. So, if strawberry can produce well in Bangladesh it can play a vital role in our economy. It is a new addition in our crop rotation. It is a new crop genetic resource for Bangladesh. Strawberry has much contribution in medicine. If strawberries are produced in high amount in Bangladesh, then strawberry depended many industry will be established. It will provide a great job opportunity for the poor people of Bangladesh. As it is seasonal plant, seeds have to collect or cultures have to maintain for next season. Recent media report showed that commercial cultivation of strawberry of different genotypes is possible in Bangladesh. However, strawberries are getting popularity in Bangladesh but its fruits are imported in our country from abroad and sold in the super market of Dhaka and other big cities at the rate of Tk. 1200-1800 per kg. So, development of strawberry varieties suitable for Bangladesh condition is not only save our foreign currency reserve but also will generate revenue for local farmer. To achieve this goal we need rapid, efficient and large scale multiplication of these strawberry genotypes.

Behaviours of the pathogen of crown rot disease depends upon its nutritional response, phytopathogenic organisms express a similarity in broader behaviour for their basic nutritional needs, yet, they maintain their individuality for the choice of specific substances (Lilly and Barnett, 1951).

It is now well established that phytopathogens show greater diversities in their ability to utilize the same elements from different nutrient media. This selective property of pathogen has resulted in the formulation of many natural, semi synthetic and synthetic culture media. These culture media always contain essential elements needed for the proper and sporulation of the pathogen. Moreover, the suitability of a particular medium varies with fungi. Though, media differ with respect to constituents and concentrations (Lilly and Barnett, 1951), the selection of basal medium becomes indispensable for the physiological studies of fungi.

The growth of pathogen is profoundly affected by environment also temperature, light and pH are important environmental factors which influence the growth and development of the pathogen.

Various methods for controlling such diseases have been investigated including the use of resistant varieties (Brisa *et al.*, 2007), chemical control, cultural practices (Punja *et al.*, 1986), plant volatile compounds (El-Mougy *et al.*, 2007), plant extracts (Kumar and Tripathi, 1991) and biological control, particularly with species of *Trichoderma* and *Gliocladium* (Ristaino *et al.*,1994).

Disease control with chemical is very much popular because of its quick action, broad spectrum activity and easy availability to the growers. Different chemicals have been used successfully in controlling the crown rot of strawberry (Steven and Natalia, 2009; Illis, 2004; Ivanovic, 1992, 2005; Legard, 2000; Smith, 1998; Shamim *et al.*, 2009; Delp and Milholland, 1980). But now a days people are very conscious about environmental pollution due to excessive and injudicious use of chemicals.

Chemical control may not be effective because the pathogen often forms hard over wintering structures (acervuli) which enable the pathogen to survive as dormant under a wide range of unfavorable environmental conditions. Moreover, problems like leaching degradation, environmental pollution and killing the non target organisms are also associated with chemical control. Host resistance is broken down easily by the frequent appearance of new virulent strains. However, the application of large quantities of chemicals in agriculture has the potential to exert toxic effects on humans and wild life as well as to cause environmental pollution. Mainly due to these reasons, biological control strategies have been adopted extensively.

Thus, there has been a growing interest on research of possible use of plant extracts for control of pest and diseases in agriculture which is less harmful to human health and environment. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials. Plant metabolites and plant based 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.

Different species of *Trichoderma* have been used widely against many plant pathogens. *Trichoderma* is a filamentous fungus distributed in species like *T. viride*, *T. harzianum, T. hamatum* and *T. asperelum* (Khuls *et al*., 1999). *Trichoderma* has multiple mechanisms for control of pathogens (Benitez *et al*. 2004). *Trichoderma* spp. has a wide host range and so is strongly antagonistic to fungal pathogens like *Pythium, Rhizoctonia, Fusarium, Botrytis, Sclerotium, Colletotrichum,* nematodes and many other plant pathogens (Harman, 1996). Winidham *et al*. (1986) reported that *Trichoderma* increased plant growth by the production of a growth stimulating factor. Leinhos and Buchenauer (1992) concluded that fungi release toxic metabolites and enzymes into the medium in which they grow. Benitez *et al.* (2004) isolated chemicals like harzianic acid, tricholin and viridine, which play a very important role in antagonistic behavior

of *Trichoderma.* Biological control of plant pathogens using antagonistic fungi and plant extracts, therefore, assume more significance and it has drawn special attention of the plant pathologists all over the world.

1.10. OBJECTIVES OF THE PRESENT INVESTIGATION

The present study has been planned aiming at the following objectives:

1. Survey of fungal diseases of strawberry in Bangladesh.
2. To find out and identify of the fungal disease causal pathogens in strawberry.
3. Chemical and biological studies of the main causal pathogen of crown rot.
4. Acquisition of antagonists for control of the disease.
5. Identification of locally available medicinal plants for the control of the pathogen.
6. To develop an effective protocol for control of the identified pathogen.

CHAPTER II
REVIEW OF LITERATURE

Crown rot is the major disease of strawberry caused by *Colletotrichum gloeosporioides*. Presently very little literature is available in Bangladesh on crown rot of strawberry caused by *C. gloeosporioides*. Hence, the available literature on crown rot of strawberry and other crops is being reviewed here under.

History

Colletotrichum was first reported infecting strawberry in Florida by Brooks in 1931. He reported a disease of stolons and petioles that were caused by *Colletotrichum fragariae.* Subsequintly he also reported that it caused a wilt and crown rot in 1935. In the 1960 he was found that *C. gloeosporioides* could also cause a crown rot and wilt of strawberry and this species has since become the predominant cause of Colletotrichum crown rot in Florida (Legard, 2000b). Later described as *Glomerella cingulata*, the teleomorph of *C. gloeosporioides were* reported to cause crown rot in Florida (Howard *et al*. 1984). Butler (1918) reported *C. gloeosporioides* for the first time in India causing leaf spot in coffee. While, McRae (1924) reported that it was causal organism of mango anthracnose. Singh and Chohan (1972) reported fruit rot of pomegranate due to *Glomerella cingulata* and rotting was noticed from fruit end portion. The fungus has been reported on various fruit crops (Rawal, 1990).

Symptomatology

Anthracnose crown rot of strawberry caused by C. *gloeosporioides* and is first apparent by the wilting of the youngest leaves in the day. The young wilted leaves may appear to recover and become turgid in the evenings; however, most will wilt and die after a few days (Smith, 1998). *Colletotrichum* spp. also causes

leaf spots (Howard *et al*., 1992; Maas and Palm, 1997; Smith, 1998). Black leaf spot, typically caused by *C. fragariae* and *C. gloeosporioides*, is characterized by gray or light black spots, usually not necrotic, peppered across the top surface of the strawberry leaflets. *C. acutatum* more typically causes irregular leaf spot, the primary symptom of which is the appearance of necrotic black lesions at the tip of the leaflets. All three *Colletotrichum* spp. also causes flower blights and fruit rots (Smith, 1998). Fully open flowers are much more susceptible than closed buds (Smith, 1993).

Survey and surveillance on incidence of crown rot disease

Sudarshan (1975) stated that survey and surveillance form the basis for any successful plant protection strategy. Plant protection to be successful depends upon early detection of the disease followed by timely adoption and application of preventive measures.

Phillips and Golzar (2008) conducted roving survey during September to September of 2005 and 2006 respectably in major strawberry growing areas of Western Australia (WA) to know the incidence and severity of crown rot. He recorded a high rate of crown rot with typical vascular discolouration in unhealthy plants collected from the field.

Sonawane *et al.* (1994) mentioned that extent of field loss in pomegranate due to biotic and abiotic factors and they recorded 44-64% fruit disease incidence in January-February flowering crop compared to other seasons.

Mandhare *et al.* (1996), conducted survey in Nashik district of Maharashtra in India during December. Most of the gardens of pomegranate (cv. Ganesh) were severely affected by fruit spot to the extent of 40-60%. Venkataravanappa (2002) reported that in field survey of northern and southern districts of Karnataka showed diverse incidence form of anthracnose on mango, such variation in

disease incidence was usually attributed to environmental variations and variability in pathogenic fungus.

Amarjit Singh *et al*. (2007) reported appearance of disease in severe form on crops in rainy season compared to winter. The disease varied from 0.17 to 68.51 percent in different guava growing areas and was more prevalent in Ludhiana as compared to Hoshiarpur and Patiala districts.

Prashanth (2007) conducted roving survey during August to September of 2006 in major pomegranate growing areas of north Karnataka to know the incidence and severity of anthracnose. He recorded maximum disease incidence in August-September which was attributed to susceptibility of cultivars and favorable environmental conditions. Anthracnose appeared to be in severe form in the all pomegranate growing areas, which ranged from 18.14-35.84% of severity on a tree.

Benagi *et al.* (2009) conducted roving survey during ambiabahar and hastabahar during August to January of 2008-2009 in major pomegranate growing areas of north Karnataka to know the incidence and severity of diseases. Plantations of at least three years and above were selected for survey. Bacterial blight appeared to be in severe form in the all pomegranate growing areas which ranged from 0.67-94.80% of severity on a tree and they also noticed 18% incidence of anthracnose disease.

Single spore culture

Naik (1985) and Bhat (1987) purified the culture by single spore isolation after isolating *C. gloeosporioides* from infected tissues of betel vine and cashew respectively. Similarly, Ekbote (1994) and Sudhakar (2000) purified the *C. gloeosporioides* culture isolated from the infected tissues of mango and stylosanthes respectively by single spore isolation. Venkataravanappa (2002) and Prashanth (2007) purified the culture by single spore isolation after isolating

C. gloeosporioides from infected tissues of mango and pomegranate respectively using two percent water agar media.

Pathogenecity

Sehgal *et al*. (1965) sprayed *C. capsici* spore suspension obtained from 15 days old culture on coriander plants before and after flowering. They noticed the development of symptoms 12 days after inoculation only on the inflorescence, which later extended to the adjacent foliage. Similarly, Latham and Williams (1983) inoculated the apple fruit with perithecial culture of *Glomerella cingulata* under laboratory condition and observed the production of lesions with orange conidial mass, which later became grey then, black after 10-12 days incubation.

Amusa and Alabi (1996) isolated *C. gloeosporioides* from infected pepper (*Capsicum*) and its pathogenecity were confirmed. Anthracnose of bell pepper caused by *C. capsici* was observed from 3 to 15%. The most severe disease occurred on ripened fruits and pathogenecity was confirmed by tooth prick inoculation method (Roy *et al*., 1997).

Venkataravanappa (2002) reported that pathogenecity was proved by spraying spore suspension (10^6 spores/ml) of fungus on five month old mango seedlings and infection occurred twelve days after inoculation. Prashanth (2007) proved pathogenecity by artificial inoculation (pin prick method) of fungus on pomegranate fruit.

Morphological studies

The genus *Colletotrichum* was described by Corda (1831-32) under the name *Colletotrichum* a single species. Later, he changed the name as *C. gloeosporioides.* Saccardo (1884) and Potebinia (1910) placed *Colletotrichum* in Melanconiales and Acervulaes respectively.

Penz (1884) first time described *C. gloeosporioides*. Later in 1924, McRae described the morphological characteristics of *C. gloeosporioides*.

Earlier reports (Small 1926) revealed that *C. gloeosporioides,* which has been considered as conidial stage of *Glomerella cingulata.* This fungus has a wide host range belonging to various families *viz.*, anacardiaceous, musaceae, palmaceae, coricaceae, vitaceae sapotaceae etc (Bilgrami *et al*., 1979). Bhat (1987) reported conidial measurement as 5.5-6.7 × 3.0-4.2 µm.

Mordue (1971) enumerated the morphological characters of *C. gloeosporioides*. The acervuli were usually setose or glabrous, round to elongate or irregular in shape and attained as much as 500.0 µm in diameter, conidia were hyaline, cylindrical, acetate, uninucleate, measuring 9.0-24.0 × 36.0 µm formed on hyaline or fairly brown cylindrical phialidic conidiophores on potato dextrose agar. The conidia varied in size and shape than those on the host.

Sutton (1980) revealed that conidia of *C. gloeosporioides* were hyaline, aseptate prior to germination, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9.0-24.0× 6.0-12.0µm. *C. gloeosporioides* fungus with grayish white to dark gray on potato dextrose agar and produced aerial mycelium ranging from a thick mat to sprase or tufts of mycelium. Conidia were hyaline, unicellular and either cylindrical with obtuse ends or ellipsoidal with a rounded apex narrow, truncate base. They measured 7.0-20.0 µm × 2.5-5.0 µm and formed hyaline brown conidiophores in acervulli that were irregular in shape and about 500.0 µm in diameter. Setae were 6.0-8.0 × 20.00 µm, one to four septate, brown and slightly swollen at the base and tapered at the apex. Orange slimy conidial masses can be formed as the acervulli matured (Litz, 1997).

Rajesh (1999) recorded the morphological characters of *C. gloeosporioides* isolated from cashew. He reported acervulus were numerous, dark and measured 5.5-12.0 µm in diameter. Setae were dark brown, irregularly arranged in the acervulus. Conidiophores were single celled, hyaline and non septate, closely

packed together in rows. Conidia are oblong to cylindrical with rounded ends, hyaline, non septate with one or two vacuoles, measured 8.0- 10.0×3.5-4.0 µm.

Venkataravanappa (2002) reported that conidia of *C. gloeosporioides* were oblong or cylindrical, one celled, hyaline straight, one to two oil globules were observed in the conidium. Conidia on the culture media were found to be in reddish mass.

Prashanth (2007) reported that mycelial colour varied from white to light red color. The growth was flat to raise fluffy and sparse. Pigmentation in fungi also varied in different media i.e. brown to black and light pink to orange.

C. capsici obtained from chilli host and culture recorded the acervulli about 53.3- 136.4µm in host and 71.0-161.9µm in culture, setae 150.0µm long in and conidia 16.0-30.0×2.5-4.0 µm in size (Vinaya, 2008).

Cultural studies on different media

Sonada *et al*. (1974) reported that conidia of *C. gloeosporioides* grown either on potato dextrose agar or V-8 juice agar appeared pink in mass. Whereas, Lenne and Sonada (1978) found that *C. gloeosporioides* was produced bright orange spores mass on oat meal agar. Lande and Utikar (1978) reported that abundant mycelial growth and sporulation of *Drecheslera rostrata* causes fruit spot of pomegranate observed on PDA, M-2 agar, Czapek (Dox) agar and Coon's medium. Yashoda *et al*. (1993) studied the growth phase of *C. gloeosporioides* causing anthracnose of areca nut and observed that the fungus reached the maximum growth after 10 days of inoculation.

Ekbote *et al*. (1997) evaluated 10 different synthetic and non synthetic media for sporulation and growth of *C. gloeosporioides*. Among the tested media maximum mycelial growth of *Glomerella cingulata* was recorded on Richard's, Brown's and potato dextrose agar followed by Czapek (Dox) agar.

Sudhakar (2000) reported that maximum mycelial growth was recorded in five media (Sabouraud dextrose agar, Richard's agar, Brown's agar, potato dextrose agar and oat meal agar) and they did not differ significantly. The least mycelial growth was recorded in Asthana and Hawker's 'A' medium. Further, he reported that in liquid media the maximum mycelial weight was recorded in Richard's broth (288.33 mg) and least in Asthana and Hawker's 'A' broth (166.66 mg) after 16 days of incubation.

Prashanth (2007) reported that among none/semi synthetic media maximum mycelial growth and good sporulation of the fungus was recorded on PDA followed by oat meal agar while least growth and poor sporulation was observed on host leaf extract media. Among the synthetic media, maximum growth and fair sporulation were recorded on Richard's agar and least growth with poor sporulation was recorded on Czapek's agar. Vinod and Benagi (2009) worked on papaya anthracnose caused by *C. gloeosporioides* and he reported that among different solid media Richard's agar showed good growth and sporulation.

Udhayakumar and Usha Rani (2010) reported that among the 10 culture media tested, PDA was found to be best in mycelial growth (84.8mm), mycelial dry weight (625.4mg) and excellent in acervuli production of *C. gloeosporioides,* while least on water agar.

Utilization of carbon sources

Durairaj (1956) observed that among the various carbon sources tested, sucrose was most efficient utilized by *C. capsici* followed by glucose and mannose.

Thind and Randhawa (1957) found that *C. capsici* grew best on media having dextrose, sucrose, maltose, starch and tartaric acid. Similarly, glucose as a best carbon source for *C. gloeosporioides* was reported by many worker (Chandra and Tandon, 1962 and Lal and Tandon, 1968). Result of investigations of Verma (1979) indicated that the total absence of sucrose checked the growth of all

species of *Colletotrichum*. The mycelial growth was positively correlated with increased concentration of glucose. In general, higher amount of sucrose supported better growth but only little amount of sucrose was needed for better sporulation especially for *C. gloeosporioides* and *C. atrameniarium*.

Naik (1985) reported that sucrose was best carbon source for the growth of *C. gloeosporioides* of betel vine followed by glucose, dextrose, citric acid and mannitol. Ekbote (1994) observed maximum growth of the fungus obtained in sucrose (443.00 mg) as a carbon sources, followed by glucose (414.66 mg) and least growth was observed on lactose (131.00 mg).

Sangeetha and Rawal (2008) reported that mannitol was found to be the best source of carbon for the growth *C. gloeosporioides* of mango followed by fructose and sucrose. Heavy sporulation was observed where maltose was used as carbon source followed by moderate sporulation in fructose and lactose.

Udhayakumar and Usha Rani (2010) studied to different carbon sources; the pathogen (*C. gloeosporioides*) produced maximum mycelial growth and mycelial dry weight when the basal medium was supplemented with manitol (79.5mm and 590.8mg) as a carbon source.

Deshmukh *et al.* (2012) observed that eight carbon sources, starch and xyllose proved to be the best for the growth and sporulation of *C. gloeosporioides* followed by glucose and sucrose.

Utilization of nitrogen sources

Durairaj (1956) found that among the nitrogen source tried on *C.capsici*, ammonium phosphate supported maximum mycelial growth followed by organic nitrogen sources (urea and asparagines), ammonium oxalate and potassium.

Mishra and Mahmood (1960) reported that asparagines at 0.2 % nitrogen were found to be good source. Further they observed that for sporulation, peptone

appeared to be the best followed by potassium nitrate and sodium nitrate. Singh and Shankar (1971) in their studies on growth and sporulation of *C. capsici* from betel vine found that L-leucine was the best among the different nitrogen sources. Rajak (1983) reported that potassium nitrate was best inorganic nitrogen source and asparagine was best organic nitrogen source for the growth of *C. gloeosporioides.*

Naik (1985) reported that potassium nitrate supported the maximum growth of *C. gloeosporioides* on betel vine followed by Dimethioine, L-leucine, glycine and histidine.

Ekbote (1994) reported that potassium nitrate (387.66 mg) supported the maximum growth followed by sodium nitrate (376.00 mg), L-Asparagines (341.66 mg) and ammonium nitrate showing least growth (175.33).

Sangeeth and Rawal (2008) reported that among the different nitrogen sources tested, ammonium nitrate supported good growth and sporulation of *C. gloeosporioides*. Potassium nitrate and sodium nitrate also showed good growth but with moderate sporulation.

Udhayakumar and Usha Rani (2010) studied to different nitrogen sources, the pathogen (*C. gloeosporioides*) produced maximum mycelial growth and mycelial dry weight when the basal medium was supplemented with ammonium nitrate (86.6mm and 680.8mg) as a nitrogen source.

Deshmukh *et al.* (2012) observed that 10 different nitrogen sources, potassium nitrate proved to be the best for the growth and sporulation of *C. gloeosporioides*.

Effect of hydrogen ion concentrations

Satter and Malik (1939) observed fairly good growth of *Glomerella cingulata* in pH range from 4 to 9 and optimum pH was 6.9.

Durairaj (1956) found that the growth of *C. capsici* increased with increased in pH of the medium and the optimum pH was 7.0. The role of pH on the growth of phytopathogenic fungi has been reviewed by Tandon (1961). The growth of fungi optimum pH ranges between 5.5 and 7.0. These pathogens can however, grow at a wider range of pH, especially the acidic side (4.0).

Verma (1969) reported that maximum growth of *C. gloeosporioides* was found at an optimum pH of 6.0. Singh and Shankar (1971) obtained the highest growth of *G. cingulata* at pH 5 followed by 4, 6, 7, 8, 9 and 10. Thind and Madan (1979) observed its growth on a neutral alkaline pH range.

Rajak (1983) found that pH of 7.0 was optimum for *C. gloeosporioides.* Naik (1986) and Hegde (1986) observed that maximum growth of *C. gloeosporioides* at a pH 6.0 and 6.5 respectively.

Ekbote (1994) observed maximum growth was at a pH level of 6.5 followed by 6.0. The least growth was recorded at a pH level of 4.0. Ashoka (2005) found that maximum dry weight at a pH 6.0 (414.33 mg).

Kamanna (1996) reported that maximum growth of *C. gloeosporioides* was found at an optimum pH of 6.0.

Udhayakumar and Usha Rani (2010) reported that among the pH levels tested, 7.0 was the best for the mycelial growth (88.3mm) and mycelial dry weight (730.5 mg) of *C. gloeosporioides.*

Deshmukh *et al.* (2012) observed that maximum dry mycelial weight and sporulation of *C. gloeosporioides* at pH 5.5 and pH 6.5 in liquid medium.

Effect of temperatures on *Colletotrichum gloeosporioides*

Sattar and Malik (1939) reported that the best growth of *Glomerella cingulata* was at 25-29°C and the minimum and maximum range of temperatures were 10-

15°C and 35°C respectively in their study. Rajak (1983) also reported the temperature of 25°C as the optimum for the growth of *C. gloeosporioides*.

Naik (1985) reported that 20-30°C as optimum temperature range for *C. gloeosporioides* and maximum growth was observed at 25°C.

Smith and Black (1988) reported that artificial inoculation with *C. fragariae* on strawberry plants, showed higher severity rate of disease at temperature 35°C and 100 percent relative humidity for 48 hours.

Bhat (1991) reported that growth of *C. gloeosporioides* gradually increased as temperature increased from 10-30°C. Red pigmentation of *C. acutatum* was observed at 32°C on potato dextrose agar (Gunnel and Gubler, 1992).

Yashoda *et al*. (1993) conducted *in vitro* experiment on growth of *C. gloeosporioides* (*Glomerella cingulata*) and obtained maximum growth after 10 days incubation on potato dextrose broth, with optimum pH in the range 5.0-6.5 and optimum temperature in the range 25-35°C.

Mesta (1996) reported a temperature 30°C for growth of *C. capsici.* Similar results were also reported by Vinaya (2008).

Sudhakar (2000) found that pathogen could grow well at temperature of 20 to 30°C and relative humidity of 95 percent. Similar results were also reported by Estrada *et al.,* 2000; Prasanna Kumar, 2001 and Prashanth, 2007.

Venkataravanappa (2002) reported that highest spore germination (98.69%) of *C. gloeosporioides* was recorded at 25°C and the least spore germination was recorded at 10 and 40°C.

Sangeetha and Rawal (2010) reported that anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most important diseases of mango. During survey, the disease samples of fruits affected by anthracnose were collected from Arambakam, Dapoli, Hassan, Hessarghatta, Lucknow,

Raichur, Tiruvur, Tumkur and isolations were made. Studies were conducted to find out the temperature requirement of different isolates by incubating them at 15, 20, 25 28 and 30°C. The study indicated that the temperature of 25°C was found to be good for the growth of Aramkabam, Lucknow and Tiruvur isolates. Maximum growth of Dapoli, Hessarghatta and Tumkur isolates were recorded at 28°C whereas 30°C supported good growth of Hassan and Raichur isolates, nine days after inoculation. As regards to sporulation, Dapoli, Hessarghatta and Raichur isolates were good at 28°C whereas, 25°C supported good sporulation of Lucknow and Tiruvur isolates. Hassan and Tumkur isolates showed moderate sporulation at 28°C and 25°C supported moderate sporulation of Arambakam.

Vinod *et al.* (2009) reported that 30°C is required for the good growth of *C. gloeosporioides* causal agent of anthracnose of papaya. Pandey *et al.* (2012) observed that the range of temperature 20-30°C was found optimum for the growth and sporulation of *C. gloeosporioides* causal agent of anthracnose of mango.

Effect of light on *Colletotrichum gloeosporioides*

Choudhuary (1936) reported that continuous light or darkness was found to inhibit sporulation of *C. graminicola*. Where as cultures exposed to alternate light and darkness were found to sporulate earlier and more conspicuous. Minussi and Kimati (1978) reported that *C. graminicola* of sorghum isolated from four localities sporulated abundantely under continuous light.

Growth and sporulation of *C. graminicola* at different light exposures *in vivo* and *in vitro* tests showed that diurnal light exposure favored growth and sporulation of the pathogen. Disease was more when the pathogen was exposed to diurnal light compared to continuous light or darkness (Mishra and Siradhana, 1980).

Mesta (1996), Sudhakar (2000) Prashanth (2007) and Vinod *et al* (2009) reported that exposure of *Colletotrichum* spp. to alternate cycles of 12 hours light and 12 hours dark showed maximum growth and sporulation.

Venkataravanappa (2002) reported that exposure of *C. gloeosporioides* to alternate cycles of light and darkness recorded maximum mycelial growth followed by continuous darkness.

***In vitro* evaluation of plant extracts**

In recent years, the increasing use of potentially hazardous fungicides in agriculture has been the subject of growing concern of both environmental and public health authorities. Integration of chemicals, plant extracts, and biotic agents along with resistance for managing the plant diseases has been considered as novel approach as it requires low amount of chemicals, by reducing the cost of control as well as pollution hazards with minimum interference of biological equilibrium (Papavizas, 1973).

Ark and Thompson (1959) demonstrated the aqueous and organic solvent extracts of garlic had potent fungicidal and bactericidal activity against several plant pathogens.

Pathak and Jain (1970) reported the antifungal activity of leaf extracts of tulsi. Jetti *et al*. (1987) observed that leaf extracts of *Polyalthia longifolia* inhibited the growth of *C. gloeosporioides in vitro*.

Chauhan and Joshi (1991) tested the efficacy and persistence of 14 plant extracts and Carbendazim in controlling anthracnose of mango fruit caused by *C. gloeosporioides*.

Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for more than two weeks when fruits were inoculated and were significantly better than the other plant extracts tested (Chauhan and Joshi, 1991). Similarly castor oil

(5%), eucalyptus oil (1%), garlic bulb, *Zingiber officinale*, mango, turmeric and lantana leave also significantly controlled the disease. Persistence was the maximum with Carbendazim (0.05%) even in the pulp, followed by castor oil (10%), garlic bulb and leaves (Chauhan and Joshi, 1991).

Tulsi (*Ocimum sanctum* L.) leaf extract was found to check spore germination, growth, and total proteins and pectolytic and cellulolytic enzymes of various rot pathogens (Saini and Pathak, 1991; Patel, 1991; Patil *et al.,* 1992; Vyas, 1993; Godara and Pathak, 1995).

Escopalao and Silvestre (1996) tested fifteen medicinal plant extracts *in vitro* against *C. gloeosporioides* cause of anthracnose of mango fruits. Only the extracts of komoneigue (fruit) and garlic vine (leaves) crude extracts inhibited the growth of the fungus. Both extracts were effective at 1:10 and 1:100 dilutions and were comparable to the Benomyl chemical check.

Ashashivapuri *et al.* (1997) observed the ethanol extract of *Allium cepa* L., *Allium sativum* L., *Azardirachta indica* Jess, *Calotropis procera* L., *Datura stramonium* L., *Ocimum sanctum* L., *Polyalthia longifolia* Benth and Hook, *Tegetus erecta* L., *Vinca rosea* L. and *Withamia somnifera* L. showed fungi toxic properties against *C. capsici*.

Imtiaj *et al.* (2005) found that antifungal activities of 13 plant extracts were tested against conidial germination of *C. gloeosporioides*. conidial germination of *C. gloeosporioides* was completely inhibited in Curcuma longa (leaf and rhizome), Tagetes erecta (leaf) and Zingiber officinales (rhizome) after 15 minutes of incubation respectively.

Prasanna *et al.* (2006) evaluated three plant extracts against *C. gloeosporioides*, among these *Ocimum* leaf extracts was found to be best in inhibiting the fungus.

Roico *et al.* (2007) evaluated crude extracts of leaves and stems of 40 different plant species against *C. gloeosporoides* causing anthracnose of papaya. The

better antifungal effect observed with crude plant extract of night-blooming jasmine and cherimoya after 14 or 18 hours incubation, determined by optical microscopic and spectrophotometric methods.

Babu *et al.* (2008) reported that extraction of plant parts with ethanol and acetone were more inhibitory than water extractions to fruit rot pathogen *C. gloeosporioides*. Extracts of *Azadirachta indica*, *Tagtes erecta* and *Annona squamosa* were effective in inhibiting the growth of the fungus.

Shovan *et al.* (2008) reported that plant extracts of garlic, ginger, onion and neem at three different concentrations (5%, 10% and 20%) were evaluated against the radial growth and mycelial dry weight of *C. dematium.* Among the four plant extracts, garlic extract at 20% concentration was appeared to be best in inhibiting the mycelial growth and mycelial dry weight of the test pathogen followed by onion, ginger and neem extracts and each extracts were significantly different from each other.

Jadav *et al.* (2008) reported that garlic bulb (10%) extract was effective in inhibiting the growth of *C. gloeosporioides.* Prashanth *et al.* (2008) evaluated the plant extracts of eucalyptus, garlic extract, dhatura leaf extract, tulsi leaf extract and *Polyalthia longifolia* leaf extract inhibited the growth of *C. gloeosporoides* the causal organism of pomegranate anthracnose.

Vinod *et al*. (2008) evaluated crude extracts of different plant species against *C. gloeosporoides* causing anthracnose of papaya. Among the different botanicals, 7.5% extract *of Lantana camera* was found to be superior (45.54%) in respect of inhibition of mycelial growth followed by turmeric extract (40.73%). Marinus *et al.* (2010) reported that among the plant extracts, *Allium sativum* (10%) and *Azadirachta indica* (10%) demonstrated the highest inhibition of mycelial growth of *C. gloeosporioides.*

Sreelatha and Bhagynarajan (2008) reported that *Ocimum sanctum* was effective at all the tested concentrations inhibiting the mycelial growth of *C. gloeosporioides.* Watve *et al.* (2009) reported that maximum inhibition was achieved due to neem leaf extract (78.15%) followed by garlic (58.89%), sadamuli (57.04%) and tulsi (55.93%) and the least colony diameter was observed in glyricidia (25.93%) against jatropha leaf spot caused by *C. gloeosporioides*.

Mukherjee *et al*. (2011) evaluated the anti fungal effects of some plant extracts namely tobacco leaf, keora seed, keora, mahogoni, gaint indian milky weed, garlic and ginger at different concentrations (30%, 40%, 50%, 60% and 70%) on the growth and development of *C. gloeosporioides* causal agent of anthracnose of mango. The growth inhibition increased with the increase of concentration of all the plant extracts. The highest mycelial growth inhibition (74.35%) was observed in case of garlic extracts at 70% concentration. Garlic extract at 50% and 60% concentration were also effective than other treatments.

Rahman *et al*. (2011) observed that hundred percent inhibition of conidial germination and shortest germ tube formation of *Colletotrichum capsici* were recorded in *Azadiracta indica* (leaf), *Ocimum sanctum* (leaf) and *Curcuma longa* (rhizome) extracts after 4 to 24 hours of incubation. *Lantana camara* (leaf) and *Colocasia antiquorum* (leaf) extracts exhibited less inhibitory against the pathogen.

***In vitro* evaluation of cowdung and cow urine**

Basak and Lee (2002) reported that cow urine was inhibitory to mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum*, *F. solani* f. sp. *cucurbitae* and *Sclerotinia sclerotiorum* (Lib.) that cause diseases in cucumber. They also reported positive response of fresh cow urine and cowdung in suppression of mycelial growth of *F. solani*, *F. oxysporum* and *S. sclerotiorum*.

Jabin (2003) reported the inhibitory activity of cow urine against *F. semitectum* and further complete inhibition (100%) of the mycelial growth of the pathogen by use of cow urine mixed with leaf extracts of *Calotropis procera*, *Vitex negundo* L. and *Crescentia alata*.

Priya and Kurucheve (2005) studied the effect of animal excrements on the conidial germination of *Cercospora personata* (Berk. and Curt.) Among the animal excrements tested cow urine at 10%, cowdung at 20% and cow urine plus cowdung (1:1) at 2.5% concentration recorded complete inhibition of conidial germination. In control, maximum conidial germination of 96.0% was recorded.

Selvi and Kurucheve (2005) evaluated the effect of natural products like sheep urine at 5%, buffalo urine at 20%, hen litter and goat dung at 100%. Results indicated that hen litter reduced the production of poly galacturonase (PG) up to 88.61%, polygalacturonase trans-eliminate (PGTE) with 90.83 and pectin trans-eliminate (PTE) with 83.63% than the other products tested.

Nargis *et al*. (2006) reported that the extracts of *Adhatoda vasica* Ne., *Zingiber officinale* L., *Vinca rosea* and *Azadirachta indica* Juss. in combination with cowdung, *Calotropis procera* and cow urine posses high ability to inhibit conidial germination of *B. sorokiniana* which might be used for controlling phytopathogens of crop plants.

Basak *et al*. (2002) noticed that two bio-matters cow urine was found more effective than that of cowdung in conidial germination of *Fusarium solani*. No germination of conidia was recorded after one hour of incubation in any medium whereas in cow urine germination of conidia was not also observed even after 2 hours of incubation. After 7 hours of incubation out of 200 conidia of *F. solani* 28 in cow urine and 64 in cowdung were germinated while in control total germinated conidia was 185. In case of percentage inhibition of conidial germination the highest percentage (100%) was recorded in cow urine after 2 hours of incubation followed by 3 hours (96.0%), 4 hours (91.0%) and 6 hours

(89.4%). During the test on inhibition of mycelial growth, the highest percentage (62.8%) was recorded in cow urine potato dextrose agar (CUPDA) medium tested after 4 days of incubation, followed by 3 days (60.5%), 5 days (56.5%) and 2 days (55.0%).

Raja *et al*. (2006) noticed that animal urine containing high nitrogen significantly reduced the *Macrophomina phaseolina* (Tassi), *Fusarium oxysporum* f sp. *Lycopersici* and *Rhizoctonia solani* Kuhn.

***In vitro* evaluation of phytohormones**

Alam *et al*. (2002) noticed that phytohormone IAA had 100% inhibition of conidial germination against *C. gloeosporioides* at 0.005 to 0.006% concentrations for an immersion after 5-30 minutes. Rest of the hormones also had good effects against *C. gloeosporioides.*

***In vitro* evaluation of antagonists**

Deshmukh and Raut (1992) reported that *T. harzianum* and *T. viride* over grew colonies of *C. gloeosporioides* and *T. harzianum* was more aggressive than *T. viride.*

Mederios and Menezes (1994) reported that a paired culture method in petriplates *C. gloeosporioides* showed a high degree of sensitivity to *T. harzianum*, *T. polysporum* (Linkex Pers.) and *T. pseudokoningi.*

Mishra and Narain (1994) reported that the four isolate of *Gliocladium virens* and single isolate of *Streptoverticillium* inhibited radial growth of *C. gloeosporioides* by the cell free culture filtrates of the bioagents.

Trichoderma spp. were reported to be effective against guava fruit rot pathogens like *Lasiodiplodia theobromae*, *C. gloeosporioides*, *Pestalotiopsis versicolar*, *Phomopsis psidii* and *Rhizopus arrhizus* (Majumdar and Pathak, 1995). Gud (2001) found that among seven antagonists evaluated *T. viride* (66.4%) proved

highly antagonistic against *C. gloeosporioides* followed by *Gliocladium virens* (58.67%).

The antagonistic ability of *T. viride, T. harzianum, T. logidrachytum, Gliocladium virens, Aspergillus niger, Pseudomonas florescence* and *Bacillus subtilis* were tried *in vitro* against *C. gloeosporioides* causing leaf spot in turmeric by dual culture technique. All the bioagents proved inhibitory to the growth of pathogen. In dual culture technique, significantly the maximum inhibition (66.40%) was recorded in *T. viride* (Patel and oshi, 2001).

Santha Kumari (2002) observed that the isolates of T_1 and T_2 of *T. harzianum* and the isolates of A_1 and A_2 of *Aspergillus niger* were found effective in inhibiting the growth of *C. gloeosporioides* causing anthracnose of black pepper under *in vitro* condition.

Shirshikar (2002) reported that culture and culture filtrate of *T. viride* was more effective than *T. harzianum* in inhibiting the mycelial growth of *Botryodiplodia theobromae* and *C. gloeosporioides.*

Ashoka (2005) tested six biocontrol agents and noticed that maximum reduction in colony growth was observed in *T. harzianum* (64.65%) followed by *T. viride* (55.38%) and *T. virens* (54.50%) which were on par with each other.

Shovan *et al.* (2008) reported that a total of 20 *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops were screened against *C. dematium* following dual plate culture technique. The screened isolates of *Trichoderma* showed significantly variable antagonism ranging from 50.93 to 89.44% reduction of the radial growth of *C. dematium.* Among the promising antagonists, the isolate T_3 of *T. harzianum* showed the highest 89.44 % inhibition of mycelial growth of *C. dematium.*

Prashanth *et al.* (2008) reported that *T. viridae, T. harazianum, Pseudomonas fluorescence, Bacillus subtillis were* found to be effective antagonistic which inhibited the mycelial growth of *C. gloeosporioides.*

Hussain *et al.* (2008) found that treatments with any of the species of *Trichoderma* integrated with 300 mg Mancozeb/L PDA were effective and did not allow any growth of the pathogen. When Mancozeb was used alone at 100 mg/L PDA or 300 mg/L PDA, it reduced the growth of the pathogen by 49.21% and 100%, respectively, as compared to the control. The cultures of *T. viride*, *T. harzianum* and *T. hamatum* reduced the growth of *C. gloeosporioides* by 28.00%, 50.40% and 58.41%, respectively, when used alone. The dose of 300 mg Captan/L PDA reduced the colony diameter by 31.74%, while its integration with *T. hamatum* reduced the growth of the pathogen by 75.07%. It is concluded that Mancozeb is more effective than Captan against the pathogen. *Trichoderma* spp. is also effective in controlling the growth of *C. gloeosporioides* whether used alone or in integration with the fungicides.

Jadav *et al.* (2008) reported that *T. viride* and *T. harzianum* were found to be more effective antagonist which inhibited mycelial growth of *C. gloeosporioides* causing leaf spot of kokum.

Vinaya (2008) reported that *T. viridae, T. koningii T. harzianum Pseudomonas fluorescens, Bacillus subtillis* were found to be effective bioagent which inhibited mycelial growth of *C. capsici.*

Vinod *et al.* (2009) reported that *T. viride* was found to be effective which inhibited mycelial growth of *C. gloeosporioides* causing anthracnose of papaya.

Watve *et al.* (2009) reported that *T. harzianum* (83.33%) followed by *T. viridae* (77.78%) and *Bacillus subtillis* (77.78%) were found to be effective which inhibited mycelial growth of *C. gloeosporioides* causing leaf spot of jatropha.

Ajith *et al.* (2010) observed that all the selected *Trichoderma* spp. has potential to inhibit mycelial growth of *C. capsici*. The volatile compounds produced form all the selected *Trichoderma* species showed 30 to 67% inhibition of *C.capsici*, However non-volatile compounds or culture filtrate from *Trichoderma viride* at 3%-4% concentration shows complete mycelial inhibition of the test fungi. *Trichoderma harzianum, T. saturnisporum* and *T. reesei* also have the ability to control growth of *C.capsici* by 21 to 68% at a concentration of 50% culture filtrate.

Ngullie *et al.* (2010) reported that in *vitro* studies indicated that *Trichoderma viride* and *Pseudomonas fluorescens* were very effective in inhibiting mycelial growth of *C. gloeosporioides*.

Sobowale *et al.* (2010) found that *T. longibrachiatum* significantly inhibited the growth of *C. gloeosporioides* ($P > 0.0001$). Growth inhibition of *C. gloeosporioides* by *T. longibrachiatum* was better in inoculating antagonist before pathogen than in the other two pairing methods ($P = 0.05$). *C. gloeosporioides* had significant addition of radial mycelia only between days 1 and 2 as well as days 2 and 3 after pairing (DAP) before contact was made with *T. longibrachiatum.* Addition of mycelia mass of *C. gloeosporioides* slowed down significantly by the day upon contact with *T. longibrachiatum* ($P = 0.05$, $R_2 = 0.86$). F value for day after pairing (DAP) was also highly significant ($P > 0.0001$).

Rahman *et al*. (2011) observed that different concentrations and different day-old metabolites of five *Trichoderma* strains were significantly affected to inhibit of conidial germination and germ tube growth of *C. capsicci*. The 100% inhibitions of conidial germination and shortest germ tube formation was exhibited at 2000 mg/l concentration of 30 days old metabolites of *T. harzianum* IMI-392433 and the lowest inhibition of conidial germination and longest germ

tube formation was recorded at 1000 mg/l concentrations of 10 days old metabolites of *T. pseudokoningii* IMI-392431after 4 to 24 hours of incubation.

***In vitro* evaluation of fungicides**

Bernard and Schrader (1984) found Chlorothalonil as most effective fungicide against *C. gloeosporioides*. Naik and Hiremath (1986) reported the complete inhibition of mycelial growth of *C. gloeosporioides* with Blitox (0.1, 0.2 and 0.3%) followed by Foltaf and Cuman L (0.3%). Bhat (1991) reported Captofal, Mancozeb, Copper oxy chloride and Carbendazim inhibited the conidial germination of *C. gloeosporioides*.

Gullino *et al*. (1984) found the agent responsible for a black fruit rot of strawberry in Italy was identified as *Colletotrichum gloeosporioides.* The influence of temperature and pH on the growth of the pathogen is reported. Among the fungicides tested, Prochloraz, followed by Captafol, Chlorothalonil, Captan, Dichlofluanid, Thiram and Tolclofos-methyl, gave the best results in vitro.

Lonsdale (1992) recommended the pre flowering application of Copper oxy chloride followed by monthly application of Copper oxy chloride from fruit set onwards for effective in control of mango anthracnose.

Ali *et al.* (1993) reported that complete inhibition of mycelial growth of *C. gloeosporioides* of tea was found in case of Bavistin and Tilt at the concentration of 100 and 200 ppm respectively followed by Folicur 400 ppm and Calixin 1500 ppm.

Koelsch *et al*. (1995) tested fungicides against *C. gloeosporioides* of Periwinkle and Propiconazole inhibited maximum growth (96%) and Thiophenate methyl with Mancozeb partially growth (50%).

Mesta (1996), and Hegde (1998), reported that Carbendazim (0.1%), Chlorothalonil (0.1%), Bitertinol (0.1%) and Mancozeb (0.1%) were more effective in inhibiting *C. capsici* under laboratory conditions.

Ekbote *et al*. (1996) reported that among the six fungicides tested, Derosal gave 100% inhibition of mycelial growth at 0.05 and 0.10% concentration, while Indofil M-45 gave 100% inhibitions at 0.3% concentration. The least percent inhibition of mycelial growth was observed in Kavach at all the tested concentration.

Tomy (1997) reported that among six fungicides tested against *C. gloeosporioides* causing black leaf spot in mulberry. Manocozeb, Carbendazim and Copper oxychloride proved to be effective in inhibiting mycelial growth of pathogen.

Banik *et al*. (1998) evaluated two systemic (Carbendazim and Thiophanate methyl) and three non systemic (Captan, Ziram and Chlorothalonil) fungicides against *C. gloeosporioides*. They indicated that carbendazim and thiophanate methyl gave the best control followed by Ziram and Captan.

Dinesh Kumar (1998) reported that Bavistin (500 ppm) reduced the fruit rot and increased TSS (Total soluble solids), while acidity and ascorbic acid contents were decreased steadily with an increase in duration of storage.

Jamadar *et al.* (1998) tested the various fungicides for control of pomegranate fruit spots (*C. gloeosporioides*). Among the treatments, spraying with Mancozeb along with Carbendazim was most effective in reducing the incidence of fruit spot recording the disease reduction of 88 .9%.

Srinivasan and Gunasekaran (1998) reported that Contaf (Hexaconazole) at all the four (0.10, 0.15, 0.20, and 0.40%) concentrations completely inhibited the mycelial growth, while Indofil M-45 at 0.50% concentration inhibited only up to 88%.

Gaikwad (2000) reported that seven sprays of fungicide, Carbendazim (0.1%) and Mancozeb (0.2%) was found to be effective for controlling leaf and fruit spot of pomegranate.

Patel and Joshi (2002) observed that Carbendazim (Bavistin 50% WP), Thiophanate methyl (Topsin-M 75% WP), Propiconazole (Tilt 25% EC) at 250, 500 and 1000 ppm, Hexaconazole (Contaf 5% EC) at 750, 1000 and 1500 ppm and Tricyclazole (Beam 75% EC) at 500 and 1000 ppm observed 100% inhibition of *C. gloeosporioides* causing leaf spot of turmeric.

Imtiaj *et al.* (2005) found that five fungicides such as Cupravit, Bavistin, Dithane M-45, Thiovit and Redomil were tested against conidial germination of C. gloeosporioides. Dithane M-45 and Redomil were the most effective when the conidia were immersed for 10-20 minutes at 500-1000 ppm concentrations.

Abhishek and Verma (2007) evaluated fungicides against *C. gloeosporioides* causing anthracnose disease of Mango. Bavistin and Topsin-M proved to be most effective at the concentration of 100µg/ml.

Kumar *et al*. (2007) observed that fungicidal resistance /sensitivity six isolates (*C. gloeosporioide*) were studied using four systemic fungicides *viz*., Carbendazim-50ppm, Thiophanate- methyl-50 ppm, Propiconazole-25 ppm and Hexaconazole-25 ppm and two non-systemic fungicides *viz*., Mancozeb-1000 ppm and Copper oxychloride-1000 ppm in poisoned food technique. All isolates were highly sensitive to sensitive for systemic fungicides except Cg_3 which was moderately resistant to Thiophanate-methyl. Isolates Cg_1, Cg_3 and Cg_6 were highly sensitive, Cg_5 and Cg_7 were resistant and Cg_2 and Cg_4 were highly resistant to Mancozeb. All isolates were resistant to Copper oxychloride.

Shovan *et al.* (2008) reported that five fungicides namely Tilt-250 EC, Vitavax-200, Rovral 50 WP, Dithane M-45 and Cupravit were evaluated at 100, 200 and 400 ppm for their efficacy against the mycelial growth and mycelial dry weight

of *C. dematium*. The complete inhibition was obtained with Tilt-250 EC at all the selected concentrations. The highest concentration of Vitavax-200 inhibits 77.41% and 83.45% mycelial growth and mycelial dry weight respectively and significantly superior to Rovral 50 WP at the highest concentration. Dithane M-45 and Cupravit were found to be significantly inferior against the test pathogen.

Vinod *et al.* (2008) evaluated 10 fungicides tested at different three concentration (0.05, 0.1 and 0.15%), Carbendazim found to be effective among the overall the tested chemicals and gave 100% mycelial growth inhibition of *C. gloeosporioides* at all the concentrations.

Prashanth *et al.* (2008) reported that among the non systemic fungicides, combiproduct Carbendazim + Mancozeb recorded highest percent inhibition of mycelial growth (89.23%) of fungus, which was followed by Propineb (87.78%) and the least inhibition of fungus was recorded in Chlorothalonil (53.78%) at 0.1 percent concentration. Among four systemic fungicides maximum percent inhibition of growth of *C. gloeosporioides* was observed in Difenconazole (90.78%) and Propiconazole (90.78%) which were on par with each other and was followed by Carbendazim (88.89%) while least percent inhibition of fungus was recorded in Iprobenfos (75.99%) at 0.1% concentration.

Shamim *et al.* (2009) reported that the efficacy of five commercial fungicides, Bavistin DF, Dithane M-45, Sulcox 50 WP, Corzim 50 WP and Rovral 50 WP were tested against *Colletotrichum gloeosporioides* of strawberry. Bavistin inhibited mycelial growth completely and was followed in efficacy by Dithane M-45.

Vinod *et al.* (2009) reported that Carbendazim found to be effective among the overall the tested chemicals and gave 100% mycelial inhibition of *C. gloeosporioides.* Watve *et al.* (2009) reported that Carbendazim (0.1%), Propiconazole (0.1%), Difenconazole (0.1%) and Copper oxychloride (0.3%)

inhibited the growth and sporulation to the extent of 100% followed by Bordeaux mixture (1%), Tridemefon (0.1%), and Mancozeb (0.1%)

Field evaluation of plant extracts

Ganapathy and Narayanaswamy (1990) reported that neem oil collected from *Azadirachta indica* and leaf extract of *Nerium odorum* reduced the incidence of rust, late leaf spot and ring mosaic of groundnut. Usman *et al*. (1991) found that neem seed kernel extract (2%) was most effective in controlling rust and late leaf spot of groundnut.

Rovesti *et al*. (1992) found equal effects of aqueous neem kernel extract and Sulphur against *Sphaerotheca fulignea* (Schlecht) pallacci on courgetles, *Erysiphe graminis* on wheat and *E. graminis* on barley when applied both before and after inoculation of pathogen. Further, they have also reported significant control of *P. recondita* on Wheat with neem seed kernel extract.

Shivashankar and Kadam (1993) reported that spraying of neem leaf extract (2%) in combination with recommended fungicides recorded the numerical superiority in reducing leaf spot and rust incidence in groundnut

Adiver *et al*. (1995) evaluated aqueous neem leaf extract under field condition for control of late leaf spot and rust diseases of groundnut and showed that the aqueous neem leaf extract had effect on reducing the disease and increased yield.

Patil (1996) reported that the addition of neem seed kernel and/or amaranthus leaf extracts in the spraying schedule along with Propiconazole found to be effective in reducing the severity of sunflower rust at all the stages of crop growth. AUDPC values were less in the plant extracts applied plots compared to control. When the fungicide applied with the plant extract in sequence, the disease severity and AUDPC values were more or less on par with the fungicide application alone. Maximum C:B ratio obtained in Propiconazole-Amaranthus

propiconazole (1:3.02) followed by Propiconazole-Nimbicidinepropiconazole (1:80) spray schedule.

Suresh *et al*. (1997) extracted two limonoids from the neem tree (*A. indica*) and tested antifungal activity using groundnut rust (*Puccinia arachidis* Speg.) as a bioassay system. The extract compounds reduced the number of pustules and consequently the disease severity.

Ghewande (1989) reported that the extracts of *Azadirachta indica* Juss and *Lawsonia inermis* Syn. L. alba were found effective in controlling rust of groundnut in addition to increasing yield by 15.42 percent. In case of bean rust, neem extract has given 90% control when applied before the plants exposed to the fungus. However, it worked poorly once rust was established (Locke, 1990).

Anahosur *et al*. (2000) observed that use of nimbicidine (0.5%) inter mixed with Hexaconazole (0.1%) sprays reduced the disease index and thereby increase the yield.

Charigkapakorn (2000) reported that crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil could restrict growth of the anthracnose fungus. Among the biofungicides used against the fungus *Colletotrichum* spp. On chilli fruit, found that the most effective control was sweetflag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage.

Dubey (2002) reported that soil application of karanj cake (2.5%) with spraying of karanj leaf extract (2.5%) followed by karanj cake (2.5%) application with spraying of subabul leaf extract (2.5%) and only soil application of karanj cake showed the best performance as they increased seed germination and grain yield

of urdbeen and mungbean and decreased seedling mortality and disease intensity (web blight).

Muralidharan *et al*. (2003) reported that among the different plant derived products most of them reduced disease incidence and increased grain yield harvests. The degree of disease reduction and yield increase were most pronounced when these products were applied to control blast and sheath blight disease.

Field evaluation of cow urine

Sridhar *et al*. (2002) reported that application of 50 ml of cow urine in 500 ml of water spraying on plants in early morning reduces the virus, fungus and bacterial incidence in vegetable crops.

Selvaraj (2003) reported that foliar spray of agnihotra ash at 200 g dissolved in one litre cow urine and 10 litres of water five times at 10 days interval had increased the yield of cabbage (38.8%) and reduced the leaf spot incidence.

Kannan *et al.* (2005) reported that combined application of soil drenching and foliar spray of sheep urine at 10% found to be most effective in reducing the incidence of groundnut stem rot to 9%. With regard to yield the same treatment registered the maximum yield of 1655 kg per ha. whereas controlled plot recorded 1053 kg per ha. Manikandan (2005) observed that spraying of 200 ml of cow urine mixed with two liters of water was found effective in controlling the brinjal damping off in nursery.

Vijaylakshmi *et al*. (2005) reported the effective control of chilli leaf spot by 10 percent cow urine spray once in 10 days thrice followed by half liter cow urine along with half liter sour butter milk mixed with nine liters of water once in seven days twice.

Raja *et al*. (2005) stated that animal urine treatments were significantly superior over fungicides and control in respect of reduced sheath blight disease intensity and increased grain yield. Among the treatments buffalo urine spray at 10 percent concentration showed minimum disease intensity and maximum grain yield followed by cow urine and sheep urine at 10% concentration. The total and ortho-dihydroxy phenol content has profoundly influenced by animal urine treatments and significantly increased the Peroxidase, Polyphenol oxidase and Phenylalanine ammonia lyase activities.

Kannan *et al*. (2007a) reported that in pot and field studies, foliar spray of combined application of buffalo urine and sheep urine (1:1 v/v) at 5% concentration on peanut have completely inhibited the mycelial growth, production and germination of sclerotia of *Sclerotium rolfsii* when compared to control and chemical fungicide, mancozeb (0.05%). Subsequently it has increased the pod yield.

Patil (2008) observed that among the seven commercially available neem based products tested *viz*., neem oil, margotricure, nimbicidine and neem gold at 0.5% and wanis at 1.0%, sprayed thrice at an interval of 10 days starting from the onset of disease were found promising in reducing the soybean rust severity with significant increase in seed yield and 100 seed weight. Among the plant products highest B:C ratio (2.74) was recorded in neem oil followed by margotricure (1.12) and mimbicidine (0.96). Three sprays of either cow urine (1:10), cow milk (1:10), vermiwash (1:2) and panchagavya (3%) at 10 days interval starting from the onset of disease were found to be best among the different ITK's tested in reducing the rust severity and increasing the grain yield (Patil, 2007).

Field evaluation of antagonists

Freeman *et al*. (2001) reported that *Trichoderma* species have been applied to control of anthracnose disease of strawberry. Prasad *et al.* (2002) found significantly higher seed yield when field soil was treated with *T. harzianum* and

T. viride against root rot of chickpea. Increased root development and yield were also observed in betel vine, gladiolous, sunflower, mustard, *Chrysanthemum*, tomato, maize, sugarcane, groundnut and chickpea by the application of *T. harzianum* (Singh *et al*. 2007).

Babu *et al.* (2008) reported that *T. viride* and plant growth promoting rhizobacteria reduced the growth of *C. gloeosporioides* of banana fruit rot effectively. Mukhtar (2008) observed the highest vigour index when okra seeds were treated with *T. harzianum*.

Padder *et al.* (2010). Screened three bioagents (*Trichoderma viride, T. harzianum* and *Gliocladium virens*) and five biopesticides (Achook, Neemgold, Wannis, Spictaf and Neemazal) were evaluated under *in vitro* and *in vivo* conditions against *Colletotrichum lindemuthianum*. All the three antagonistic fungi caused significant inhibition of mycelial growth, maximum being with *T. viride* (69.21%) followed by *T. harzianum* (64.20%). Among the biopesticides tested at four concentrations, Wanis applied 1000 µl/ml caused maximum inhibition of 82.12 percent followed by Spictaf (52.85%). *T. viride* and Wanis 1000 µl/ml were most effective in reducing the seed borne infection. Integration of bioagents with Bavistin showed that disease can be effectively managed with seed dressing either with Bavistin or biopesticide followed by foliar treatment with fungicide or biopesticide.

Ferdousi *et al.* (2010) reported that application of *T. harzianum* IMI 392432 significantly ($p = 0.05$) suppressed the Alternaria fruit rot disease as compared to *Alternaria tenuis* (T_2) treatment and improved both growth and yield. The treatment T_4 (*T. harzianum* IMI-392432 + *A. tenuis*) was most effective in reducing disease percentage (72.27%) as compared to *A. tenuis* (T_1) treatment. The highest seed germination rate (85.56%) and the highest growth and yield (12.5 g/plant) was also recorded in the same treatment (T_4), followed by T_5 (*T. harzianum* IMI-392433 + *A. tenuis*), T_6 (*T. harzianum* IMI-392434 + *A. tenuis*),

T_2 (*T. virens* IMI-392430 + *A. tenuis*), and T_3 (*T. pseudokoningii* IMI-392431 + *A. tenuis*) treatment, while single treatment with *A. tenuis* significantly decreased these values.

Ngullie *et al.* (2010) observed that field evaluation of effective plant extracts, antagonists and fungicide, revealed that spraying with *T. viride* (2%) showed a maximum disease reduction of 61.41% followed by *P. fluorescens,* (58.10%).

Kashem *et al.* (2011) reported that application of *T. harzianum* (TG-2) showed the highest inhibition of the foot and root rot disease of lentil pathogen in field condition. The lowest foot and root rot incidence (6.9%), highest seed germination (82.08%), maximum plant stand (93.12%) and the highest seed yield (3726.67 kg ha^{-1}) were recorded in plots where the isolate TG-2 was applied.

Soytong *et al.* (2010) observed that all extracts and compounds inhibited the growth of *C. gloeosporioides* strain WMF01, with average ED50 values between 1 to 50 ppm. Applications of bioproducts of *Chaetomium*, *Penicillium* and *Trichoderma* a mixture of those bioproducts in a powder formulation and a chemical control were conducted in the field to control anthracnose disease of 5-varieties of grape. All bioproducts significantly reduced the disease incidence on leaves, twigs and fruits of grape in all varieties as compared to the chemical control.

Field evaluation of fungicides

Anthracnose was effectively controlled by spraying with Carbendazim (0.1%) or Topsin-M (0.1%) or Chlorothalonil (0.2%) at 14 days intervals until harvest. Benlate (0.2%) and Dithane Z-78 (0.2%) are extremely toxic to fungus in culture. However, these have not been tested in the field (Bose *et al.*, 1953).

Fungicides as a spray or dip, with a food-grade wax has also shown to be effective in reducing anthracnose. This is a common practice especially for fruits shipped to overseas markets (Akamine and Arisumi, 1953).

Tandon and Singh (1968) reported that two sprays of Zineb or Bordeaux mixture at the time of flowering and continued up to harvest at 14 days intervals for control of anthracnose of mango.

Desai (1998) conducted field studies in Karnataka and reported that by spray of Kitazin at 0.05% to 0.2% gave effective control (97.78-99.5%) of anthracnose caused by *Colletotrichum gloeosporioides* on pomegranate.

Hegde (1998), *in vivo* evaluation of fungitoxicants revealed that Propiconazole (0.05%), Mancozeb (0.2%), Hexaconazole (0.05%) and Triademefon (0.05%) were more effective in inhibiting the growth of *C.capsici.* Jamadar *et al.* (1998) reported that combi product like Mancozeb 0.2% + Carbendazim 0.05% was more effective in controlling fruit spot incidence over control followed by Bordeaux mixture, reduced the disease by more than 88% against control.

Navale *et al.* (1998) evaluated the efficacy of fungicides against the fruit spot of Pomegranate in Ambiabahar. Ziram at the rate of 0.25% was found to be cheaper than the remaining fungicides tested, which recorded least percent disease index (0.17%).

Gaikwad (2000) reported that seven sprays of fungicides Carbendazim (0.1%) and Mancozeb (0.1%) pomegranate caused by *C. gloeosporioides.* Jahagirdar *et al.* (2000) found that three sprays with Kitazin at the rate of 0.2 % was found to be effective in checking the incidence of anthracnose of pomegranate (5.5%) as compared to farmers method (16.3%). Sanders *et al*. (2000) reported the pre harvest application of Copper oxychloride and Benomyl which gave a significant reduction of inoculums level in the field.

Chhata and Kumawat (2001) studied the management of bacterial and fungal fruit spot of pomegranate two years on farmer field and reported that four sprays of Bavistin (0.1%) + Streptocycline (0.04%) at an interval of 15 days reduced the PDI to 10.42 where as untreated control recorded 71.21 PDI.

Strobilurin fungicides Azoxystrobin, Trifloxystrobin and Pyraclostrobin have recently been labeled for the control of anthracnose of chilli (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003).

Gaikwad *et al.* (2002) tested efficacy of Mancozeb (0.25%), Copper oxychloride (0.25%), Carbendazim (0.10%), Chlorothalonil (0.25%) Mancozeb + Carbendazim (0.3 %) and Bordeaux mixture (1.00%) in controlling fruit rot of custard apple caused by *C. gloeosporioides* (*Glomerella cingulata*) and recorded better control of the pathogen as compared to the control. Spraying with Bordeaux mixture resulted in the highest disease control (88.28%) and consequently, in the highest fruit yield (11.49 t/ha), fruit weight (181.42 g), returns (Rs. 94,587 ha^{-1}), additional income over the control (Rs. 82,224 ha^{-1}) and benefit: cost ratio (1: 5.9).

Gurudatt *et al*. (2003) reported that addition of nimbicidine in the spray schedule along with Hexaconazole, Propiconazole and Triadimefon were found effective in reducing the soybean rust severity with increasing seed yield, 100 seed weight and B: C ratio.

Raghuwanshi *et al.* (2004) tested eight fungicides along with check (non sprayed) against leaf and fruit spot of pomegranate caused by *C. gloeosporioides* and found that Carbendazim at 0.1% was found to be effective reducing the percent disease index (4.83) as compared to other fungicides tested.

Prasanna Kumar *et al.* (2006) reported that Carbendazim (0.1%) treatment showed lowest percent disease index followed by Tricyclazole, Benomyl and Copper oxychloride against *C. gloeosporioides* of mango anthracnose.

Boonratkwang *et al*. (2007) reported that *Trichoderma* species have been applied to control *Colletotrichum* species in chilli. Patel *et al.* (2007) evaluated the efficacy of fungicides on the leaf and fruit spots in pomegranate. Among different fungicides Thiophenate methyl and Copper oxychloride showed highest leaf and fruit intensity and Difenconazole and Propiconazole showing lowest fruit and leaf spot intensity

Babu *et al.* (2008) reported that *T. viride* and plant growth promoting Rhizobacteria reduced the growth of *C. gloeosporioides* of banana fruit rot effectively. Prashanth *et al.* (2008) reported the efficacy of Difenconazole, Propiconazole and Iprobenfos against *C. gloeosporioides* in managing the anthracnose of pomegranate.

Navale *et al.* (2009) reported that spraying of 0.1% Difenconazole against *C. gloeosporioides* causing anthracnose of pomegranate showed least percent disease intensity, maximum percent disease control and highest fruit yield followed by 0.1 % Propiconazole. Patel *et al.* (2009) reported that Carbendazim sprayed fruits showed highest percent disease control over non sprayed fruits and Propineb showed lowest percent disease control against *C. gloeosporioides* of pomegranate.

CHAPTER III
MATERIALS AND METHODS

The details of materials used and the methodology followed during the course of investigation are described here:

3.1. MATERIALS

3.1.1. Plant Materials Used

Strawberry plants: The strawberry cultivar RABI-3 (*Fragaria× annanasa* Duch.) was used in the present investigation. The fungal infected strawberry plants were collected from the different areas (Tangail, Jamalpur, Mymensingh, Sherpur, Naogaon, Joypurhat, Bogra, Natore, Pabna, Gaibandha, Rangpur, Dinajpur and Rajshahi) of Bangladesh during February, 2010 to May, 2012. The infected plants were collected in the polythene bags and labeled properly with date for future references. The fungal infected plants were identified studying symptoms of the diseases and confined using indicator plants (Natalia, 2006 and Anon., 2010, 1996). Then they were used for fungal pathogen isolation. Among the isolated pathogens crown rot disease causal pathogen (*Colletotrichum gloeosporioides*) were used for conducting different experiments in the present investigation.

Locally available medicinal plants: The locally available medicinal plants were collected from Rajshahi University Campus, Mirzapur and Meherchandi areas of the Rajshahi city and the bulbs of onion and garlic and rhizome of ginger were collected from the local market of Rajshahi. The local name, scientific name and used parts at the present investigation of the collected medicinal plants are mentioned in the **Table 3.1.**

Table 3.1. The local name, scientific name and parts used of the collected medicinal plants.

Local name	Scientific name	Used parts
Akanda	*Calotropis procera* R.Br.	Leaf
Apang	*Achiranthes aspera* L.	Leaf
Arjun	*Terminalia arjuna* Wt and Arn.	Leaf
Assamlata	*Eupatorium odoratum*	Leaf
Basak	*Adhatoda vasica*	Leaf
Bishkatali	*Polygonum orientale* L.	Leaf
Dhatura	*Datura metel* L.	Leaf
Eucalyptus	*Eucalyptus citriodora*	Leaf
Garlic	*Allium sativum* L.	Bulb
Ginger	*Zingiber officinale* Rose.	Rhizome
Lantana	*Lantana camara* L.	Leaf
Lemon	*Citrus aurantifolia*	Leaf
Mehedi	*Lawsonia inermis L.*	Leaf
Nayntara	*Vinca rosea* L.	Leaf
Neem	*Azadirachta indica* A. Juss.	Leaf and Bark
Onion	*Allium cepa* L.	Bulb
Sajna	*Moringa oleifera* Lamk.	Leaf
Thankuni	*Centella asiatica L.*	Leaf
Tulsi	*Ocimum sanctum L.*	Leaf
Wood-apple	*Aegle marmelos*	Leaf
Marigold	*Tagetes erecta*	Leaf
Papaya	*Carica papaya*	Leaf
Alamanda	*Alamanda carthartica*	Leaf

3.1.2. Chemicals Used

Fungicidal chemicals: 13 fungicides were used in the present study. There were collected from the local market of Rajshahi city. The name of fungicides and there active ingredients and manufacturer are presented in **Table 3.2.**

Table 3.2. The name of fungicides and their active ingredients and manufacturer.

Fungicides	Active ingredients	Manufacturer
Bavistin DF	50% Carbendazim	BASF, Germany.
Cupravit 50 WP	50% Copper oxychloride	Bier A. G. Liverkugen, Germany.
Dithane M-45	80% Mancozeb	DaoAgro-science, Brazil.
Ridomil WG	40 g Metalexil/Kg + 640 g Mancozeb/Kg	Syngenta production, France.
Rovral 50 WP	500 g Eprodion /Kg	Bier Thi. Com. Ltd., Thailand.
Secure 600 WG	100 g Fenamidon/Kg + 500 g Mancozeb/Kg	Bier crop science, SA France.
Tilt 250 WP	250 g Propiconazole/l	Syngenta crop protection, A. G. casol, Switzerland.
Score 250 EC	250 g Fenoconazole/l	Syngent Agro. A., France.
Hayconazole 5 EC	50 g Hexaconazole/l	Tegros chemicals India Ltd., India.
Folicur 250 EC	250 g Tetraconazole/l	Bier crop science, SA France.
Thiovit 80 WG	800 g Sulfer/Kg	Syngenta Agro. A, France.
Antracol 70 WP	70 g Propeneb/Kg	Bier crop science Ltd., India.
Sulcox 50 WP	500 g Copper oxychloride/Kg	Chemiskey producten gacel shaft, Germany.

Subsequently, the above mentioned fungicides viz. Bavistin DF, Cupravit 50 WP, Dithane M-45, Ridomil WG, Rovral 50 WP, Secure 600 WG, Tilt 250 WP, Score 250 EC, Hayconazole 5 EC, Folicur 250 EC, Thiovit 80 WG, Antracol 70 WP and Sulcox 50 WP were designated Bavistin, Cupravit, Dithane, Ridomil, Rovral, Secure, Tilt, Score, Hayconazole, Folicur, Thiovit, Antracol and Sulcox respectively.

Surface sterilizing agents: 0.1% $HgCl_2$ solution and 70% ethanol were used as surface sterilizing agents and savlon were used as detergent.

Other chemicals: Lactic acid, Methilated sprit, Indole-3 butyric- acid (IBA), α-Naphthalene acetic acid (NAA), 6-Benzyl adenine (BA), Indole-3- acetic acid (IAA), 2,4-Dichlorophenoxyacetic acid (2,4-D).

3.1.3. Other Materials Used

The plant materials and chemicals were used for different experiments contained within various types of culture vessels, the whole constituting a culture system. The culture vessels such as petridishes (90 mm), test tubes (150 × 25 mm), bottles (120 × 50 mm), conical flasks (250 ml, 1000 ml), measuring cylinders, glass rods, beakers, pipette pumps, cotton plugs, rubber bands, filter paper, aluminum foils, marker pen, spirit lamp, forceps, needle, sharp blade, firebox, microscope, electronic balance, autoclave, pH meter, magnetic stirrer, haemocytometer, centrifuge machine, incubator, desiccators, thermometer, laminar airflow cabinet, spray machine etc. were also used in the present investigation.

3.2. METHODS

The methods involved in the present investigation are described under the following heads:

3.2.1. Preparation of Culture Media

The different culture media were used for the present investigation. The constituents and their amounts of one litre culture media were given bellow:

Potato dextrose agar (PDA) medium

Constituents	Amounts
Peeled and sliced potatoes	200.00 g
Dextrose	20.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Richard's medium

Constituents	Amounts
Surose	50.00 g
Potassium nitrate (KNO_3)	10.00 g
Potassium hydrogen phosphate ($K_2HPO_4.7H_2O$)	5.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	2.50 g
Ferric chloride ($FeCl_3$)	0.02 g
Distilled water	1000.00 ml

Czapek's medium

Constituents	Amounts
Surose	30.00 g
Sodium nitrate ($NaNO_3$)	2.00 g
Potassium hydrogen phosphate ($K_2HPO_4.7H_2O$)	1.00 g
Magnesium sulphate ($MgSO_4.\ 7H_2O$)	0.50 g
Ferrous sulphate ($FeSO_4.\ 7H_2O$)	0.01 g
Potassium chloride (KCl)	0.05 g
Distilled water	1000.00 ml

Sabouraud's medium

Constituents	**Amounts**
Glucose	20.00 g
Agar	20.00 g
Peptone	10.00 g
Distilled water	1000.00 ml

Host medium

Constituents	**Amounts**
Strawberry plant extracts	200.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Corn meal agar medium

Constituents	**Amounts**
Cornmeal	20.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Yeast extracts medium

Constituents	**Amounts**
Yeast extracts	25.00 g
Agar	20.00 g
Distilled water	1000.00 ml

Soil medium

Constituents	**Amounts**
Soil	100.00 g
Agar	20.00 g
Distilled water	1000.00 ml

pH of the media: In all tests the pH of the media was adjusted to 6.5 using a digital (TOA, Japan) pH meter with the help of 0.1N HCl or 0.1N NaOH (where necessary) before addition of agar.

Addition of agar: After the adjustment of pH, agar 20 g/l was added. Then the medium was heated for 6-7 minutes in a microwave oven (Shimuju, Japan) to melt agar completely.

Sterilization: Finally, the culture vessels containing media were autoclaved at 15 lb/sq inch pressure and at the temperature of 121°C for 20 minutes to insure sterilization. Then the vessels with the media were allowed to cool at vertically and then mark with a glass marker pen and stored in the culture room for ready use.

3.2.2. Preparation of Dye Solutions

Preparation of lacto phenol: Lacto phenol solution was used as a mounting medium and it was consisted of the following composition (Ainsworth, 1963).

Constituents	Amounts
Phenol crystals	20.00 g
Lactic acid	20.00 ml
Glycerol	40.00 ml
Distilled water	1000.00 ml

After weighing the constituents were taken in a conical flask and then distilled water added. The flask was shaken well till a homogenous solution was obtained.

Preparation of lacto-phenol cotton blue: One gram of cotton blue added to 100 ml of lacto phenol and shaken well till it was dissolved. The solution of lacto-phenol cotton blue was stored in a cool dark place.

3.2.3. Preparation of Clearing Reagent

Throughout the present investigation corning glassware and chemicals supplied by E. Mark and BDH were used. All glassware's were cleaned with a solution of Potassium dichromate and Sulphuric acid in the following proportions (Ricker and Ricker, 1963).

Constituents	Amounts
Potassium dichromate ($K_2Cr_2O_7$)	60.00 g
Sulphuric acid (H_2SO_4)	60.00 ml
Water (H_2O)	1000.00 ml

3.2.4. Preparation of 0.1% Mercuric Chloride Solution

Mercuric chloride solution is used for surface sterilization of the diseased sample. It was shaken well until dissolved.

Constituents	Amounts
Mercuric chloride ($HgCl_2$)	100.00 mg
Distilled water	100.00 ml

3.2.5. Culture Techniques and Isolation of the Pathogens

3.2.5.1. Sample collection, preparation and surface sterilization

Sample having typical symptoms of fungal diseases of strawberry were collected from different areas of Bangladesh**.** After collection, the diseased materials were washed in running tap water. Then the infected portion was cut into small pieces along with some healthy portion and selected for surface sterilization. Surface sterilization was carried out by dipping the infected plant parts in 0.1% $HgCl_2$ solution with gentle shaking for 1-2 minutes followed by 3-5 times washing with sterile distilled water in front of running laminar airflow cabinet.

3.2.5.2. Preparation of humid chamber

The humid chamber used for the incubation of the diseased plant parts and it was consisted of a petridish with its lids. Before used, the petridishes were clean and sterilized by flaming method. The inner wall of the petridishes and its lid were lined with previously sterilized filter papers, which were then moistened with sterilized distilled water. The excess water was poured out and the plate with its lid was used as humid chamber. Described number of diseased materials of suitable size from each sample lots collected from the field was incubated using a separate humid chamber.

3.2.5.3. Inoculation techniques

All inoculations and aseptic manipulations were carried out in a running laminar airflow cabinet. The cabinet was switched on for half an hour before used for cleaned with 70% ethanol to reduce the chances of contamination. All instruments like scalpels, needle, forceps, tiles, petridishes etc. were covered with aluminum foil paper and sterilized by steam sterilization method. During working time, these were again sterilized by 70% ethanol dip and flaming method inside the inoculation chamber. To ensure complete aseptic condition both hands were also wiped by 70% ethanol.

After sterilization, the infected portions were taken out using flamed forceps and dried between filter papers finally transferred to PDA media/ humid chamber. The infected portions were placed sufficiently apart, so as to prevent them from touching each other. In each culture vessel 1-3 infected explants were inoculated. After inoculation, the culture vessels were labeled by glass marker pen, than the vessels were ready for incubation.

3.2.5.4. Culture incubation

The inoculated culture vessels were incubated in a growth chamber providing a special culture environment. The vessels were placed on the shelves of a culture

rack in the growth chamber. It may be mentioned specially that, all cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance of 40-30 cm from the culture shelves. The cultures were maintained at 28 ± 2°C with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark. The cultures were incubated for 7 days and the growth of the fungus was observed periodically. The pathogens isolated from infected parts of host plants. The pathogens were transferred to PDA slants and sub cultured on PDA medium for the use of next experiments.

3.2.5.5. Single spore isolation of the pathogens and their culture

10 ml of clear 2% filtered water agar was poured into sterile petriplates and allowed to solidity. Dilute spore suspension was prepared in sterile distilled water and from this two ml of spore suspension was spread uniformly on the water agar plates and the excess suspension was drained off aseptically from the plates. The plates were incubated at 28 ± 1^0C for a few hours. They were examined frequently under the microscope for well isolated germinating spore and were then marked with ink on the glass surface of the plate. These marked agar areas were cut and transferred to PDA slants and incubated at 28 ± 1^0C.

3.2.6. Preparation of Slides

A suitable portion of culture from PDA plate was selected under a microscope and taken out the help of forceps and needles and put in one or two drops of lacto-phenol on clean slide. It was then gently warmed by heating and cooling over a low flame of sprit lamp for 6 to 8 times, but was never allowed to boil. Whenever needed, the material was stained with small quantity of lacto-phenol cotton blue. A clean cover glass was placed over the materials; excess fluid was removed by soaking with blotting paper and examined under compound microscope.

3.2.7. Identification of the Isolated Pathogens

The pathogens isolated from infected parts of host plants describe above. The pathogens were transferred to PDA slants and sub cultured on PDA medium for the identification. The isolated pathogens were identified with the help of keys outlined by Sutton (1980).

3.2.8. Proving the Pathogenecity

Pathogenecity of the isolated organism was proved in the field condition by inoculation method. The strawberry plants were grown in an earthen pot containing loamy soils. The plants were inoculated by spraying a conidial suspension with an atomizer. The inoculated plants were covered with polythene bags and incubated at 28 ± 2°C for two days. Then the inoculated plants were transferred to the Botanical garden of Rajshashi University. Water was added everyday to keep them under moister condition after removing polythene bags. Observation was made after 10-12 days. When the host plants had developed characteristic lesion and compared with maturity developed symptoms recorded before. The pathogen was reisolated from the diseased portion and compared with the original cultures to prove the Koch's postulates.

3.2.9. Maintenance of the Culture

The isolated and identified pathogens were sub cultured on PDA slants and allowed to grow at 28 ± 1^0C for one week. Such slants were preserved in refrigerator at 5^0C and renewed once in two months.

3.2.10. Morphological Studies of the Crown Rot Disease Causal Pathogen (*C. gloeosporioides*)

A loopful of culture of *C. gloeosporioides* obtained from twelve days old culture was placed on the slide and mixed thoroughly with lacto phenol to obtain uniform spread. A cover slip was placed over it, length and breadth of 100 spores was measured under high power objective calibrated micrometer. The

average size of the spores was calculated. Similarly, the spores produced in the crowns were also measured and the average size was calculated.

3.2.11. Growth Characters of *C. gloeosporioides* on Different Media

For experiment with solid media, 20 ml of media were poured into after sterilization under aseptic condition, in sterilized 90 mm petridis. Petriplates were inoculated with 5 mm disc taken from the 10 days old pure culture. Each treatment was replicated three times. The measurement of mycelial growth of the colony were taken at intervals of 10 days and expressed in mm. Increase of circular colonies, the diameter of the colony was measured in two direction at right angles to each other, whereas in case of irregular colonies the measurement was taken along the longest and the shortest directions and the average was taken as growth of the colony (Brown, 1923). On the other hand, potato dextrose agar (PDA), Richard's, Sabouraud's, Czapek's, corn meal, yeast extracts, soil and host media were used for observation of growth of *C. gloeosporioides*.

3.2.12. Effect of Carbon Sources on Mycelial Growth of *C. gloeosporioides*

To determine the most readily utilizable source of carbon by the fungus *C. gloeosporioides,* carbon sources were inoculated in the Richard's medium which was taken as a basal medium and prepared by dissolving the ingredients except influence of abiotic factors in fungal growth carbon source (sucrose). Various sources of carbon such as sucrose, lactose, starch, glucose, mannitol, fructose and sorbitol were used. The quantity of starch (complex polysaccharides) taken was similar to that of sucrose. The different carbon sources dissolved separately in the medium 20 ml of sterilized Richard's agar was poured into sterilized petri plates. Plates without carbon source served as absolute control. Each treatment was replicated three times. These petriplates were inoculated aseptically with 5 mm diameter culture block of mycelium obtained from the 7 days actively growing pure culture with the help of sterilized cork borer. Inoculated petri plates were incubated at room temperature (28 ± 2°C). The mycelial growth was

recorded at 10 days after inoculation and sporulation was also counted from the plate of three replications in each treatment (Verma and Prasad, 1975; Sonai Rajan and Muthukrishnan, 2010). The sporulation was graded as follows:

Excellent = >75, Good = 50 -75, Moderate = 25 - 50, Poor = 1-25 and

No conidia = 0

3.2.13. Effect of Nitrogen Sources on Mycelial Growth of *C. gloeosporioides*

This experiment was conducted to find out the source of nitrogen which can be most efficiently utilized by the fungus for its growth and sporulation. Richard's medium was taken as the basal medium. A 20 ml of sterilized Richard's medium was poured into sterilized petriplates (90 mm diameter). Sodium nitrate in the basal medium was replaced by various sources of nitrogen *viz*., sodium nitrate, ammonium sulphate, potassium nitrate, calcium nitrate, peptone, urea and L-Aspergine. The different nitrogen sources dissolved separately in the medium. Plates without nitrogen source served as absolute control. Each treatment was replicated three times. The rest of the procedure adopted was similar to the experiment of carbon sources (Verma and Prasad, 1975).

3.2.14. Effect of pH on Mycelial Growth of *C. gloeosporioides*

Effect of pH on the growth of fungus was tested at 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. 20 ml of the medium were poured after sterilization under aseptic condition onto sterilized 90 mm petriplates. Petriplates were inoculated with 5 mm disc taken from the 10 days old pure culture. Each treatment was replicated three times. The measurement of mycelial growth were taken at 10 days and expressed in mm. The measurement of mycelial growth was performed followed by Brown (1923).

3.2.15. Effect of Temperatures on Mycelial Growth of the Pathogen

Effect of temperatures on the growth of fungus was tested at 15, 20, 25, 30 and 35°C. 20 ml of potato dextrose agar medium were poured after sterilization under aseptic condition in sterilized 90 mm petridishes. Petriplates were inoculated with 5 mm disc taken from the 10 days old pure culture. Each treatment was replicated three times. The measurement of mycelial growth were taken at 10 days and expressed in mm and this measurement of mycelial growth was done following Brown (1923).

3.2.16. Effect of Light on Mycelial Growth of *C. gloeosporioides*

To study the effect of light requirement PDA medium was used in this experiment. 20 ml of solid medium were inoculated and exposed to different lengths of light hours *viz*., alternate cycles of continuous 12 hours lighting and 12 hours darkness in an environmental chamber. Petriplates were inoculated with 5 mm disc taken from the periphery of 10 days old pure culture. Each treatment was replicated three times and incubated for 10 days. Mycelial growth was obtained as described earlier and results were analyzed statistically.

3.2.17. Method of Plant Extracts Preparation

Fresh plant material were collected and washed first in tap water and then in distilled water. 100 gram of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth.

3.2.18. Effect of Plant Extracts on Mycelial Growth of *C. gloeosporioides*

To study the effect of 24 plant extracts against the growth of *C. gloeosporioides* following poison food technique as described by Begum and Bhuiyan (2006). Five, 10, 15, 20 and 25 ml of stock solution were mixed with 95, 90, 85, 80 and 75 ml of sterilized molten PDA media respectively, so as to get 5, 10, 15, 20 and

25 percent concentrations. The medium was thoroughly shaken for uniform mixing of extract. 20 ml of medium was poured into each of the 90 mm sterile petri plates. Each plate was seeded with mycelium of five mm size disc from periphery of actively growing culture were cut out by cork borer and one such disc was placed at the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 28 ± 1ºC temperature for ten days and mycelial growth was taken when maximum growth was occurred in the control plates. The efficacy of plant products or botanicals was expressed as percent of mycelial growth over the control which was calculated by using the formula suggested by Sundar *et al.* (1995).

$$\% \text{ inhibition} = x - y / x \times 100$$

Where, x = mycelial growth of control plate,

y = mycelial growth on treated plate.

3.2.19. *In Vitro* Evaluation of Cow Urine and Cowdung

Cow urine: In this test, cow urine potato dextrose agar (CUPDA) medium was used. Urine was added with PDA in different (5, 10, 15, 20 and 25%) concentrations and sterilized. 20 ml of each medium was poured aseptically into petriplates of 90 mm diameter. Five mm disc from an actively growing zone of 10 days old culture was placed upside down at the centre of the solidified medium and were incubated at 28 ± 1ºC. Each treatment was replicated thrice. The measurements of the colony were taken when the maximum growth was attained. % of inhibition of mycelial growth was calculated.

Cowdung: For this test, cowdung potato dextrose agar (CDPDA) medium was used. Cow dung was added with PDA in different (5, 10, 15, 20 and 25%) concentrations. The medium were inoculated and mycelial growth was recorded as described above.

3.2.20. *In Vitro* Evaluation of Phytohormones

The experiment of mycelial growth inhibition was carried out following Miah *et al*. (1990). Five phytohormones (IAA, NAA, IBA, BA and 2,4-D) were used in this test. Phytohormones were mixed with potato dextrose agar (PDA) medium in 20, 50, 100, 200 and 500 ppm concentrations. The phytohormones were tested against *C. gloeosporioides* on the PDA using poisoned food technique under *in vitro* condition as described earlier.The percent inhibition of growth of the test fungus was calculated by following method as given by Brown (1923).

3.2.21. *In Vitro* Evaluation of Antagonists Against Tested Pathogen.

In vitro evaluation of antagonists against *C. gloeosporioides.* The efficacy of 15 antagonists was tested against *C. gloeosporioides* for mycelial growth inhibition on the potato dextrose agar medium using dual culture and culture filtrate technique.

List of antagonists used against *C. gloeosporioides* are mentioned below:

i) Th1 – Th10 = *Trichoderma harzianum* of soil isolated; ii) *T. harzianum;* iii) *T. virens;* iv) *T. viride;* v) *T. hamatum;* vi) *T. pseudokoningii*

3.2.22. *In Vitro* Evaluation of Antagonists by Dual Culture

Antagonists were evaluated for their efficacy through dual culture technique (Dennis and Webster (1971). The antagonists and the test fungus were inoculated side by side on a single petridis containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen separately. They were incubated for 10 days. The diameter of the colony of both antagonists and the pathogen was measured in two directions and average was recorded. Percent inhibition of growth of the test fungus was calculated by using the formula of Sundar *et al.* (1995).

3.2.23. *In Vitro* Evaluation of Antagonists by Culture Filtrate Technique

The 15 antagonists were grown in potato dextrose broth at 28 ± 2°C with inter mittent shaking at 150 rpm. The metabolites were collected after 30 days and filtered.The sterilized filtrates were amended in PDA to make 35% concentration in petri plates. The solidified agar plates were inoculated at the centre with 5 mm diameter mycelial disc of the pathogen and incubated at 28 ± 2°C for 10 days. The plates without filtrate served as control. The mycelial growth was measured and percent inhibition of mycelial growth was calculated.

3.2.24. *In Vitro* Evaluation of Fungicides

The efficacy of 13fungicides was tested against *C. gloeosporioides* for mycelial growth inhibition on the PDA medium using poisoned food technique (Dhingra and Sinclair, 1985). Five concentrations (50, 100, 250, 500 and 1000 ppm) were used in this experiment. Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar so as to get the desired concentration of fungicide. Later, 20 ml of the poisoned medium was poured into sterile petriplates. Mycelial disc of 5 mm size from actively growing culture of the fungus were cut by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then such plates were incubated at 28 ± 2°C for 10 days and mycelial growth was measured. The efficacy of a fungicide was expressed as per inhibition of mycelial growth over control that was calculated by using the formula suggested by Sundar *et al*. (1995) as described earlier.

3.2.25. Field Evaluation of Plant Extracts, Cow Urine, Antagonists and Chemical Fungicides Against Crown Rot Disease of Strawberry

A plot experiment in randomized block design and three replications was conducted from 2010-2012 at the Parila, Boyalia, Rajshahi and Botanical

garden, Rajshahi university, Rajshahi. The experiment was conducted to evaluate the efficacy of plant extracts, cow urine, antagonists, and fungicides that were effective under *in vitro* conditions. The recommended strawberry genotype RABI-3 was used under field condition. Plantlets were planted at 40 cm × 35 cm distance on 50 cm wide and 350 cm long raised bed. The soil of the experimental plots was specially amended with cowdung and coarse sand (1: 1 v/v). Urea -TSP-MP was applied at the rate 75-60-75 kg/ha.

3.2.26. Preparation and Application of Spore Suspensions

Mycelial disc (5 mm diameter) of *Trichoderma* isolates and *C. gloeosporioides* were obtained from 7 days old culture and separately transferred to 50 ml PDA in a 250 ml conical flask and incubated at 28°C. After incubation, 30 ml of sterile distilled water was added to each culture and the flasks were shaken at 50 rpm for 30 min in an orbital shaker. Then the content of each conical flask was filtered through sterile muslin cloth. The culture filtrate, containing the spores, was collected, and a concentration of 5×10^5 spores/ml (*Trichoderma* isolates) and 3×10^5 spores/ml *(C. gloeosporioides)* was obtained by dilution with sterilized distilled water. The inoculum prepared in tap water was uniformly sprayed in the evening hours to all the treatment plots at 42 days after planting. In all the treatments totally four sprays were given at 45, 60, 75 and 90 days after planting except in one, where the antagonist was inoculated before the pathogen. The control treatment was maintained by spraying tap water. Soil was moistened as and when necessary. Weeding was done three times during the whole crop growing period.

3.2.27. Treatments Combinations

The experiments were designed with the following combinations:

T_0 = Control (*C. gloeosporioides* alone)

T_1 = Tilt (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_2 = Bavistin (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_3 = Hayconazole (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_4 = Dithane (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_5 = Antracol (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_6 = Folicur (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_7 = Cupravit (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_8 = Thiovit (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_9 = Score (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{10}= Rovral (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{11} =Ridomil (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{12} = Secure (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{13} = Sulcox (1000 ppm) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{14} = Garlic bulb extract (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{15} = Mehedi leaf extract (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{16} = Neem leaf extract (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{17} = Sajna leaf extract (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{18} = Apang leaf extract (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{19} = *Trichoderma harzianum* (5×10^5 spores/ml) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{20} = *Trichoderma* isolatesTH1 (5×10^5 spores/ml) + *C. gloeosporioides* (3×10^5 spores/ml)

T_{21} = Cow urine (25%) + *C. gloeosporioides* (3×10^5 spores/ml)

3.2.28. Data Collection on Field Evaluation

Ten plants were selected randomly from each replication and the data were collected on crown rot disease incidence, canopy size (cm^2) per plant, number of fruits per plant and fruit weight per plant at different days after plantation. After planting of strawberry plantlets fruits were collected from the field within 60-120 days. Ripe fruits were harvested twice a week at 100% red epidermis. Canopy size (cm^2) was measured at 80 days after plantation.

3.2.29. Technique of Data Analysis

The collected data were analyzed by following biometrical technique developed by Mather (1949) based on the mathematical models of Fisher *et al.* (1932) and those of Hayman (1958), Kempthorne (1957) and Allard (1960). The techniques used are described under the following heads:

Mean: Data on individual observation were added together and divided by the total number of observations and the mean was obtained as follow:

$$\text{Mean, } \overline{X} = \frac{\sum x_i}{N}$$

Where, $\overline{X}$ = Average number of observations

Σ = Summation

x_i = Individual reading recorded on each observation

N = Number of observations.

Standard error of mean (SE): Instead of taking one sample, several samples are taken to estimate the standard deviations of the deferent samples. The sample variations were measured by Standard Error (or Standard Error of mean) which was determined as follows:

$$SE = \frac{S}{\sqrt{N}}$$

Where, $S = \sqrt{\frac{\sum X_1^2 - \frac{(\sum X_1)^2}{N}}{N-1}}$

X_1 = Total number of observations

Σ = Summation; S = Standard deviation.

Test of least significant difference (LSD): To test the least significant difference between two means the following formula was used:

$$LSD = \sqrt{\frac{2 \times MSe}{n}} \times t \quad at \quad 5\% \quad level \text{ of significant}$$

Here, Mse = Mean square of error

n = Number of observations

t value at 5% level = Value at 5% significant level from 't' table.

CHAPTER IV
RESULTS

The present investigation was conducted on various aspects on crown rot disease of strawberry with reference to survey and surveillance of fungal diseases. Details of the results so far obtained from each of the experiments are presented under different heads:

4.1. Survey and Surveillance of Fungal Diseases

Roving survey was undertaken during the period from 2010 to 2012 to assess the fungal diseases of strawberry in major growing areas of Bangladesh and the results are described below:

4.1.1. Fungal diseases of strawberry in Bangladesh

Many fungal diseases of strawberry were noticed in Bangladesh **(Plate 4.1- 4.3).** These diseases were leaf spot (*Mycosphaerella fragariae*), leaf blotch (*Gnomonia comari*), leaf scorch (*Diplocarpon earliana)* leaf blight (*Dendrophoma obscurans*), downy mildew (*Peronospora potentillae*), powdery mildew (*Sphaerotheca macularis),* Alternaria fruit rot (*Alternaria tenuissima*), anthracnose fruit rot (*Colletotrichum acutatum*), fruit rot (*Mucor* spp.), Phomopsis soft rot (*Phomopsis obscurans*), gray mold (*Botrytis cinerea*), Rhizopus rot or leak (*Rhizopus stolonifer*), crown rot (*Colletotrichum gloeosporioides*), root rot/black root rot (*Fusarium oxysporum*) and Verticillium wilt (*Verticillium albo-atrum*) etc. The name of these diseases and their identified causal pathogens were presented in **Table 4.1.**

Table 4.1. Fungal diseases of strawberry in Bangladesh.

Name of diseases	Name of causal pathogens	
	Main pathogens	Other pathogens
Fruit diseases		
Alternaria fruit rot	*Alternaria tenuissima*	-
Antracnose fruit rot	*Colletotrichum acutatum*	-
Fruit rot	*Mucor* spp.	*Aspergillus* sp., *Penicillium* spp.
Phomopsis soft rot	*Phomopsis obscurans*	-
Gray mold	*Botrytis cinerea*	-
Rhizopus rot (leak)	*Rhizopus stolonifer*	-
Leaf diseases		
Leaf spot	*Mycosphaerella fragariae*	
Leaf blotch	*Gnomonia comari*	*Gnomonia fragariae*
Leaf scorch	*Diplocarpon earliana*	-
Leaf blight	*Dendrophoma obscurans*	-
Downy mildew	*Peronospora potentillae*	-
Powdery mildew	*Sphaerotheca macularis*	-
Crown and root diseases		
Crown rot	*Colletotrichum gloeosporioides*	*Colletotrichum fragariae*
Black root rot	*Fusarium oxysporum*	*Pythium* spp.
Verticillium wilt	*Verticillium albo-atrum*	-

4.1.2. Symptoms, occurrence and prevalence of damage of fungal diseases of strawberry in Bangladesh

The summarized results on this study are presented in **Table 4.2** and **Plate 4.1 - 4.3**. Among the fruit diseases anthracnose fruit rot, fruit rot and Rhizopus rot or leak were common fruit diseases under my survey areas which prevalence of damage was intensive. Leaf spot was the main leaf disease and it was commonly occurred and prevalence of damage was little. On the other hand, root rot and Verticillium wilt were the common and intensive diseases. Among the diseases in the present surveyed areas, crown rot was a common and very intensive disease.

Table 4.2. Symptoms, occurrence and prevalence of damage of fungal diseases of strawberry in Bangladesh.

Name of diseases	Affected parts of plant	Symptoms	Occurrence	Prevalence of damage
Fruit diseases				
Alternaria fruit rot	fruits	fruit rot	occasional	little
Anthracnose fruit rot	fruits	fruit rot	common	intensive
Fruit rot	fruits	fruit rot	common	intensive
Phomopsis soft rot	fruits	fruit rot	general	moderate
Gray mold	fruits and blooms	fruit rot	general	moderate
Rhizopus rot (leak)	fruits	fruit rot	common	intensive
Leaf diseases				
Leaf spot	leaves	leaf spot	common	moderate
Leaf blotch	leaves and fruits	leaf spot and fruit rot	general	moderate
Leaf scorch	leaves and runners	leaf spot	general	moderate
Leaf blight	leaves and runners	leaf spot	general	moderate
Downy mildew	leaves	leaf spot	occasional	little
Powdery mildew	leaves and fruits	leaf roll	common	little
Crown & root diseases				
Crown rot	crowns	plant death	common	very intensive
Black root rot	roots	stunting and plant death	common	intensive
Verticillium wilt	roots and crowns	plant wilt	common	intensive

4.1.3. Incidence (%) of *C. gloeosporioides* and other pathogens isolated from infected crowns

District wise incidence of *Colletotrichum gloeosporioides* and other pathogens in strawberries were surveyed during 2010-2012 and presented in **Table 4.3** and **Fig. 4.1-4.2.** The maximum 77.77% incidence of *C. gloeosporioides* was recorded in Rajshahi district followed by Sherpur (77.14%), Dinajpur (75.00%) and Natore (70.00%).The lowest 57.14% incidence of *C. gloeosporioides* was found in Rangpur district. The maximum 42.66% other pathogens incidence was recorded in Rangpur district followed by Gaibandha (40.80%), Jamalpur (40%) and Mymensingh (39.24%). The lowest other pathogen incidence of 22.23% was found in Rajshahi district.

Table 4.3. Incidence (%) of *Colletotrichum gloeosporioides* and other pathogens isolated from infected crowns of strawberry plant.

Name of districts	Number of infected crowns	Incidence (%) of *C. gloeosporioides* and other pathogens	
		C. gloeosporioides	Other pathogens
Rajshahi	35	77.77	22.23
Natore	10	70.00	30.00
Joypurhat	8	62.50	37.50
Bogra	9	66.66	33.34
Gaibandha	10	59.20	40.80
Rangpur	7	57.14	42.86
Dinajpur	8	75.00	25.00
Tangail	6	66.66	33.34
Mymensingh	5	60.76	39.24
Sherpur	9	77.14	22.46
Jamalpur	5	60.00	40.00

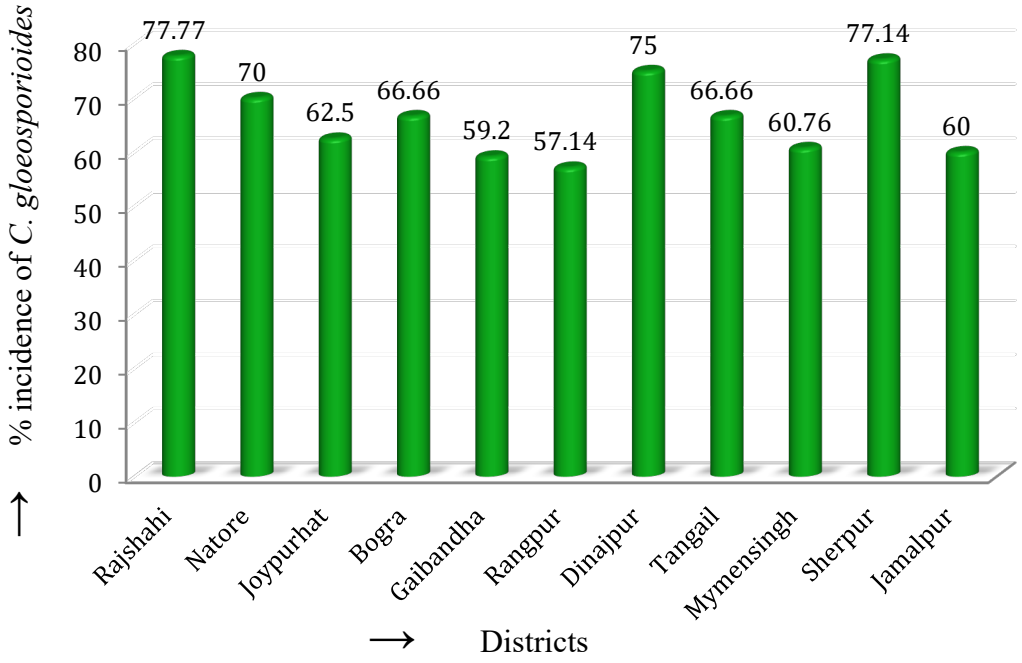


Fig. 4.1: Incidence (%) of *Colletotrichum gloeosporioides* isolated from infected crowns of strawberry.

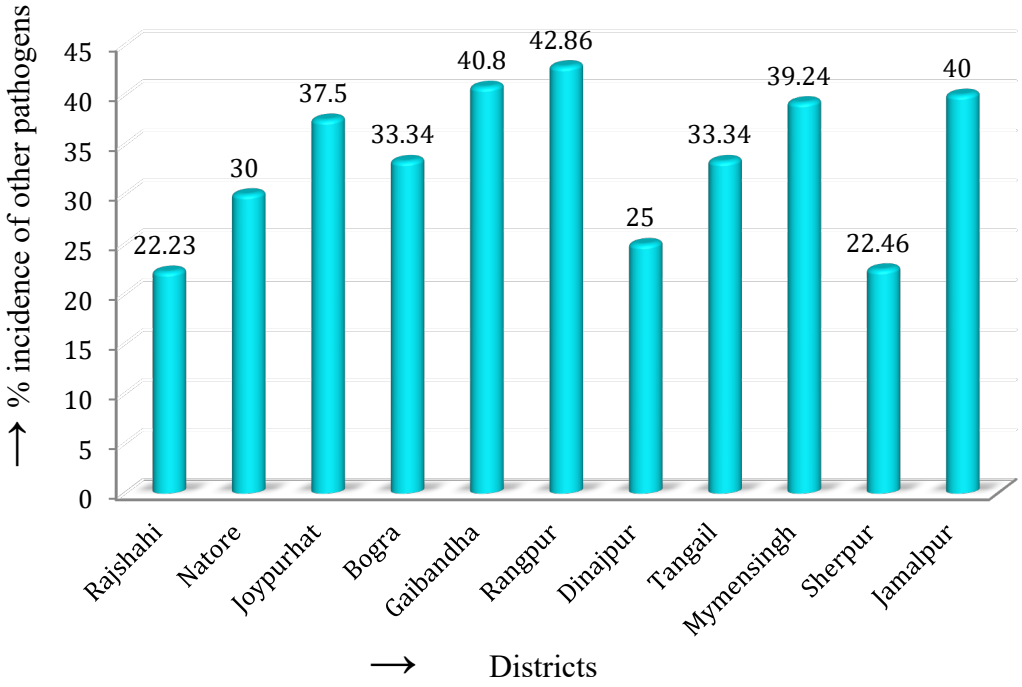
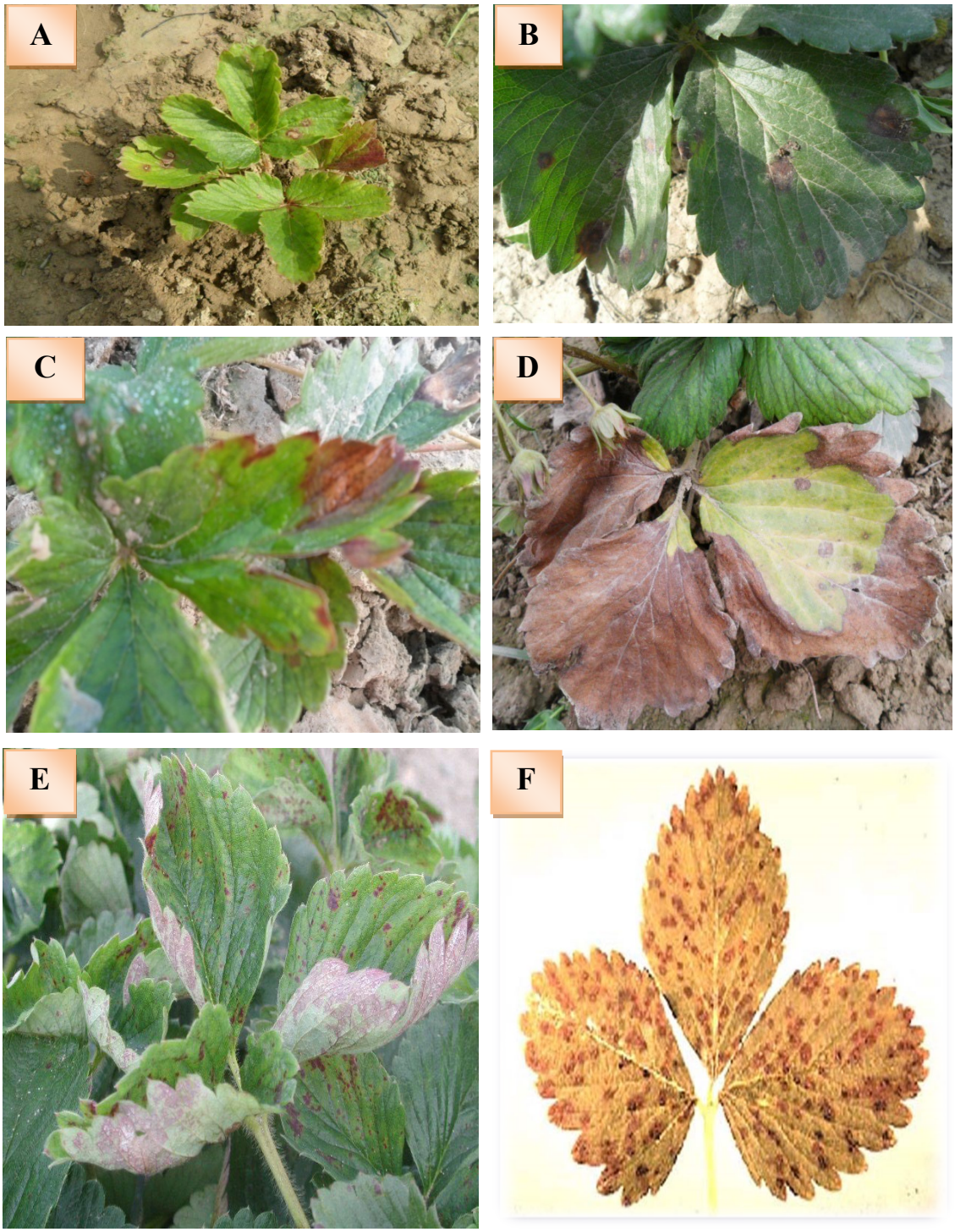


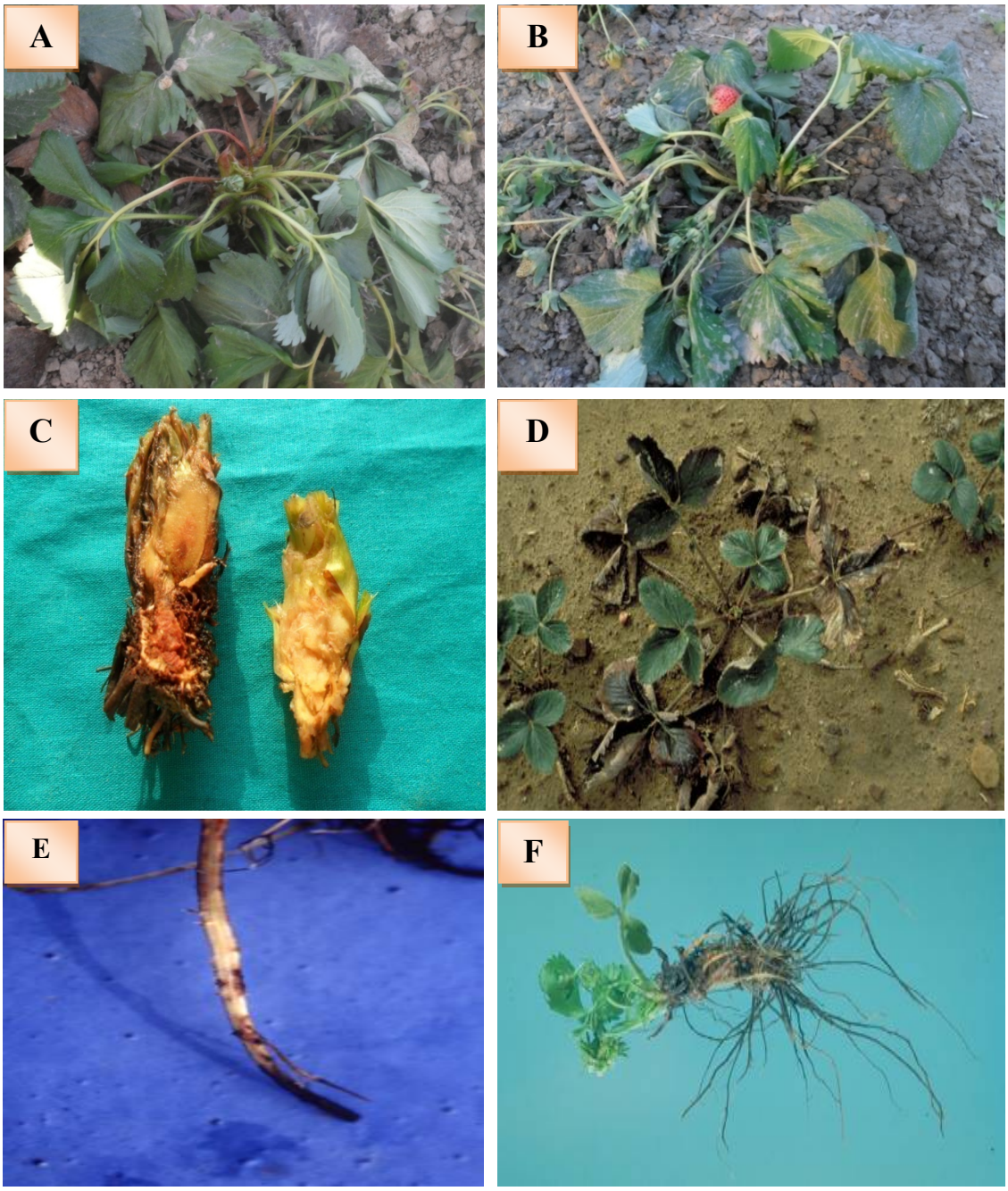
Fig. 4.2: Incidence (%) of other pathogens isolated from infected crowns of strawberry.

PLATE 4.1



Photographs showing leaf diseases of strawberry. **A-B.** Leaf spot, **C-D.** Leaf blight, **E.** Powdery mildew and **F.** Leaf scorch.

PLATE 4.2



Photographs showing crown and root diseases of strawberry. **A-B.** Crown rot disease infected plants, **C.** Longitudinal section of crowns (left site showing infected crown with radish brown colour and right site showing healthy crown), **D.** Verticillium wilt and **E-F.** Black root rot**.**

PLATE 4.3

Photographs showing fruit diseases of strawberry. **A.** Rhizopus rot, **B.** Gray mold, **C.** Fruit rot, **D.** Anthracnose fruit rot, **E.** Alternaria fruit rot and **F.** Different fruit diseases.

4.2. Various Aspects on Crown Rot Disease of Strawberry

4.2.1. Symptomatology

Crown rot or vascular collapse in strawberries is caused by the soil borne fungus *C. gloeosporioides.* The fungus moves into the crown from petiole or stolon lesions, on as an infection from spores washed by rain or irrigation into the center bud. Plant infected with virulent strains initially show signs of water stress and subsequently collapse. This process may be relatively rapid, taking only two or three days at the high temperature. Under low temperature, plants may show signs of stress and subsequently recover for weeks before collapsing. Cutting through crown tissue of infected plants lengthwise reveals a reddish brown colour. Infected plants from nurseries may grow normally for some time before symptoms occur. There are typically no lesions on foliage or stolons in production fields, although under green house condition or in summer nurseries necrosis on stolons, lesions on fruit or black leaf spots may be visible.

4.2.2. Identification of the crown rot disease causal pathogen (*C. gloeosporioides*)

The crown rot disease causal pathogen was identified up to species level based on their morphological and cultural characters.

4.2.3. Taxonomic position of the causal organism

Division - Mycota
Subdivision - Eumycota
Class - Deuteromycetes
Order - Melanconiales
Family - Melanconiaceae
Genus - *Colletotrichum*
Species - *Colletotrichum gloeosporioides.*

4.2.4. Morphology of the *C. gloeosporioides* on host tissue

The overall results on this experiment are presented in **Table 4.4** and **Plate 4.4.**

Microscopic examination of infected tissue revealed that acervuli were circular and measured from 116.4 to 264.7µm. The acervulus was covered with a mucilaginous mass containing numerous conidia. Conidia were hyaline, single celled and smooth walled. They were oblong or cylindrical and measured 6.0 - 11.2 × 2.0 - 4.0 µm. There were no setae on host tissue**.**

4.2.5. Morphology of the *C. gloeosporioides* on culture medium

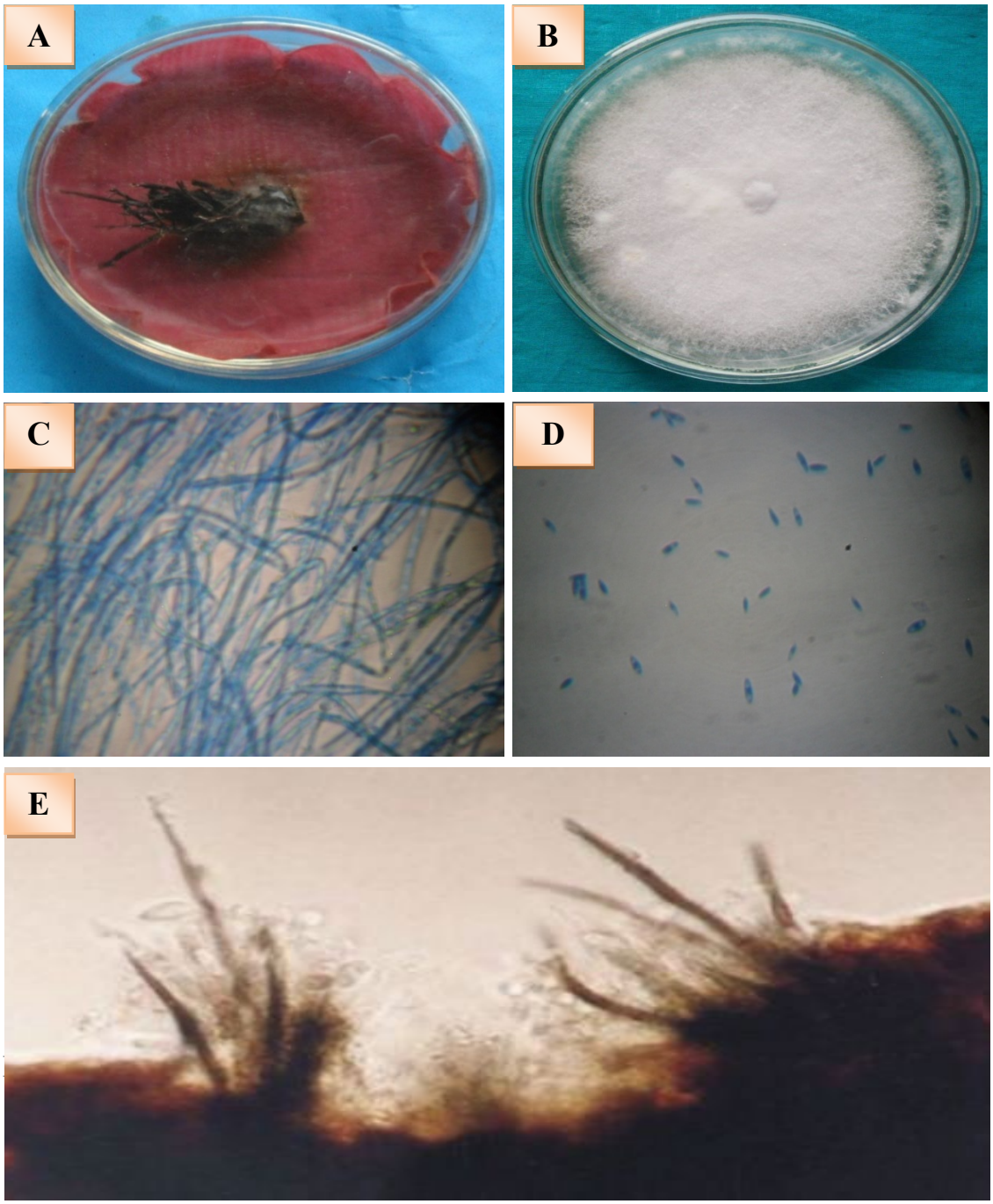
The overall results on this experiment are presented in **Table 4.4** and **Plate 4.4.**

The fungal colony from 10 days old culture on PDA media was white to dull white with smooth margins. The mycelium was hyaline, superficial, septete and branched. The aerial mycelium was white to dull white. Sporulation was abundant with maximum fruiting bodies at the centre of the plate as against mycelium growth towards the periphery. The acervuli from the culture measured 129.0 - 281.4 µm. The conidia from the culture measured 9.0 - 20.0 × 3.0 - 7.5 µm. Setae were arising through this mass were erect, measuring 37.0 - 89.0 × 1.4 - 4.0 µm and were dark brown to black. Based on the morphology of the pathogen was identified as *C. gloeosporioides*.

Table 4.4. Morphological characteristics of *C. gloeosporioides.*

Characters	Measurment in µm			
	On culture medium		On host	
	Range	Average	Range	Average
Acervuli	129.0 - 281.4	193.19	116.4 - 264.7	198.54
Conidia	9.0 - 20.0 × 3.0 - 7.5	13.04 × 4.81	6.0 -11.2 × 2.0 - 4.0	8.66 × 2.94
Setae	37.0 - 89.0 × 1.4 - 4.0	51.13 × 2.4	-	-

PLATE 4.4



Photographs showing morphology of *C. gloeosporioides.* **A.** Culture in host, **B.** Culture in PDA medium, **C.** Mycelia, **D.** Conidia and **E.** Acervuli.

4.2.6. Cultural characteristics of *C. gloeosporioides* on different media

Diversity in cultural and morphological characters of *C. gloeosporioides* were studied in eight culture media at room temperature 28 ± 1°C and the results obtained are presented in **Table 4.5, Fig. 4.3 - 4.4** and **Plate 4.5.**

The mycelial growth, colony characters and sporulation of the pathogen was recorded, when the maximum growth was attained of the tested pathogen. The effect of different culture media on the growth of fungus differed significantly. The maximum mycelial growth of *C. gloeosporioides* was recorded on PDA (82.00 mm) and Richard's agar (81.55 mm) media, which were found to be significantly superior to all other media followed by Corn meal agar (79.77 mm) and soil media (79.44 mm).The next best medium were found to be Yeast extract medium (78.11 mm) which were on par with each other followed by host leaf extract (74.00 mm) and Czapek's agar medium (73.77 mm). The lowest mycelial growth (66.55 mm) was recorded in Sabouraud's agar medium.

Mycelium colour varied from white to black. The growth varied from flat, raised fluffy to sparse. Pigmentation in the media also varied from brown to black and light pink to orange. Sporulation also showed greater variations in different media, ranging from excellent to poor sporulation. The excellent sporulation was recorded from PDA medium and good sporulation was in Richard's agar and Corn meal agar media. The poor sporulation noticed in Sabouraud's agar medium.

Table 4.5. Effect of media on mycelial growth and sporulation of *C. gloeosporioides.*

Name of media	Mycelial growth (mm) of *C. gloeosporioides* in different media ($\overline{X}$ ± SE)	Sporulation
PDA	82.00 ± 0.23	86.44 ± 0.21
Richard's	81.55 ± 0.24	71.55 ± 0.23
Czapek's	73.77 ± 0.27	32.22 ± 0.27
Corn meal agar	79.77 ± 0.27	70.77 ± 0.31
Sabouraud's	66.55 ± 0.17	23.00 ± 0.25
Yeast cxtract	78.11 ± 0.30	41.11 ± 0.22
Host leaf extract	74.00 ± 0.23	45.88 ± 0.31
Soil	79.44 ± 0.17	28.33 ± 0.26
LSD value at 5%	0.737	0.658

The sporulation (Conidia/microscopic field (40 xs) were graded as follows:

Excellent = >75, Good = (50 - 75), Moderate = (25 - 50)

Poor = (1-25) and No conidia = 0

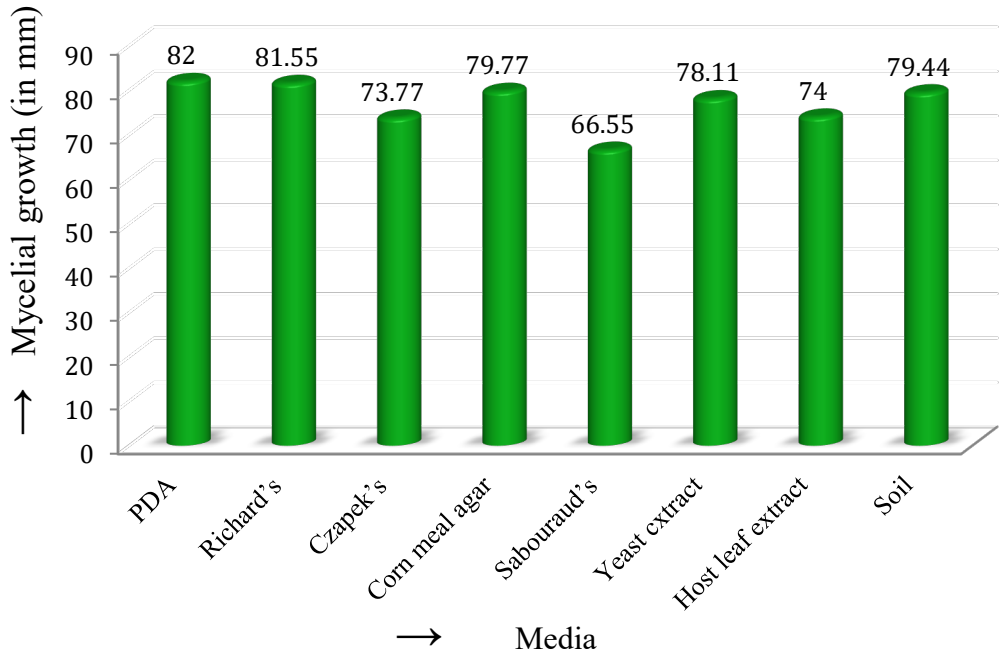


Fig. 4.3: Mycelial growth of *C. gloeosporioides* in different media.

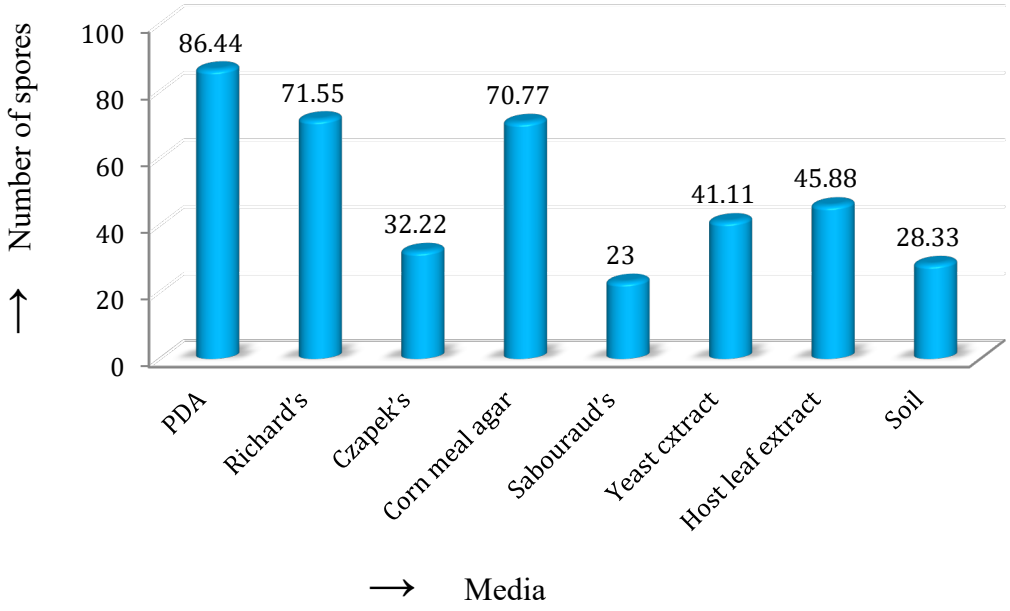


Fig. 4. 4: Sporulation of *C. gloeosporioides* in different media.

4.2.7. Effect of carbon sources on mycelial growth and sporulation of *C. gloeosporioides*

The seven carbon sources were tested in solid Richard's agar medium to know their effect on the growth and sporulation of the *C. gloeosporioides*. Carbon free Richard's agar medium was used as a control medium. The summarized results on this experiment are presented in **Table 4.6, Fig. 4.5 - 4.6** and **Plate 4.5.** The results indicated that maximum mycelial growth was in glucose containing medium (70.22 mm) which was significantly higher than that of other carbon sources. The next in order of merit was sucrose (57.22 mm), mannitol (50.77 mm), starch (46.55 mm), fructose (46.11 mm), sorbitol (45.77 mm) and lactose (44.11 mm) containing media. On the contrary the lowest growth was recorded in control medium as compared to carbon containing sources.

The excellent sporulation was recorded in glucose and sucrose containing media and good sporulation was in starch containing medium.

Table 4.6. Effect of carbon sources on mycelial growth and sporulation of *C. gloeosporioides.*

Carbon sources	Mycelial growth (mm) of *C. gloeosporioides* in different carbon sources ($\overline{X}$ ± SE)	Sporulation
Glucose	70.22 ± 0.22	87.99 ± 0.24
Sucrose	57.22 ± 0.22	89.11 ± 0.31
Mannitol	50.77 ± 0.22	42.66 ± 0.27
Lactose	44.11 ± 0.26	21.55 ± 0.22
Starch	46.55 ± 0.24	64.44 ± 0.21
Fructose	46.11 ± 0.26	23.22 ± 0.26
Sorbitol	45.77 ± 0.27	19.33 ± 0.29
Control	23.33 ± 0.16	0.0
LSD value at 5%	0.720	0.821

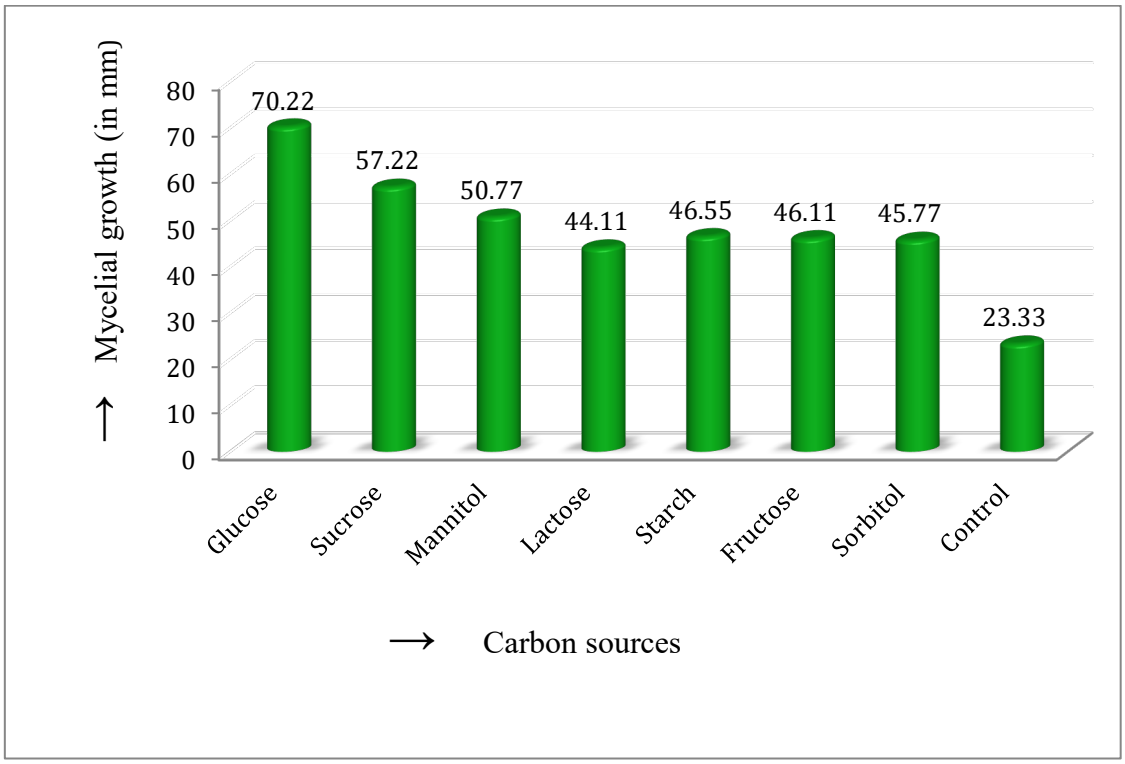


Fig. 4.5: Mycelial growth of *C. gloeosporioides* in different carbon sources.

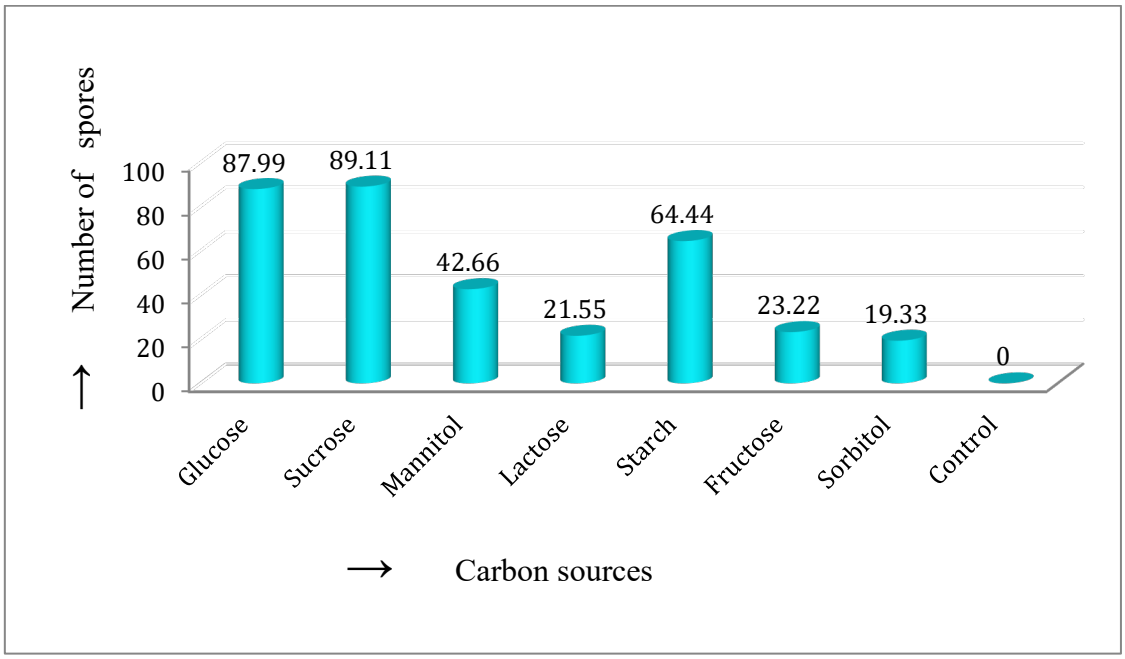


Fig. 4.6: Sporulation of *C. gloeosporioides* in different carbon sources.

4.2.8. Effect of nitrogen sources on mycelial growth and sporulation of *C. gloeosporioides*

The seven nitrogen sources were tested in solid Richard's agar medium to know their effects on the growth and sporulation of the *C. gloeosporioides*. Nitrogen free Richard's agar medium was used as a control medium. The results are presented in **Table 4.7, Fig. 4.7 - 4.8** and **Plate 4.5.** From the table it is noted that maximum growth of *C. gloeosporioides* was found in potassium nitrate (59.66mm) which was significantly higher from other nitrogen sources. The next in order of merit was ammonium sulphate (53.22mm), sodium nitrate (50.77mm), calcium nitrate (46.44 mm), while peptone (42.22 mm) showed moderate growth of the pathogen. The rest of nitrogenous sources showed lower growth as compared to control.

Sporulation of *C. gloeosporioides* was excellent in potassium nitrate and ammonium sulphate containing media, while good sporulation was recorded in peptone and calcium nitrate. L-Aspergine and urea containing media exhibited the poor sporulation of the fungus.

Table 4.7. Effect of nitrogen sources on mycelial growth and sporulation of *C. gloeosporioides*.

Nitrogen sources	Mycelial growth (mm) of *C. gloeosporioides* in nitrogen sources ($\overline{X}$ ± SE)	Sporulation
Potassium nitrate	59.66 ± 0.23	83.66 ± 0.23
Calcium nitrate	46.44 ± 0.29	61.22 ± 0.20
Sodium nitrate	50.77± 0.22	45.77 ± 0.32
Ammonium sulphate	53.22 ± 0.22	81.33 ± 0.27
Peptone	42.22 ± 0.22	60.00 ± 0.25
L-Aspergine	32.22 ± 0.22	24.11 ± 0.21
Urea	29.77 ± 0.22	22.88 ± 0.29
Control	14.55 ± 0.17	0.0
LSD value at 5%	0.684	0.710

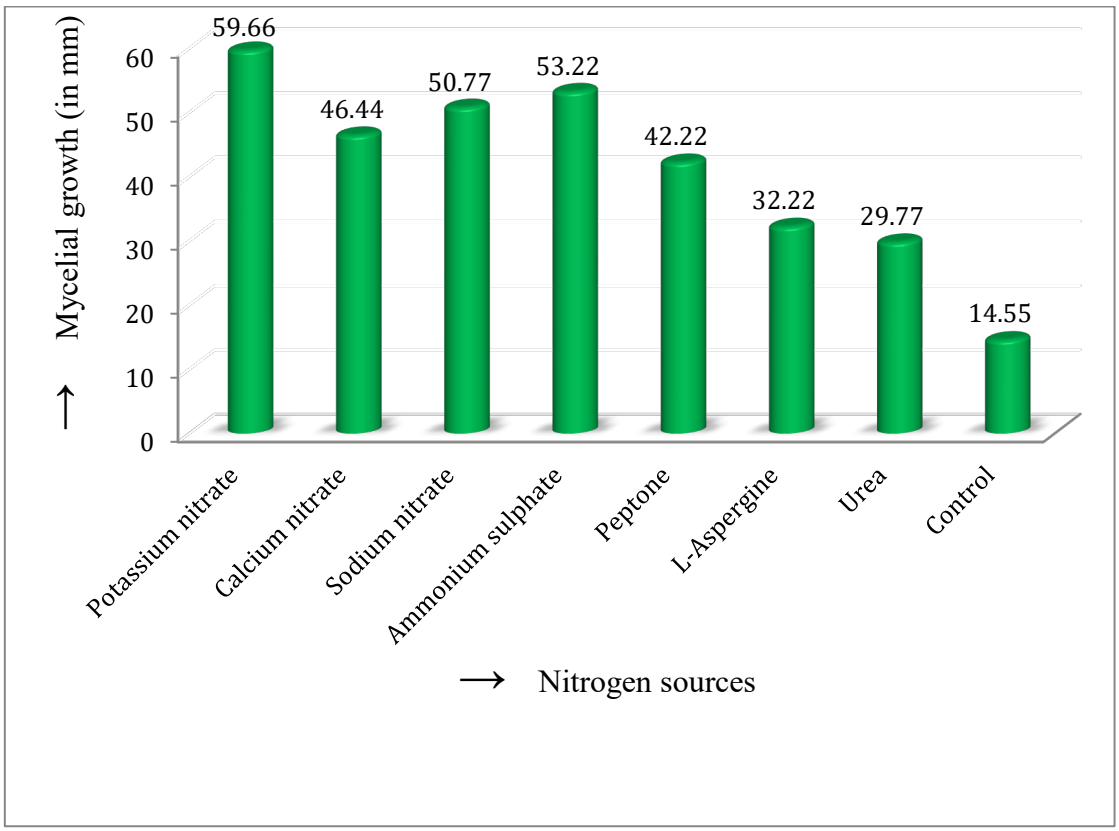


Fig. 4.7: Mycelial growth of *C. gloeosporioides* in different nitrogen sources.

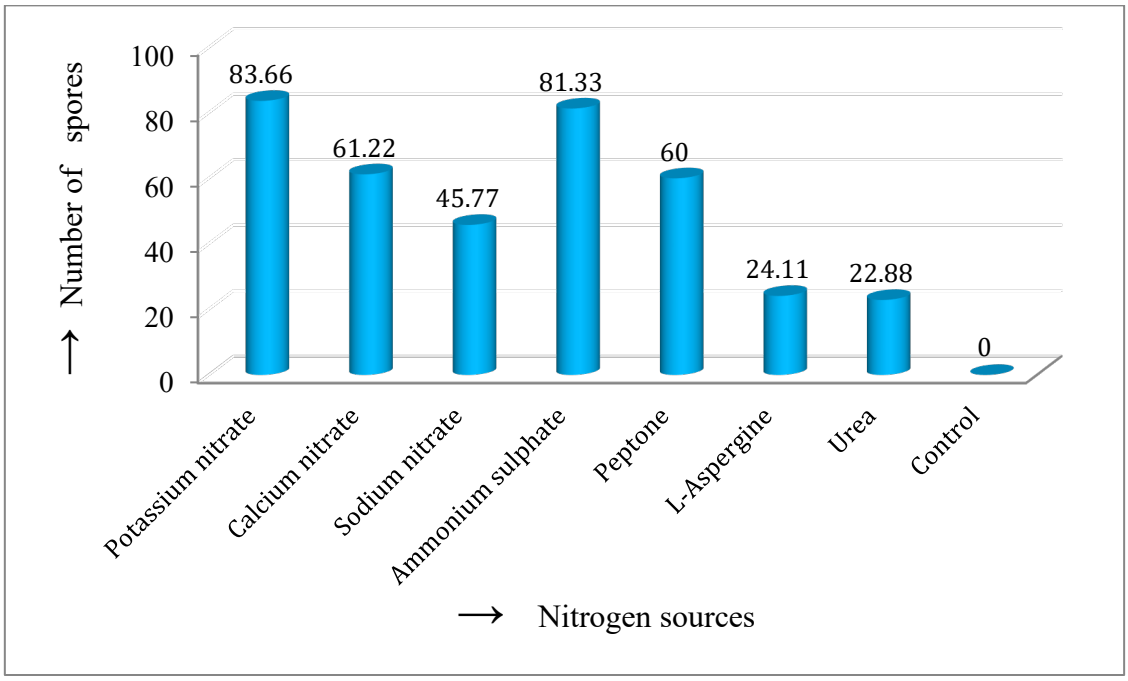


Fig. 4.8: Sporulation of *C. gloeosporioides in* different nitrogen sources.

PLATE 4.5: Photographs showing mycelial growth of *C. gloeosporioides* in

A. Different media:

Upper line (from left to right): PDA, Richard's, Czapek's and Corn meal agar media.

Lower line (from left to right): Sabouraud's, Yeast extract, Host leaf extract and Soil media.

B. Carbon sources:

Upper line (from left to right): Mannitol, Starch, Fructose and control.

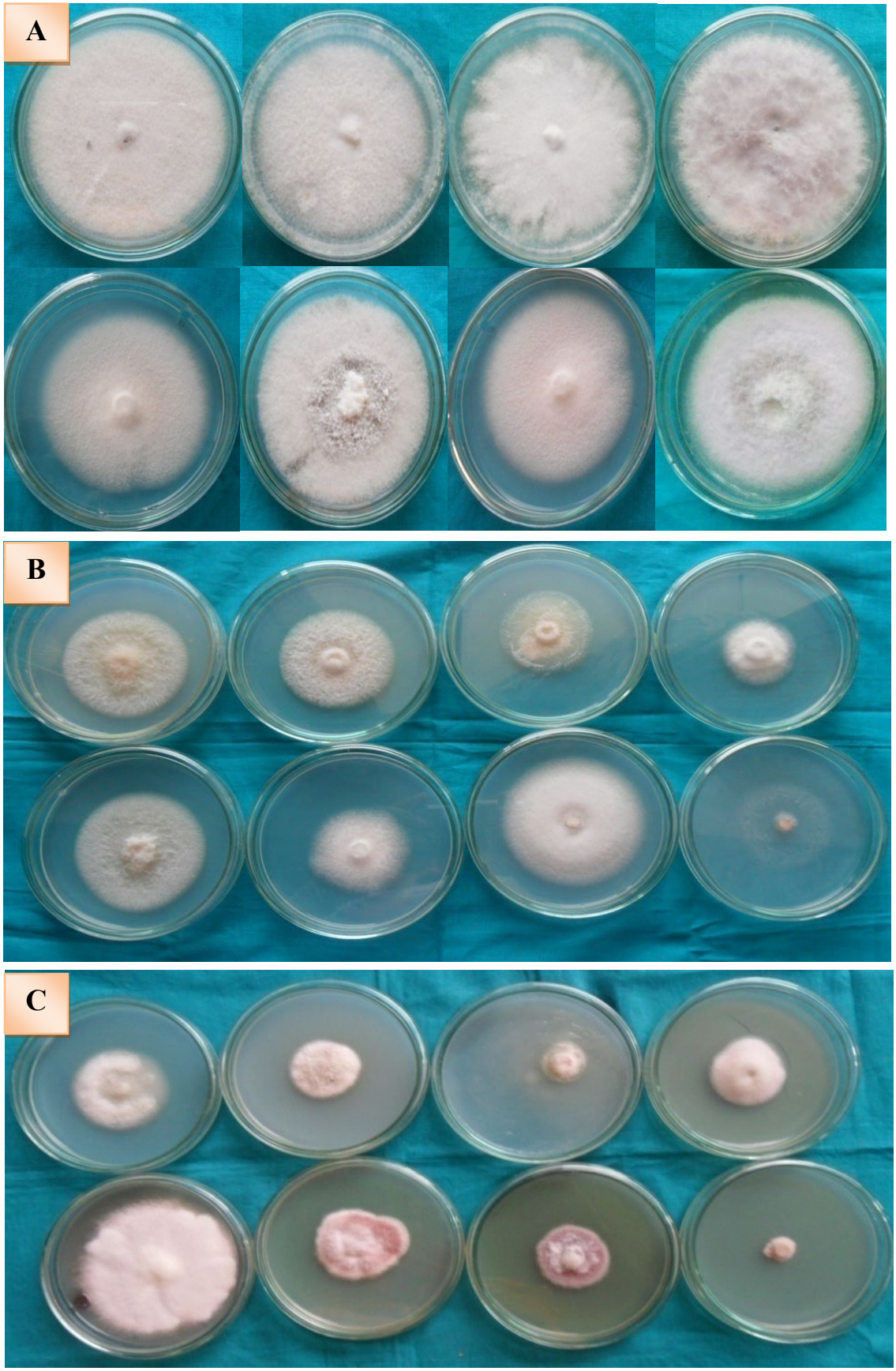
Lower line (from left to right): Sucrose, Lactose, Glucose and Sorbitol.

C. Nitrogen sources:

Upper line (from left to right): Ammonium sulphate, Peptone, Urea and Calcium nitrate

Lower line (from left to right): Potassium nitrate, Sodium nitrate, L-Aspergine and control.

PLATE 4.5



4.2.9. Effect of pH on *C. gloeosporioides*

The effect of pH on the growth of *C. gloeosporioides* was studied. The results obtained are presented in **Table 4.8, Fig. 4.9 - 4.10** and **Plate 4.6.**

The data from **Table 4.8** indicate that the effects of different pH levels on the growth of *C. gloeosporioides* were significant. The maximum growth of the fungus was observed at a pH level of 6.5 (88.11 mm) followed by 6.0 (84.22 mm), 7.0 (82.44 mm), 5.5 (78.11 mm), 5.0 (64.22 mm), 7.5 (77.11 mm) and the lowest growth was noticed in case of pH 4 (40.44 mm). The fungus produced excellent sporulation in pH 6.0 and 6.5, good sporulation was observed in pH 7, while poor sporulation was recorded in pH 4.5.

Table 4.8. Effect of pH levels on mycelial growth and sporulation of *C. gloeosporioides* after 10 days of incubation.

pH levels	Mycelial growth (mm) of C. gloeosporioides in different pH level ($\overline{X}$ ± SE)	Sporulation
4.0	40.44 ± 0.17	0.0
4.5	50.66 ± 0.23	21.27 ± 0.18
5.0	64.22 ± 0.22	44.22 ± 0.28
5.5	78.11 ± 0.26	47.66 ± 0.21
6.0	84.22 ± 0.22	83.55 ± 0.25
6.5	88.11 ± 0.26	88.11 ± 0.31
7.0	82.44 ± 0.17	69.77 ± 0.26
7.5	77.11 ± 0.26	45.33 ± 0.22
LSD value at 5%	0.720	0.843

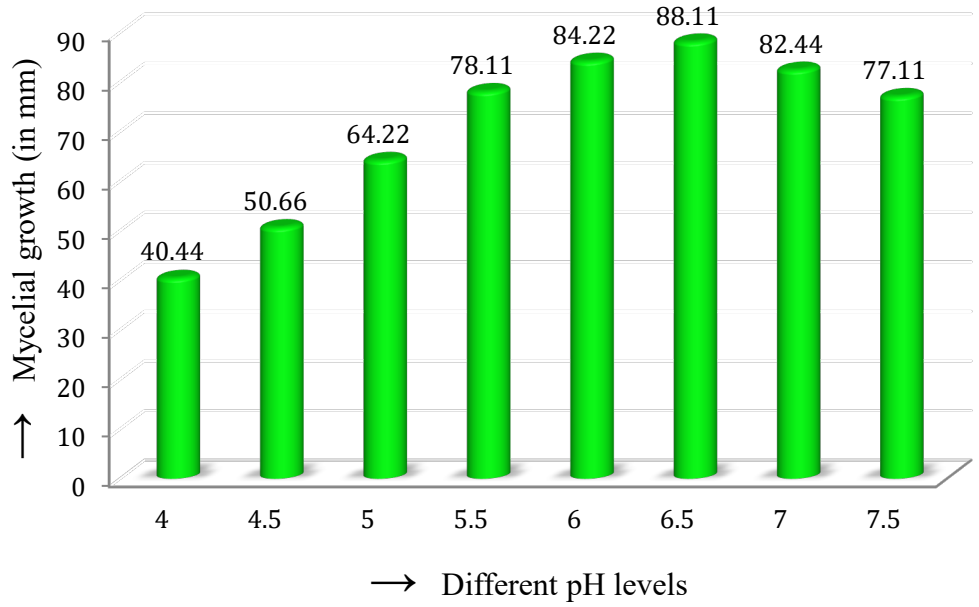


Fig. 4.9: Mycelial growth of *C. gloeosporioides* in different pH levels.

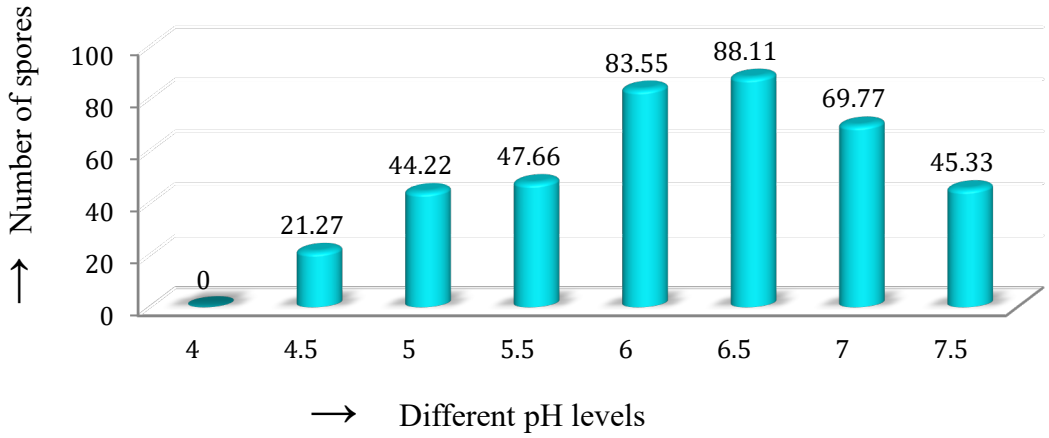


Fig. 4.10: Sporulation of *C. gloeosporioides in* different pH levels.

4.2.10. Effect of temperatures on *C. gloeosporioides*

C. gloeosporioides was grown on PDA medium at six temperature levels *viz.,* 10, 15, 20, 25, 30 and 35°C to know the optimum temperature required for maximum mycelial growth and sporulation. The results obtained are presented in **Table 4.9, Fig. 4.11 - 4.12** and **Plate 4.6.**

Growth of *C. gloeosporioides* on PDA showed gradual increase with the increase of temperature from 10 to 30°C and later declined with further increase in temperature. The maximum mycelial growth was recorded at 30°C (87.11 mm), which was significantly superior to all other temperatures tested followed by 25°C (84.33 mm), 35°C (74.11 mm) and 20°C (67.11 mm) but lowest mycelial growth was recorded at 10°C temperature (32.22 mm). The growth at all the temperatures was statistically differences from each other. The sporulation was excellent at 30°C, good at 25°C and moderate at 35°C. Poor sporulation was recorded at 20°C and no sporulation at 10 and 15°C.

Table 4.9. Effect of temperature levels on mycelial growth and sporulation of *C. gloeosporioides* after 10 days of incubation.

Temperatures (°C)	Mycelial growth (mm) of *C. gloeosporioides* in different temperatures ($\overline{X}$ ± SE)	Sporulation
10	32.22 ± 0.22	0.0
15	50.44 ± 0.17	0.0
20	67.11 ± 0.26	21.55 ± 0.32
25	84.33 ± 0.23	72.22 ± 0.23
30	87.11 ± 0.26	85.88 ± 0.21
35	74.11 ± 0.26	48.66 ± 0.27
LSD value at 5%	0. 546	0.748

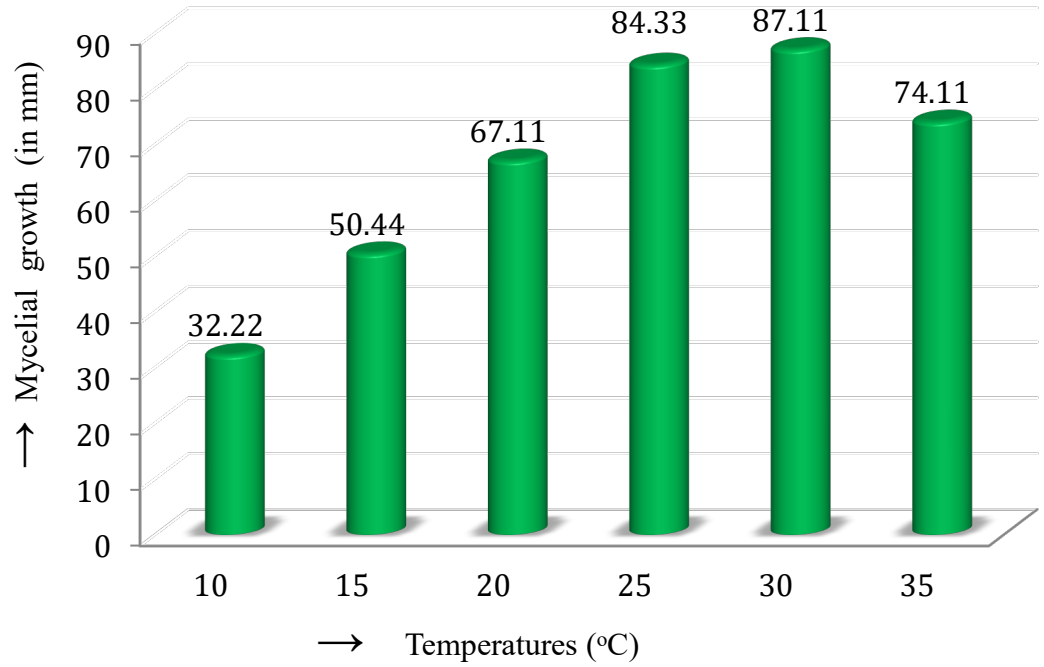


Fig. 4.11: Mycelial growth of *C. gloeosporioides* in different temperatures.

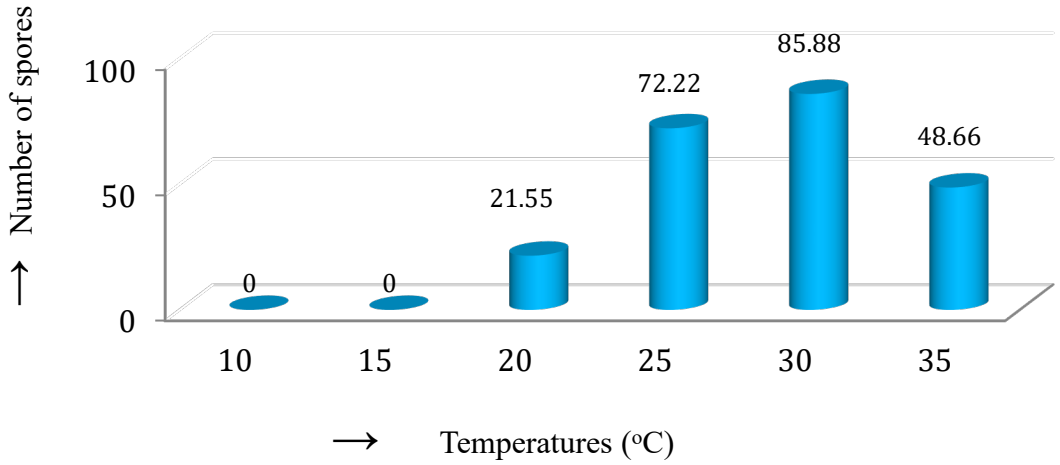


Fig. 4.12: Sporulation of *C. gloeosporioides in* different temperatures.

4.2.11. Effect of light and darkness on growth and sporulation of *C. gloeosporioides*

The experiment was conducted to study the effect of light on growth of *C. gloeosporioides* by exposing the culture to alternate cycle of 12 hours light and 12 hours darkness followed 12 hours darkness and 12 hours light. Continuous lighting followed by continuous darkness for 10 days was noticed and results are presented in **Table 4.10, Fig. 4.13 - 4.14** and **Plate 4.6.**

Alternate cycles of 12 hours lighting and 12 hours darkness recorded maximum mycelial growth of 86.22 mm which was on par with alternate cycle's darkness and lighting 85.77 mm both recorded excellent sporulation. But there was significantly reduced mycelial growth when exposed continuous light and continuous dark. The lowest mycelial growth of 54.88 mm was recorded at continuous darkness with poor sporulation. Pigmentation of the fungus also varied from white to pink.

Table 4.10. Effect of light growth and sporulation of *C. gloeosporioides* after 10 days of incubation.

Light durations	Mycelial growth (mm) of *C. gloeosporioides* ($\overline{X}$ ± SE)	Sporulation
Continuous lighting of 24 hours	57.77 ± 0.22	24.11 ± 0.29
Continuous darkness of 24 hours	54.88 ± 0.26	21.66 ± 0.24
Alternate cycles of 12 hours lighting and 12 hours darkness	86.22 ± 0.22	89.44 ± 0.31
Alternate cycles of 12 hours darkness and 12 hours lighting	85.77 ± 0.27	87.88 ±0.25
LSD value at 5%	0.769	0.632

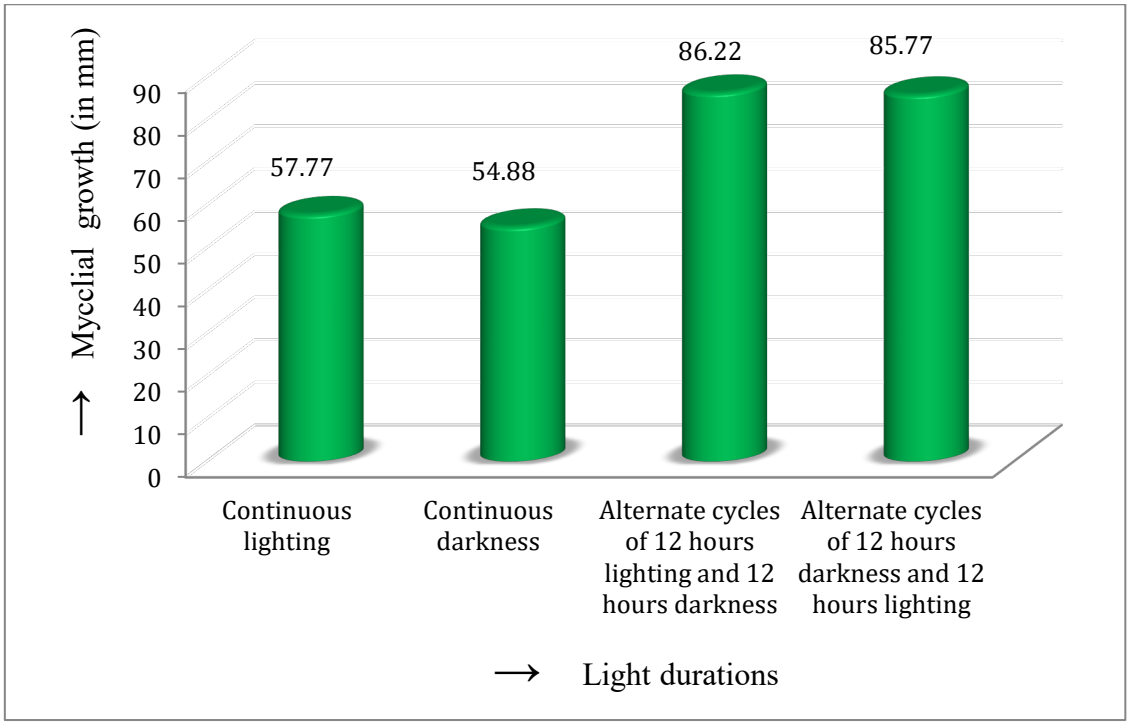


Fig. 4.13: Mycelial growth of *C. gloeosporioides* in different light durations.

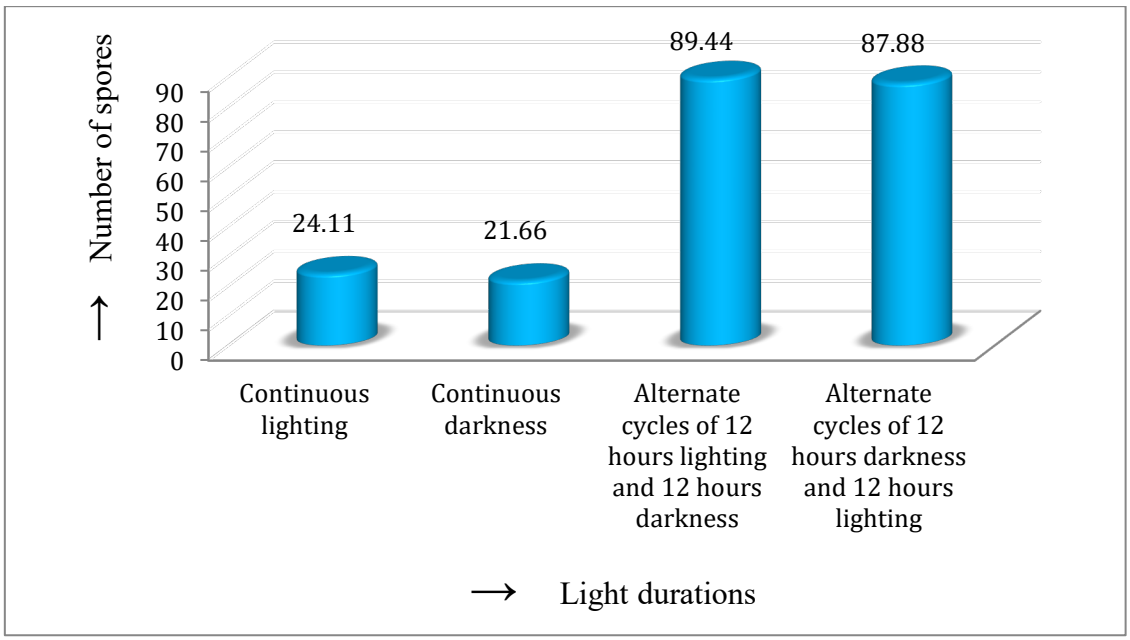


Fig. 4.14: Sporulation of *C. gloeosporioides in* different light durations.

PLATE 4.6: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing

A. Different pH levels:

Upper line (from left to right): 4.0, 4.5, 5.0 and 5.5 pH.

Lower line (from left to right): 6.0, 6.5, 7.0 and 7.5. pH.

B. Different temperatures:

Upper line (from left to right): 10, 15 and 20°C.

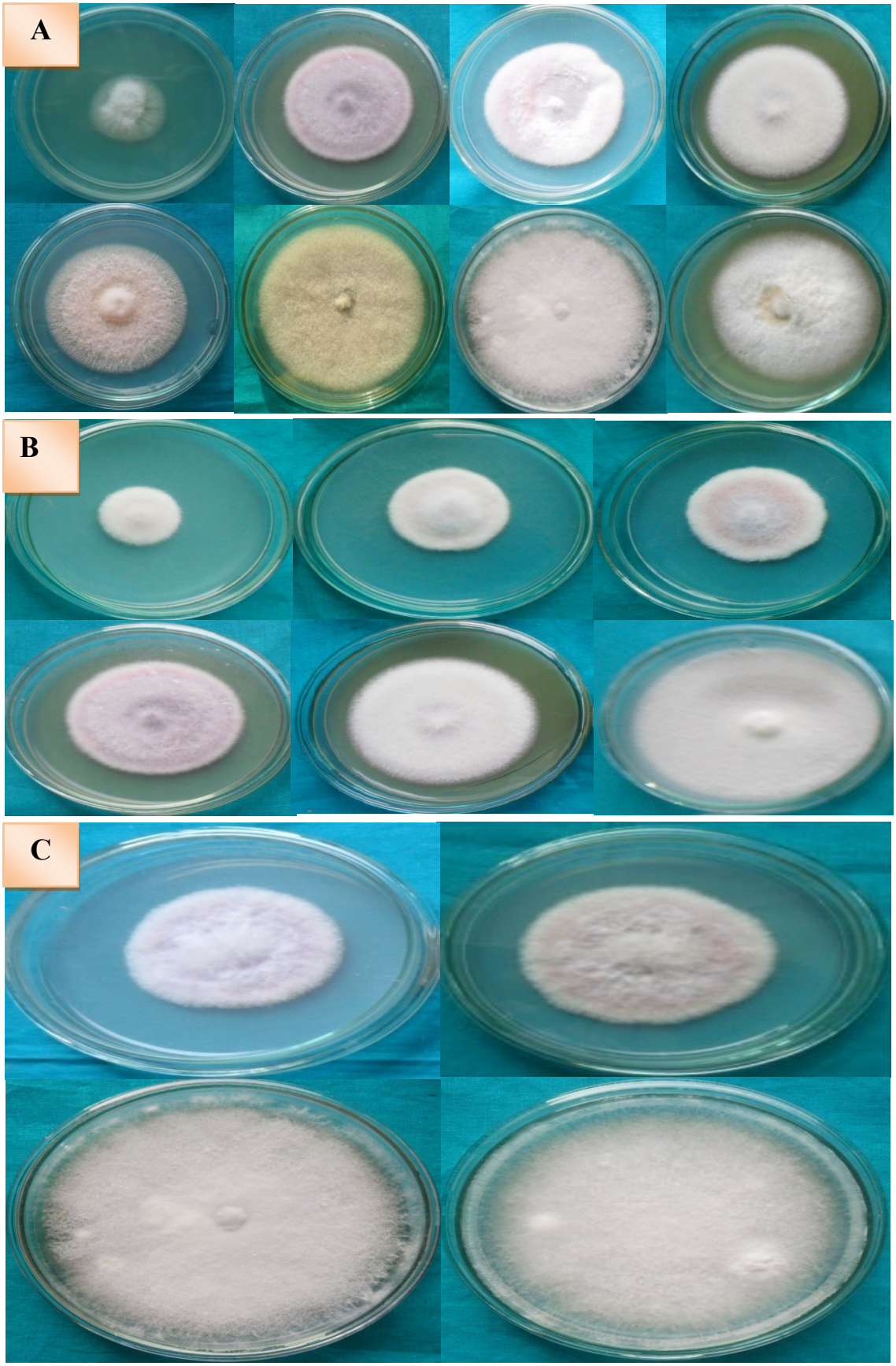
Lower line (from left to right): 25, 30 and 35°C.

C. Different light durations:

Upper line (from left to right): Continuous lighting and continuous darkness.

Lower line (from left to right): Alternate cycles of 12 hours lighting and 12 hours darkness and alternate cycles of 12 hours darkness and 12 hours lighting.

PLATE 4.6



4.2.12. *In vitro* evaluation of plant extracts against *C. gloeosporioides*

Plant derivatives possessing fungicidal properties are gaining worldwide interest as alternatives or as supplements for the existing fungicides. These products will help in reducing cost, environmental hazards and development of resistance by pathogen to fungicides. Keeping this in view an experiment was conducted to assess the antifungal activity of 24 plant extracts against *C. gloeosporioides* and the results are presented in **Table 4.11, Fig. 4.15** and **Plate 4.7 - 4.9.**

From the mean results of five concentrations of plant extracts it is revealed that effects of plant extracts on the fungal growth inhibition were significant. Mehedi leaf extract was 100% effective in inhibiting of mycelial growth which was significantly superior overall other plant extracts evaluated. The second highest mycelial growth inhibition was observed in garlic bulb extract (66.53%) followed by arjun (62.33%) leaf extract. Further in order of merit the mycelial growth inhibition was observed in apang (55.40%), sajna (54.11%), alamanda leaf (51.64%) and ginger rhizome extracts (51.35). The lowest growth inhibition of *C. gloeosporioides* was obtained from akanda (9.28%).

At 25% concentration of plant extracts, 100 % inhibition of mycelial growth was recorded in mehedi leaf extract followed by garlic bulb extract (90.33%). The second highest mycelial growth inhibition was observed in arjun (66.77%) and dhutra (66.00%) leaf extract. Further wood apple, akanda and thankuni leaf extract showed less mycelial inhibition of 20.55, 20.77 and 24.77% respectively.

At 20% concentration of plant extracts, 100% inhibition of mycelial growth was recorded in mehedi leaf extract. The second highest mycelial growth inhibition was observed in garlic bulb extract (77.66%) followed by arjun leaf extract (70.11%).

At 15% concentration of plant extracts, 100% inhibition of mycelial growth was recorded in mehedi leaf extract. The second highest mycelial growth inhibition was observed in garlic bulb extract (67.33%) followed by arjun leaf extract

(62.33%). The lowest mycelial growth inhibition was recorded in akanda leaf extract (6.55%).

At 10% concentration of plant extracts, 100% inhibition of mycelial growth was recorded in mehedi leaf extract. The next highest mycelial growth inhibition was observed in arjun leaf extract (60.44%) followed by garlic bulb extract 52.55%).

At 5% concentration of plant extracts, 100% inhibition of mycelial growth was recorded in mehedi leaf extract. The second highest mycelial growth inhibition was observed in arjun leaf extract (52.00%) followed by apang leaf extract (47.22%). The lowest mycelial growth inhibition was recorded in akanda leaf extract (1.77%).

In general, 24 plant extracts showed antifungal activity against mycelial growth of *C. gloeosporioides* at all the concentrations tested. All extracts at 25% were significantly superior over 5, 10, 15 and 20% concentrations. However, percent inhibition was reduced with decreased concentrations of plant extracts.

Table 4.11. *In vitro* evaluation of plant extracts against mycelial growth of *C. gloeosporioides.*

Name of plants	Mycelial growth inhibition (%) of *C. gloeosporioides* in different concentrations of plant extracts ($\overline{X}$ ± SE)					
	5	10	15	20	25	Mean
Garlic	44.77±0.22	52.55±0.29	67.33±0.33	77.66±0.23	90.33±0.33	66.53±0.28
Onion	20.88±0.26	34.11±0.48	38.00±0.33	41.11±0.26	42.00±0.40	35.22±0.34
Ginger	35.11±0.26	49.33±0.23	51.77±0.27	56.55±0.29	64.00±0.23	51.35±0.24
Neem	33.00±0.23	35.88±0.20	38.11±0.26	45.33±0.16	52.77±0.27	41.02±0.22
Neem	42.22±0.22	45.00±0.28	50.11±0.38	53.88±0.20	56.22±0.22	49.48±0.26
Eucalyptus	37.22±0.27	41.00±0.28	42.00±0.28	45.44±0.29	55.33±0.23	44.20±0.27
Biskatali	17.55±0.17	20.11±0.26	26.66±0.23	30.11±0.26	35.77±0.22	26.04±0.22
Apang	47.22±0.22	52.00±0.23	56.33±0.16	60.22±0.22	61.22±0.22	55.40±0.21
Assamlata	31.44±0.29	40.00±0.28	41.55±0.24	45.11±0.26	50.22±0.27	41.66±0.26
Marigold	24.22±0.22	32.44±0.29	35.66±0.23	40.88±0.26	50.22±0.27	36.68±0.25
Lemon	28.00±0.28	35.33±0.16	41.66±0.23	51.11±0.26	52.55±0.29	41.73±0.23
Papaya	13.77±0.27	21.11±0.26	23.55±0.41	27.88±0.26	34.77±0.27	24.22±0.29
Sajna	45.33±0.16	50.44±0.17	52.66±0.33	60.33±0.16	61.77±0.27	54.11±0.21
Arjun	52.00±0.28	60.44±0.29	62.33±0.16	66.77±0.32	70.11±0.26	62.33±0.24
Dhutra	41.11±0.26	42.55±0.37	47.00±0.23	51.11±0.26	66.00±0.28	49.55±0.28
Alamanda	39.55±0.24	45.88±0.26	53.55±0.17	56.66±0.23	62.55±0.29	51.64±0.23
Mehedi	100	100	100	100	100	100
Thankuni	15.11±0.26	17.55±0.17	21.22±0.22	23.22±0.22	25.66±0.23	20.55±0.22
Tulsi	21.11±0.26	23.00±0.28	27.33±0.16	50.44±0.17	57.55±0.17	35.88±0.20
Wood apple	7.11±0.26	11.00±0.23	14.11±0.26	16.77±0.27	20.55±0.24	13.91±0.25
Lantana	13.88±0.26	21.00±0.23	27.55±0.24	31.00±0.23	34.33±0.16	25.55±0.21
Akanda	1.77±0.22	03.77±0.27	6.55±0.24	13.55±0.24	20.77±0.27	9.28±0.24
Nayntara	20.44±0.17	25.88±0.26	40.33±0.23	41.88±0.26	45.77±0.22	34.86±0.22
Basak	29.00±0.28	37.00±0.28	42.00±0.28	45.11±0.26	49.11±0.26	40.44±0.27
LSD at 5%			0.781			-

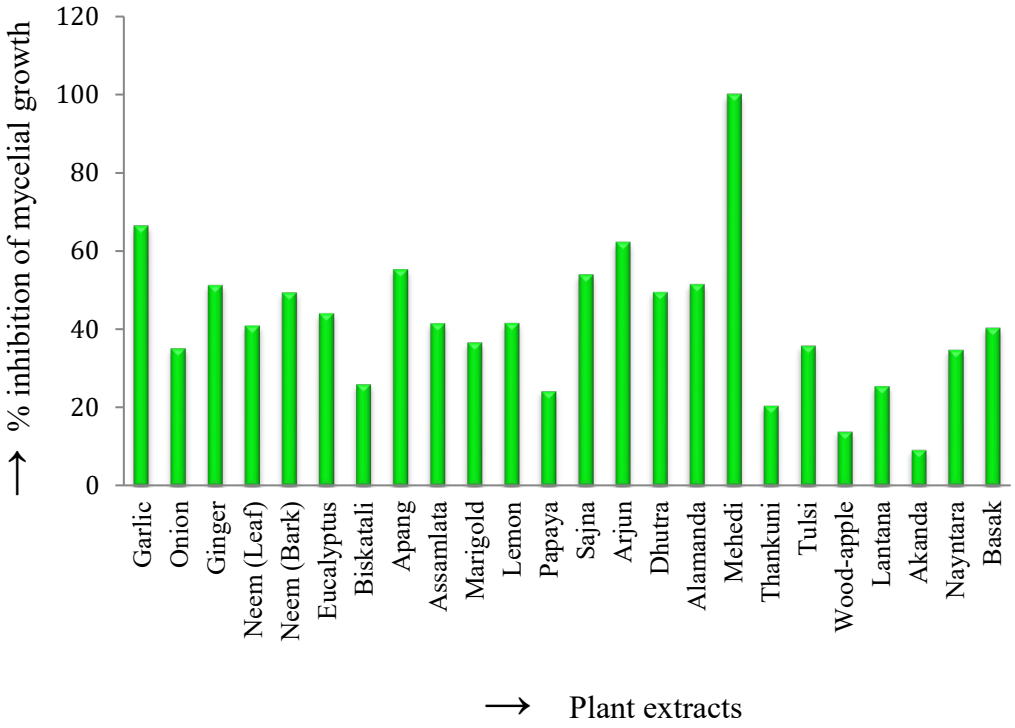


Fig. 4.15: The graphs are mean of mycelial growth inhibition (%) of *C. gloeosporioides* at five concentrations of different plant extracts.

PLATE 4.7: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of plant extracts.

A. Garlic bulb extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

B. Onion bulb extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

C. Ginger bulb extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

D. Neem leaf extract:

Upper line (from right to left): 10%, 5% concentrations and control.

Lower line (from right to left): 25%, 20%, and 15% concentrations.

E. Neem bark extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

F. Eucalyptus leaf extract:

Upper line (from right to left): 10%, 5% concentrations and control.

Lower line (from right to left): 25%, 20%, and 15% concentrations.

G. Biskatali leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

H. Apang leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

PLATE 4.7

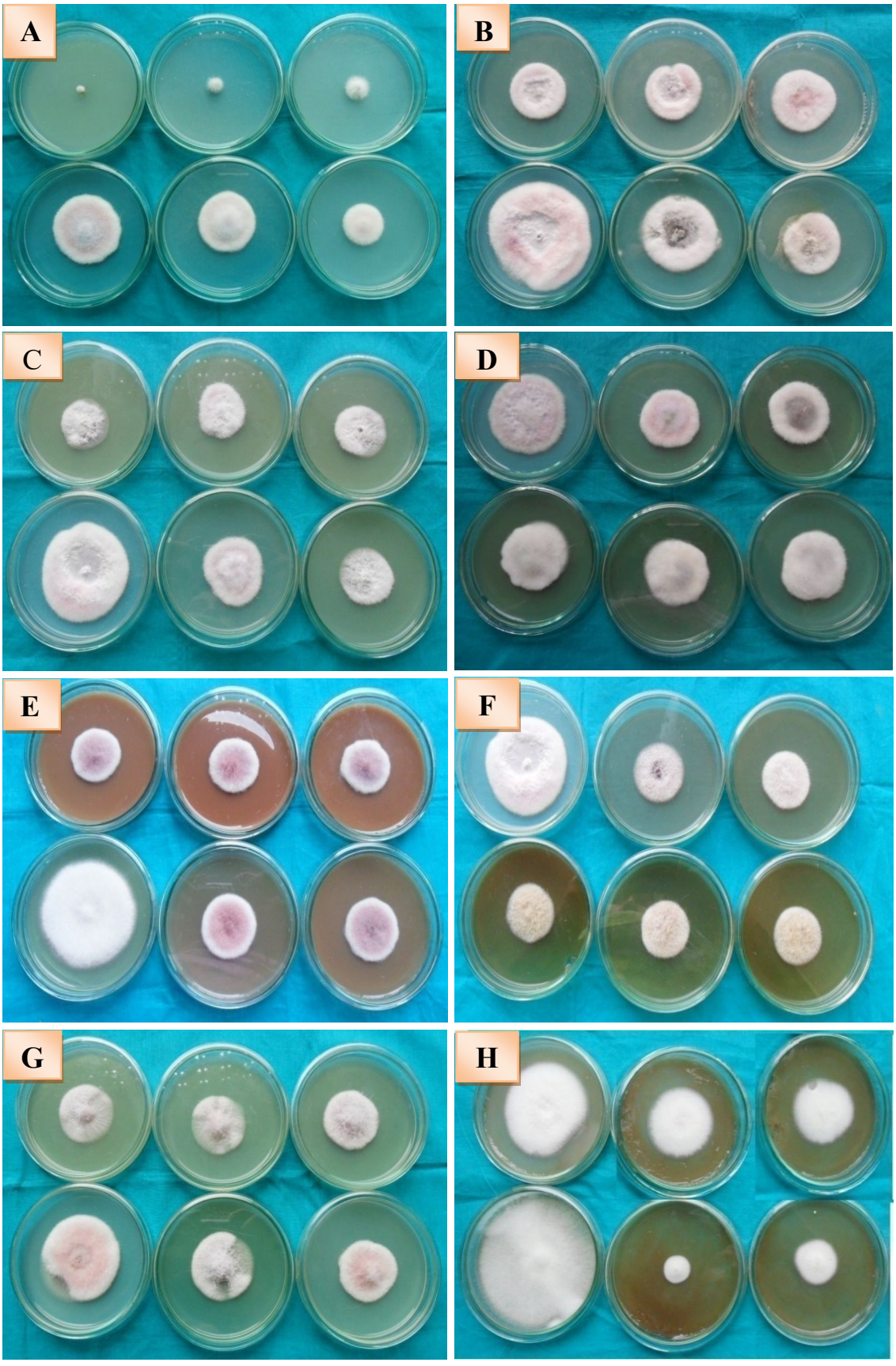


PLATE 4.8: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of plant extracts.

A. Assamlata leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

B. Marigold leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

C. Lemon leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

D. Papaya leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

E. Sajna leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

F. Arjun leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

G. Dhutra leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

H. Alamanda leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

PLATE 4.8

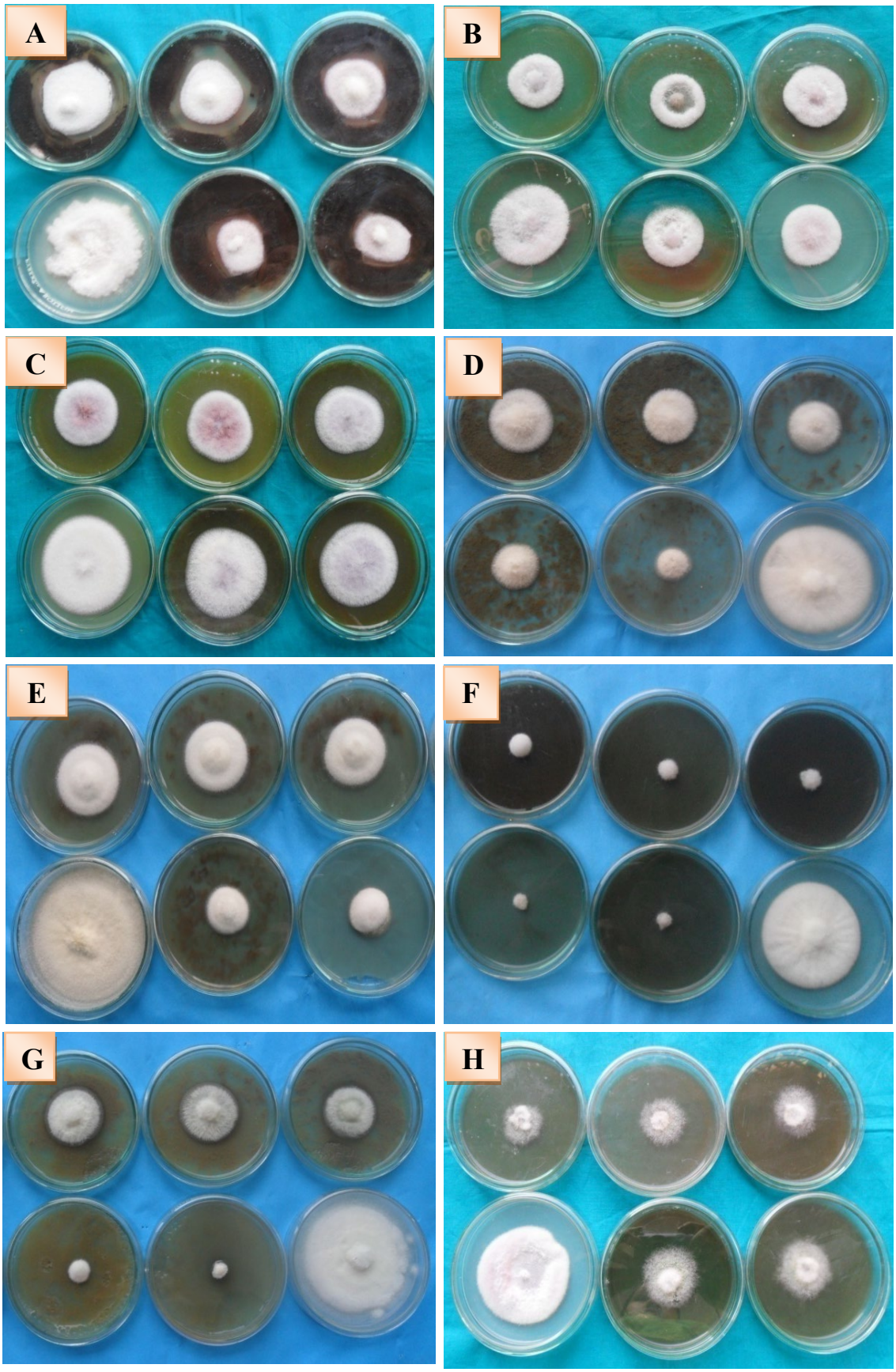


PLATE 4.9: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of plant extracts.

A. Mehedi leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

B. Thankuni leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

C. Tulsi leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

D.Wood apple leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

E. Lantana leaf extract:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

F. Akanda leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

G. Nayantara leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

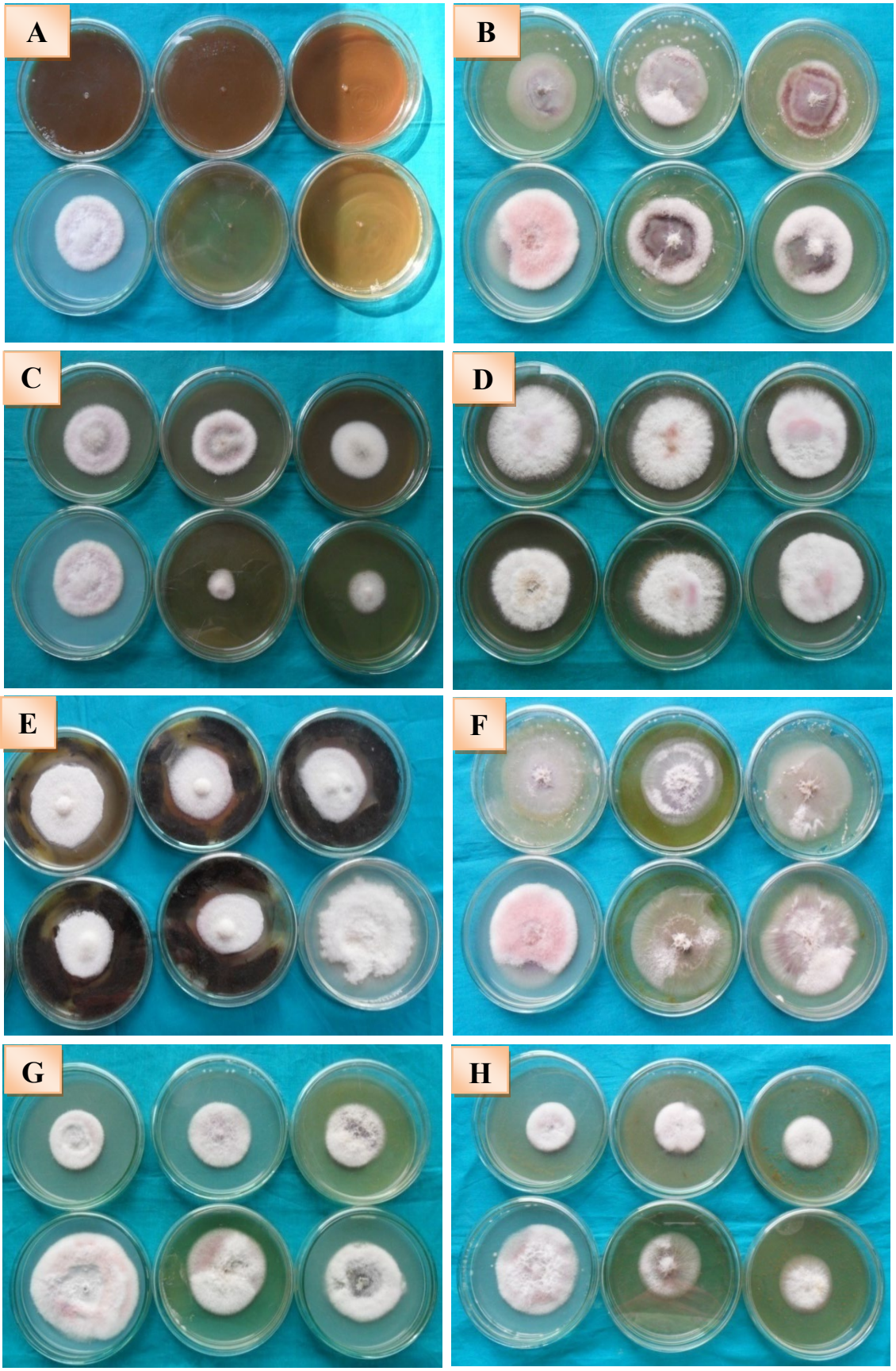
Lower line (from right to left): 10%, 5% concentrations and control.

H. Basak leaf extract:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

PLATE 4.9



4.2.13. *In vitro* evaluation of cowdung and cow urine against *C. gloeosporioides*

The efficacy of cow urine and cowdung on suppression of mycelial growth and their percentage inhibition are shown in **Table 4.12, Fig. 4.16** and **Plate 4.10.**

Results indicate that cow urine showed significantly higher inhibition activity against the selected fungal pathogen than that of cowdung. After 10 days of incubation the highest mycelial growth inhibition (62.55%) was recorded in PDA medium containing 25% cow urine followed by 20% cow urine containing medium (55.55% inhibition). On the other hand among the five concentrations of cowdung 25% concentration was higher effective and maximum mycelial growth inhibition (41.88%) was recorded in this concentration which was followed by 20% cowdung containing medium (34.44% inhibition).

Table 4.12. *In vitro* evaluation of cowdung and cow urine against mycelial growth of *C. gloeosporioides.*

Treatments	Concentrations (%)	% inhibition of mycelial growth of *C. gloeosporioides* ($\overline{X} \pm$ SE)
Cow urine	5	34.88 ± 0.26
	10	37.22 ± 0.27
	15	41.55 ± 0.24
	20	55.55 ± 0.24
	25	62.55 ± 0.29
Cowdung	5	14.22 ± 0.22
	10	20.55 ± 0.24
	15	23.00 ± 0.33
	20	34.44 ± 0.29
	25	41.88 ± 0.26
LSD value at 5 %	-	0.769

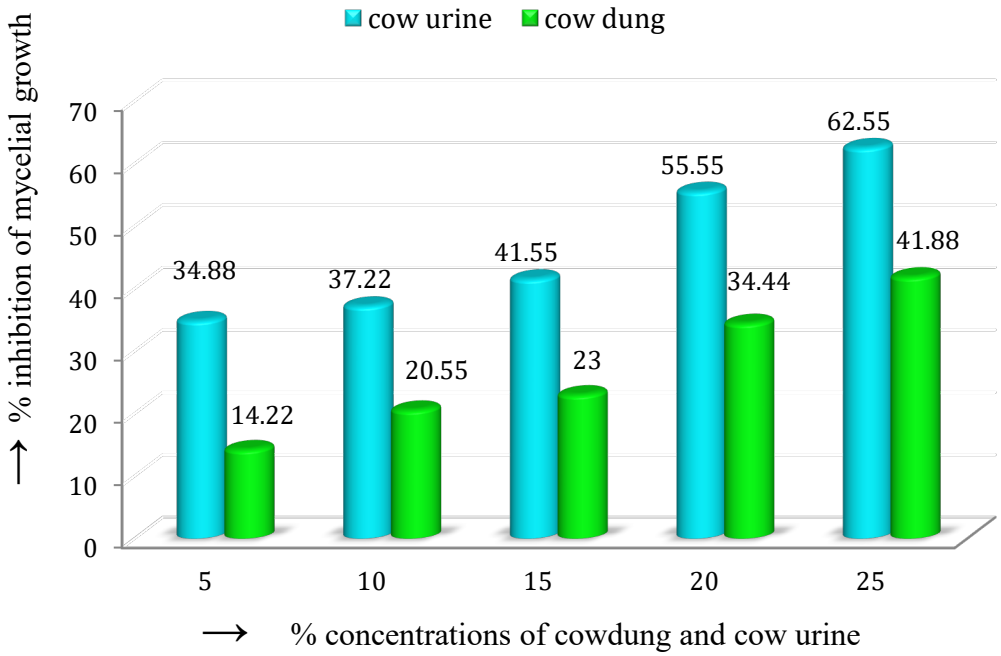
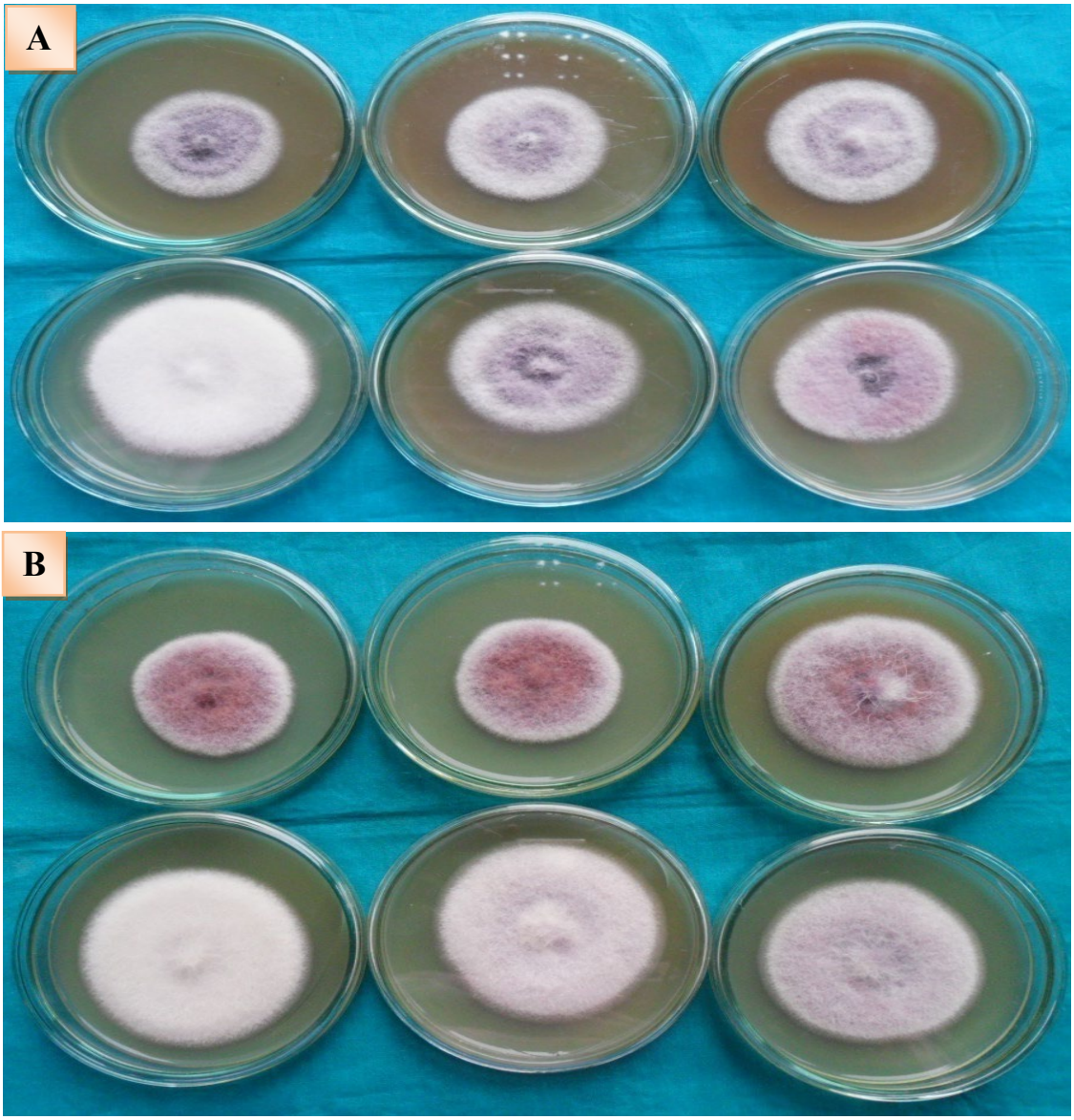


Fig. 4.16: Mycelial growth inhibition (%) of *C. gloeosporioides* in PDA medium containing different concentrations of cow urine and cowdung.

PLATE 4.10



Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of cow urine and cowdung.

A. cow urine:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

B. cowdung:

Upper line (from left to right): 25%, 20%, and 15% concentrations.

Lower line (from right to left): 10%, 5% concentrations and control.

4.2.14. *In vitro* evaluation of phytohormones against *C. gloeosporioides*

Screening of phytohormones was done against *C. gloeosporioides* under laboratory condition by following poisoned food. Data with respect to inhibition of mycelial growth of *C. gloeosporioides* at five concentrations of phytohormones were recorded and percent inhibition is presented in **Table 4.13, Fig. 4.17** and **Plate 4.11.**

From the mean results of five concentrations of phytohormones it is observed that effect of phytohormones on the fungal growth was significant. Among five phytohormones, maximum inhibition of growth of *C. gloeosporioides* was observed in NAA (93.66%) which was significantly superior to all other phytohormones followed by 2,4-D (88.00%), IAA (87.95%), and IBA (81.20%).The lowest 63.84% inhibition of fungus was recorded in BA

Among the tested five concentrations, 500 ppm concentration of all phytohormones was significantly superior to 200, 100, 50 and 20 ppm concentrations. Hundred percent inhibition of mycelial growth of the fungus was recorded at 500 ppm concentration of NAA, IAA, 2,4-D and IBA. The lowest 70.77% inhibition of mycelial growth was recorded at 500 ppm concentration of BA. At 200 ppm concentration, hundred percent inhibition of mycelial growth of the fungus was recorded in NAA, IAA and 2,4-D followed by IBA (89.90%) remained on par with each other. The lowest percent inhibition of mycelia growth was recorded in BA (68.00%). At 100-500 ppm concentration, hundred percent inhibition of mycelial growth of the fungus was recorded in NAA. The lowest inhibition of mycelial growth was recorded in BA (64.22%). Further 2, 4-D, IAA and IBA were on par with each other. At 50 ppm concentration, maximum mycelial growth inhibition of the fungus was recorded in NAA (92.00%) followed by IAA (77.33%) and 2,4-D (76.44%). The lowest mycelial growth inhibition was recorded in BA (60.11%).

At 20 ppm concentration, maximum inhibition of mycelial growth of the fungus was recorded in NAA (76.33%). The lowest inhibition of mycelial growth was noticed in BA (56.11%). Further 2,4-D, IAA and IBA were on par with each other. Similarly, NAA at 100-500 ppm concentrations and IAA and 2,4-D at 200-500 ppm concentrations and IBA at 500 ppm concentration were remained on par with each other.

Table 4.13. *In vitro* evaluation of phytohormones against mycelial growth of *C. gloeosporioides.*

Phyto-hormones	% inhibition of mycelial growth at different concentrations (ppm) ($\overline{X}$ ± SE)					
	20	50	100	200	500	Mean
IAA	75.22±0.22	77.33±0.33	87.22±0.22	100	100	87.95±0.15
2,4-D	71.11±0.26	76.44±0.29	92.44±0.29	100	100	88.00±0.16
BA	56.11±0.26	60.11±0.26	64.22±0.22	68.00±0.28	70.77±0.27	63.84±0.28
NAA	76.33±0.33	92.00±0.28	100	100	100	93.66±0.12
IBA	63.66±0.33	71.11±0.26	81.33±0.33	89.88±0.26	100	81.20±0.23
LSD at 5%	0.692					

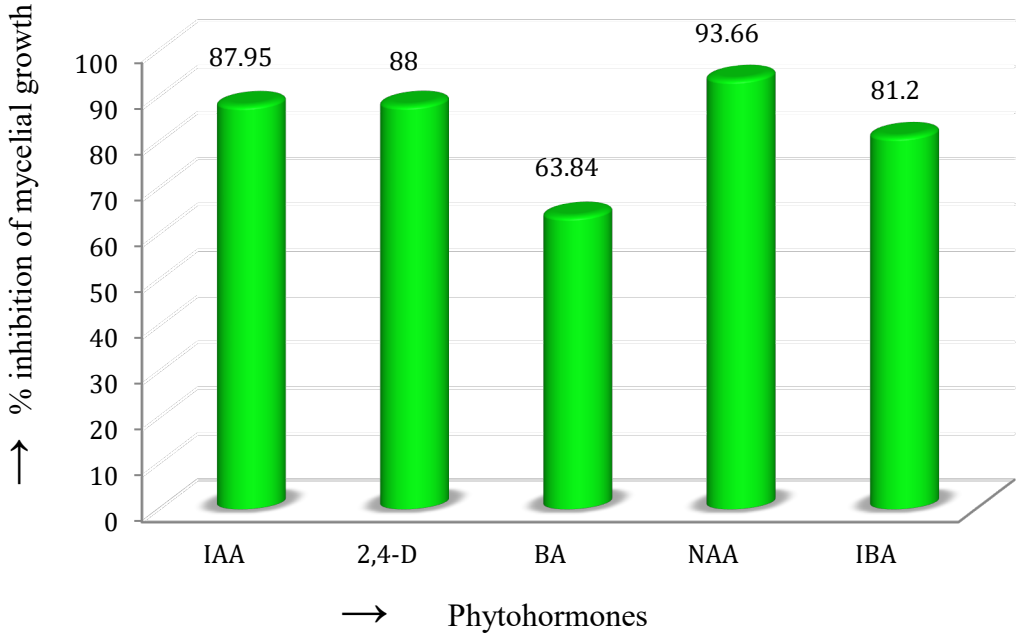


Fig. 4.17: The graphs are mean of mycelial growth inhibition (%) of *C. gloeosporioides* at five concentrations of different phytohormones.

PLATE 4.11: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of phytohormones.

A. IAA:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

B. 2, 4-D:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

C. BA:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from right to left): 20%, 25% concentrations and control.

D. NAA:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

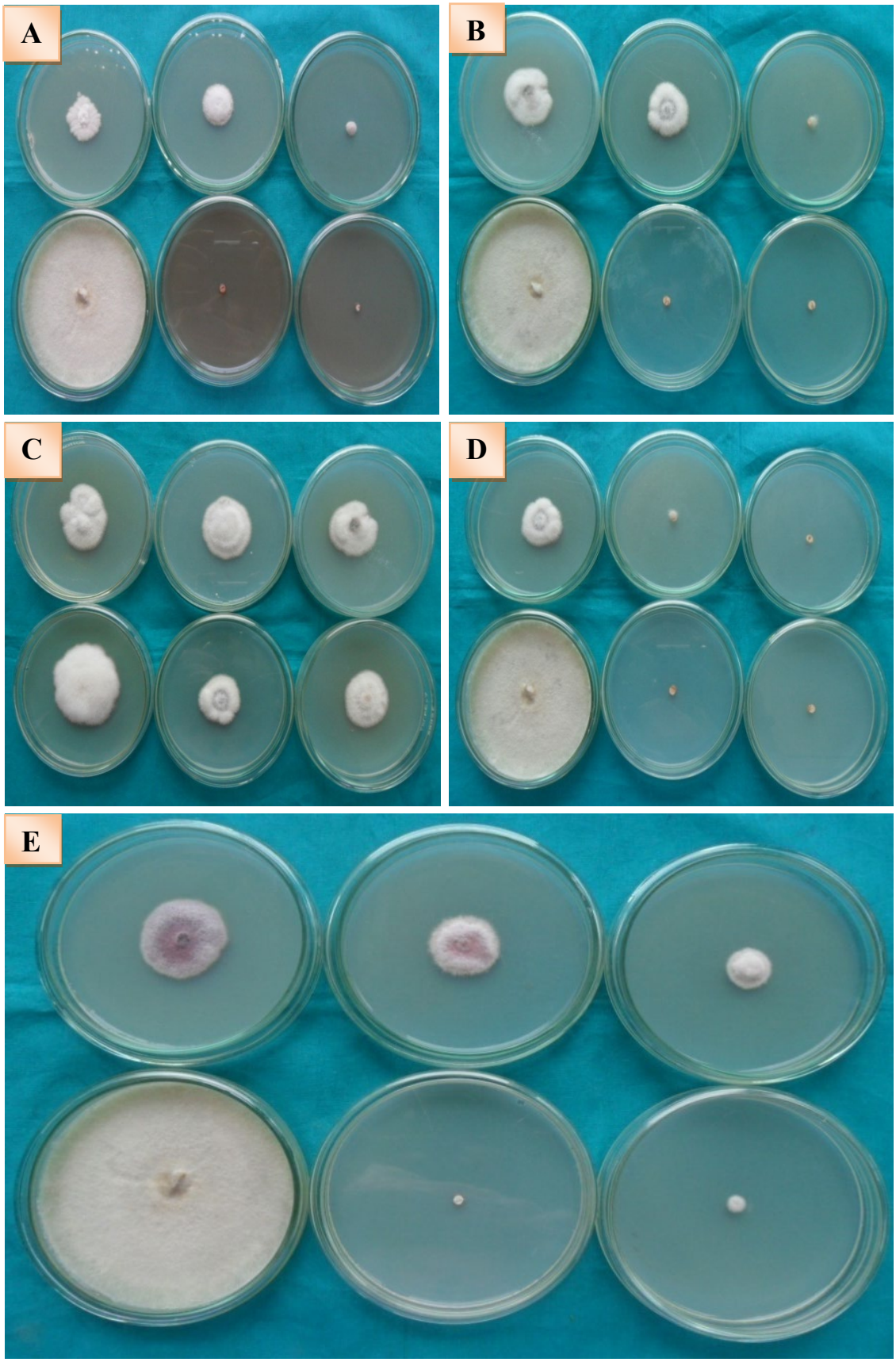
Lower line (from left to right): 20%, 25% concentrations and control.

E. IBA:

Upper line (from left to right): 5%, 10%, and 15% concentrations.

Lower line (from left to right): 20%, 25% concentrations and control.

PLATE 4.11



4.2.15. *In vitro* evaluation of antagonists against *C. gloeosporioides* in dual culture

15 antagonists were tested against *Colletotrichum gloeosporioides.* The results are presented in **Table 4.14, Fig. 4.18** and **Plate 4.12.** The results revealed that all the antagonists significantly reduced the growth of *C. gloeosporioides* either by over growing or by exhibiting inhibition zones. Most of the antagonists inhibited mycelial growth of *C. gloeosporioides* by fast and over growing nature as observed in antagonists. After measuring the mycelial growth of *C. gloeosporioides,* it was noticed that maximum reduction in mycelial growth was found in *T. harzianum (*80.22%) which was significantly superior overall the antagonists tested. The second highest reduction in mycelial growth was recorded for isolates Th1 *(*77.66%) followed by *T. virens* (73.88%), *T. viride* (73.11%) isolates Th6 (71.11%), Th8 (70.88%) and Th2 (70.33%). Further the best in inhibiting the mycelial growth of fungus was isolates Th5 *(*68.11%), Th9 (67.11%), *T. hamatum (*64.66%), Th4 (64.11%), Th3 (57.11%), *T. pseudokoningii* (55.77%), Th7 (54.77%) and Th10 (50.22%).

Table 4.14. *In vitro* evaluation of antagonists against *C. gloeosporioides* in dual culture technique.

Aantagonists	Sources of antagonists	% inhibition of mycelial growth ($\overline{X}$ ± SE)
Th1	Soil	77.66 ± 0.23
Th2		70.33 ± 0.23
Th3		57.11 ± 0.26
Th4		64.33 ± 0.23
Th5		68.11 ± 0.26
Th6		71.11 ± 0.26
Th7		54.77 ± 0.32
Th8		70.88 ± 0.26
Th9		67.11 ± 0.26
Th10		50.22 ± 0.22
T. harzianum	Plant pathology Lab, RU	80.22 ± 0.22
T. virens		73.88 ± 0.26
T. viride		73.11 ± 0.26
T. hamatum		64.66 ± 0.23
T. pseudokoningii		55.77 ± 0.22
LSD value at 5%	-	0.757

*Th = Isolates of *T. harzianum*

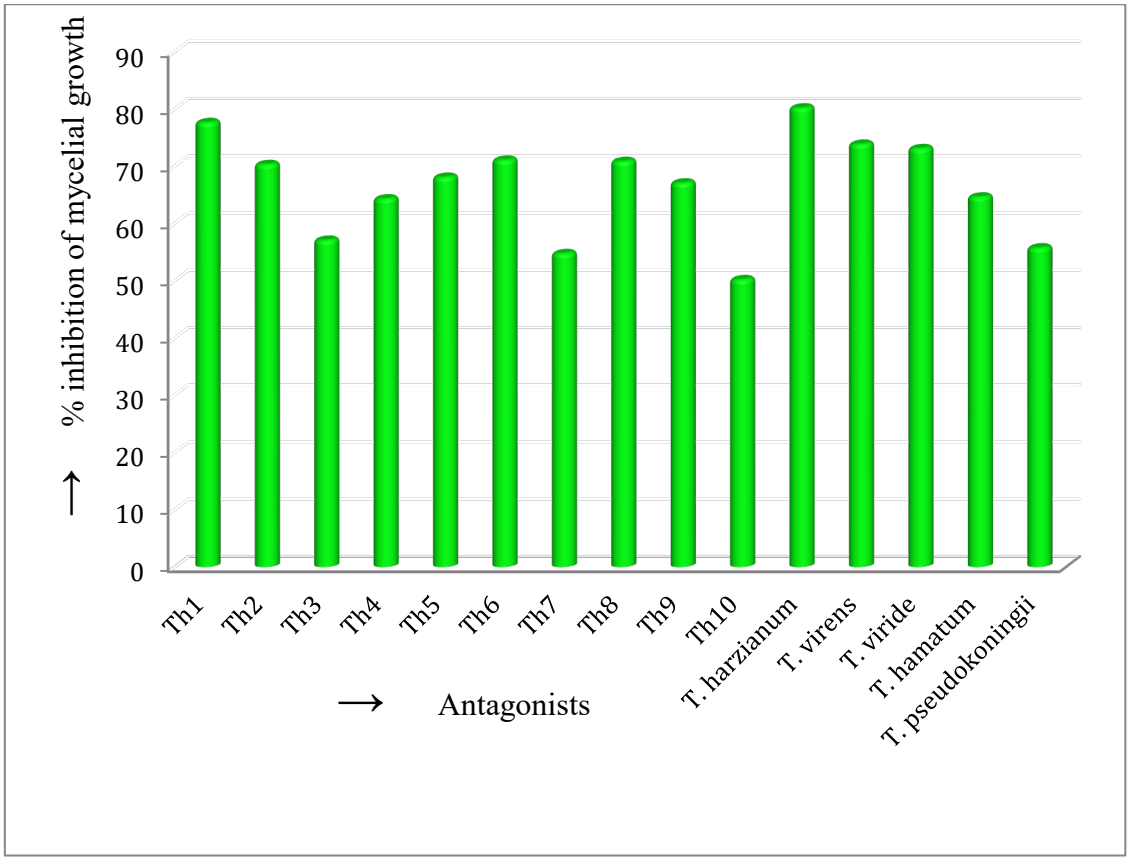


Fig. 4.18: Mycelial growth inhibition (%) of *C. gloeosporioides* in PDA medium containing different antagonists by dual culture technique.

PLATE 4.12: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different antagonists by dual culture .

A. Upper line (from left to right): Th1, Th2 and Th3 antagonists.

Lower line (from right to left): Th4, Th5 antagonists and control.

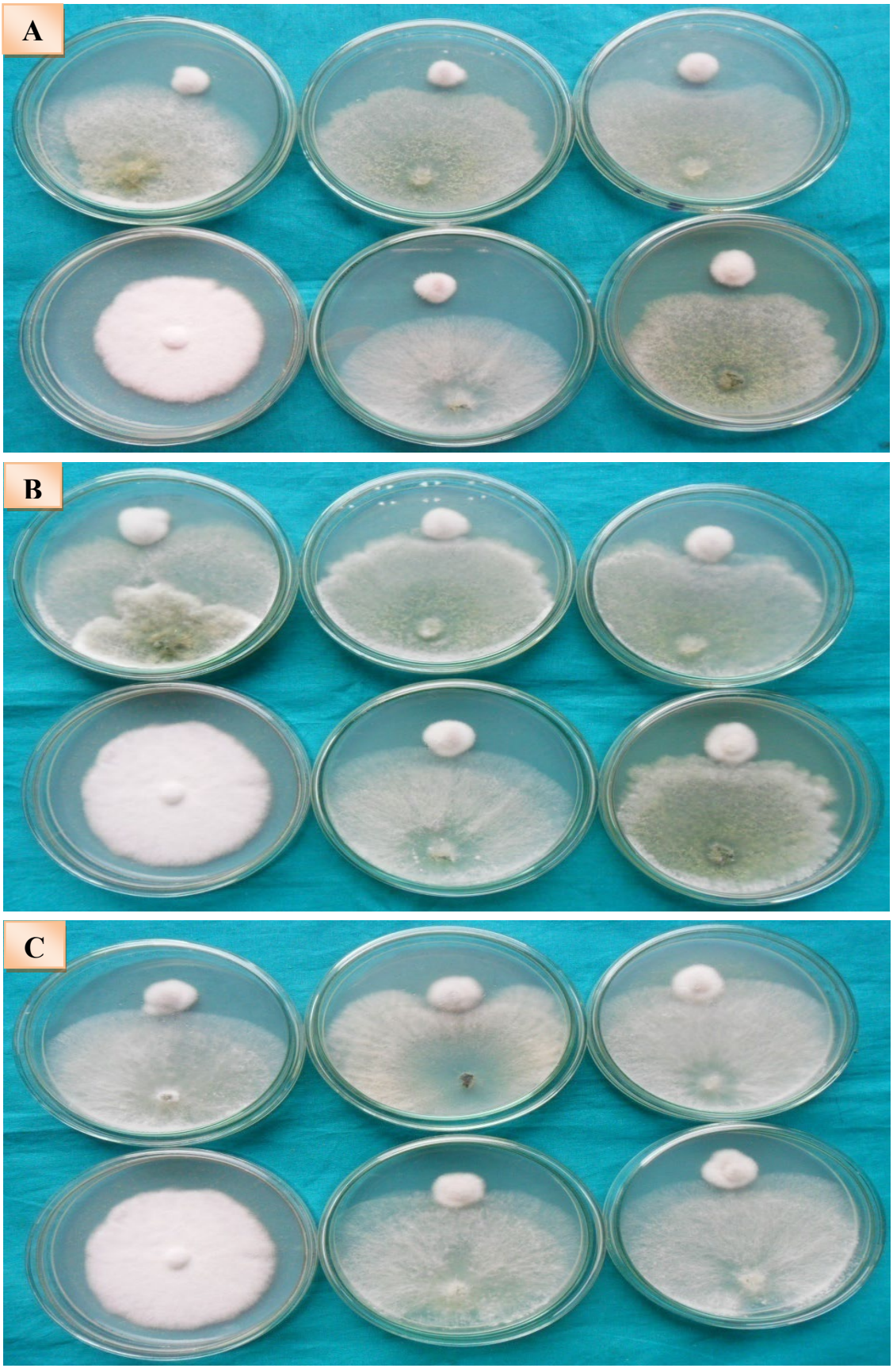
B. Upper line (from left to right): Th6, Th7 and Th8 antagonists.

Lower line (from right to left): Th9, Th10 antagonists and control.

C. Upper line (from left to right): T. harzianum, T. virens and T. viride antagonists.

Lower line (from right to left): *T. hamatum*, *T. pseudokoningii* and control.

PLATE 4.12



4. 2.16. *In vitro* evaluation of antagonists against *C. gloeosporioides* in culture filtrate

The results on this experiment are shown in **Table 4.15, Fig. 4.19** and **Plate 4.13.** 15 antagonists were produced inhibitory substances which are evident from the growth inhibition of the tested pathogen. Among these antagonists, *T. harzianum* proved to be the most effective antagonist inhibiting the growth of the pathogen by 85.22% and significantly higher compared to the other antagonists. The next best in inhibiting the mycelial growth of fungus was isolates Th1 (82.44%) followed by Th6 (81.11%), Th8 (79.78%) and *T. virens* (79.33%). The antagonist's isolate Th8 and *T. virens* revealed equal results statistically. Further the best in inhibiting the mycelial growth of fungus was isolate Th2 (77.22%), *T. viride* (76.22%), isolates Th5 (76.00%), Th9 (73.55%), Th4 (71.11%), Th7 (68.00%) *T. hamatum* (64.22%), isolate Th3 (62.11%) and *T. pseudokoningii* (61.00%). The antagonists *T.viride* and isolate Th5 revealed equal results statistically. The lowest effective antagonistic organism was isolate Th10 which inhibited the growth of the pathogen by 57.55%.

Table 4.15. *In vitro* evaluation of antagonists in culture filtrate against *C. gloeosporioides.*

Name of antagonists	Sources of antagonists	% inhibition of mycelial growth of *C. gloeosporioides.* ($\overline{X}$ ± SE)
Th1		82.44 ± 0.29
Th2		77.22 ± 0.27
Th3		62.11 ± 0.26
Th4		71.11 ± 0.26
Th5		76.00 ± 0.28
Th6	Soil	81.11 ± 0.26
Th7		68.00 ± 0.40
Th8		79.88 ± 0.26
Th9		73.55 ± 0.24
Th10		57.55 ± 0.24
T. harzianum	Plant Pathology	85.22 ± 0.22
T. virens	Lab, RU	79.33 ± 0.23
T. viride		76.22 ± 0.22
T. hamatum		64.22 ± 0.22
T. pseudokoningii		61.00 ± 0.28
LSD value at 5 %	-	0.799

*Th = *T. harzianum* isolates of soil

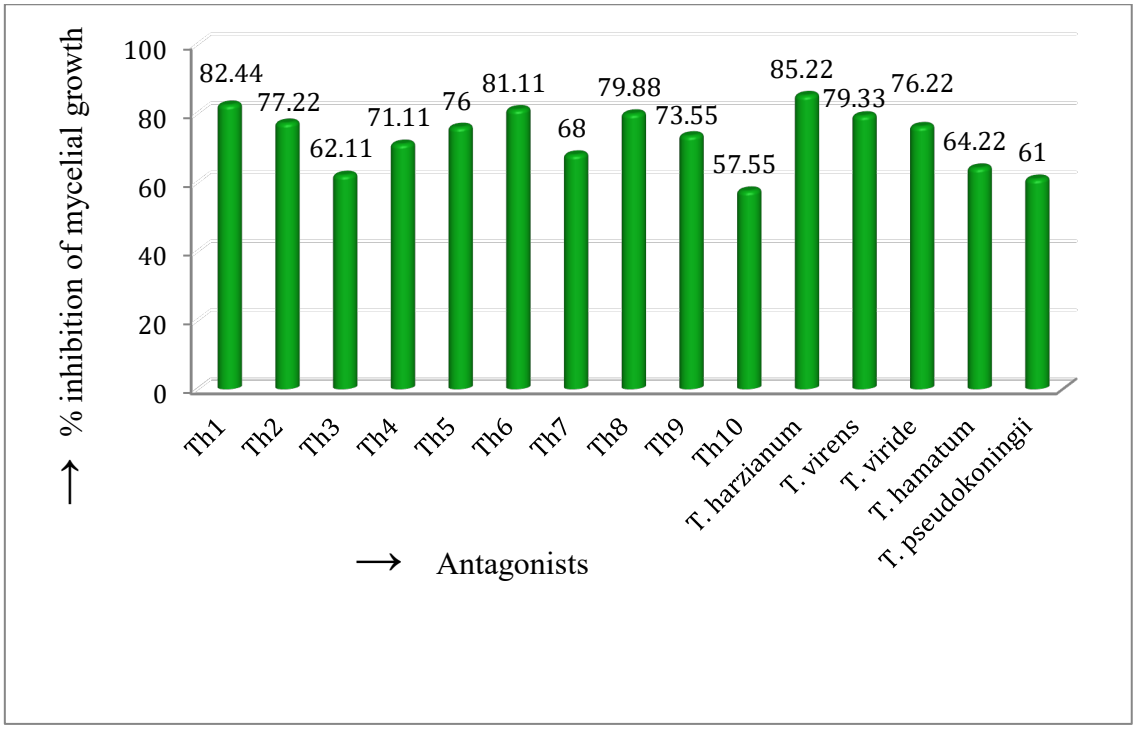
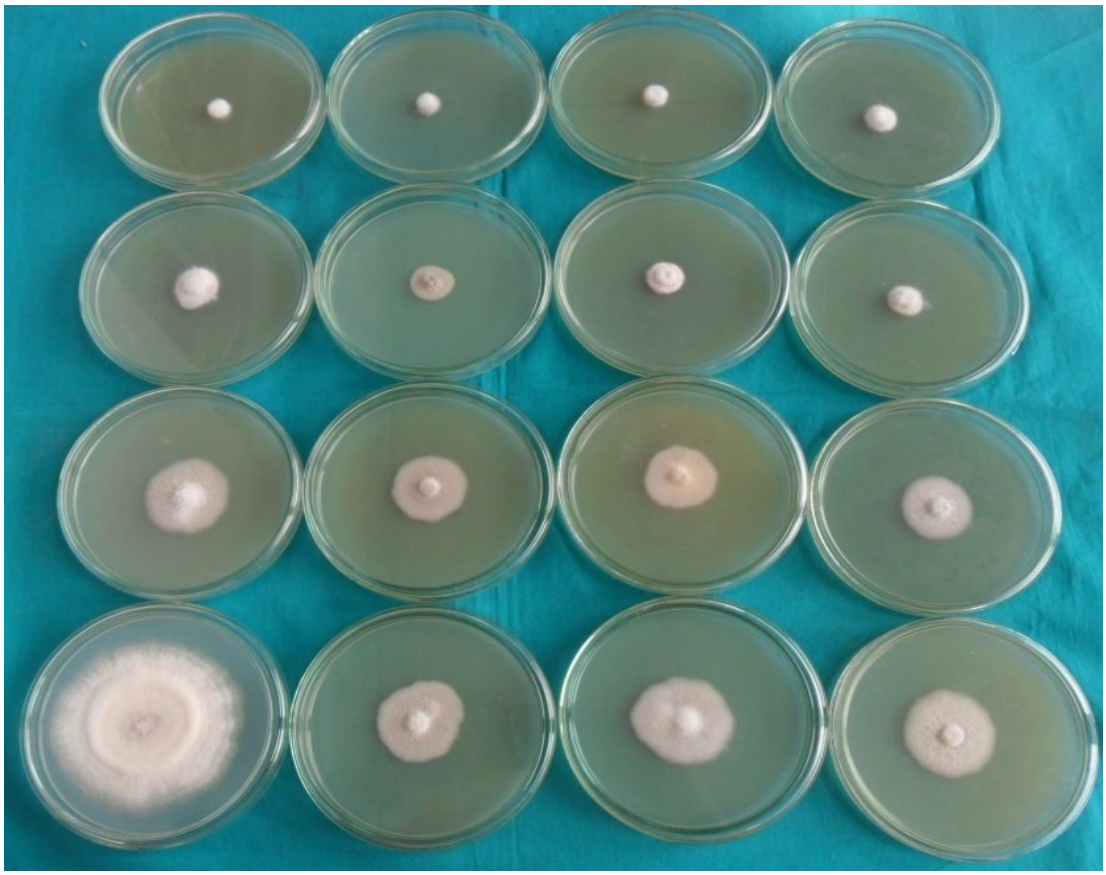


Fig. 4.19: Mycelial growth inhibition (%) of *C. gloeosporioides* in PDA medium containing different antagonists by culture filtrate technique.

PLATE 4.13



Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different antagonists by culture filtrate technique.

1st line (from left to right): *T. harzianum*, Th1, Th6 and Th8 antagonists.

2nd line (from left to right): *T. virens*, Th2, *T. viride* and Th5 antagonists.

3rd line (from left to right): Th9, Th4, Th7 and *T. hamatum* antagonists.

4th line (from right to left): Th3, *T. pseudokoningii,* Th10 antagonists and control.

4.2.17. *In vitro* evaluation of fungicides against *C. gloeosporioides*

Screening of fungicides was done against *C. gloeosporioides* under laboratory condition by following poisoned food. Data with respect to inhibition of mycelial growth at five concentrations of fungicides were recorded and percent inhibition is presented in **Table 4.16, Fig. 4.20** and **Plate 4.14 - 4.15.**

From the mean results of five concentrations of fungicides it is revealed that fungicides, concentrations and their interaction differed significantly with respect to inhibition of the mycelial growth of *C. gloeosporioides.*

Among 13 fungicides, maximum inhibition of mycelial growth was found in Bavistin (98.17%) and Folicur (98.04%) which were significantly superior to all other fungicides. It was followed by Tilt (86.17%), Hayconazole (85.97%), Score (81.44%), Rovral (64.64%) and Ridomil (50.97%). The lowest inhibition of fungus was recorded in Cupravit (14.42%).

Among the tested five concentrations, 1000 ppm concentration of all fungicides was significantly superior to 500, 250, 100 and 50 ppm concentrations. In this concentration 100% inhibition of mycelial growth of the fungus was recorded in Bavistin, Folicur, Score and Hayconazole followed by Tilt (90.11%), Rovral (80.22%) and Ridomil (63.22%).

At 500 ppm concentration, 100% inhibition of mycelial growth of the fungus was recorded in Bavistin and Folicur followed by Hayconazole (90.22%), Tilt (89.33%) and Score (85.11%) remained on par with each other. The lowest mycelial growth inhibition was recorded in Cupravit (17.88%).

At 250 ppm concentration, 100% inhibition of mycelial growth of the fungus was recorded in Bavistin and Folicur. The lowest inhibition of mycelial growth was recorded in Cupravit (13.77%). Further Tilt, Hayconazole and Score were on par with each other.

At 100 ppm concentration, 100% inhibition of mycelial growth of the fungus was recorded in Bavistin and Folicur followed by Tilt (84.00%), Hayconazole (81.11%) and Score (74.66%). The lowest inhibition of mycelia growth was recorded in Cupravit (12.00%).

At 50 ppm concentration, maximum percent inhibition of mycelial growth of the fungus was recorded in Bavistin (90.88%) and Folicur (90.22%). The lowest inhibition of mycelial growth was recorded in Cupravit (8.66%).

Table 4.16. *In vitro* evaluation of different fungicides against mycelial growth of *C. gloeosporioides.*

Fungicides	% inhibition of mycelial growth at concentrations (ppm)					
	50	100	250	500	1000	Mean
Bavistin	90.88 ±0.26	100	100	100	100	98.17±0.05
Rovral	53.66 ±0.23	56.88±0.26	64.77±0.22	70.88±0.26	80.22±0.22	64.64±0.24
Cupravit	8.66 ±0.23	12.00±0.28	13.77±0.27	17.88±0.26	20.55±0.24	14.42±0.25
Score	71.88 ±0.26	74.66±0.23	80.44±0.24	85.11±0.26	100.00	81.44±0.19
Tilt	81.00 ±0.28	84.00±0.28	86.66±0.23	89.33±0.16	90.11±0.26	86.17±0.24
Antracol	24.77 ±0.22	28.00±0.28	31.11±0.26	34.11±0.20	34.77±0.22	30.51±0.23
Folicur	90.22 ±0.22	100	100	100	100	98.04±0.04
Ridomil	42.11 ±0.26	46.66±0.33	50.22±0.22	55.33±0.16	63.22±0.22	50.97±0.23
Dithane	17.77 ±0.27	20.22±0.22	26.11±0.20	30.44±0.17	33.11±0.20	25.33±0.21
Hayconazole	77.11 ±0.26	81.11±0.26	84.66±0.23	90.22±0.22	100	85.97±0.19
Secure	26.00 ±0.23	30.44±0.17	35.22±0.22	41.22±0.22	45.77±0.22	35.44±0.21
Thiovit	20.22 ±0.22	22.44±0.29	25.77±0.22	26.77±0.27	29.88±0.26	24.82±0.25
Sulcox	25.33 ±0.16	34.55±0.24	41.22±0.22	44.22±0.22	47.33±0.33	38.35±0.23
LSD at 5 %				0.675		

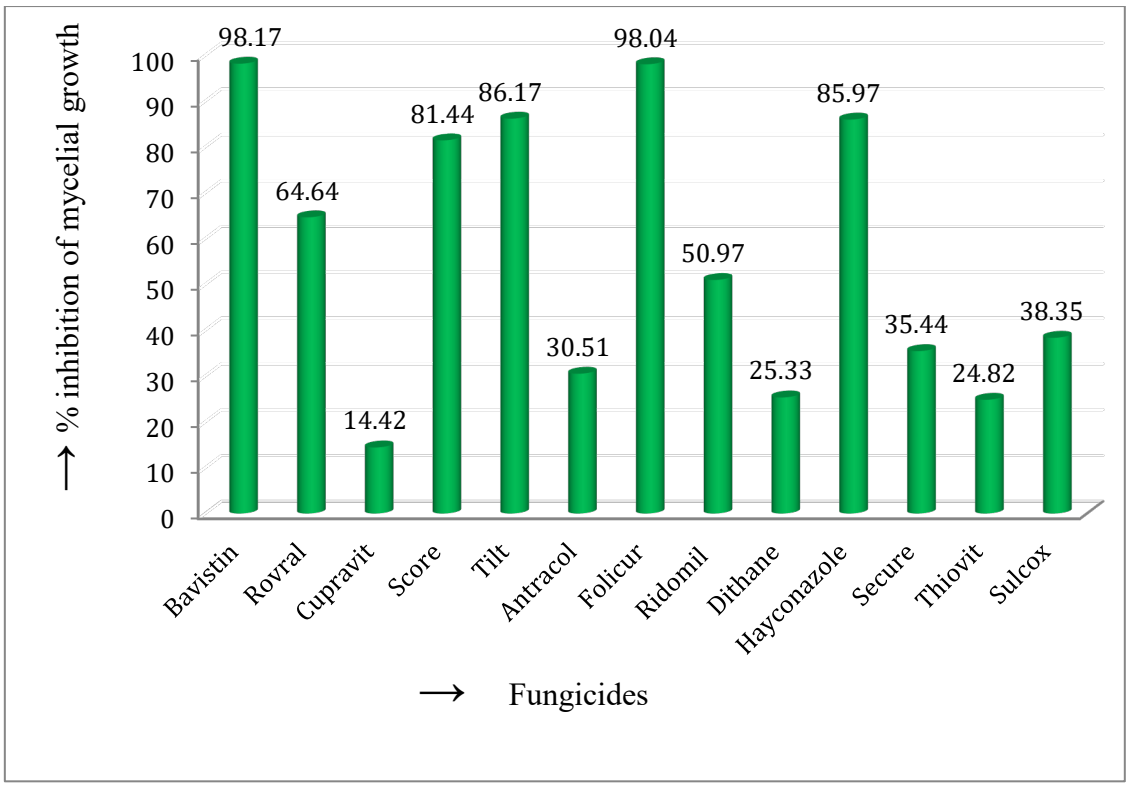


Fig. 4.20: The graphs are mean of mycelial growth inhibition (%) of *C. gloeosporioides* at five concentrations of different fungicides.

PLATE 4.14: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of fungicides.

A. Bavistin:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

B. Rovral:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

C. Cupravit:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

D. Score:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

E. Tilt:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

F. Antracol:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

PLATE 4.14

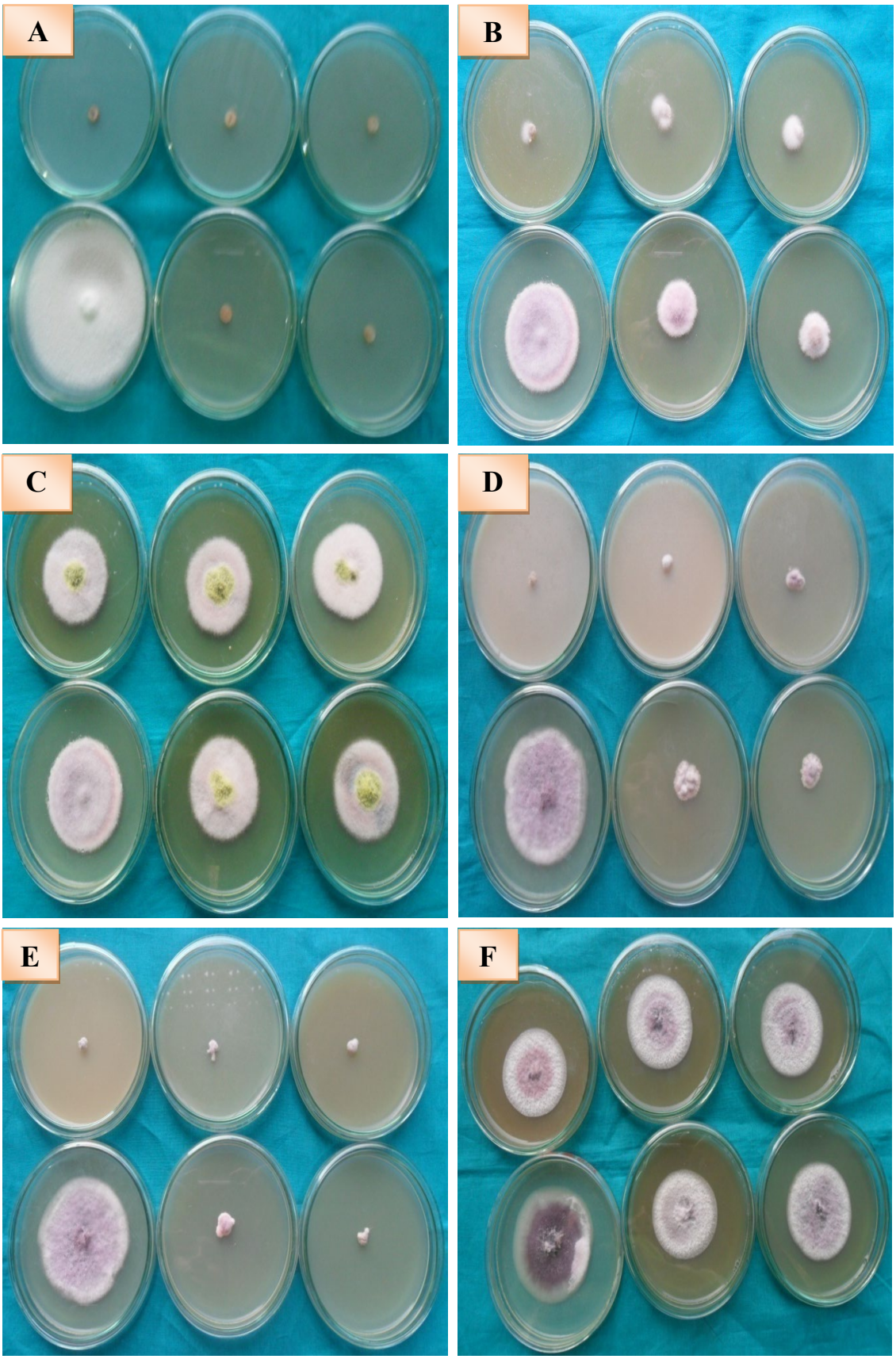


PLATE 4.15: Photographs showing mycelial growth of *C. gloeosporioides* in PDA medium containing different concentrations of fungicides.

A. Folicur:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

B. Ridomil:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

C. Cupravit:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

D. Secure:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

E. Hayconazole:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

F. Thiovit:

Upper line (from right to left): 100, 50 ppm concentrations and control.

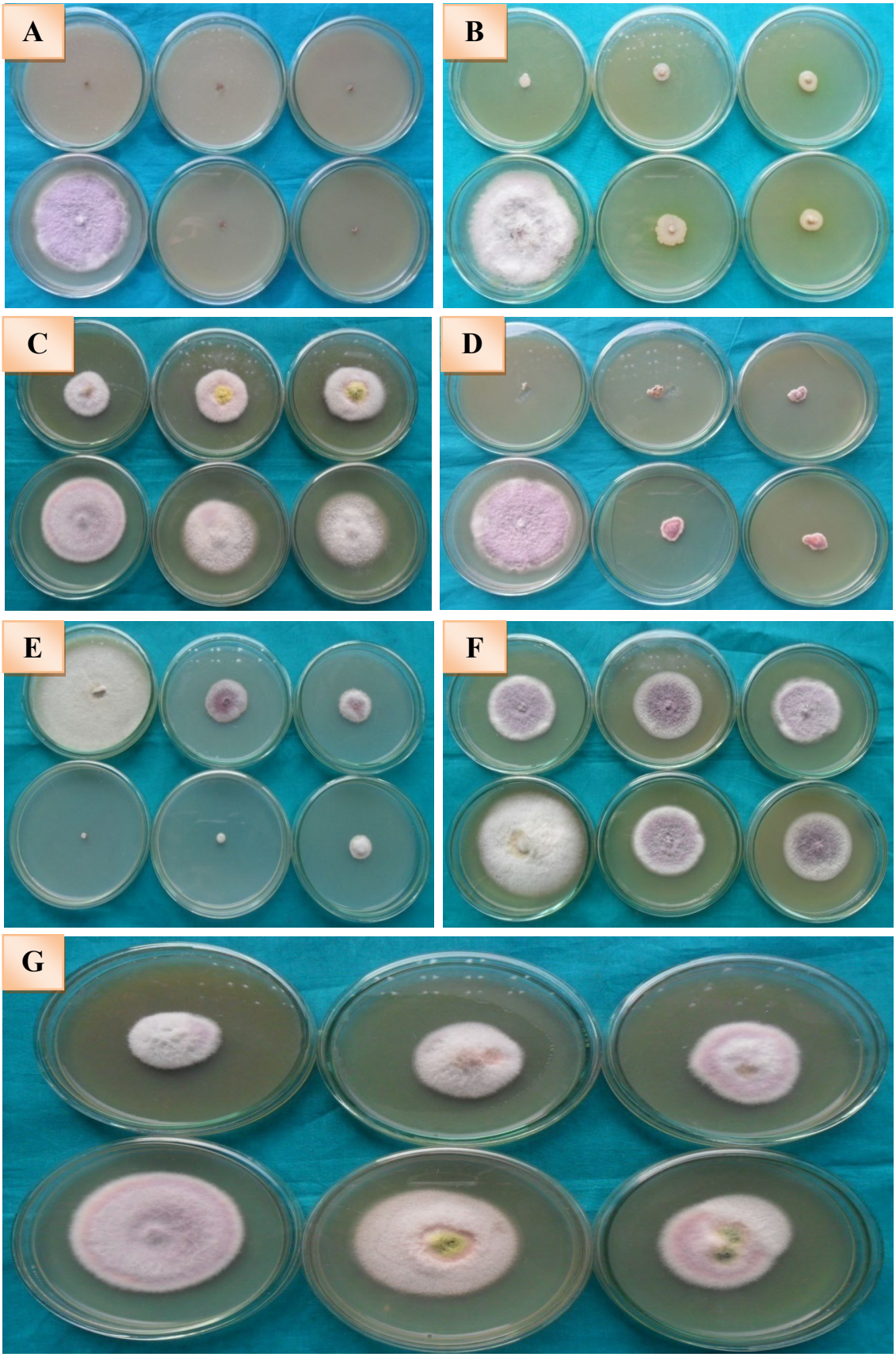
Lower line (from left to right): 1000, 500 and 250 ppm concentrations.

G. Sulcox:

Upper line (from left to right): 1000, 500 and 250 ppm concentrations.

Lower line (from right to left): 100, 50 ppm concentrations and control.

PLATE 4.15



4.2.18. Field evaluation of plant extracts, cow urine and antagonists against crown rot disease of strawberry

Five plant extracts (Garlic, Neem, Sajna, Mehedi and Apang), two antagonists and cow urine were selected for field evaluation. In the field condition, all the treatments were found significantly superior to the control in reducing the incidence of crown rot in the both years. Pooled data of two years were collected from each treatment. The results on this experiment are described under separate heads**:**

Disease incidence

In the field trials, no crown rot disease incidence was recorded for garlic, mehedi and *T. harzianum* treated plants **(Table 4.17** and **Fig. 4.21 - 4.22)**. The little disease incidence was noticed in neem (10.22%) and sajna (16%) leaf extracts treated plants as compared to 62.88% in the control. It was followed by apang leaf extract (19.88%) and Th1 isolates (20.33%) treated plants. Among the treatments, cow urine confined the highest crown rot incidence (25.88%).

Table 4.17. Crown rot disease incidence in plant extracts, cow urine and antagonists treated plants under field conditions.

Treatments	Concentrations (%)	% crown rot disease incidence ($\overline{X} \pm SE$)	% disease decrease than that of control
Garlic	25	0.0	100.00
Neem	25	10.22 ± 0.23	83.74
Sajna	25	16.00 ± 0.26	74.55
Mehedi	25	0.0	100.00
Apang	25	19.88 ± 0.23	68.38
Cow urine	25	25.88 ± 0.26	58.84
Th1	5×10^5 spores/ml	20.33 ± 0.22	67.66
T. harzianum	5×10^5 spores/ml	0.0	100.00
Control	-	62.88 ± 0.21	0.0
LSD value at 5 %	-	0.564	-

Th1=Soil isolate.

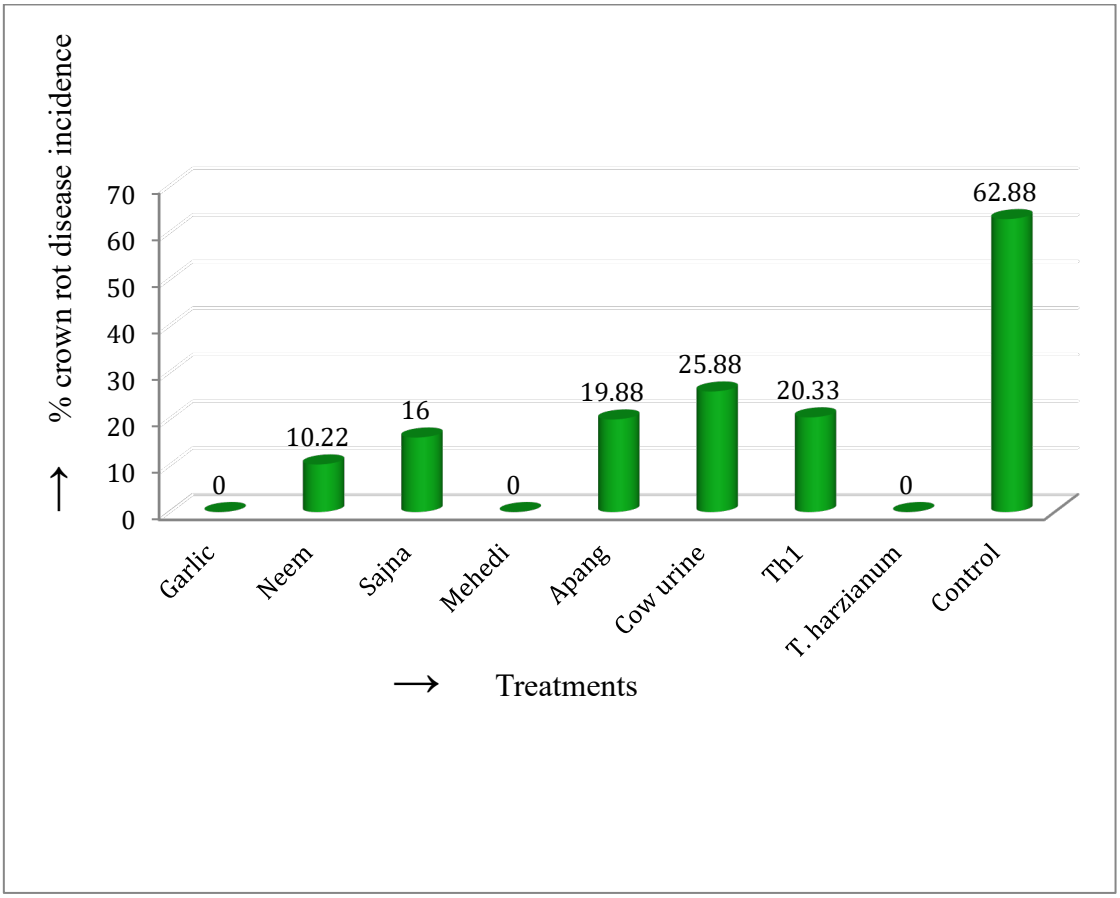


Fig. 4.21: Crown rot disease incidence in different treatments.

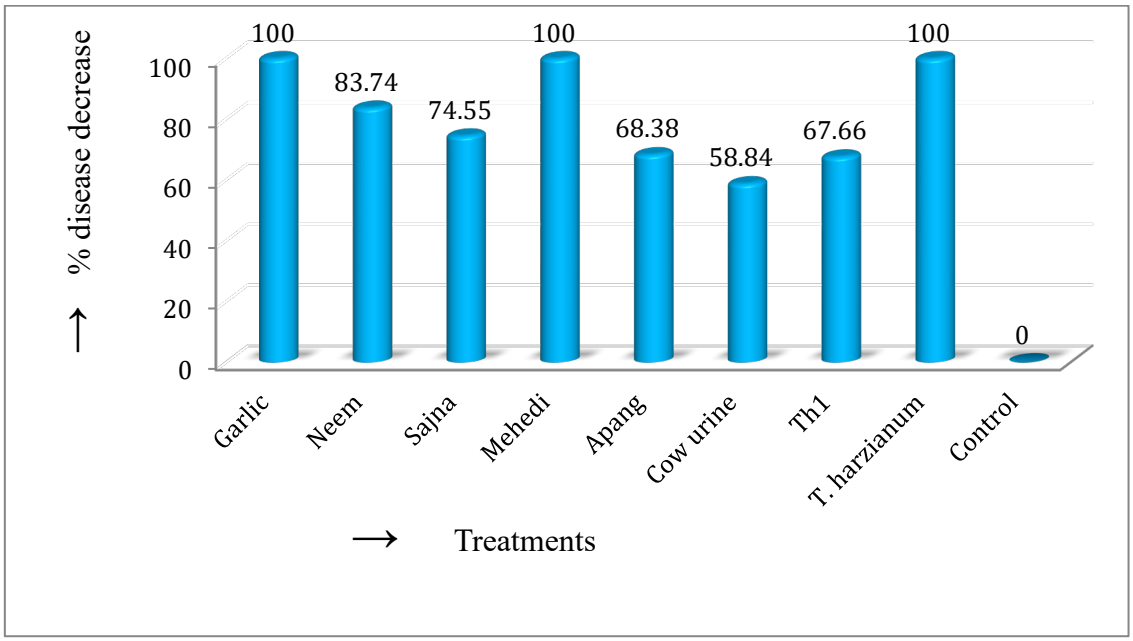


Fig. 4.22: % of crown rot disease decrease in different treatments than that of control.

Canopy size per plant

The results obtained are presented in **Table 4.18** and **Fig. 4.23 - 4.24.** All the treatments were found significantly superior to the control in increasing the canopy size of strawberry plants in the both years. The highest canopy size 534.64 cm^2 was obtained in plots where the treatment *T. harzianum* was applied followed by the treatments garlic bulb extract (523.98 cm^2), neem (514.07 cm^2) and sajna leaf extracts (509.82 cm^2) as compared to 233.24 cm^2 per plant in the control. The next canopy size was observed in apang leaf extract (433.05 cm^2) which was followed by the treatments Th1 isolate (405.35 cm^2) and cow urine (389.84 cm^2). The lowest canopy size (315.48 cm^2) was found in mehedi leaf extract treated plants than that of other treatments.

Table 4.18. Canopy size of plant extracts, cow urine and antagonists treated plants under field conditions.

Treatments	Concentrations (%)	Canopy size (cm^2) per plant ($\overline{X} \pm SE$)	% increase canopy size than that of control
Garlic	25	523.98 ± 6.98	124.65
Neem	25	514.07 ± 2.61	120.40
Sajna	25	509.82 ± 1.33	118.58
Mehedi	25	315.48 ± 1.38	35.25
Apang	25	433.05 ± 1.13	85.66
Cow urine	25	389.84 ± 2.88	67.14
Th1	5×10^5 spores/ml	405.35 ± 2.70	73.79
T. harzianum	5×10^5 spores/ml	534.64 ± 4.68	129.22
Control	-	233.24 ± 3.70	0.0
LSD value at 5 %	-	9.935	-

Th1=Soil isolates

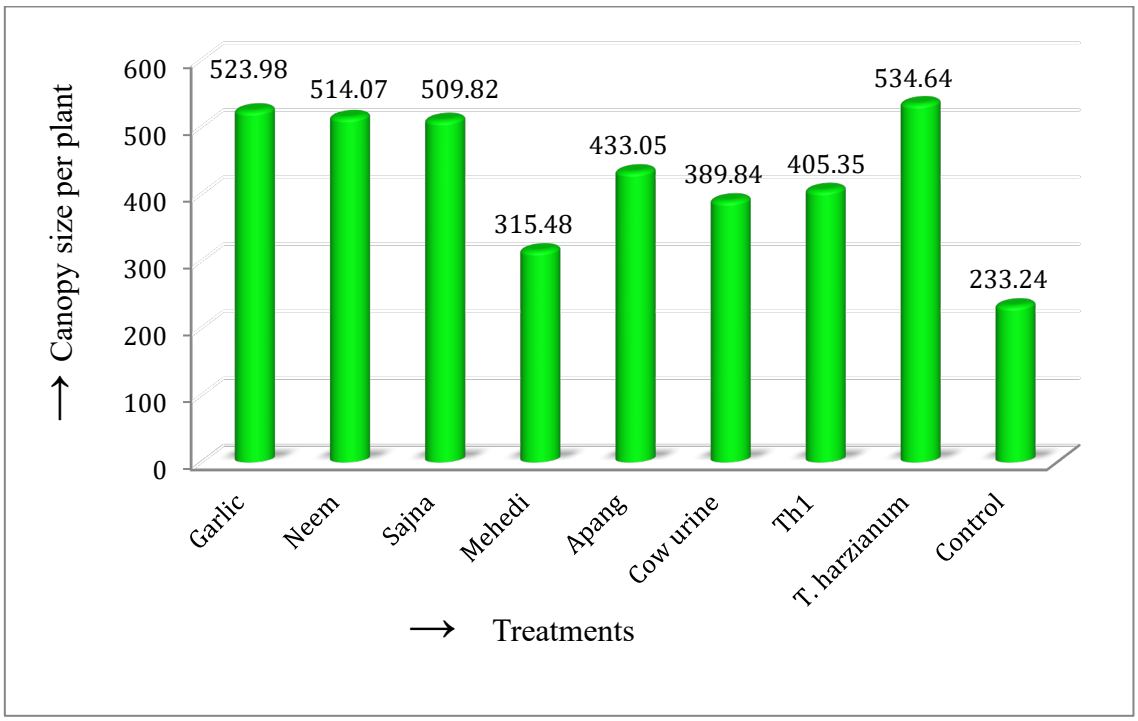


Fig. 4.23: Canopy size (cm^2) per plant in different treatments.

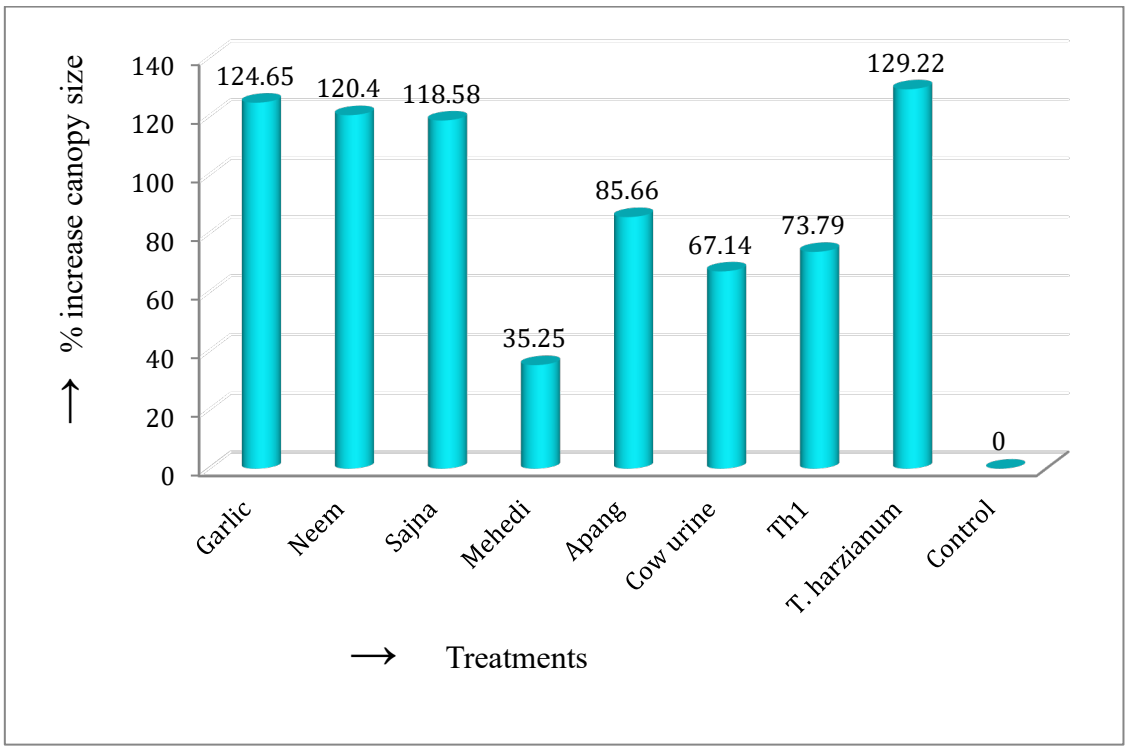


Fig. 4.24: % of canopy size (cm^2) increase in different treatments than that of control.

Number of fruits per plant

From the **Table 4.19** and **Fig. 4.25 - 4.26** it appears that *T. harzianum* treated plants exhibited the highest number of fruits (11.12) per plant as compared to 5.33 in the control plants. It was followed by garlic bulb extract (10.88), neem (10.33), sajna (9.55) and apang leaf extracts (9.20) treated plants. The number of fruits per plant 9.18 and 8.80 was recorded in cow urine and Th1 isolate treated plants respectively. The lowest number (6.28) of fruits per plant was observed in mehedi leaf extract treated plants which was statistically different from the other treatments. All the treatments were found significantly superior to the control plants in increasing the number of fruits per plant of strawberry in the both years.

Table 4.19. Efficacy of plant extracts, cow urine and antagonists on the number of fruits per plant against crown rot disease of strawberry under field conditions.

Treatments	Concentrations (%)	Number of fruits per plant ($\overline{X}$ ± SE)	% fruits number increase than that of control
Garlic	25	12.62 ± 0.87	92.08
Neem	25	12.32 ± 0.67	87.51
Sajna	25	11.46 ± 1.29	74.42
Mehedi	25	8.28 ± 1.19	26.02
Apang	25	11.20 ± 1.29	70.47
Cow urine	25	10.18 ± 1.12	54.94
Th1	5×10^5 spores/ml	10.80 ± 1.01	64.38
T. harzianum	5×10^5 spores/ml	13.12 ± 1.03	99.69
Control	-	6.57 ± 0.24	0.0
LSD value at 5 %	-	0.949	-

Th1=Soil isolate.

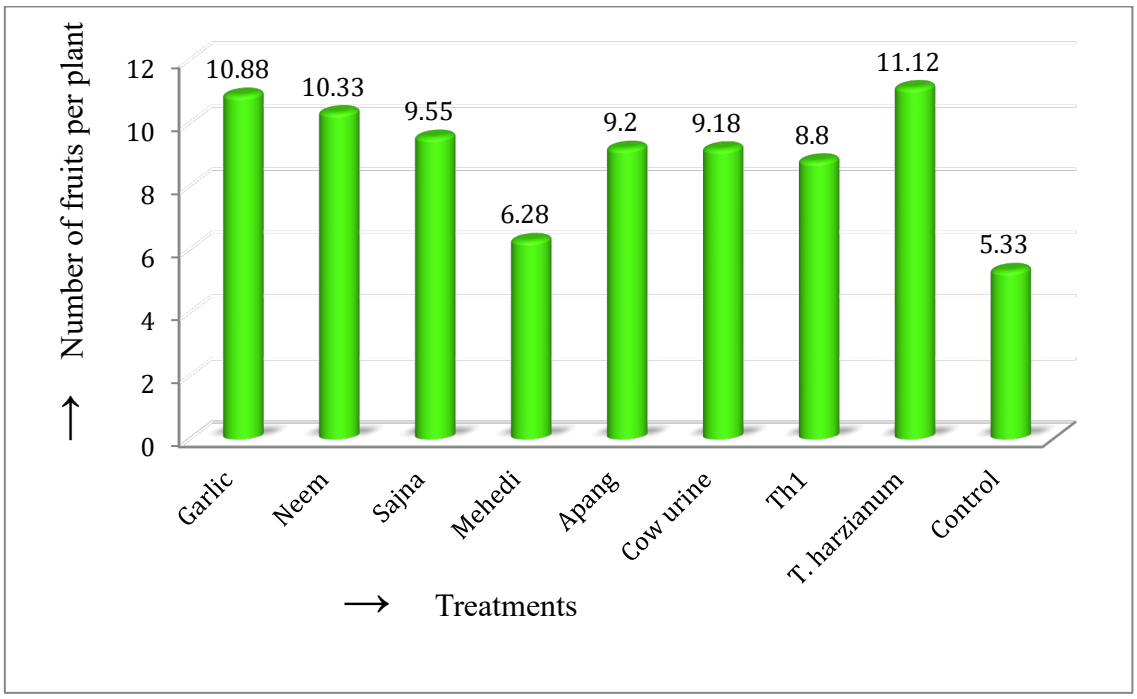


Fig. 4.25: Number of fruits per plant in different treatments.

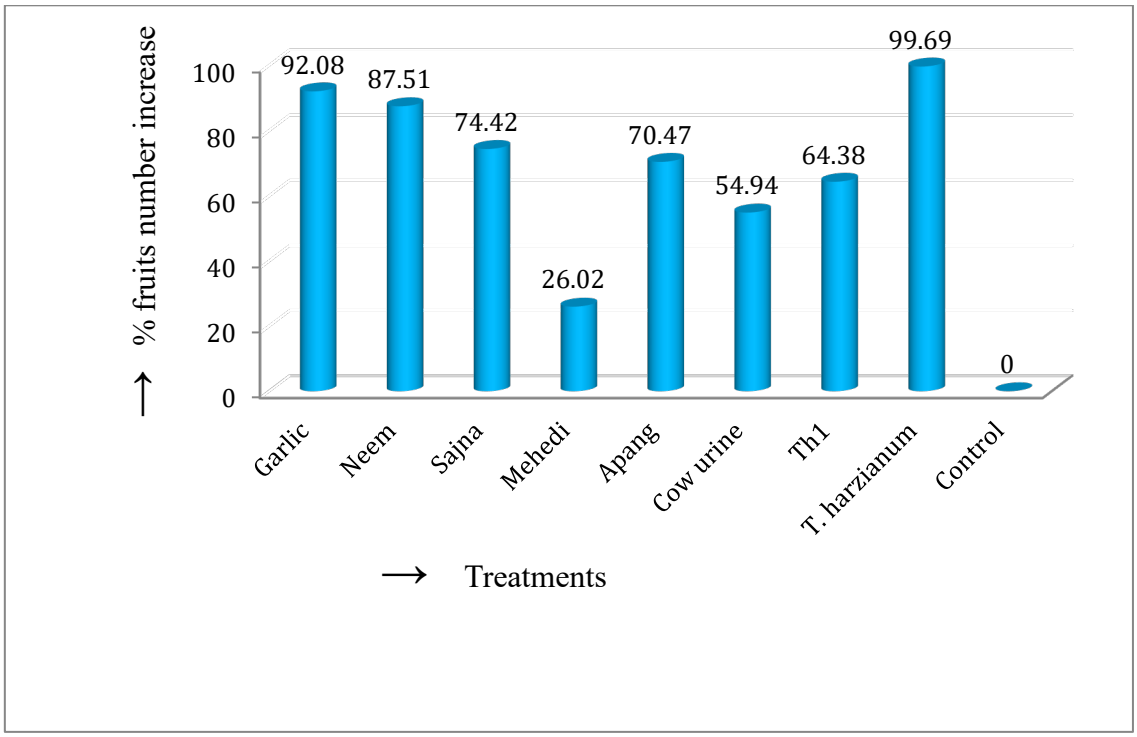


Fig. 4.26: % of fruit number increase in different treatments than that of control.

Fruit weight (g) per plant

The summarized results on this experiment are presented in **Table 4.20** and **Fig. 4.27 - 4.28. S**ignificantly higher fruit weight per plant was noticed in treatments treated plants than that of control plants. A spray with *T. harzianum* resulted in the highest fruit weight per plant of 151.53 g as compared to 63.88g in the control. It was followed by garlic bulb extract (149.29 g), neem (145.99 g), sajna (139.22 g) and apang leaf extracts (136.34 g). The fruit weight per plant 129.41 g and 123.96 g was recorded in cow urine and Th1 isolate treated plants respectively. The lowest fruit weight was observed in mehedi leaf extract treated plants (80.00 g) which was statistically different from the other treatments.

Table 4.20. Fruit weight (g) of plant extracts, cow urine and antagonists treated plants under field conditions.

Treatments	Concentrations (%)	Fruit weight (g) per plant ($\overline{X} \pm SE$)	% yield increase than that of control
Garlic	25	149.29 ± 0.76	133.70
Neem	25	145.99 ± 0.63	128.53
Sajna	25	139.22 ± 0.93	118.03
Mehedi	25	80.00 ± 0.97	25.23
Apang	25	136.34 ± 0.72	113.43
Cow urine	25	129.41 ± 0.65	102.58
Th1	5×10^5 spores / ml	123.96 ± 0.61	94.05
T. harzianum	5×10^5 spores/ ml	151.53 ± 76	137.21
Control	-	63.88 ± 0.26	0.0
LSD value at 5 %	-	1.187	-

Th1=Soil isolate

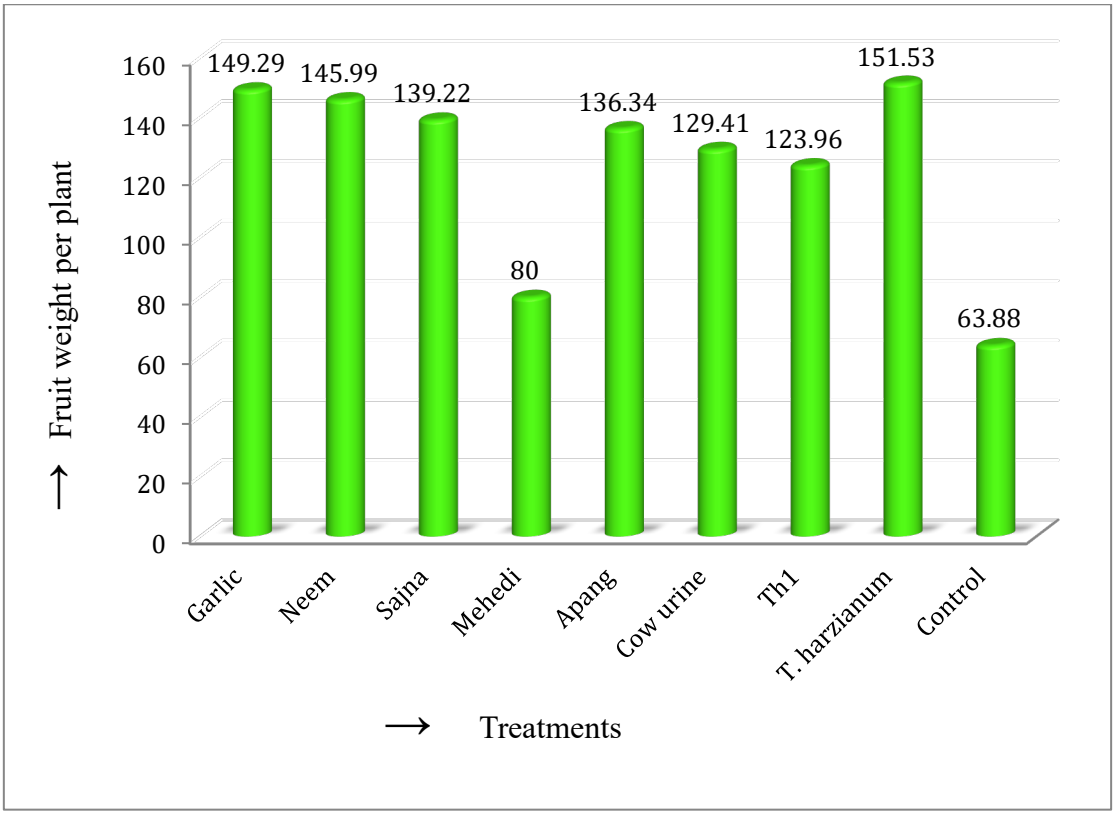


Fig. 4.27: Fruit weight (g) per plant in different treatments.

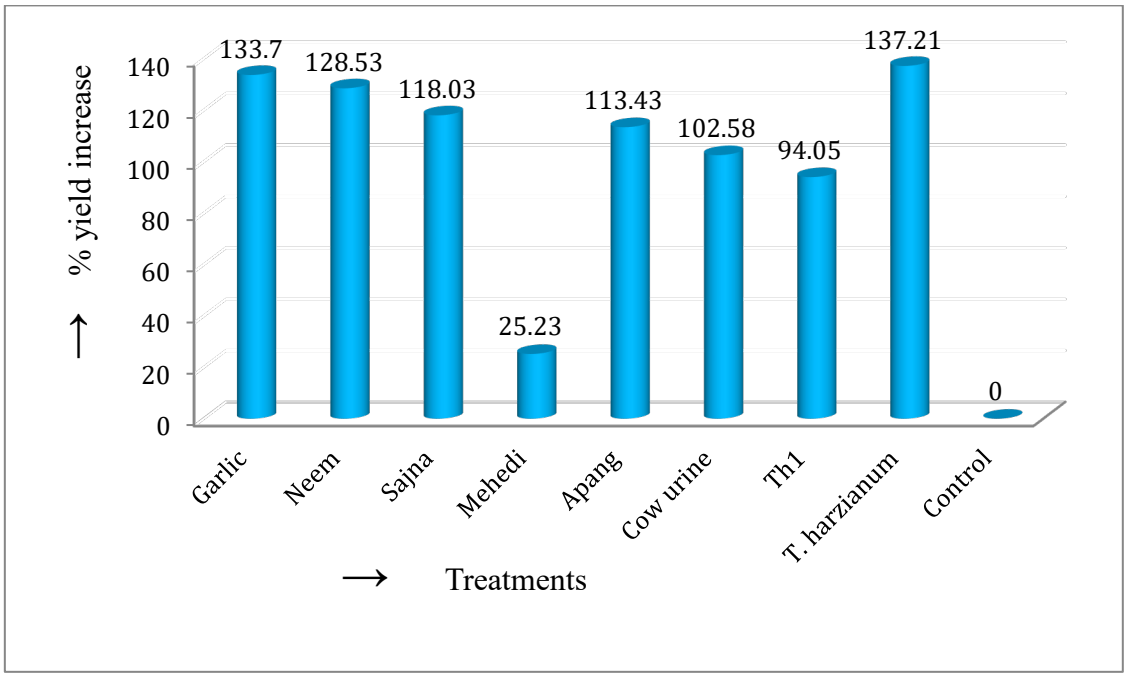


Fig. 4.28: % of yield increase in different treatments than that of control.

4.2.19. Field evaluation of fungicides against crown rot disease

13 fungicides - Bavistin DF (Carbendazim), Rovral 50WP (Eprodion), Cupravit 50WP (Copper oxychloride), Score 250EC (Fenoconazole), Tilt 250EC (Propiconazole), Antracol 70WP (Propeneb), Folicur 50EC (Tetraconazole), Ridomil 68WG (Metalexil + Mancozeb), Dithane M-45 (Mancozeb), Hayconazole 5EC (Hexaconazole), Secure 600WG (Fenamidon + Mancozeb), Thiovit 80WG (Sulfur) and Sulcox 50WP (Copper oxychloride) were used for field evaluation. In the field condition, all the fungicides were found significantly superior to the control in reducing the incidence of crown rot disease in the both years. Pooled data of two years were collected from each treatment.

Disease incidence

The results on this experiment are presented in **Table 4.21** and **Fig. 4.29 - 4.30.** In the field trials, no crown rot disease incidence was recorded for Bavistin treated plants. The little disease incidence was recorded for Folicur (3.11%), Score (6.22%), Hayconazole (9.66%), Dithane (10.00%) and Tilt (10.22%) treated plants as compared to 62.88% in the control. It was followed by Ridomil (12.88%), Secure (16.22%) and Rovral (19.88%) treated plants. Among the tested fungicides, Cupravit confined the highest crown rot incidence (35.77%). Other three fungicides - Antracol, Thiovit and Sulcox were showed 20.33, 25.88 and 30.33% crown rot incidence respectively.

Table 4.21. Crown rot disease incidence in fungicides treated plants under field conditions.

Treatments	Concentrations (ppm)	% crown rot disease incidence ($\overline{X} \pm SE$)	% disease decrease than that of control
Bavistin DF	1000	0	100.00
Rovral 50WP	1000	19.88 ± 0.23	68.38
Cupravit 50WP	1000	35.77 ± 0.22	43.11
Score 250EC	1000	6.22 ± 0.22	90.10
Tilt 250EC	1000	10.22 ± 0.22	83.74
Antracol 70WP	1000	20.33 ± 0.22	67.66
Folicur 250EC	1000	3.11 ± 0.26	95.05
Ridomil 68 WG	1000	12.88 ± 0.26	79.51
Dithane M-45	1000	10.00 ± 0.23	84.09
Hayconazole 5EC	1000	9.66 ± 0.22	84.63
Secure 600WG	1000	16.22 ± 0.23	74.20
Thiovit 80WG	1000	25.88 ± 0.26	58.84
Sulcox 50WP	1000	30.33 ± 0.26	51.76
Control	-	62.88 ± 0.21	0.0
LSD value at 5 %	-	0.682	-

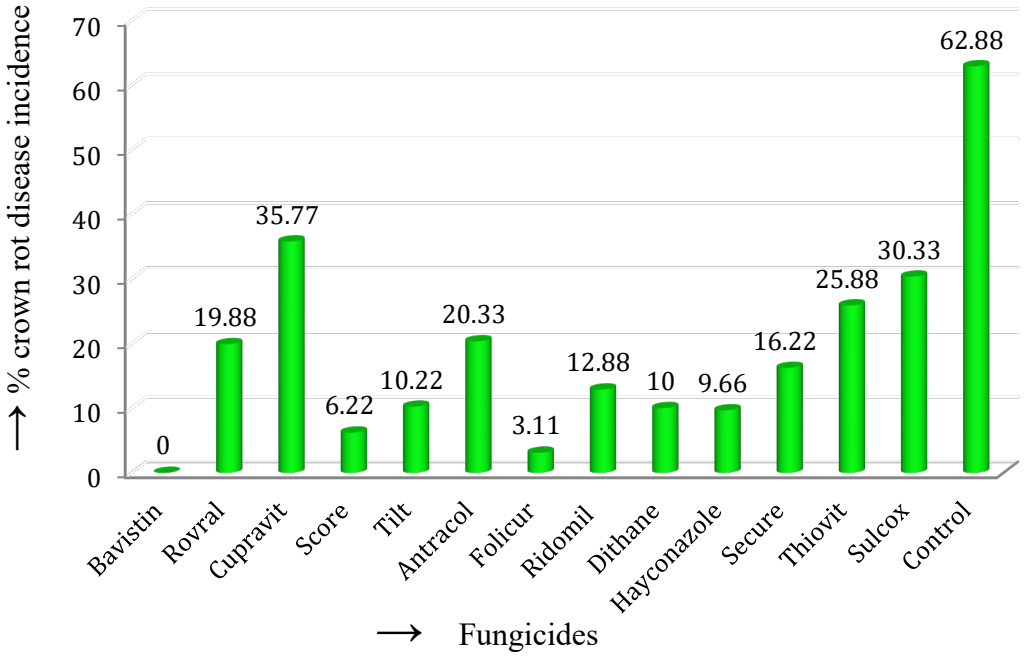


Fig. 4.29: Crown rot disease incidence in different fungicides.

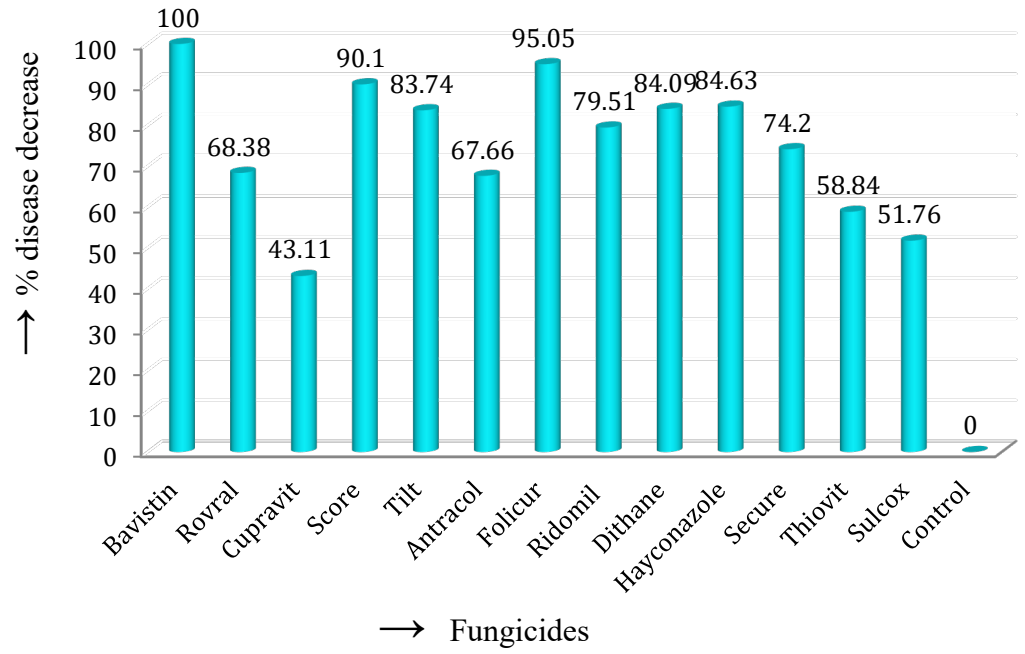


Fig. 4.30: % of crown rot disease decrease in different treatments than that of control.

Canopy size per plant

The summarized results on this experiment are presented in **Table 4.22** and **Fig. 4.31 - 4.32.** The Bavistin sprayed plot exhibited the highest canopy size of 458.11cm^2 per plant as compared to 233.24 cm^2 in the control followed by Score (426.00 cm2) and Hayconazole (384.00 cm^2) sprayed plot. The next canopy size was observed in fungicide Dithane (380.81 cm^2) which was followed by the fungicides Ridomil (360.11 cm^2), Rovral (350.33 cm^2), Tilt (347.66 cm^2), Secure (345.98 cm^2), Antracol (335.04 cm^2), Sulcox (325.66 cm^2), Thiovit (323.41 cm^2) and Cupravit (321.77 cm^2). The lowest canopy size was observed in fungicide Folicur (258.33 cm^2).

Table 4.22. Canopy size of fungicides treated plants under field conditions.

Fungicides	Concentrations (ppm)	Canopy size (cm^2) per plant ($\overline{X} \pm SE$)	% canopy size increase than that of control
Bavistin DF	1000	458.11 ± 10.59	96.41
Rovral 50WP	1000	350.33 ± 8.49	50.20
Cupravit 50WP	1000	321.77 ± 7.43	37.95
Score 250EC	1000	426.00 ± 9.58	82.64
Tilt 250EC	1000	347.66 ± 3.28	49.34
Antracol 70WP	1000	335.04 ± 4.41	43.64
Folicur 250EC	1000	258.33 ± 5.20	10.75
Ridomil 68 WG	1000	360.11 ± 4.26	54.39
Dithane M-45	1000	380.81 ± 4.72	63.26
Hayconazole 5EC	1000	384.00 ± 7.05	64.63
Secure 600WG	1000	345.98 ± 5.24	48.33
Thiovit 80WG	1000	323.41 ± 6.52	38.65
Sulcox 50WP	1000	325.66 ± 3.71	39.62
Control	-	233.24 ± 3.70	0.0
LSD value at 5 %	-	18.490	-

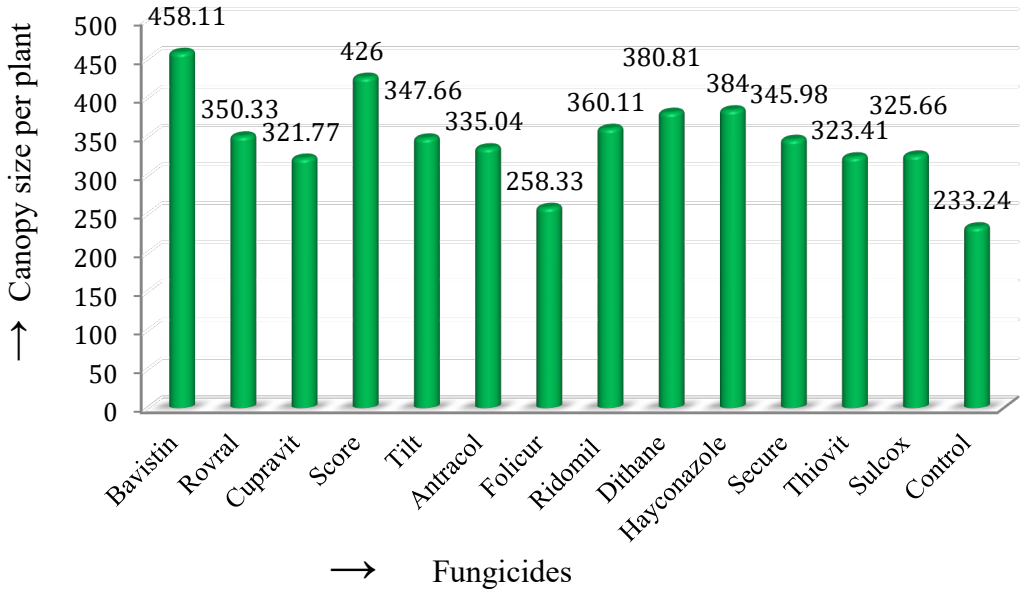


Fig. 4.31: Canopy size (cm^2) per plant in different fungicides.

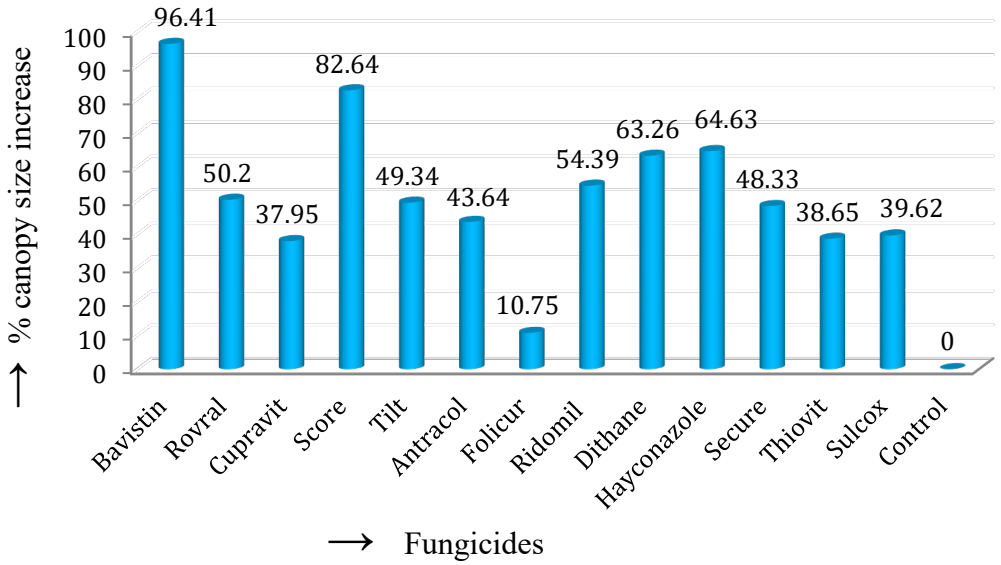


Fig. 4.32: % of canopy size (cm^2) increase in different treatments than that of control.

Number of fruits per plant

For the number of fruits per plant all the treatments were significantly superior to the control in both years **(Table 4.23** and **Fig. 4.33 - 4.34).** A spray with Bavistin resulted in the highest number of fruits per plant of 10.44 as compared to 5.33 in the control. It was followed by the fungicides Score (10.11), Hayconazole (9.88) and Dithane (9.33). Further number of fruits per plant was observed in the fungicides Ridomil (9.22), Rovral (8.88), Tilt (8.33), Secure (8.00), Antracol (7.44), Sulcox (7.22), Cupravit (6.92) and Thiovit (6.28). The lowest number of fruits per plant was observed in fungicide Folicur (6.12) treated plants.

Table 4.23. Fruits number of fungicides treated plants under field conditions.

Fungicides	Concentrations (ppm)	Number of fruits per plant ($\overline{X} \pm SE$)	% fruits number increase than that of control
Bavistin DF	1000	10.44 ± 0.29	95.87
Rovral 50WP	1000	8.88 ± 0.26	66.60
Cupravit 50WP	1000	6.92 ± 0.22	29.93
Score 250EC	1000	10.11 ± 0.26	89.68
Tilt 250EC	1000	8.33 ± 0.23	56.28
Antracol 70WP	1000	7.44 ± 0.33	39.58
Folicur 250EC	1000	6.12 ± 0.23	14.82
Ridomil 68 WG	1000	9.22 ± 0.22	72.98
Dithane M-45	1000	9.33 ± 0.23	75.04
Hayconazole 5EC	1000	9.88 ± 0.26	85.36
Secure 600WG	1000	8.00 ± 0.23	50.09
Thiovit 80WG	1000	6.28 ± 0.26	17.82
Sulcox 50WP	1000	7.22 ± 0.22	35.45
Control	-	5.33 ± 0.23	0.0
LSD value at 5 %	-	0.774	-

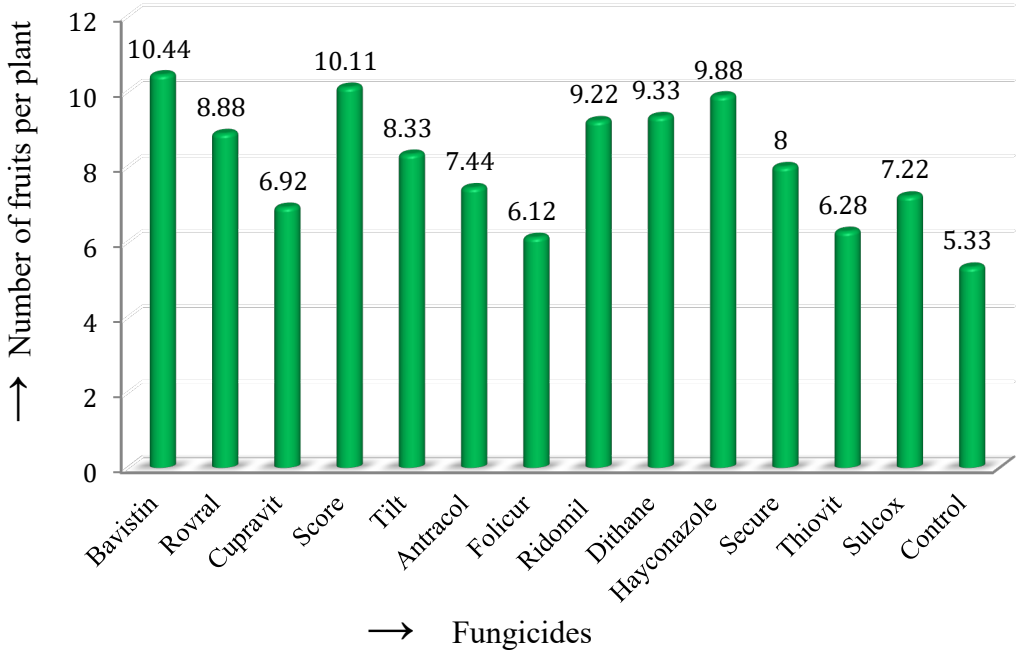


Fig. 4.33: Number of fruits per plant in different fungicides.

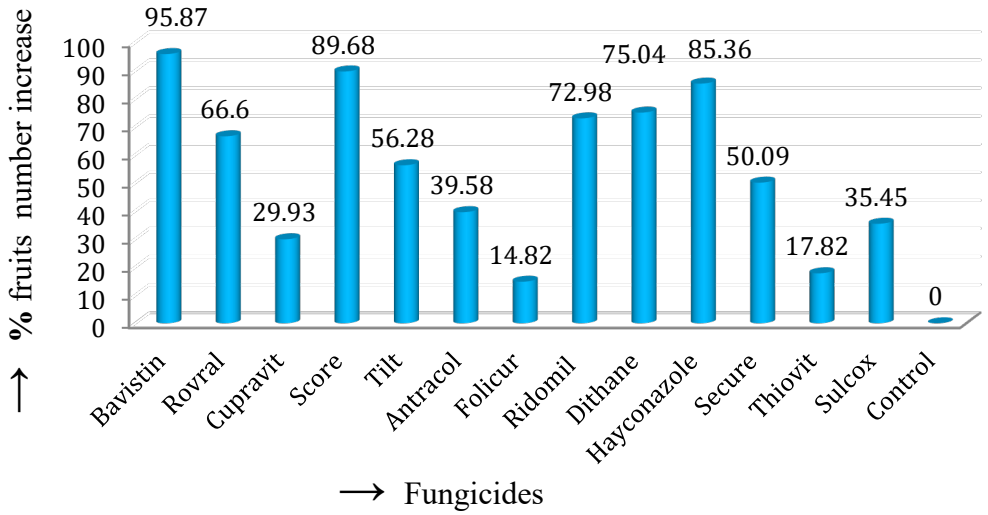


Fig. 4.34: % of fruit number increase in different fungicides than that of control.

Fruit weight (g) per plant

The Bavistin sprayed plot exhibited the highest fruit weight per plant of 148.56 g as compared to 63.88 g in the control plot **(Table 4.24** and **Fig. 4.35 - 4.36).** It was followed by the fungicides Score (143.44 g), Hayconazole (139.41 g) and Dithane (134.86 g). Further next fruit weight per plant was observed in the fungicides Ridomil (127.85 g), Tilt (121.00 g), Rovral (119.20 g), Secure (116.00 g), Thiovit (96.85 g), Sulcox (85.12 g), Antracol (83.76 g), and Cupravit (81 g).The lowest fruit weight was observed in fungicide Folicur (75.19 g).

Table 4.24. Fruits weight (g) of fungicides treated plants under field conditions.

Fungicides	Concentrations (ppm)	Fruit weight (g) per plant ($\overline{X} \pm SE$)	% yield increase than that of control
Bavistin DF	1000	148.56 ± 0.81	132.25
Rovral 50WP	1000	119.20 ± 0.98	86.59
Cupravit 50WP	1000	81.00 ± 0.57	26.80
Score 250EC	1000	143.44 ± 0.87	124.54
Tilt 250EC	1000	121.00 ± 0.57	89.41
Antracol 70WP	1000	83.76 ± 0.83	31.12
Folicur 250EC	1000	75.19 ± 0.84	17.70
Ridomil 68 WG	1000	127.85 ± 0.30	100.14
Dithane M-45	1000	134.86 ± 0.88	111.11
Hayconazole 5EC	1000	139.41 ± 0.71	118.23
Secure 600WG	1000	116.00 ± 0.45	81.59
Thiovit 80WG	1000	96.85 ± 0.88	51.61
Sulcox 50WP	1000	85.12 ± 0.46	33.24
Control	-	63.88 ± 0.26	0.0
LSD value at 5 %	-	2.110	-

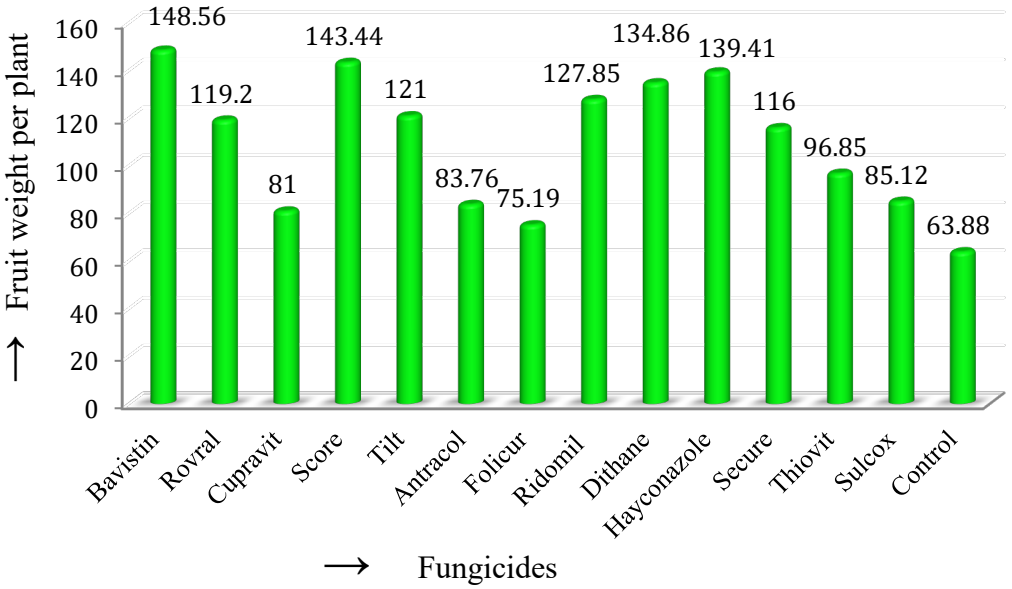


Fig. 4.35: Fruit weight (g) per plant in different fungicides.

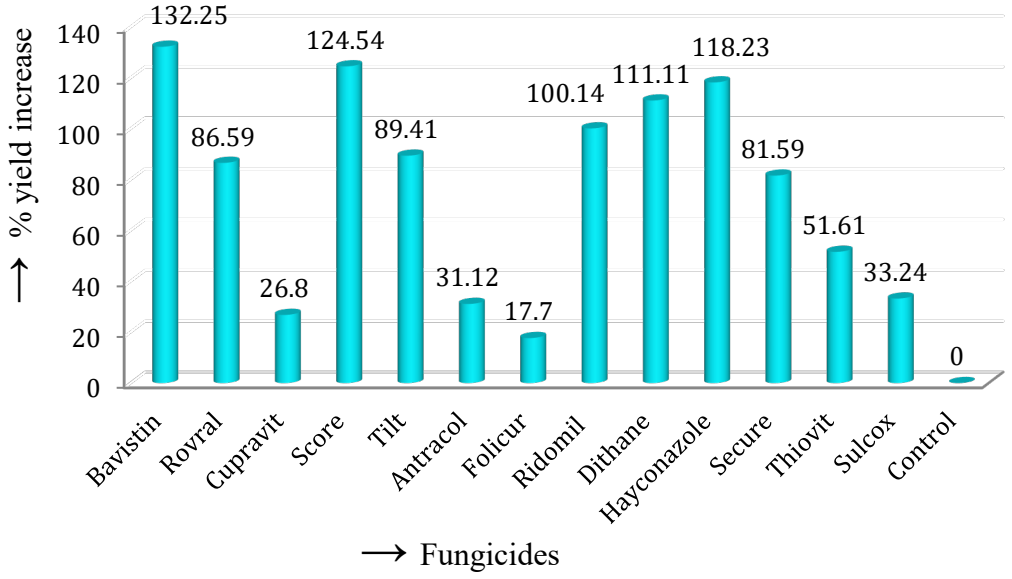
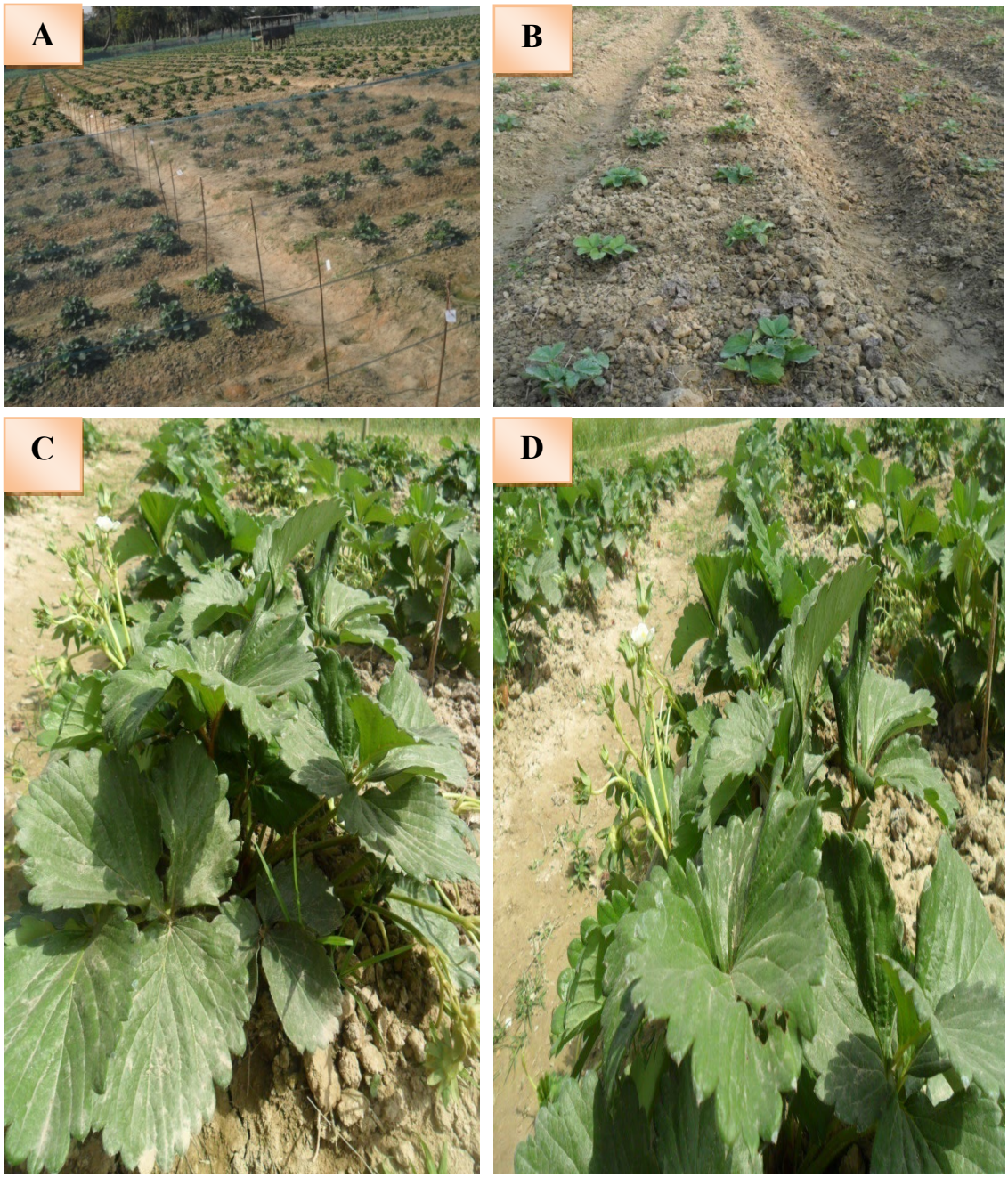


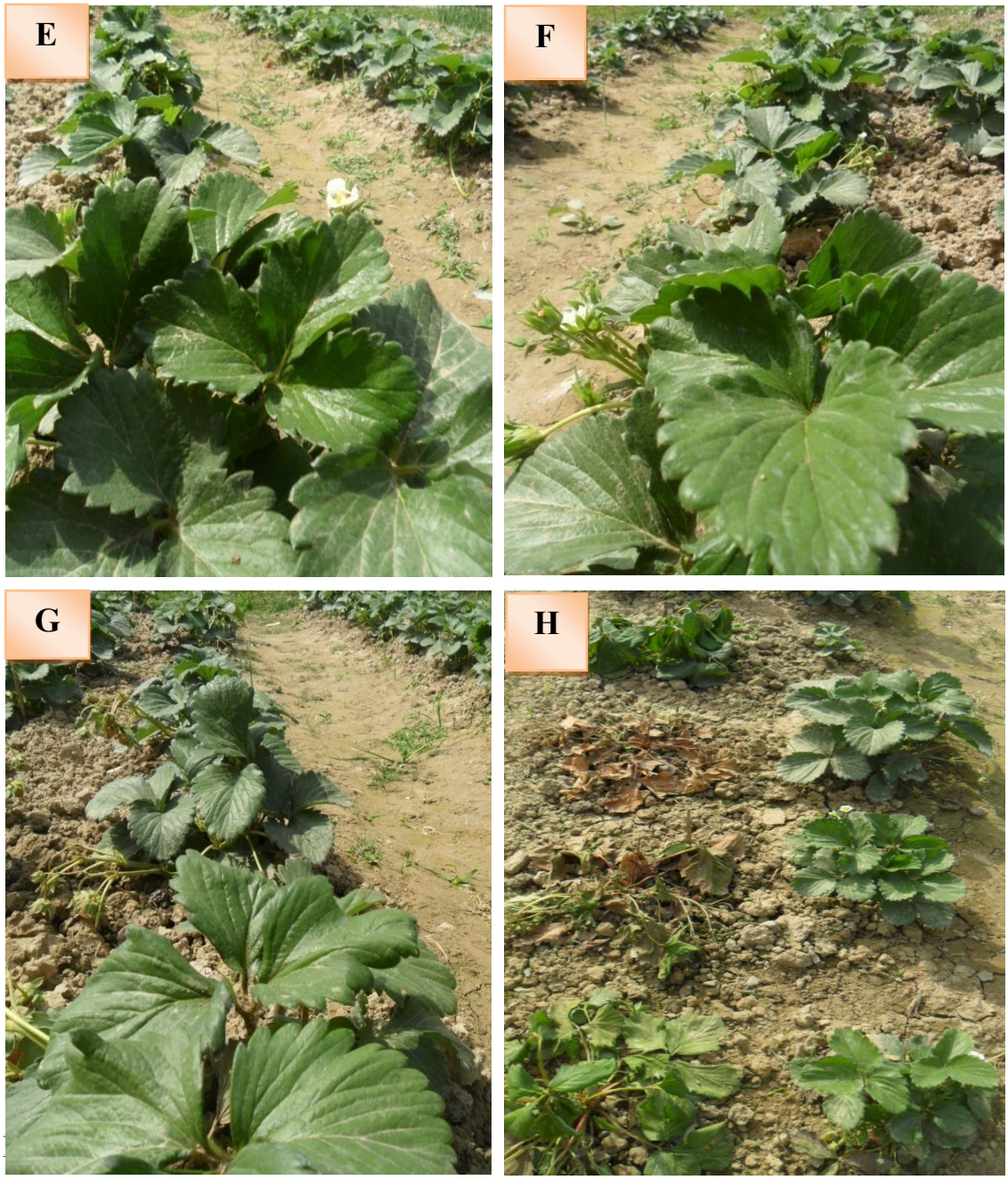
Fig. 4.36: % yield increase in different fungicides than that of control.

PLATE 4.16



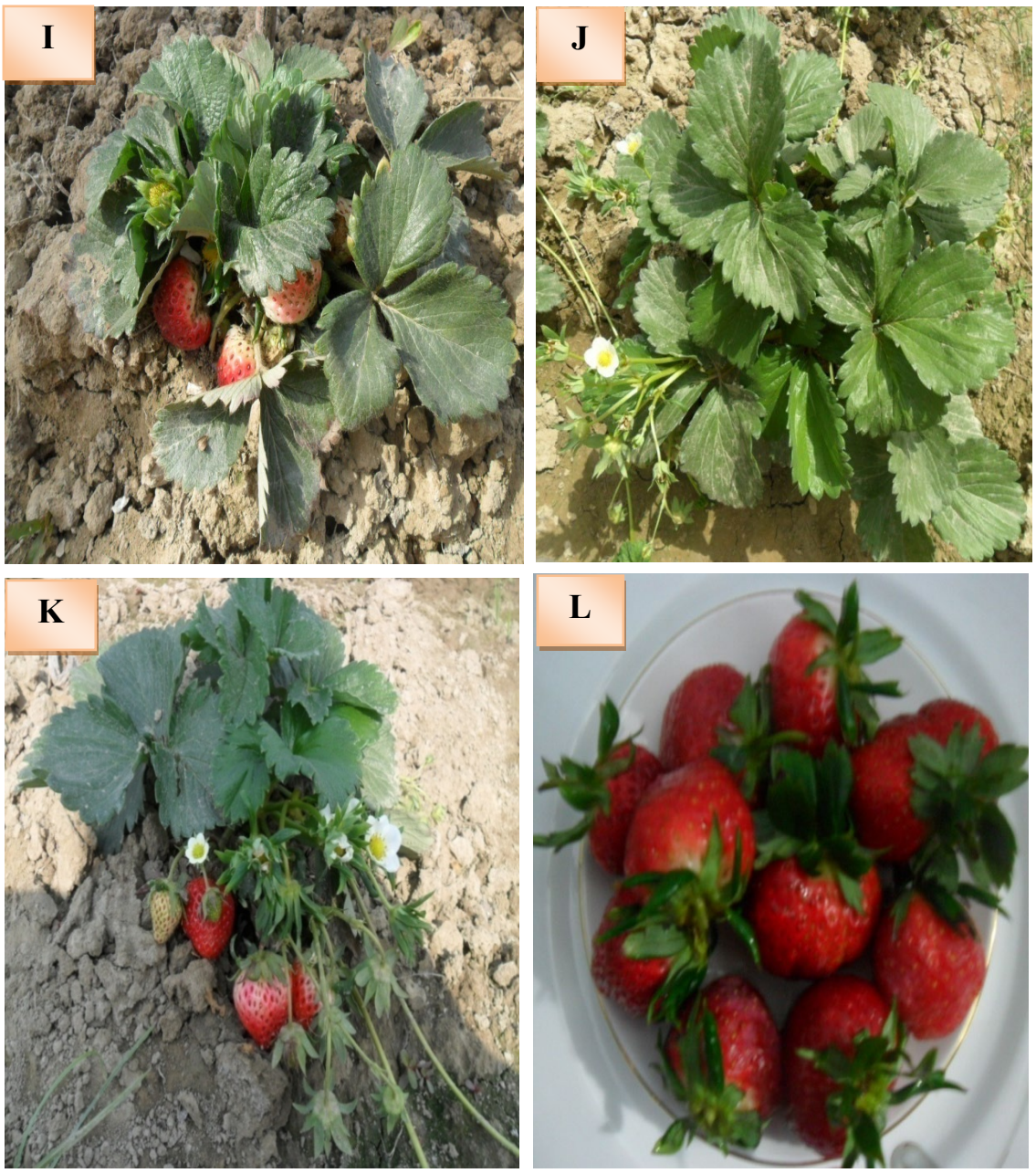
Photographs showing field evaluation of *T. harzianum* antagonist and neem leaf extract. **A.** Experimental field (1st year), **B.** Experimental field (2nd year), **C.** *T. harzianum* (antagonist) treated plants and **D.** Neem leaf extract treated plants.

PLATE 4.17



Photographs showing field evaluation of garlic bulb extract, cow urine and Bavistin fungicide. **E.** Garlic bulb extract treated plants, **F.** Cow urine treated plants, **G.** Bavistin fungicide treated plants and **H.** Control.

PLATE 4.18



Photographs showing field evaluation of Folicur fungicide. **I**. Folicur fungicide treated short strawberry plant, **J.** Strawberry plant with flowers, **K.** Strawberry plant with fruits and **L.** Harvested strawberry fruits.

CHAPTER V
DISCUSSION

Strawberry (*Fragaria* × *ananassa* Duch.) is a popular fruit all over the world where suitable soil and moisture conditions are available. It is a fruit of winter leading country. It is one of the most popular, nutritious and lovely looking fruits in the world. The production and consumption of strawberry is increasing day by day because of its food value and other importance. Strawberry suffers from several fungal diseases. Crown rot disease is one of them and this disease is a serious in strawberry.

The present investigation on crown rot disease of strawberry caused by *C. gloeosporioides* included symptomatology, isolation, identification, morphology of the fungus, pathogenecity test, cultural, nutritional, physiological studies, *in vitro* and *in vivo* evaluation of plant extracts, biomatters (cowdung and cow urine), phytohormones, antagonists and fungicides against pathogen. The results of the present investigation are discussed below:

Symptomatology

Crown rot of strawberry caused by C**.** *gloeosporioides* is first apparent by the wilting of the youngest leaves of the day. The young wilted leaves may appear to recover and become turgid in the evenings; however, most will wilt and die after a few days. Cutting through crown tissue of infected plants lengthwise reveals a reddish brown colour (Smith, 1998). Similar symptoms were reported by various workers (Horn *et al.*, 1972; Howard *et al.*, 1992; *Mass* and Palm, 1997; Phillis and Mack, 2007).

Collection, isolation and identification

The field survey of disease conducted during 2010-2012 in different districts of Bangladesh showed diverse incidence of crown rot on strawberry. Affected disease samples, showing typical crown rot symptoms were collected from different locations during survey. Upon tissue isolation the pathogen from the locality was brought into pure culture and identified as *C. gloeosporioides* based on morphological, cultural characters and pathogenecity in accordance with the description given by Ekbote (1994), Sudhakar (2000), Prasanna Kumar (2001), Venkataravanappa (2002) and Prashanth (2007). The causal organism *C. gloeosporioides* was isolated from diseased plants by following standard tissue isolation method. Further pure culture was obtained by following single spore isolation. Pure culture of fungi thus obtained was used in rest of the studies. Similar work had been done by Naik (1985) and Bhat (1987) on betel vine and forest crops respectively.

Morphology of the *C. gloeosporioides*

Identified as *C. gloeosporioides* based on colony and spore morphology which revealed that acervuli were circular to elliptical, measuring 129.0-281.4 µm, setae were erect in habit, measuring 37.0-89.0 × 1.4-4.0 µm. Conidia were hyaline, single celled and smooth walled. They were cylindrical and measuring 9.0-20.0 × 3.0-7.5 µm. These results are in agreement with that of Bose *et al.* (1953) where the morphology of the pathogen reported that the acervuli measured 115.0-467.0 µm and size of conidia varied from 11.0-16.0 × 4.0-6.0 µm. Similarly Sutton (1993) reported that the conidia of *C. gloeosporioides* were hyaline, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9.0-24.0 × 4.0-12.0µm. Holiday (1980) and Jeffries *et al.* (1990) reported that conidial size were 7.0-20.0 × 2.5-5.0 µm and setae were 40.0-80.0 × 1.7-5.0 µm on tropical crops and tropical fruit crops respectively.

Pathogenicity

In pathogenecity studies, the conidial suspension of *C. gloeosporioides* was sprayed on healthy plants and seven days after incubation typical symptoms were found. Similar results were recorded while proving pathogenecity by Bhat (1991) and Ekbote (1994) on pomegranate and mango respectively, Kota (2003) proved the pathogenecity of *C. gloeosporioides* on mango and banana.

Growth and sporulation of *C. gloeosporioides* on different solid media

Every living being required food for its growth and reproduction and fungi are not exception to it. Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory, it is necessary to furnish those essential elements and compounds in the medium, for their growth and other life processes. All media are not equally good for fungi, nor there a universal substrates or artificial medium, upon which all fungi grow. So, different media including both synthetic and nonsynthetic were tried for *C. gloeosporioides* in the present experiment. The amount of vegetative growth can be estimated by measuring mycelial growth of the colony. The former method was made use of while studying the growth of the fungus on different solid media. Among the eight solid media, maximum growth and good sporulation of the fungus was recorded on potato dextrose agar (82.00 mm) and Richard's agar (81.55 mm), which were found to be significantly superior to all other media followed by Corn meal agar (79.77 mm) and soil medium (79.44 mm), while the least growth and poor sporulation was observed in Sabouraud's agar medium (66.55 mm). Present studies are in accordance to the better performance of *C. gloeosporioides* on PDA which may be attributed to inherent complex nature of material supporting good fungal growth owing to provision of some additional nutrients as reported by Ekbote *et al. (*1997), Sudhakar (2000), Akhtar (2000), Rani and Murthy (2004) and Prashanth (2007).

Nutritional characters of *C. gloeosporioides*

Carbon occupies a unique position among the essential elements required by fungi. Almost half of the mycelial growth of fungi cells consist of carbon which is a main structural element and which also plays an important functional role (Lilly and Barnett, 1951; Biligrami and Verma, 1978).

The utilization of various carbon compounds may depend either on the activity of the fungus to utilize certain simpler forms or on its power to convert the complex carbon compound into simpler forms, which may be easily utilized. In the present study glucose supported maximum growth (70.22 mm) and excellent sporulation of *C. gloeosporioides* followed by sucrose (57.22 mm), and mannitol (50.77 mm).The lowest growth and poor sporulation was reported in case of lactose (44.11 mm). Glucose and sucrose are the best source of energy and comes under complex sugar. Sucrose being major component of photosynthetic plants is generally utilized as good source by most of plant pathogenic fungi (Lilly and Barnett, 1951) and similar observation were also made by Naik (1986). Glucose also promoted growth in fungi, since it is a simple sugar (Chandra and Tandon, 1962). Fairly good growth was noticed in soluble starch also indicating the presence of enzyme having power to break down starch into simple sugar, which can be utilized easily. Among the eight carbon sources, lactose supported least growth of the fungus, since it is an uncommon sugar for plant pathogenic fungi. Over and above, glactocydase a enzyme required for breaking down lactose to glactose and glucose had not been commonly reported in fungi, but adoptive enzyme system which enables fungi to grow on such a common sugars have been discussed (Cochrane, 1958). Yang *et al*. (2000) reported glucose was the best carbon source for mycelial growth and sucrose was the best for sporulation of *C. musae* from banana. Deshmukh *et al*. (2012) reported that starch and xylose were the best for carbon source for mycelial growth and sporulation of *C. gloeosporioides* Penz. and Sacc. from Indian bean.

Nitrogen, a compound of protein is an essential element and like carbon, it is also used by fungi for functional as well as structural purposes. But all the sources of nitrogen are not equally good for the growth of fungi. The fungus *C. gloeosporioides* showed variation in its ability to utilize different nitrogen sources. It utilized potassium nitrate more efficiently and was a better nitrogen source than any other nitrogen sources tested. Similar observation has been made by Rajak (1983), Naik (1985) and Ekbote (1994) in case of *C. gloeosporioides.* The nitrate compounds are excellent nitrogen sources for imperfect fungi and also ascomycetes (Bilgrami and Verma, 1978).

In the present study potassium nitrate supported maximum growth (59.66 mm) and excellent sporulation of fungus. The next nitrogen sources were ammonium sulphate (53.22 mm), sodium nitrate (50.77 mm), calcium nitrate (46.44 mm) and peptone (42.22 mm). The least growth and poor sporulation was reported in L- Aspergine (32.22 mm) and urea (29.77 mm). Mishra and Mahmood (1960) noticed the maximum growth of *C. gloeosporioides* in L-Asparagine. The fact that good growth in these amino acids was indicated the direct utilization of these compounds in protein synthesis (Lilly and Barnett, 1951). The poor growth was observed in urea. Cochrane (1958) has opined that urea breaks down to ammonia during autoclaving and ammonia in high concentration is toxic to fungi. Sporulation of *C. gloeosporioides* was excellent in potassium nitrate and ammonium sulphate while good sporulation recorded in peptone and calcium nitrate. L-Aspergine and Richard's agar medium (without nitrogen) supported poor sporulation of the fungus. Purkayastha and Sengupta (1975) found peptone, casaminoacid and potassium nitrate were favorable for both sporulation and mycelial growth of *C. gloeosporioides*., incitant of jute anthracnose. Sangeetha *et al*. (2008) reported ammonium nitrate supported good growth and sporulation. Deshmukh *et al*. (2012) reported potassium was the best nitrogen source for mycelial growth and sporulation of *C. gloeosporioides* from Indian bean.

Physiological characters of *C. gloeosporioides*

The fungi generally utilize substrate in the form of solution, only if the reaction of the solution is conductive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for a better fungal growth. In the present study maximum growth of the fungus was obtained at pH 6.5 (88.11 mm) followed by pH 6.0 (84.22 mm) and pH 7.0 (82.44 mm), where as optimum pH range was found to be 5.5 to 7.5 and the least growth was observed at pH 4.0 (40.44 mm). Abe and Kono (1956) found that a pH range 5.2 to 5.8 is the best for *Glomerella cingulata*. Verma (1969) and Hegde (1986) observed maximum growth of C. gloeosporioides at pH of 6.0. Rajak (1983) and Udhayakumar (2010) claimed a pH of 7.0 as optimum for C. gloeosporioides. Katti (1981) found the maximum growth of Glomerella cingulata at pH 5.8 and optimum pH range was 5.2 to 6.4. A pH of 5.5 to 6.5 was found to be optimum for growth and sporulation of *C. falcatum*, (Ahmed and ivingaraciam, 1974). Ekbote (1994) reported that the maximum growth of fungus at 6.5 pH and optimum pH range was found to be between 5.0 to 7.5 where as, Vinod (2008) reported the maximum growth of fungus at pH 6.5. At reduced pH, the cell membrane becomes saturated with H^+ ions which limit the passage of cations. The reverse condition could be obtained when the medium is alkaline and accumulated OH^- ions present the passage of essential anions. Enzyme activity is also conditioned by the reaction of the medium, as a result, the reduced growth of fungi (Bilgrami and Verma, 1978). The role of pH on the growth of phytopathogenic fungi has been reviewed by Tandon (1961).

Among the external factors, temperature is an important factor governing distribution, growth, reproduction and survival of the fungus. Temperature affects almost every function of fungi including the growth and sporulation. Temperature has profound effect on the vegetative and reproductive activity of the fungi. Effect of temperature on mycelial growth revealed that maximum growth (87.11 mm) was at 30°C. Further increase in temperature level to 35°C

decreased mycelial growth and the least mycelial growth (32.22 mm) was observed at 10°C. However the temperature at 20-30°C was found to be optimum for growth and sporulation of *C. gloeosporioides*. This is in agreement with the observation made on *Glomerella cingulata*, *C. capsici* and *C. gloeosporioides* by various workers who noticed best growth at 25-29°C. (Sattar and Mallik, 1939; Abe and Kono, 1956; Choudhuary, 1957; Verma, 1969; Mancini *et al.*, 1973; Ekbote *et al*., 1996; Prasanna Kumar, 2001; Venkataravanappa, 2002; Prashanth, 2007; Vinaya, 2008 and Sangeetha *et al.*, 2010). Excellent sporulation of fungus was observed at 30°C and least sporulation was found at temperature 15°C and 35°C which is an important pathogenic character. The present results are also in accordance with Estrada *et al*. (1993 and 2000).

Light has a profound effect on growth and sporulation of fungus. The preliminary studies carried out in the present investigation with *C. gloeosporioides* indicated a maximum growth (86.22 mm) and excellent sporulation when it was exposed to alternate light and dark condition which was on par with 12 hours dark and 12 hours light (85.77 mm). The least mycelial growth (57.77 and 54.88 mm) and poor sporulation were recorded when *C. gloeosporioides* was exposed to continuous light and continuous dark and both were on par with each other. When exposed to alternate light and darkness it attained maximum radial growth which might be due to induction of certain metabolic process necessary for growth and sporulation of the fungus, which usually doesn't occur in continuous light. Venkataravanappa (2002), Prashanth (2007), Vinod Tasiwal (2008) and Jayalakshmi (2010) observed in their studies that the exposure of alternate light and darkness favoured good growth and sporulation as compared to continuous light and continuous dark. Similarly these studies are in agreement with the findings of Chowdhuary (1936), Mishra and Siradhana (1980), Saifulla and Ranganathaiah (1990), Kamanna (1996), Sudhakar (2000), Ashoka (2005) and Narendra Kumar (2006).

***In vitro* evaluation of plant extracts**

Extensive use of fungicides has led to various environmental problems, human health and their persistence in the fruits. To sort out these problems botanicals were tested in laboratory against *C. gloeosporioides*. Continuous use of chemical fungicides in the management of disease also brought new problems with them. Amongst them, pollution of air, water, soil, residual toxicity, development of resistance in the pathogen against chemicals there by the need to apply them more with their escalating prices and harmful effects on non target organisms. Contrary to the problems associated with use of synthetic chemicals, botanicals are environmentally non pollutive, renewable, inexhaustible, indigenously available thus readily biodegradable relatively cost effective and hence constitute as a suitable plant protection in the strategy of integrated disease management.

Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and considered as one of the components in the integrated disease management (Khadar, 1999 and Nagesh, 2000). Leaf extracts of higher plants have been considered to be a useful source of fungitoxic substances (Tripathi *et al*., 1978). Plant derivatives possessing pesticide properties are gaining worldwide interest as alternatives or as supplements for the existing pesticides (Toriyama, 1972). In the present investigation extracts of 24 plant species were evaluated in the laboratory to know the possible presence of fungi toxic substances against mycelial growth of *Colletotrichum gloeosporioides* causing crown rot disease in strawberry. The results of the present investigation showed anti-fungal activity but varied with different plant species and concentrations. Among the tested twenty four plant extracts against *C. gloeosporioides*, all the concentrations (5-25%) of mehedi leaf extract showed 100% inhibition of mycelial growth which was significantly superior to other plant extracts. 25% garlic extract showed 90.33% inhibition of mycelial growth followed by arjun (70.11%), dhutra

(66.00%), ginger (64.00%) and allamanda (62.55).The least mycelial growth was observed in wood-apple (7.11%) at 5% concentration. Effectiveness of eucalyptus leaf, garlic bulb and *Ocimum* leaf extract against *C. gloeosporioides* is supported by the work of Prashanth (2007). The toxicity of *Allium cepa*, *A. sativum, Ocimum sanctum*, *Datura stromonium*, *Polyalthia longifolia, Tagetas errecta* and *Vinca rosea* on has been reported by Ashashivapuri *et al*. (1997).The fungicidal spectrum of neem has already been investigated by Singh and Pande (1966) and Parveen and Alam (1993). Antifungal properties of neem was also established by Ghewande (1989), Ganapathy and Narayanaswamy (1990), Patil (2008) and Rahejha and Thakore (2002). In effectiveness of tulsi leaf extract against *C. gloeosporioides* is supported by the work of Patel and Joshi (2001), where in they reported that tulsi leaf extract was in effective in inhibiting mycelial growth of the fungus.

***In vitro* evaluation of cowdung and cow urine**

Cow urine and cowdung are the two important bio matters that are extensively used by the growers as manure for production of different crops. It is also believed that cow urine and cowdung have some medicinal properties that are employed in ayurvediic or unani (herbal) medicine for curing many human diseases. It is pertinent to mention here that the role of cow urine and cowdung in controlling plant pathogens. So the results of the present experiment will provide a new technology for controlling plant pathogens instead of using different chemical fungicides that are very hazardous to both environment and soil structure.

In the present study, cowdung and cow urine were tested at five concentrations. Cow urine showed better inhibitive activity against the selected fungal pathogen than that of cowdung. In case of inhibition of mycelial growth the highest percentage (62.55%) was recorded in cow urine medium tested at 25% concentration followed by twenty percent (55.55%) concentration. For inhibition

of mycelial growth the least percentage (41.88%) was recorded in cowdung medium tested at 25% concentration followed by twenty percent (34.44%) concentration. In a research study Basak and Lee (2001, 2002) proved that cow urine and cowdung had some effectiveness in suppression of conidial germination and mycelial growth of *F. oxysporum* f. sp. c*ucumerinum* causing Fusarium wilt of cucumber plants. Complete suppression of mycelial growth of *S. sclerotiorum and Bipolaris sorokiniana* may be possible if different herbal plant extracts are added with fresh cow urine and cowdung before application (Basak and Lee, 2002; Nargis *et al.*, *2006)*.

***In vitro* evaluation of phytohormones**

The continuous and indiscriminate use of chemicals to manage diseases results in accumulation of harmful chemical residues in the soil, water and fruits. Use of bioagents, now days, is the best and has been most emphasized and widely accepted practice as it is environmentally safe and can overcome the residual problems associated with heavy use of fungicides for management disease. Hence, the present investigation was taken up to screen the phytohormones for effective management of crown rot disease.

In the present study five phytohormones were tested at five concentrations. Among phytohormones NAA was completely (100%) inhibiting the growth of *C. gloeosporioides* at three concentrations (100, 200 and 500 ppm other hand IAA and 2,4-D were completely (100%) inhibiting at two concentrations (200 and 500 ppm). IBA was completely (100%) inhibiting growth only at 500 ppm concentration. The least mycelial growth was recorded in BA at all concentrations (20-500ppm). Sheshtawi and Kiss (1975) reported that in the laboratory MCPA and 2,4-D did not affect the development of the cereal pathogen *F. graminearum* at a concentration of 1000 ppm. Gentle and Bovio (1986) reported application of growth regulator alfa naphthyl acetic acid (NAA) before inoculation of tomato plants with *F.oxysporum f. sp. Lycopersi,* delayed

expression and development of wilt symptoms. Michniewich and Rozej (1987) and Melinda and Stevenson (1991) have pointed out that auxin acts as a fungal growth and development controlling factor, while its role in the growth and development processes may vary in different species. NAA reduced mycelial growth rate of fungi (Al-Masri *et al.*, 2002; Michniewich and Rozej, 1987). NAA increases the resistance of potato plants to early blight (Melinda and Stevenson, 1991). Alam *et al.* (2002) reported that the phytohormone IAA had great effect (100% inhibition) against *C. gloeosporioides* at 0.005 to 0.006% concentrations for an immersion after 5-30 minutes. Alam *et al.* (2004) also observed that the plant extracts and phytohormones were showed *in vitro* effects on mycelial growth of anthracnose causing fungus.

In vitro evaluation of antagonists

The synthetic fungicides are widely used by the farmers to eradicate pathogens but it results in environmental hazards and harmful side effects on human being and animals. The chemical fungicides not only develop fungicide resistant strains but also accumulate in food and ground water as residues. In order to overcome such hazardous control strategies, scientists, researchers from all over the world paid more attention towards the development of alternative methods which are, by definition, safe in the environment, non toxic to humans and animals and are rapidly biodegradable, one such strategy is use of biocontrol agents to control fungal plant diseases. *Trichoderma* have long recognized as agents for the control of plant disease and for their ability to increase plant growth and development. The antagonistic nature might be due to antibiosis, nutrient competition and cell wall degrading enzymes. Among the biocontrol agents, *Trichoderma* is most promising and effective biocontrol agent. *Trichoderma* as antagonist controlling wide range of microbes was well documented and demonstrated for more than seven decades ago (Weindling, 1934), but their use under field conditions came much later (Chet *et al*., 1997), and their mechanism of myco parasitism is much more complex, involves

nutrient competition, hyper parasitism, antibiosis, space and cell wall degrading enzymes. In the present investigation 15 antagonists were tested against *C. gloeosporioides.* Both volatile and non volatile (culture filtrate) compounds from *Trichoderma* were evaluated for growth of tested pathogen.

For dual culture, it was noticed that maximum reduction in mycelial growth was observed in *T. harzianum (*80.22%) which was significantly superior overall the tested antagonists. The second highest reduction in mycelial growth was recorded for isolate Th1 *(*77.66%) followed by *T. virens* (73.88%) and *T. viride* (73.11%). For culture filtrate, among 15antagonists *T. harzianum* proved to be the most effective (85.22%) antagonist inhibiting the growth of the pathogen and it was significantly higher compared to the other antagonists. The next in order of merits in inhibiting the mycelial growth of fungus was isolate Th1 (82.44%) followed by Th6 (81.11%), Th8 (79.78 %) and *T. virens* (79.33%). The least effective antagonistic organism was isolate Th10 which inhibited the growth of the pathogen by 57.55%. From the results it is evident that volatile compounds from *T. harzianum* suppress the mycelial growth of *C. gloeosporioides* and found effective when compare to others. The earlier studies also revealed that antimicrobial metabolites produced by *Trichoderma* is effective against a wide range of fungal phytopathogens eg., *Fusarium oxysporum, Rhizoctonia solani, Curvularia lunata, Bipolaris sorokiniana and C. lagenarium, C. acutatum, C.gloeosporioides* (Ajith *et al.*, 2010; Padder *et. al*., 2010; Svetlana *et. al*., 2010; Dennis and Webstar, 1971; Mortuza, 1997; Kumar and Satyavir, 1998; Kaur *et. al*., 2006). A large variety of *Trichoderma* produced by volatile secondary metabolites such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogens (Vey *et al*., 2001). Similar results were obtained by Deshmukh and Raut (1992), Santha (2002), Patel and Joshi (2001) and Raheja and Thakore (2002) in *C. gloeosporioides.* The antagonism of *Trichoderma* spp. against many fungi is mainly due to production at acetaldehyde compound (Robinson and Park, 1966 and Dennis and Webster, 1971). This may also be the reason for its antagonistic effect on *C.*

gloeosporioides. Pyke and Dictz (1960) found ermadin as major volatile antibiotic produced by *Trichoderma* spp., which suppresses several plant pathogens. Godtfredsen *et al.* (1965), Mandhare *et al.* (1996) and Prashanth (2007) reported that *Trichoderma* spp. was the most effective antagonist to inhibit the anthracnose of pomegranate.

***In vitro* evaluation of fungicides**

In the absence of resistant cultivars, use of fungicides to manage the disease is an old age practice. When there is outbreak of epidemic for any reason perhaps use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates evaluation of fungicides under *in vitro* conditions to know their efficacy and initiate spray schedule in field conditions. *In vitro* evaluation of new synthetic molecules of fungicides is very much necessary before they are tried under field condition.

In the present study 13 fungicides were tested at five concentrations. Among all the fungicides Bavistin and Folicur were completely (100%) inhibiting the growth of *C. gloeosporioides* at four concentrations (100, 250, 500 and 1000 ppm). Hayconazole and score were completely (100%) inhibiting growth only at concentration of 1000 ppm followed by Tilt (90.11%) and Rovral (80.22%). Similar results were also obtained by Biradar (2002). Carbendazim (Bavistin) was found to be very effective in the other investigations conducted by Shamim *et al*., 2009; Orlikowski and Wajdyla, 1991; Tomy, 1997; Meah and Khan, 1986 and Kumbhar and Caudhary, 1979. Carbendazim and Triademefon are the best fungicides for inhibiting the growth of *C. gloeosporioides*. Carbendazim and Benomyl are a group of Benzimidazole fungicide and they interfere the production of energy and cell wall synthesis of fungi (Nene and Thapliyal, 1982). Further, they also reported the effectiveness of Triazole, which inhibit sterol biosynthesis pathway in fungi. According to Davidse (1986) reported

Carbendazim induced nuclear instability by disturbing the mitosis and meiosis.Tilt-250 EC and Rovral 50WP were most effective against *Colletotrichum* spp. (Shovan *et al*., 2008) and Dithane-45 was noted as poor performing fungicides (Alabi *et al*., 1986; Hossain, 1989; Rahman *et.al*., 1993; Islam *et.al*., 2002 and Sharif, 2005).

***In vivo* evaluation of plant extracts**

Five plant extracts, cow urine, two antagonists and 13 fungicides were field evaluated for their efficacy on crown rot disease of strawberry. Among the five plant extracts, no crown rot disease incidence was recorded from garlic and mehedi leaf extracts treated plants. The little disease incidence was recorded for neem (10.22%) and sajna (16%) as compared to 62.88% in the control. The highest canopy size (523.98 cm^2) per plant was obtained in plots where the treatment garlic bulb extract was applied followed neem (514.07 cm^2) and sajna leaf extract (509.82 cm^2) as compared to control (233.24 cm^2). The garlic bulb extract sprayed plot showed the highest number of fruits per plant (10.88) as compared to 5.33 in the control. It was followed by neem (10.33), sajna (9.55) and apang leaf extracts (9.20) treated plants. A spray with garlic bulb extract resulted in the highest fruit weight per plant of 149.29 g as compared to 63.88 g in the control. It was followed by neem (145.99 g), sajna (139.22 g) and apang leaf extracts (136.34 g). Mehedi leaf extract treated plants were not healthy due to their discoloration of leaves as well as canopy size; number of fruits and fruit weight per plant were decreased. This is an agreement with the observations of various workers who noticed very effectiveness of garlic extract in controlling the anthracnose pathogen *Colletotrichum* spp. in different crops (Singh *et al*., 1997; Mala *et al*., 1998; Harbant *et al*., 1999 and Shovan *et al*., 2008). Miah *et al.* (1990) observed that extracts of garlic, ginger and neem were effective in controlling *Drechslera oryzae.* Ngullie *et al.* (2010) reported that spraying with garlic extract were fruit rot incidence decreased and yield increased in chilli. Effectiveness of eucalyptus leaf, garlic bulb and *Ocimum* leaf extract against *C.*

gloeosporioides is supported by the work of Prashanth (2007). The toxicity of *Allium cepa*, *A. sativum, Ocimum sanctum*, *Datura stromonium*, *Polyalthia longifolia, Tagetas errecta* and *Vinca rosea* has been reported by Ashashivapuri *et al*. (1997) on bloom stage. Natarajan and Lalithakumari (1987) reported that the antifungal activity of the leaf extract of *Lawsonia inermis* on D. oryzae was tested at 1: 40 dilution (EC50 concentration) by measuring the growth. The antifungal substance contained in leaf of *Lawsonia* identified as 2-dhydroxy-1, 4 naphthoquinone. Under *in vivo* condition, foliar spray of the leaf extract effectively controlled disease than the seed treatment with fungicides.

***In vivo* evaluation of cow urine**

The cow urine as well as milk contains microorganisms, which were useful as Lactic acid, bacteria are used as digestive. These organisms also produce substances, which has wide antibiotic activity. Some of the organisms may produce substances, which can either kill or inhibit the growth of microbes involved in food poisoning. Urine of cow contains microorganisms, which probably help the growth of plants. Fresh cow urine exhibits antimicrobial activity. Cow urine especially that from pregnant cows was rich in growth hormones and helps in crop growth (www.ciks.org). Cow urine rich in uric acid, a source of nitrogen (Singh, 1996) was readily soluble and liquid form was one of the important compounds in panchagavya and was readily available to the plants directly influencing the nitrogen content of leaves (Salatin, 1993 and Sharma, 1976). Panchagavya eliminates the imbalances in physical, chemical and biological processes due to the cosmic energy produced by stirring of the stock solution. The basic elements of growth were harmonized by this energy, which refreshes the growth process (Sundaraman *et al*., 2001).

The crown rot incidence 25.88%, canopy size 389.84 cm^2, number of fruits per plant 9.18 and fruit weight per plant 129.41g were recorded for the spray with cow urine at 25%. Selvaraj (2003) reported that foliar spray of agnihotra ash at

200 g dissolved in one litre cow urine and 10 litres of water five times at 10 days interval had increased the yield of cabbage (38.8%) and reduced the leaf spot incidence. Raja *et al*. (2005) stated that animal urine treatments were significantly superior over fungicides and control in respect of reduced sheath blight disease intensity and increased grain yield. Under field condition 1:10 dilution of cow urine was found effective in reducing the disease intensity in different crops which has been well documented by several researchers (Sridhar *et al*., 2002; Manikandan, 2005; Vijayalakshmi *et al*., 2005 and Patil, 2007).

***In vivo* evaluation of antagonists**

Among the antagonists, a spray with *Trichoderma harzianum* resulted in no crown rot incidence, the highest canopy size (534.64 cm^2) per plant, number of fruits per plant (11.12) and fruit weight per plant (151.53 g) as compared to the control. This is in agreement with the reports of earlier studies (Sujatha Bai, 1992; Jeyalakshmi, 1999). The antagonism of *Trichoderma* spp. against many fungi is mainly due to production at acetaldehyde compound (Robinson and Park, 1966 and Dennis and Webster, 1971). This may also be the reason for its antagonistic effect on *C. gloeosporioides*. Sultana (1991) obtained up to 81.60% higher lentil seed yield when they were treated with *T. harzianum*. Sumitra and Gaikward (1995) obtained increased shoot and root length in *Trichoderma* treated plots. *T. harzianum* when applied as a seed treatment on potatoes, frequently increased both size and yield (Harman, 2000). Biswas and Das reported that against seedling disease *Trichoderma* is superior as seed coating. Prasad et al.(2002) found that soil treated with *T. harzianum* showed 61.5% disease control in chickpea while Kashem *et al*. observed seed < 30% disease control in lentil. Inoculation with *Trichoderma* isolates reduced the incidence of root rot and increased seed germination in lentil (Vyas and Mathur, 2002). Prasad *et al.* (2002) found significantly higher seed yield when field soil was treated with *T. harzianum* and *T. viride* against root rot of chickpea. Increased root development and yield were also observed in betel vine, gladiolous,

sunflower, mustard, *Chrysanthemum*, tomato, maize, sugarcane, groundnut and chickpea by the application of *T. harzianum* (Singh *et al*., 2007). Porras *et al*. (2003) reported *Colletotrichum* crown rot of strawberry controlled by *Trichoderma* spp. Freeman *et al*. (2001) reported *Trichoderma* species have been applied to control anthracnose of strawberry. Boonratkwang *et al*. (2007) reported *Trichoderma* species have been applied to control *Colletotrichum* species in chilli.

***In vivo* evaluation of fungicides**

No crown rot disease incidence was recorded for Bavistin treated plants. The little disease incidence was recorded for Folicur (3.11%), Score (6.22%), Hayconazole (9.66%), Rovral (10.00%) and Tilt (10.22%) as compared to 62.88% in the control. It was followed by Ridomil (12.88%), Secure (16.22%) and Dithane (19.88%).

The yield was dependent on canopy size. When the canopy size was increased yield was also increased. The highest canopy size 458.11cm^2 per plant was obtained in plots where the fungicide Bavistin sprayed followed by the fungicides Score (426.00 cm^2) and Hayconazole (384.95 cm^2) as compared to 233.24 cm^2 in the control. The next canopy size was observed in fungicide Dithane (380.81cm^2) followed by the fungicides Ridomil (360.11cm^2), Rovral (350.33 cm^2) and Tilt (347.66 cm^2).

A spray with Bavistin resulted in the highest number of fruits per plant of 10.44 as compared to 5.33 in the control. It was followed by the fungicides Score (10.11), Hayconazole (9.88) and Dithane (9.33). Further number of fruits per plant was observed in the fungicides Ridomil (9.22), Rovral (8.88), Tilt (8.33), and Secure (8.00). The Bavistin sprayed plot exibited the highest fruit weight per plant of 148.56g as compared to 63.88g in the control. It was followed by the fungicides Score (143.44 g), Hayconazole (139.41g) and Dithane(134.). Further next fruit weight per plant was observed in the fungicides Ridomil (127.85 g),

Tilt (121.00 g), Rovral (119.20 g) and Secure (116.00 g). The lowest canopy size (258.33 cm^2), number of fruits per plant (6.12) and fruit weight per plant (75.19 g) were observed for Folicur treated plants but the plants were exhibited lower crown rot incidence (3.11%) and they were shorter with dark green leaves than untreated plants. Similar results were observed by Smith (2008). The results are in agreement with findings of several workers (Abhishek and Verma, 2007; Desai, 1998; Gaikwad, 2000; Hegde, 1998; Jahagirdar *et al*., 2000; Jamadar et al., 2007; Navale *et al*., 2009; Patel *et al*., 2007; Prasanna *et al*., 2001; Prashanth *et al*., 2008; Raghuwamshi *et al*., 2004; Venkataravanappa, 2002). Gaikwad (2000) reported that seven sprays of fungicides Carbendazim (0.1%) and Mancozeb (0.2%) was found to be effective for controlling leaf and fruit spot of pomegranate caused by *C. gloeosporioides.* The efficacy of Bavistin against *C. gloeosporioides* pathogen was reported by several workers (Mishra, 1988; Raju and Rao, 1989; Azad *et al*., 1992). Hegde (1998), *in vivo* evaluation of fungitoxicants revealed that Propiconazole (0.05%), Mancozeb (0.2%), Hexaconazole (0.05%) and Triademefon (0.05%) were more effective of *C. capsici.*

CHAPTER VI
SUMMARY

The present investigation was conducted on various aspects on crown rot disease of strawberry with reference to survey and surveillance of fungal diseases. The results obtained are summarized below:

Many fungal diseases of strawberry were noticed in Bangladesh**.** These diseases were leaf spot, leaf blotch, leaf scorch, leaf blight, downy mildew, powdery mildew*,* Alternaria fruit rot, anthracnose fruit rot, fruit rot, Phomopsis soft rot, gray mold, Rhizopus rot or leak, crown rot, root rot or black root rot and Verticillium wilt etc.

An extensive roving survey was carried out in different districts of Bangladesh to assess the severity of crown rot disease on strawberry. This study revealed that crown rot disease is a common and very intensive disease of strawberry in Bangladesh. The study also revealed that the highest incidence of causal pathogen *C. gloeosporioides* was at Rajshahi district (77.77%) followed by Sherpur (77.14%), while the lowest pathogen incidence was recorded at Rangpur (57.14%) district followed by Gaibandha (59.20%).

Crown rot caused by *Colletotrichum gloeosporioides*, plants infected with virulent strains initially show signs of water stress and subsequently collapse. Cutting through crown tissue of infected plants lengthwise reveals reddish brown colour.

The colony morphology in general indicated that fungus on potato dextrose agar produced grayish white mycelium and hyaline conidiophores. The conidia were cylindrical, hyaline, single celled with oil globules in the centre and measured as 9.0-20.0 × 3.0-7.5 and acervulus as 129.0-281.4 μm.

Pure culture of this fungus was obtained by following single spore culture technique from the diseased crowns. This fungal culture was found to be pathogenic to strawberry plants.

The fungus *C. gloeosporioides* exhibited diversified cultural characters on solid media. Among the solid media maximum mycelial growth of the fungus (82.00 mm) was recorded on potato dextrose agar, whereas, the least on Sabouraud's agar.

Among the carbon sources tested, glucose was found to be the best for the growth and sporulation of *C. gloeosporioides* than that of other carbon sources and lactose was the least utilized. Among the nitrogen sources tested, potassium nitrate was found to be the best for growth and sporulation of *C. gloeosporioid*es followed by ammonium sulphate.

C. gloeosporioides grew at all pH levels tested, however, the maximum growth and excellent sporulation of fungus was obtained at pH 6.5, while optimum range of pH was found to be 5.5 to 7.5.

Temperature of 30°C was found to favour the growth of the fungus in solid media which recorded the highest mycelial growth (87.11 mm) with excellent sporulation, whereas, the least mycelial growth (32.22 mm) with poor sporulation was recorded at 10°C.

The light also plays an important role in growth of *C. gloeosporioides* the maximum mycelial growth (86.22 mm and 85.77 mm) was recorded when it was exposed to both 12 hours alternate light followed by 12 hours dark and followed by 12 hours light and the least mycelial growth of the fungus (54.88 mm) was recorded when exposed to continuous dark.

Among the twenty four plant extracts, 100 % inhibition of mycelial growth of fungus was recorded in mehedi leaf extract followed by garlic (90.33%) and

arjun (70.11%) leaf extracts. The biomatter cow urine at 25% found to be effective in the management of crown rot disease of strawberry.

Among the tested phytohormones, 100% inhibition of mycelial growth of the pathogen was recorded at 100, 200 and 500 ppm concentrations of NAA followed by IAA and 2,4-D at the concentrations of 200 and 500 ppm and IBA at 500 ppm concentration.

15 antagonists were tested against *C. gloeosporioides* following dual culture and culture filtrate technique. The results showed that *T. harzianum* has potential to inhibit the mycelial growth of *C. gloeosporioides*.

Out of 13 fungicides tested, the highest 100% inhibition of mycelial growth of *C. gloeosporioides* was recorded at 100, 250, 500 and 1000 ppm concentrations of Bavistin and Follicur followed by Hayconazole, Score, Tilt and Rovral at the concentrations of 1000 ppm.

In the field evaluation of plant extracts, garlic bulb extract (25%) was most effective on crown rot disease of strawberry and recorded higher yield (149.29 g per plant) followed neem leaf extract (145.99 g per plant). On the other hand the antagonist *T. harzianum was* found to be most effective in field condition recorded higher yield (151.53 g per plant).

Among the different fungicides, Bavistin at 1000 ppm was most effective and recorded higher yield (148.56 g per plant) followed by Score (143.44 g per plant) Hayconazole (139.41 g per plant) and Dithane (134.86 g per plant).

CHAPTER VII
REFERENCES

Abe, T. and M. Kono. 1956. Studies on the anthracnose of tea bush. II Physiological characters and pathogenicities of leaves. Sci. Rept. Fac. Agric. Saikvo. Univ., **8:** 81-88.

Abhishek, S. and K. S. Verma. 2007. *In vitro* cross pathogenecity and management of *Colletotrichum gloeosporioides* causing anthracnose of mango. Ann. Pl. Protec. Sci., **15(1):** 186-88.

Adiver, S. S., K. Giriraj and K. H. Anahosur. 1995. Neem leaf extract versus fungicides for control of foliar diseases of groundnut. Karnataka J. Agric. Sci., **8:** 69-73.

Hussain, A., F. Raziq and H. Khan. 2008. *In vitro* integrated control of *Colletotrichum gloeosporioides* with biological and chemical agents. Sarhad J. Agric., **24(**1).

Ahmed, H. U. and G. G. Divingaraciam. 1974. Growth and sporulation of *Colletotrichum falcatum* at different temperature, pH and light condition. Philippines Agric., **57:** 379-382.

Ainsworth, G. C. 1963. Ainsworth and Bisby's Dictionary of Fungi. 5th ed. Commonwealth Mycol. Inst. Kew, Surrey, England, p. 663.

Ajith, P. S. and N. Lakshmidevi. 2010. Effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. Nature. Sci., **8 (9)**.

Akamine, E. K. and T. Arisumi. 1953. Control of post harvest storage decay of fruits of papaya (*Carica papaya* L.) with special reference to the effect of hot water. Proc. Am. Soc. Hort. Sci., **61:** 270-274.

Akhtar, K. P. 2000. Fresh potato extract the best source for the growth of *Colletotrichum gloeosporioides* causing anthracnose of mango and

Fusarium subglutinans isolated from malformed inflorescence of mango. Pakistan J. Phytopathol., **12:** 134-136.

Akter, N., M. F. Begum, S. Alam and M. S. Alam. 2006. Inhibitary effect of different plant extracts, cowdung and cow urine on conidial germination of *Bipolaris sorokiniana.* J. Biosci., **14:** 87-92.

Alabi, O., A. M. Emechebe and P. D. Tyagi. 1986. Laboratory and green house evaluation of fungicides for the control of brown blotch of cow pea. Samaru J. Agric. Res. Nigeria, **4:** 25-33.

Alam, S., M. S. Banu, M. F. Ali, A. Nargis, M. R. Islam and M. S. Alam. 2002. *In vitro* inhibition of conidial germination of *Colletotrichum gloeosporioides* Penz. by fungicides, plant extracts and phytohoromones. Pakistan J. Biol. Sci., **5(3):** 303-306.

Alam, S., K. D. Han, H. hur, J. Oukshim, K. C. Chang, T. Soolee and M. W. Lee. 2004. *In vitro* effects of plant extracts and phytohormones on mycelial growth of anthracnose fungi. Korean Soc. Mycol., **32 (3):**134.

Alexander, S. A. and C. M. Waldenmaier. 2002. Management of anthracnose in bell pepper. New Fungicide and Nematicide Data commit. Am. Phytopathol. Soc., **58:** 49.

Allard, R. W. 1960. Principles of plant breeding. Jahn Wiley and Sons. New York.

Al-Masri, M. I., M. S. Ali Shtayeh, Y. Elad, P. Tudzynski and R. Barakat. 2002. Effect of plant growth regulators on white mould (*Sclerotinia sclerotiorum*) on bean and cucumber. J. Phytopathol., **150:** 481-487.

Amusa, N. A. and A. A. Alabi. 1996. Host range of *Colletotrichum gloeosporioides* from *Glyricidia sepium* and its implication in crop production. Crop Res., **11:** 359-363.

Anonymous, 2010. List of strawberry diseases. Wikipedia, the free Encyclopedia. Available from online.

Anonymous, 1996. IPM: Reports on plant diseases. Available from http://ipm. Illinois.edu/diseases/series700/rpd702/index.html.

Anahosur, K. K., G. M. Hegde, and K. Anil Kumar. 2000. Integrated management of soybean rust. **In:** Ann. Meet. Symp. Emerging Trends in Pl. Dis. Mgt, Indian Phytopathol, Soc., South Zone, Bangalore, December, 7-8.

Ark, P. A. and J. P. Thompson. 1959. Control of certain diseases of plant with antibiotics and garlic (*Allium sativum* L.). Pl. Dis. Rept., **43:** 276.

Ashashivapuri, O. P., Sharma and S. L. Jhamana. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. J. Mycol. Pl. Pathol., **27:** 29-31.

Ashoka, S. 2005. Studies on fungal pathogens of vanilla with special reference to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Azad P. 1992. Efficacy of certain fungitoxicants against *Colletotrichum capsici* (Syd) Butler and Bisby, the incitant of ripe fruit rot of chilli. J. Assam Sci. Soc., **34 (2):** 34-39.

Babu, L. J., H. N. Gour and S. Pankaj. 2008. Efficacy of phytoextracts and bioagents against *Colletotrichum gloeosporioides* causing banana fruit rot. J. Mycol. Pl. Pathol., **38(3):** 635-637.

Banik, A. K., S. A. K. M. Kaiser and R. S. Dhua. 1998. Evaluation of some systemic and nonsystemic fungicides against *Colletotrichum gloeosporioides* and *Diplodia natalensis* causing post harvest fruit rot of mango. J. Soils. Crops, **25:** 119- 122.

Basak, A. B. and M .W. Lee. 2001. Comparative efficacy and *in vitro* activity of cow urine and cowdung for controlling Fusarium wilt of cucumber. Abstract published in the Korean Soc. Pl. Pathol. Ann. Meet. Int. Conf., held on the 25-30th October, Kyongju, Korea, p.49

Basak, A. B. and M. W. Lee. 2002. *In vitro* inhibitory activity of cow urine and cowdung of *Fusarium solani* f. sp. *cucurbitae*. Mycrobiol., **30 (1):** 51-54.

Basak, A. B., M. W. Lee and T. S. Lee. 2002. Inhibitive activity of cow urine and cowdung against *Sclerotinia sclerotiorum* of cucumber. Mycrobiol., **30 (3):** 175-179.

Benagi, V. I., M. R. Ravikumar, S. B. Gowdar and B. B. Basavaraj. 2009. Survey on diseases of pomegranate in northern Karnataka. Paper presented in; 2nd Int. Symp. pomegranate and minor mediterranean fruit crop. Univ. Agric. Sci., Dharwad, India, June, 23-27, p. 135.

Benitez, T., A. M. Rincon., M. Carmenlimon and A. C. Codon. 2004. Biocontorl mechanisms of *Trichoderma* strains. Int. Microbiol., **7:** 249-260.

Bernard, E. L. and R. A. Schrader. 1984. Anthracnose of *Acacia* in Florida. Occurrence and fungicidal control. Proc. Florida. State Hort. Sci., **97:** 244-247.

Bhat, N. M. 1987. Studies on foliar fungal disease of forest crops in and around Dharwad. M.Sc. (Agric.) Thesis, Univ. Agric. Sci. Dharwad, India.

Bhat, N. 1991. Studies on fruit rot of pomegranate caused by *Colletotrichum gloeosporioides* and *Aspergillus niger*. M.sc. (Agric) Thesis, Univ. Agric. Sci. Dharwad, India.

Biligrami, K. S. and R. N.Verma. 1978. Physiology of fungi, Vikas Publishing House, Pvt. Ltd., New Delhi., p. 493.

Biradar, S. V. 2002. Studies on *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. *Botryodiplodia theobromae* Pat. and *Fusarium semitectum* Berk. and Rav. causing fruit rot of custard apple (*Annona squamosa* L.). M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Biswas, M. K, M. Hossain, M. B. Ahmed, U. K. Roy, R. Karim, M. A. Razvy, M. Salahin and R. Islam. 2007. Multiple shoots regeneration of strawberry under various colour illuminations. American-Eurasian J. Sci. Res., **2(2):** 133-135.

Boonratkwang, C., C. Chamswarng and W. Intanoo. 2007. Effect of secondary metabolites from *Trichoderma harzianum* strain pm9 on growth inhibition of *Colletotrichum gloeosporioides* and chilli anthracnose control. Proc. 8th Nation. Pl. Protec. conf., Phisanulok, Thailand Naresuan Univ., pp. 323-336.

Bose, S. K., G. S. Sindhar, and B. N. Pande. 1953. Studies on the die-back disease of mango in the Tarai region of Kumaon. Prog. Hort., **5:** 41-53.

Brisa, R., M. A. Fernando, G. S. Asuncion, M. R. Noime, P. E. Arturo and M. D. Jose. 2007. The gene coding for a new transcription factor of *Fusarium oxysporium* is only expressed during infection of common bean. Fun. Genet. Biol., **44:** 864-876.

Brooks, A. N. 1931. Anthracnose of strawberry caused by *Colletotrichum fragariae.* Phytopathol., **21:** 739-744.

Brooks, A. N. 1935. Anthracnose and wilt of strawberry caused by *Colletotrichum fragariae.* Phytopathol., **25:** 973-974.

Brown, W. 1923. Experiments on the growth of fungi on culture media. Ann. Bot., **37:** 105-129.

Butler, E. J. 1918. Fungi and Disease in Plants. Thacker Spink and Co. Calcutta., p. 547.

Chandra, S. and R. N. Tandon. 1962. The nutrition of *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. Mycopathol., **18:** 213-214.

Chhata, L. K. and G. L. Kumawat. 2001. Chemical control of fruit spot disease in pomegranate. J. Mycol. Pl. Pathol., **31(1):**124.

Chauhan, H. L. and H. U. Joshi. 1991. Evaluation of phyto-extracts for control of mango fruit anthracnose. Proc. Nation. Symp. Botanical Pesticides in Integrated Pest Management. CTRI, Rajahmundry., p. 21-22.

Chet I., J. Inbar and I. Hadar. 1997. Fungal antagonists and mycoparasites. **In:** The mycota IV: Environmental and Microbial Relationships. Wicklow DT and Soderstorm B, eds., pp. 165-184.

Chowdhuary, S. 1936. A disease of *Zea mays* caused by *Colletotrichum graminicola* (Ces.) Wilson. Indian J. Agric. Sci., **6:** 833-843.

Chowdhuary, S. 1957. Studies on the development and control of fruit rot of chillies. Indian Phytopathol., **10:** 55-62.

Cochrane, V. M. 1958. Physiology of fungi, John Willeg and Sons, Inc. New York, p. 524.

Corda, A. C. I. 1831-1832. Strum's *Deutsclands* flora Nurnberg.

Darrow, G. M. 1966a. The strawberry: History, Breeding and Physiology. Holt, Rinehart and Winston, New York.

Darrow, G. M. 1966b. The strawberry: Holt, Rinehart and Winston. Canada.

Davidse, L. C. 1986. Benzimidazoles, fungicides mechanism of action and biological impact. Ann. Rev. Phytopathol., **24:** 43-65.

Delp, B. R. and R. D. Milholland. 1980. Control of strawberry anthracnose with Captafol. Am. Phytopathol. Soc., **64:** 11.

Dennis, C. and J. Webster. 1971. Antagonistic properties of species group of *Trichoderma* II production of non-volatile antibiotics. Transactions of British Mycol. Soc., **57:** 41-48.

Dennis, C. and J. Webster. 1971. Antagonistic properties of species groups of *Trichoderma* III. Production of volatile antibiotics. Transactions of British Mycol. Soc., **51:** 363-369.

Desai, S. A. 1998. Efficacy of O, O-disopropyl-S-benzyl thiophosphate: a systemic fungicide against anthracnose of pomegranate in Karnataka. Karnataka J. Agric. Sci., **11(4):** 1092-1093.

Deshmukh, P. P. and J. G. Raut. 1992. Antagonism by *Trichoderma* spp. on five plant pathogenic fungi. New Agriculturist, **3:** 127-130.

Deshmukh, A. J., B. P. Metha, A. N. Sabalpara and V. A. Patil. 2012. *In vitro* effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing anthrancnose of Indian bean. J. Biopest, **5:** 46-49.

Dhingra, O. D. and J. B. Sinclair. 1985. Basic plant pathology methods. CRC press, Inc. Boca Raton, Florida. pp.132-163.

Dubey, S. C. 2002. Efficacy of some oil cakes and plant extracts against web blight of urdbean and mungbean caused by *Thanatophorus cucumeris*. J. Mycol. Pl. Pathol., **32:** 158-161.

Durairaj, V. 1956. Growth of *Colletotrichum capsici* in pure culture. J. Indian Bot. sci., **35:** 409-413.

Ekbote, S. D. 1994. Studies on anthracnose of mango (*Mangifera indic*a L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Ekbote, S. D., G. M. Padaganur and K. H. Anahosur. 1996. *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides*. Karnataka. J. Agric. Sci., **9(2):** 359-360.

Ekbote, S. D., G. M. Padaganur, M. S. Patil and S. N. Chattannavar. 1997. Studies on the cultural and nutritional aspects of *Colletotrichum gloeosporioides* the causal organism of mango anthracnose. J. Mycol. Pl. Pathol., **27:** 229-230.

El-mougy, S. N., G. E. Nadia, and M. M. Abdel Kader. 2007. Control of wilt root rot incidence in *Phaseolus vulgaris* by some plant volatile compounds. J. Pl. Protec. Res., **47:** 255-265.

Escopalao, W. M. and J. C. Silvestre. 1996. Evaluation of 15 medicinal plants extracts for fungicidal property against *Colletotrichum gloeosporioides* Penz. Tausiraj anthracnose of mango fruits. Philippines Phytopathol., **32(2):** 130.

Estrada, A. B., J. C. Dodd and P. Jeffries. 1993. Effects of environment on the *in vitro* growth and development of *Colletotrichum gloeosporioides* isolates from the Philippines. Acta Hort., **341:** 360-370.

Estrada, A. B., J. C. Dodd and P. Jeffries. 2000. Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. Pl. Pathol., **49:** 608-610.

Ferdousi, M. B., M. A. Rahman and M. F. Alam. 2010. Biological control of Alternaria fruit rot of chilli by *Trichoderma* species under field conditions. Mycobiol. **38(2):** 113-117.

Fisher, R. A., F. R. Immer and O. Iedin. 1932. The genetical interpretation of statistics of the third degree of study of quantitative inheritance. Genet., **17:** 107-124.

Freeman, S. and T. Katan. 1997. Identification of *Colletotrichum* species responsible for antracnose and root necrosis of strawberry in Israel. Phytopathol., **87:** 516-521.

Freeman, S., Y. Nizani, S. Dtatan, S. Even and T. Sando. 1997. Control of *Colletotrichum acutatum* in strawberry under lab, green house and field conditions. Pl. Dis., **81:** 749-752.

Freeman, S., D. Minz, M. Maymon and A. Zveibil. 2001. Genetic diversity within *Colletotrichum acutatum* sensu Simmonds. Phytopathol., **91(6):** 586-592.

Gaikwad, A. P. 2000. Synergy between carbendazim and mancozeb in controlling leaf and fruit spots of pomegranate. J. Maharashtra Agric. Univ., **25(2):** 165-167.

Gaikwad, A. P., G. N. Salunkhe and C. A. Nimbalkar. 2002. Efficacy of fungicides against fruit rots of custard apple. J. Maharashtra Agric. Univ., **27(1):** 70-72.

Ganapathy, T. and P. Narayanaswamy. 1990. Effect of plant products on the incidence of major diseases of groundnut. Internation Arachis Newslett., **7:** 20-21.

Gentle, I. A. and M. Bovio. 1986. Fusarium wilts severity and ethylene evolution in tomato plants after treatment with tri fluralin and naphthyl acetic acid. Zeitschrift-fur-pflanzenkra-nkheiten-und-pflanzenschuta, **93:** 624-631.

Ghewande, M. P. 1989. Management of foliar diseases of groundnut (*Arachis hypogaea* L.) using plant extracts. Indian J. Agric. Sci., **59:** 133-134.

Godara, S. L. and V. N. Pathak. 1995. Effect of plant extract on post harvest rotting of sweet orange fruit. Proc. Global Conf. Advan. Res. Pl. Dis. Mgt. RCA, Udaipur. February, 12-17, p.172.

Godtfredsen, W.O. and S. Vagedal. 1965. Trichodermin, a new sesquiterpene antibiotic. Acta Chemical Scandinavica, **19:** 1088-1102.

Gud, M. B. 2001. Studies on fungal diseases of mango (*Mangifera indica* L.) fruits. M.Sc. (Agric.) Thesis, KKV, Dapoli, Maharashtra, India.

Gullino, M. L., M. L. Romano and A. Garibaldi.1984. Identification and response to fungicides of *Colletotrichum gloeosporioides* incitant of strawberry black rot in Italy. Pl. Dis., **69:** 608-609.

Gunnel, P. S. and W. D. Gubler. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. Mycologist, **84:** 157-165.

Gurudatt, M. H., K. H. Anahosur, and S. Kulkarni. 2003. Nimbicidine a botanical to manage soybean rust. In: Ann. Meet. Symp. Rec. Dev. Diagnosis and Mgt. Pl. Dis. Meet. Global Challenges. Dept. Pl. Pathol., Univ. Agric. Sci., Dharwad, p. 43.

Guttridge, C. G. 1985. *Fragaria ananassa*, **In:** A. H. Halevy (ed.) CRC handbook of flowering, CRC Press, Boca Raton, Fla., **3:** 16-33.

Hancock, J. F., D. H. Scott, and F. J. Lawrence. 1996. Strawberries. **In:** Janick and Moore, J. N. (eds.). Fruit Breeding Vine and Small Fruits. John Wiley and Sons. New York, **11:** 419-470.

Harbant, S., V. Korpraditskul, H. Singh, K. Vichai, P. P. Singh and R. C. Saxena. 1999. Evaluation of some plant extracts for the control of *Colletotrichum capsici* (SYD.) Butler and Bisby, the causal agent of chilli anthracnose. *Azadirachta indica* A. Juss.131-138

Harman, G. E. 1996. *Trichoderma* for biological control of plant pathogens: from basic research to commercialized products. Cornell Common Wealth Conf. on Biocontrol, pp. 11-13.

Harman, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Pl. Dis., **84:** 377-393.

Hayman, B. I. 1958. The seperation of epistatic from additive and dominance variation in generation means. Heredity, **12:** 370-390.

Hegde, G. M. 1998. Studies on fruit rot of chilli caused by *Colletotrichum capsici* (Sydow) Butler and Bisby in Karnataka. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Hegde, Y. R. and R. K. Hegde. 1986. Studies an anthracnose of areca nut (*Areca catechu* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Pl. Pathol. Newsletter, **4:** 24-27.

Holiday, P. 1980. Fungus Disease of Tropical Crops. Cambridge Univ. Press, Cambridge.

Horn, N. L., K. R. Burnside and R. B. Carver. 1972. Control of the crown rot phase of strawberry anthracnose through sanitation breeding for resistance and benomyl. Pl. Dis. Rep., **56:** 515-519.

Hossain, M. M. 2007. Strawberry cultivation in Bangladesh (Bangla). Sarker Publications, 38, Banglabazar, Dhaka-1100. Int. J. Sustain. Crop Prod., **4:** 5.

Hossain, S. 1989. Survey and chemical control of guava anthracnose. Abstract of Thesis (1966-1990). Dept. of Pl. Pathol. Bangladesh Agric. Univ., Mymensingh, p. 13.

Howard, C. M. 1983. Black leaf spot phase of strawberry anthracnose caused by *Colletotrichum gloeosporioides (C. fragariae)* Pl. Dis., **67:** 1144-1146.

Howard, C. M. and E. E. Albregts. 1984. Anthracnose of strawberry fruit caused by *Glomerella cingulata* in Florida. Pl. Dis., **68:** 824-825.

Howard, C. M., J. L. Mass, C. K. Chandler and E. E. Albergts. 1992. Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. Pl. Dis., **76:** 976-981.

Imtiaj, A., S. A. Rahman, S. Alam, R. Parvin, K. M. Farhana, S. B. Kim and T. S. Lee. 2005. Effect of fungicides and plant extracts on the conidial germination of *Colletotrichum gloeosporioides* causing mango anthracnose. Mycobiol, **33(4):** 200-205.

Ivanovic, M. and D. Ivanovic. 1992. Study of *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. causal agent of sour cherry anthracnose and the effect of some fungicides on this *in vitro Zastia bilja*, **43:** 211-218.

Ivanovic, M. and D. Ivanovic. 2005. Bolesti vocaka ivinoveloze injihovo suzbijanje, Poljoprivredni fakultet, Zemun, p. 400.

Illis, M. A. 2004. Using fungicides for control of anthracnose fruit rot on strawberry in Ohio, Ohio fruit growers society web, India, p. 173.

Islam, M. M., D. M. M. Rahman and A. H. M. Sharif. 2002. *In vitro* evaluation of fungicides against *Colletotrichum piperis* isolated from anthracnose disease of betel vine. B.Sc. Thesis. Dept. of Agrotech., Khulna Univ, Khulna, Bangladesh, p. 90.

Jabin, M. 2003. *In vitro* inhibitory activity of cow urine, cowdung and some medicinal plant extract against *Fusarium semitectum* Berk and Ravi of chilli. M. Sc. Thesis, Dept. of Bot. Chittagong Univ., Bangladesh, p. 9-10.

Jadav, S. K., M. P. Diwakar, U. K. Sawant and J. J. Kadam. 2008. Management of leaf spot disease of kokum incited by *Colletotrichum gloeosporioides*. J. Pl. Dis. Sci., **3(2):**193-196.

Jahagirdar, S., S. T. Yenjerappa, M. R. Ravikumarand and S. A. Deasi. 2000. Performance of kitazin 48% EC against anthracnose of pomegranate. Pestology, **24(9):** 11-12.

Jamadar, M. M., M. K. Shaikh and R. A. Balikai. 1998. Chemical control of pomegranate fruit spot. Advan. Agric. Res. India, **10:** 13-15.

Jamadar, M. M. 2007. Etiology, epidemiology and management of anthracnose of grapevine. Ph.D. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Jamadar, M. M. and D. R. Patil. 2007. Bio-efficacy of new formulations against leaf per fruit spot of pomegranate. Karnataka J. Agric. Sci., **20(4):** 865-866.

Jayalakshmi, K. 2010. Studies on anthracnose of pomegranate caused by *Colletotrichum gloeosporioides (*Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Jefferies, P., J. C. Dodd, M. J. Jegerand and R. A. Plumbley. 1990. The biology and control of *Colletotrichum* species on tropical fruit crops. Pl. Pathol., **39:** 343-366.

Jetti, A., D. S. Iyengar, S. N. Rao and U. T. Bhalerao. 1987. Antifungal spectrum of leaf extracts of *Polyalthia longifolia*. Pesticides, **20:** 29.

Jeyalakshmi, C. and K. Seetharaman. 1999. Variation in *Colletotrichum capsici* isolates causing fruit rot and die-back of chilli. J. Soils Crops, **4:** 88.

Kamanna, B. C. 1996. Epidemiology and control of anthracnose diseases of coffee incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Ph.D. (Agric.) Thesis, Univ. Agric. Sci., Bangalore, India.

Kannan, C., P. Balabaskar, T. Suthinraj, J. Kathiravan and V. Kurucheve. 2005. Evaluation of animal excrements against stem rot incident of groundnut. **In:** Nation. Symp. Modern Conf. Pl. Dis. Mgt. Global Trust (MCPDMGT) and IPS Ann. Meet (South zone), Annamalai Univ. December, 29-30, Abstracts, p. 17.

Kannan, C., V. Kurucheve and J. Raja. 2007. Animal urine mediated induced acquired systemic resistance in peanut against *Sclerotium rolfsii*. J. Mycol. Pl. Pathol., **37 (1):** 202.

Kannan, C., V. Rajkumar and J. Raja. 2007a. Management of stem rots disease of peanut by use of animal urine. ISOR National Seminar, Jan., pp. 29-31.

Kashem, M. A., I. Hossain and M. K. Hasna. 2011. Use of *Trichoderma* in biological control of foot and root rot of lentil. Int. J. Sustain. Crop Prod. **6(1):** 29-35.

Katti, S. B. 1981. Studies on anthracnose of *Coriandrum sativum* L. caused by *Glomerella cingulata* (Stonem) Spauld and Schrenk. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Bangalore, India.

Kaur, M., O. P. Sharma and P. N. Sharma. 2006. *In vitro* effects of *Trichoderma* species on C*olletotrichum capsici* causing fruit rot of chilli (*Capsicum annuum* L.), Indian Phytopathol., **59:** 243-245.

Kempthorne, O. 1957. An introduction to genetic statistics. Jahn Willey and Sons. New York, p. 545.

Khadar, S. K.1999. Management of foliar disease of groundnut (*Archis hypogaea* L.) with special reference to botanicals. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Khuls, K., E. Leeckfeldt, T. Borner and E. Gueho. 1999. Molecular re-identification of *Trichoerma* isolates. Medical Mycol., **37:** 25-33.

Koelsch, M. C., J. C. Cole, S. L. Broembsen Von and S. L. Broembsen. 1995. Effectiveness of selected fungicides in controlling foliar diseases of common periwinkle (*Vinca minor* L.). Hort. Sci., **30:** 554-557.

Kota, V.R., 2003, Biological management of postharvest fungal diseases of major fruits. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Kumar, A. and S. C. Tripathi. 1991. Evaluation of the leaf juice of some higher plants for their toxicity against soilbrone pathogens. Pl. and soil, **132:** 297-301.

Kumar, A. and Satyavir. 1998. Evaluation of biocontrol agents against red rot (*Colletotrichum falcatum*) of sugarcane. Association of Applied Biologists, pp. 72-73

Kumar, N. P. G. 2006. Studies on anthracnose of sorghum caused by *Colletotrichum graminicola* (Ces.) Wilson. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Kumar, A. S., Reddy, N. P. E., Reddy, K. H. and Devi, M. C. 2007. Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, India. Pl. Pathol. Bull., **16:** 157-160.

Kumbhar, G. B. and K. G. Caudhary. 1979. Storage decay of sweet orange and control measures. J. Maha. Agric. Univ., **4:** 230-231.

Lal, B. and R. N. Tandon. 1968. Utilization of monosaccharides by three isolates of *Colletotrichum capsici* (Syd.) Utilization of monosaccharides Penz. Proc. Indian Acade. Sci., **38:** 1-8.

Lande, P. S. and P. G. Utikar. 1978. Sudies on fruit spot of pomegranate caused by *Drechslera rostrata.* Indian J. Mycol. Pl. Pathol., **8:** 205.

Latham, A. J. and J. C. Williams. 1983. Cultural characteristics and pathogenecity of *Glomerella cingulata* isolates from apples in albama. Pl. Dis., **67:** 1065-1068.

Legard, D. E. 2000a. Colletotrichum crown rot (anthracnose crown rot) caused by *Colletotorichum fragariae* and *Colletotrichum gloeosporioides*. Univ. Florida, Gulf Coast Res. and Edu. Wing.

Legard, D. E. 2000b. *Colletotrichum* diseases of strawberry in Florida, pages 292-299 **In:** *Colletotrichum*: Host specificity, pathology and host-pathogen intetraction. D. Prusky, S. Freeman and M. B. Dickman Ed. Am. Phytopathol. Soc. Sr. Paul, MN.

Leinhos, G. M. E. and H. Buchenauer. 1992. Inhibition of rust diseases of cereals by metabolite products of *Verticillium chlamydosporium.* J. Phytopathol., **74:** 1240-1244.

Lenne, J. M. and Sonada, R. M., 1978, Occurrence of *Colletotrichum dematium* f.sp. *trunctata* on *Stylosanthes* sp., Pl. Dis. Rep., **62:** 641-644.

Lewis, I. M. L. and S. A. Miller. 2003. Evaluation of fungicides and biocontrol agents for the control of anthracnose on a green pepper fruit. New Fungicide and Nematicide Data Commit. Am. Phytopathol. Soc., **58:** 62.

Lilly, V. G. and H. L. Barnett. 1951. Physiology of the fungi. McGraw Hill Book Company, Inc., New York, p. 464.

Litz, R. E. 1997. The mango botany, production and uses. Common Wealth Agricultural Bureau International, Library of Congress Cataloging in Publication Data, pp. 257- 280.

Lonsdale, J. H. 1992. Pre harvest fungicidal sprays for the control of post harvest diseases of mango. Year Book South African Mango Growers Association, **12:** 28-31.

Locke, J. C. 1990. Activity of extracted neem seed oil against fungal plant pathogens. In: Proc. Res. Pl. Workshop on Bot. Pest Control Project. 6-10 August. 1984, Los Banos, Philippines, pp. 132-136.

Maas, J. L. and M. E. Palm. 1997. Occurrence of anthracnose irregular leaf spot, caused by *Colletotrichum acutatum* on strawberry in Maryland. Adv. Strawberry Res., **16:** 68-70.

Majumdar, V. L. and V. N. Pathak. 1995. Biocontrol of post harvest diseases of guava fruits by *Trichoderma* spp. Acta Bot. Indica, **23:** 263-267.

Mala, S. R., M. Radha, G. Revathy and A. R. Solayappan. 1998. Garlic as a biocide for *Colletotrichum falcatum*. Indian J. Pl. Protec., **26:** 49-51.

Mancini, G., L. Russo, R. Villani and G. Remeri. 1973. *Glomerella cingulata* (Stonem). Spould and Schrenk on *Anthurium undreaum*. Rev. Pl. Pathol., **54:** 1008.

Mandhare, V. K., B. B. Pawar and S. R. Kulkarni.1996. Efficacy of fungicides against fruit spot of pomegranate. Pestology, **20(2):** 19-20.

McRae, W. 1934. Foot rot diseases of *Peper beetle* L. in Bangal. Indian J. Agric. Sci., **4:** 585-617.

Meah, M. B. and A. A. Khan. 1986. Survey of diseases of some important fruits and vegetables of Bangladesh. Dept. Pl. Pathol., Bangladesh Agric. Univ., Mymensingh. Ann. Prog. Rep., p.113.

Mederios, S. A. F. and M. Menezes. 1994. Antagoistic potential of some fungi to *Colletotrichum gloeosporioides* causal agent of cashew tree (*Anacardium occidentale*) anthracnose. Fitopathologia Brasileira, **19:** 84-89.

Melinda, J. and W. R. Stevenson 1991. A leaf disk assay for detection resistance to early blight caused by *Alternaria solani* in juvenile potato plants. Pl. Dis., **75:** 385-389.

Miah, A. T., M. U. Ahmed, N. R. Sharma, A. Ali and S. A. Miah. 1990. Antifungal activity of some plant extracts. Bangladesh J. Bot., **19(1):** 5-10.

Michniewich, M. and B. Rozej. 1987. Further studies on the role of auxin on the growth and development of *Fusarium culmorum* (W. G. Sm.) Sacc. Acta Phyvisol. Pl., **9:** 219-227.

Mesta, R. K. 1996. Studies on fruit rot of chilli caused by *C. capsici* (Sydow) Butler and Bisby in Karnataka. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Mishra, A. P. and M. Mahmood. 1960. Effect of carbon and nitrogen nutrition on growth and sporulation of *Colletotrichum capsici* (Syd.) Butler and Bisby causing anthracnose of chillies. J. Indian Bot. Sci., **39:** 314-321.

Mishra, A. P. and B. S. Siradhana. 1980. Utilization of carbon, nitrogen and vitamin by *Colletotrichum graminicola* isolates. J. Turkish Phytopathol., 9**:** 107-117.

Mishra, D. 1988. Fungicides control of anthracnose and fruit rot of chilli. Indian J. Agric. Sci., **58 (2):** 147-149.

Mishra, D. K. and A. Narain. 1994. *Gliocladium virens* and *Strptoverticillium* as sources of biocontrol of few phytppathogenic fungi. Indian Phytopathol., **47:** 236-240.

Mordue, M. 1971. Discription of pathogenic fungi and bacteria, C.M.I. Sheet No.315, Common Wealth Mycological Institute, Surrey, Kew, England, p. 2.

Mortuza, G. 1997. Effect of antagonists, alum and ultraviolet irradiation on major fruit rots of banana (*Musa sapientum* L.). College Languna, April.

Muralidharan, K., C. S. Reddy, D. Krishnaveni, and G. S. Laha. 2003. Evaluation of plant derived commercial products for blast and sheath blight control in rice. Indian Phytopathol., **56 (2):** 151-155.

Mukherjee, A., S. Khandker, M. R. Islam, Sonia and B. Shahid. 2011. Efficacy of some plant extracts on the mycelial growth of *Colletotrichum gloeosporioides*. J. Bangladesh Agric. Univ., **9(1):** 43-47.

Nagesh, G. K. 2000. Investigation on sunflower rust caused by *Puccinia helianthi* Schw. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Naik, M. K. 1985. Studies an anthracnose of betel vine (*Piper betel* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Bangalore, India.

Naik, K. S. 1986. Studies on blight of coriander (*Coriendrum sativum* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Naik, K. S., P. C. Hiremath and R. K. Hegde. 1991. Toxic metabolite production by *C. gloeosporioides* causing blight of coriander. Karnataka J. Agric. Sci., **4:** 27-31.

Natalia, P. A. 2006. Florida plant disease management guide. PDMG- **3:** 50.

Natarajan, M. R. and D. Lalithakumari. 1987. Antifungal activity of the leaf extract of *Lawsonia inermis* on *Drechslera oryaze*. Indian Phytopathol., **40:** 390-395.

Navale, A. M., K. S. Raghuwanshi, G. S. Thakare and S. G. Borkar. 2009. Chemical control of fungal leaf and fruit spot disease of pomegranate. Paper presented in; 2nd Int.. Symp. pomegranate and minor including mediterranean fruits, Univ. Agric. Sci., Dharwad, June, 23-27., p. 141.

Nene, Y. L. and P. N. Thapliya. 1982. Fungicides in plant diseases control. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, p. 163.

Ngullie, M., L. Daiho and D. N. Upadhyay. 2010. Biological management of fruit rots in the world's hottest chilli (*Capsicum chinense* Jacq.) India. J. Pl. Protec. Res., **50 (3)**.

Orlikowski, L. B. and A.Wajdyla. 1991. Occurance development and chemical control of *Colletotrichum gloeosporioides* on *Lingusyum vulgare* L.

Prove-instytutu sordunictwa- I-kwiaci astwa w-skierniewicach-seria B. Rosling-Ozuobne, **16:** 151-156.

Padder, B. A., P. N. Sharma, R. Kapil, A. Pathania and O. P. sharma. 2010. Evaluation of bioagents and biopesticides against *Colletotrichum lindemutbianum* and its integrated management in common bean. Not. Sci. Biol., **2 (3):** 72-76.

Pandey, A., L. P. Yadava, R. K. Mishra, B. K. Pandey, M. Muthukumar and U. K. Chauhan. 2012. Studies on the incident and pathogenesis of *colletotrichum gloeosporioides* penz. causes anthracnose of mango. Int. J. Sci. Nature, **3(2):** 220-232.

Papavizas, G. C. 1973. Status of biological control of soil borne pathogen. Soil Bio. Bioche., **5:** 709.

Parveen, G. and M. M. Alam. 1993. Bioactivity against plant pathogens. **In:** Neem Res. Dev. (Randhawa, N. S. and Parmar, B. S., Eds). Pub. No.3, Soc. Pesti. Sci. India, pp.144-153.

Patel, K. D. 1991. Studies on postharvest fungal diseases of guava fruits. Ph.D. Thesis, Raj. Agric. Univ., Bikaner, India.

Patel, K. D. and K. R. Joshi. 2001. Antogonistic effect of some bioagents *in vitro* against *Colletotrichum gloeosporioides* Penz. and Sacc. the causal agent of leaf spot of turmeric. J. Mycol. Pl. Pathol., **31:** 126.

Patel, K. D. and K. R. Joshi. 2002. Efficacy of different fungicides against *Colletotrichum gloeosporioides* Penz. and Sacc. the causing leaf spot of turmeric. J. Mycol. Pl. Pathol., **32:** 413-414.

Patel, S. I., J. I. Patel and J. R. Vadodaria 2007. Efficacy of fungicides against leaf and fruit spots of pomegranate caused by *Colletotrichum gloeosporioides*. J. Mycol. Pl. Pathol., **2:** 379-340.

Patel, D. S. 2009. Chemical management of fruit spot of pomegranate caused by *Colletotrichum gloeosporioides* Penz. and Sacc. Indian Phytopathol., **62(2):** 252-253.

Pathak, V. N. and J. P. Jain. 1970. Antifungal activity in leaf extracts of certain plants. Labdev J. Sci. Tech., **8:** 58.

Patil, R. K., K. D. Patel, A. Sharma and V. N. Pathak. 1992. Inhibitory effect of *Ocimum sanctum* extract on fruit rot fungi. Indian J. Mycol. Pl. Pathol., **22:** 199-200.

Patil, P. V. 1996. Studies on sunflower rust caused by *Puccinia helianthi* Schw. Ph.D. Thesis, Univ. Agric. Sci., Dharwad, India.

Patil, P. V. 2007. Eco-friendly approaches for the management of soybean rust in Karnataka. Nation. Symp. Microbial. Diver. Pl. Health. Organized by Indian Mycol. Soc., Culcutta, (Kolkata) and Dept. Pl. Path., Nadia. West Bengal. November, 29-30, p. 45.

Patil, P. V. 2008. Evaluation of botanical products against soybean rust caused by *Phakopsora pachyrhiz*i (Syd). J. Ecofriendly Agric., **3 (1):** 62-64.

Penz. 1884. Fungi Agrum. **2:** 6.

Peres, N. A., L. W. Timmer, J. E. Adaskaveg and C. J. Collell. 2005. Lifestyle of *Colletotrichum acutatum*. Pl. Dis. 89: 784-796.

Phillips, D. and S. Mack. 2007. Strawberry root and crown rot in Western Australian Plant Disease Notes., **2(1):** 145-147.

Phillips, D. and H. Golzar. 2008. Strawberry root and crown rot disease survey 2005 and 2006 seasons in Western Australia. Western Australian Agric. Author, ISSN 1833-7236.

Porras, M., B. Barrau Santos de los, C. Blanco, F. T. Arroyo and F. Romero. 2003. Combined effects of *Trichoderma* applications and soil solarization on Colletotrichum crown rot in strawberry plants. Bull. OILB/SROP, 26. **10:** 113-114.

Potebinia, A. 1910. Beitrage Dar. Micromycetam flora Miltel Russland. Ann. Mycol. Berlin, pp. 42-98.

Prasad, R. D., R. Rangeshwaran, C. P. Anuroop and H. J. Rashmi. 2002. Biological control of wilt and root rot of chickpea under field onditions. Ann. Pl. Protec. Sci., **10:** 72-75.

Prasanna, M. K. 2001. Management of post harvest diseases of mango. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Prasanna, M. K., V. B. Nargund and A. N. A. Khan. 2006. Laboratory evaluation of fungicides and botanicals against post harvest diseases in mango. Mysore J. Agric. Sci., **40(1):** 21-26

Prashanth, A. 2007. Investigation on anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. of pomegranate (*Punica granatum* L.). M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Prashanth, A., R. S. Arun, M. K. Naik, M. B. Patil and S. P. Rajesh. 2008. Evaluation of fungicides, bioagents and botanicals against pomegranate anthracnose. Indian J. Pl. Protec., **36(2):** 283-287.

Priya, J. and V. Kurucheve. 2005. Effect of animal excrement of the conidial germinate of groundnut tikka leaf spot. **In:** Nation. Symp. Mod. Conf. Pl. Dis. Mgt. Global Trust (MCPDMGT) and IPS Ann. Meet (South zone), Abstracts, p. 36.

Punja, Z. K., J. D. Carter, G. M. Campbell and E. L. Rossell. 1986. Effect of calcium and nitrogen fertilizers, fungicides and tillage practices on incidence of *Sclerotium rolfsii* on processing carrots (*Daucus carota*). Pl. Dis., **70:** 819-824.

Purkayastha, R. P. and M. Sengupta. 1975. Studies of *Colletotrichum gloeosporioides* inciting anthracnose of Jute. Indian Phytopathol., **28(4):** 454-458.

Pyke, T. R. and A. Dictz. 1960. U-Zh-963, a new antibiotic I. Discovery and biological activity. Applied Microbiol., **14:** 506-510.

Raghuwamshi, K. S., G. N. Dake, D. M. Sawant and A. L. Pharnade. 2004. Chemical control of leaf spot and fruit spot of pomegranate in hasta bahar. J. Maharashtra Agric. Univ., **30(1):** 56.

Raheja, S. and B. B. L. Thakore. 2002. Effect of physical factor, plant extracts and bio-agent on *Colletotrichum gloeosporioides* Penz., the causal organism of anthracnose of Yam. J. Mycol. Pl. Pathol., **32:** 293-294.

Rahman, M. L., A. M. Akanda and A. L. Khan. 1993. A study on the anthracnose disease of country bean. Bangladesh J. Agric. Res., **18(2):** 125-130.

Rahman, A., M. M. Rahman, A. K. Azad and M. F. Alam. 2011. Inhibitory effect of different plant extracts and antifungal metabolites of *Trichoderma* strains on the conidial germination and germ tube growth of *Colletotrichum capsici* causing chili anthracnose. Int. J. Agric. Res., **1 (1):** 20-28.

Raja, J., C. Suganthi and V. Kurucheve. 2005. Management of *Rhizoctonia solani* with animal urine. **In:** Nation. Symp. Modern Conf. Pl. Dis. Man.

Raja, J., C. Suganthi and V. Kurucheve. 2006. Use of animal urine for the management of sheath blight of rice. Phytopathol., p. 96.

Rajak, R. C. 1983. Nutritional requirements of two species of *Colletotrichum.* Acta Botanica, India, **11:** 155-160.

Rajesh, 1999. Studies on some of the forest fungal flora of parts of Western Ghats. M.Sc. (Agric) Thesis, Univ. Agric. Sci., Dharwad, India.

Raju, K. S. and G. S. Rao. 1989. Effect of combination application of Dithane M-45 with different insecticides to control fruit rot and pest complex on chilli. Indian J. Mycol. Pl. Pathol., **15 (13):** 239-246.

Rani, S. G. and K. V. M. K. Murthy. 2004. Cultural and nutritional characteristic of *Colletotrichum gloeosporioides* the causal organism in cashewnut anthracnose. J. Mycol. Pl. Pathol., **34:** 317-318.

Rawal, R. D. 1990. Fungal and Bacterial diseases of fruit crops. Paper presented **In:** Group discussion of plant pathologists working under AICRP on Horticulture crops. IIHR, Bangalore, June, 16-17., pp. 1-60.

Ricker, A. J. and R. S. Ricker. 1963. Introduction to research on plant disease. John. S. Swift. Co. Inc. New York.

Ristaino, J. B., J. A. Lewis and R. D. Lumsden. 1994. Influence of isolate of *Glocladium virens* and delivery system on biological control of southern blight on processing carrot and tomato in the field. Pl. Dis., **78:** 153-156.

Robinson, P. M. and D. Park. 1966. Volatile inhibitor of spore germination produced by Taagi. Trans. British Mycol. Soc., **49:** 639-649.

Roico, C. H. A., L. L. B. N. Laura, B. B. Silvia and B. L. Leticia. 2007. Antifungal potential of crude plant extracts on conidial germination of two isolates of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Mexican J. Phytopathol., **25(2):** 179-185.

Rovesti, L., D. I. Marco and P. Pancaldi. 1992. Effect of neem kernel extract on some Phytopathologenic fungi under green house conditions. Zeitschriftfur Pflanzen Krankheiten and Pflanzenschutz, **99 (3):** 293-296.

Roy, K. W., J. F. Killebrew and S. Ratnayake. 1997. First report of *Colletotrichum capsici* on bell pepper in Mississippi. Pl. Dis., **81:** 693.

Saccardo, P. A. 1884. Sylloge fungorum, 3, Padua.

Saifulla, M. and K. G. Ranganathaiah. 1990. Studies on the cultural behaviour of anthracnose of sorghum caused by *Colletotrichum graminicola* (Ces.) Wils., Mysore J. Agric. Sci., **24:** 52-56.

Saini, P. K. and V. N. Pathak. 1991. Economic importance of *Aspergillus* rot of mango fruits in Udaipur. Annu. Conf. Soc. Mycol. Pl. Pathol., HAU, Hisar, hold on April, 19-21, p. 36

Sakila, S., M. B. Ahmed, U. K. Roy, M. K. Biswas, R. Karim, M. A. Razvy, M. Hossain, R. Islam and A. Hoque. 2007. Micropropagation of strawberry (*Fragaria* × *ananassa* Duch.). A Newly Introduced Crop in Bangladesh. American-Eurasian J. Sci. Res., **2(2):** 133-135.

Salatin, J. 1993. One low day of manure: what's it worth, Stockmen Grass Farmer, p.11.

Sanders, G. M., L. Koresten and F. C. Whener. 2000. Survey of fungicides sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. European J. Pl. Pathol., **106:** 745-752.

Sangeetha, C. G. and R. D. Rawal. 2008. Nutritional studies of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the incitant of mango anthracnose. World J. Agric. Sci., **4(6):** 717-720.

Sangeetha, C. G. and R. D. Rawal. 2010. Temperature requirement of different isolates of *Colletotrichum gloeosporioides* isolated from mango. African J. Biotech., **9 (21):** 3086-3090.

Santha, K., P. 2002. Biocontrol of anthracnose of black pepper. J. Mycol. Pl. Pathol., **32:** 358.

Sattar, A. and S. A. Malik. 1939. Some studies on anthracnose of mango caused by *Glomerella cingulata* Stonem (S. and V.S) (*Colletotrichum gloeosporioides* Penz.) in the Punjab. Indian J. Agric Sci., **9:** 511-521.

Sehgal, S. P., A. L. Deshpande and U. S. Shrivastava. 1965. A new disease of coriander caused by *Colletotrichum* causing citrus die-back in India. Curr. Sci., **34:** 89-90.

Selvaraj, J., B. Ramaraj, K. Devarajan, N. Seenivasan, S. Senthilkumar and E. Sakthi. 2003. Effect of organic farming on growth and yield of thyme. **In:** Articles and Abstracts of Nation. Sem. Prod. Utiliz. Med. Pl., 13-14, March, 203 held at Annamali Univ. Tamil Nadu, p. 63.

Selvi, M. T. and V. Kurucheve. 2005. Effect of animal excrements on the *in vitro* production of pectolytic enzymes by *Pyricularia oryzae*. **In:** Nation. Symp. Mod. Conf. Pl. Dis. Mgt. Global Trust (MCPDMGT) and IPS Ann. Meet (South zone), Annamali Univ. Tamil Nadu, December, 29-30, Abstracts, p. 30.

Shamim, A., S. Alam, M. S. Islam and M. W. Lee. 2009. Identification of the fungal pathogen that causes strawberry anthracnose in Bangladesh and evaluation of *in vitro* fungicide activity. Korean Soc. Mycol., **37(2):** 77-81.

Sharif, A. H. M. 2005. Control of *colletotrichum capsici* causing anthracnose of chilli (*capsicum annuum* l.) by botanicals, chemicals and biocontrol

agents. Dept. Pl. Pathol, Bangabandhu Sheik Mujibur Rahman Agric. Univ., Gazipur, p. 63.

Sharma, K. C. 1976. Urea spray fertilization can bring extra yield in dwarf wheat's. Indian Fmg., **20 (5):** 31-32.

Sheshtawi, E. and E. Kiss. 1975. Influence of hormone type herbicides on the development of *Fusarium graminearum* Schwabe. Novenyvedelem, **11:** 20-22.

Shirshikar, G. S. 2002. Studies on fruit rots of mango (*Mangifera indica* L.) caused by *Botryodiplodia theobromae* Pat. and *Colletotrichum gloeosporioides* Penz. and their management. M.Sc. (Agric.) Thesis, KKV, Dapoli, Maharashtra, India.

Shivashankar, S. P. and D. N. Kadam. 1993. Efficacy of neem leaf extract against foliar diseases of groundnut. Indian Phytopathol., **45:** 72.

Shovan, L. R., M. K. A. Bhuiyan, J. A. Begum and Z. Pervez. 2008. *In vitro* control of *Colletrotrichum dematium* causing anthracnose of soyabean by fungicides, plant extracts and *Trichoderma harzianum.* Int. J. Sustain. Crop Prod. **3(3):** 10-17. Dept. Pl. Pathol., Bangabandhu Sheik Mujibur Rahman Agric. Univ., Gazipur.

Singh, R. S. and K. R. Pande. 1966. Effect of green and mature plant residues and compost on population of *Pythium aphanidermatum* in soil. Indian Phytopathol., **19 :** 367-371.

Singh, R. A. and G. Shankar. 1971. Effect of physiological factors and nutrition growth and perithecia development of *Glomerella cingulata in vitro.* Indian Phytopathol., **24:** 721-734.

Singh, R. S. and J. S. Chohan.1972. A new fruit spot disease of pomegranate. Curr. Sci., **41:** 651.

Singh, S. N., B. P. Yadav, S. K. Sinha and K. L. Ojha. 1997. Efficacy of plant extracts in inhibition of radial growth and spore germination of *Colletotrichum capsici*. J. Appli. Biol., **7:** 58-61.

Singh, A., S. Srivastava and H. B. Singh. 2007. Effect of subtracts on growth and shelf life of *Trichoderma harzianum* and its use in biocontrol of diseases. Biores.Tech., **98:** 470-473.

Singh, A., K. S.Verma and C. Mohan. 2007. Prevalence of anthracnose of guava in Punjab and its management. J. Mycol. Pl. Pathol., **37(1):**188.

Small, W. 1926. On the occurrence of a species of *Colletotrichum*. Transa. Brit. Mycol. Soc., **11:** 112-137.

Smith, B. J. and L. L. Black. 1988. Resistance of strawberry plants to *Colletotrichum fragariae* affected by environmental conditions. Pl. Dis., **72:** 834-837.

Smith, B.J. 1993. Developmental stage and temperature affect strawberry flower and fruit susceptibility to anthracnose. Phytopathol., **83:** 468.

Smith, B. J. 1998. Anthracnose leaf spot and irregular leaf spot. pp. 24-25. Anthracnose fruit rot p. 31-33. Anthracnose crown rot, pp. 46-48. **In**: J. L. Mass (ed.), Compendium of strawberry diseases. Second ed. APS Press. St. Paul. Minn.

Smith, B. J. 2008. Epidemiology and pathology of strawberry antracnose: A North American respective. Org. Hort. Sci., **43 (1):** 69-73.

Sobowale, A. A., O. A. Odeyingbo, H. O. Egberongbe, R. T. Feyisola, O. A. Ayinde and A. Adesemowo. 2010. Growth inhibition (*in vitro*) of *Colletotrichum gloeosporioides* isolated from cassava (*Manihot esculenta*) using *Trichoderma longibrachiatum*. African J. Microbiol. Res., **4(21):** 2196-2201.

Sonada, R. M., A. E. Kretschmer and J. B. Brolman. 1974. *Colletotrichum* leaf spot and stem canker of *Stylosanthes* sp. in Florida. Trop. Agric., **51:** 75-79.

Sonawane, P. C., U. T. Desal, S. H. Chaudhari and B. P. Deore. 1994. Extent of field loss in pomegranate due to biotic and abiotic factors. J. Mah. Agric. Univ., **19(1):** 161-162.

Soytong, K., W. Srinon, K. Rattanacherdchai, S. Kanokmedhakul and K. Kanokmedhakul. 2010. Application of antagonistic fungi to control anthracnose diease of grape. J. Agric. Biotech., **1:** 33-41.

Sreelatha, R. and G. Bhagyanarayan. 2008. Biocontrol efficacy of botanicals on anamorphic fungi. J. Mycol. Pl. Pathol., **38(3):** 647-649.

Sridhar, S., S. Arumugasamy, H. Saraswathy and K. Vijaylakshmi. 2002. Organic Vegetable Gardening. Centre for Indian Knowledge Systems, Chennai, p. 39.

Srinivasan, S. and M. Gunasekaran. 1998. *In vitro* assay of fungicides against pre ponderant fungi of leaf rot disease of coconut palms. Pestology, **22:** 17-22.

Steven M. K. and Natalia. 2009. Colletotrichum crown rot of strawberries. Pl. pathol., p. 238.

Sudarshan, R. A. N. 1975. Survey, surveillance and forecast in plant protection. Kisan World, **2:** 43-44.

Sudhakar. 2000. Biology and management of *Stylosanthes* anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Sujatha, B. E. 1992. Studies on fruit rot of chilli (*Capsicum annum* L.) caused by *Alternaria tenuis* Nees. M.Sc.Thesis, Tamil Nadu Agric. Univ., Madurai, India, p.173.

Sultana, N.1991. Biological control of foot and root rot of lentil with *Trichoderma harzianum* [dissertation]. Bangladesh Agric. Univ. Mymensingh.

Sumitra, R. and S. J. Gaikward. 1995. Checking Fusarium wilt of pigeon pea by biological means. J. Soil Crop., **5:** 163-5.

Sundar, A. R., N. D. Das and D. Krishnaveni. 1995. *In vitro* antagonism of *Trichoderma* spp. against two fungal pathogens of castor. Indian J. Pl. Protec. **23 (2):** 152-155.

Sundaraman, S. R., R. Selvam, R. Venkatachalam and M. Ramakrishnan. 2001. Handbook on Organic Farming, Natural way of farming movement Communication Bulletin, Prajetha NGO Network, Kongal Nagarm, Tamil Nadu.

Suresh, G., N. S. Narasimhan, S. Masilamani, P. D. Partho and G. Gopalkrishnan. 1997. Antifungal fractions and compounds from uncrushed green leaves of *Azadirachta indica*. Phytoparasitica., **25:** 33-39.

Sutton, B. C. 1980. The coelomycetes.1st ed. Commonwealth Mycol. Inst. Kew, Surrey, England.

Sutton, B. C. 1993. The genus *Glomorella* and its Anamorph *Colletotrichum* **In:** J. A. Bailey and M. J. Jeger (eds.) *Colletotrichum*: Biol, Pathol. Control, CAB, Int., Wallingford, UK., pp. 1-26.

Svetlana Zivkovic, S. Stojanovic, Z. Ivanovic, V. Gavrilovic Tatjana popovic and Jelica balaz. 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides,* Archives Biol. Sci., Belgrade, **62(3):** 611-623.

Sesan,T. E. 2006. Integrated control of Strawberry diseases. Phytopathol., **39:** 133-148.

Tandon, R. N. 1961. Physiological studies on some pathogenic fungi. Scientific Research Committee, Uttar Pradesh. India, p.80.

Thind, K. S. and H. S. Randhawa. 1957. Studies on the nutrition of *Colletotrichum capsici (*Syd.) Butler and Bisby, the incident of die-back of chillies. Curr. Sci., **26:** 17-18.

Tomy, P. 1997. Laboratory evaluation of fungicides against *C. gloeosporioides* causing black leaf spot in mulberry. Pestology, **21:** 22-23.

Toriyama, K. 1972. Breeding for resistance to major diseases in Japan. In: Pl. Breed. Pest and Dis. Mgt. London., pp. 110-115.

Tripathi, R. D., H. S. Srivastava and S. N. Dixit. 1978. A fungitoxic principles from the leaves of *Lawsonia inermis* Lam. Experimentia., **34:** 51-52.

Udhayakumar, R. and S. Usha Rani. 2010. Effect of p^H levels, carbon and nitrogen sources on the mycelial growth and bio-mas production of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Int. J. Pl. Protec. Univ. Agric. Sci. Bangalore, India, **3 (1):** 1-4.

Usman, M. R., R. Jaganathan and D. Dinakaran. 1991. Plant disease management of groundnut with naturally occurring plant products. Madras Agric. J., **78:** 152-153.

Venkataravanappa, V. and V. B. Nargund. 2002. Evaluation of different fungicides against *Colletotrichum gloeosporioides* causal organism of anthracnose of mango. Paper presented on Plant disease scenario in southern India. Univ. Agric. Sci., Bangalore, Ann. Meet. Symp. December, 19-21, p. 56.

Venkataravanappa, V. 2002. Studies on mango annthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. M.Sc. (Agric.) Thesis, Univ. Agric. Sci. Bangalore, India.

Verma, V. 1969. Effect of temperature and hydrogen ion concentration on three pathogenic fungi. Sydowia, **23:** 164-168.

Verma, M. L. 1979. Effect of sucrose concentration on growth and sporulation of 3 species of *Colletotrichum* pathogenic on chillies. Indian J. Mycol. Pl. Pathol., **9:** 130-131.

Vey, A., R. E. Hoagland and T. M. Butt. 2001. Toxic metabolites of fungal biocontrol agents. Fungi as Biocontrol Agents: Progress, Problems and Potential. Butt T. M, Jackson C and N, eds, CAB Int., Bristol., pp. 311-346.

Vijaylakshmi, K., S. Arumugasmy and S. Subhashini. 2005. Organic methods of pest and disease management Centre for Indian Knowledge Systems, p. 8.

Vinaya, H. 2008. Studies on seed borne aspects of anthracnose of chilli and its management in Karnataka. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Vinod, T.. 2008. Studies on anthracnose a postharvest disease of papaya. M.Sc. (Agric.) Thesis, Univ. Agric. Sci., Dharwad, India.

Vinod, T. and V. I. Benagi. 2009. Studies on cultural and nutritional characters of *Colletotrichum gloeosporioides,* the causal organism of papaya anthracnose. Karnataka J. Agric. Sci., **22(4):** 787-789.

Vinod, T., V. I. Benagi, R. Yashoda Hegde., B. C. Kamanna and K. Ramachandra Naik. 2009. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides.* Karnataka J. Agric. Sci., **22(4):** 803-806.

Vyas, R. K. 1993. Economic losses, epidemiology and management of *Aspergillus* rot of mango fruit. M.Sc. (Agric.) Thesis, RAU, Bikaner, India.

Vyas, R. K. and K. Mathur. 2002. Distribution of *Trichoderma* spp. in cumin rhizosphere and their potential in suppression of wilt. Indian Phytopathol., **55:** 451-457.

Watve, Y. G., M. P. Diwakar, and J. J. Kadam. 2009. An evaluation of some bioagents and plant extracts against leaf spot of jatropha caused by *Colletotrichum gloeosporioides* Penz. J. Pl. Dis. Sci., **4(1):** 95-98.

Weinding, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathol., **24:** 1153-1179.

Winiham, T. T., Y. Elad and A. R. Baker. 1986. Amechanism for increased growth induced by *Trichoderma* spp. Phytopathol., **76:** 518-521.

Yang, Y., Tang, G. Yuan, Y. Haang, M. Liu, S. Huang, Y. Yang, G. Y. Tang, Y. Y. Huang, M. Z. Liu and S. M. Huang. 2000. Biological characteristics of *Colletotrichum musae* from banana fruits in Hainan province. Acta Phytopathololologicani. Sinica., **30(4):** 337-342.

Yashoda, R. H., R. K. Hegde and Srikant Kulkarni. 1993. Physiological studies on *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing anthracnose of areca nut. Karnataka J. Agric. Sci**., 6:** 411-412.