Studies on leaf spot of pigeonpea caused by complex of *Didymella spp*.

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Master of Science (Agriculture)
in
Plant Pathology

Submitted by

Rakhi Rathod

DEPARTMENT OF MYCOLOGY AND PLANT PATHOLOGY
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI – 221005
INDIA

I.D. No. 20412MPP015 2022 Enrolment No. 429502

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

Dr. R.K. Singh, Associate Professor
Department of Mycology and Plant Pathology

Reference No. ………………………   **Date: ……………….**

CERTIFICATE

To,

The Registrar,

Banaras Hindu University,

Varanasi-221005 (India)

Through: The Head, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, B.H.U., Varanasi.

Respected Sir,

I have great pleasure in forwarding the thesis entitled Studies on leaf spot of pigeonpea caused by complex of *Didymella spp.*submitted by Miss Rakhi Rathod , I.D. No. 20412MPP015 in Partial fulfilment of the requirements for the degree of Master of Science (Agriculture) in Plant Pathology, from the Department of Mycology and Plant Pathology Studies on leaf spot of pigeonpea caused by complex of *Didymella spp.*submitted by Miss Rakhi Rathod , I.D. No. 20412MPP015 in Partial fulfilment of the requirements for the degree of Master of Science (Agriculture) in Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University and placing on record that he has completed the requisite requirements as contained in the statutes of the university.

I certify that the entire scheme of investigation reported herein, was planned and carried out solely by the candidate under my guidance and supervision. The data presented in the thesis, to the best of my knowledge and belief, are genuine and have not been utilized for the award of other degree or distinction.

Thanking you.

Yours faithfully,

(R.K Singh)

Forwarded by

Institute of Agricultural Sciences
Banaras Hindu University, Varanasi 221005, India
Ph: +91-9450530703, Email: rakeshsingh@bhu.ac.in

BHU capital of knowledge

Study on leaf spot of pigeonpea caused by complex of *Didymella spp.*

by

Rakhi Rathod

Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science (Agriculture)
in
Plant Pathology

Department of Mycology and Plant Pathology
Institute of Agricultural Sciences
Banaras Hindu University
Varanasi – 221005 (INDIA)

ID. No. 20412MPP015 **2022** **Enrolment No. 429502**

APPROVED BY ADVISORY COMMITTEE

Chairman: **Dr. Rakesh Kumar Singh**
Professor
Department of Mycology and Plant Pathology
Institute of Agricultural Sciences, B.H.U., Varanasi

Member: **Dr. Ankita Sarkar**
Assistant Professor
Department of Mycology and Plant Pathology
Institute of Agricultural Sciences, B.H.U., Varanasi

Member: **Dr. Srinivasa N.**
Assistant Professor
Department of Entomology and agriculture zoology
Institute of Agricultural Sciences, B.H.U., Varanasi

External Examiner:

URKUND REPORT

Curiginal

Document Information

Analyzed document	thesis final.docx (D141164610)
Submitted	6/24/2022 4:31:00 PM
Submitted by	
Submitter email	rakhirathod55@gmail.com
Similarity	2%
Analysis address	cenlib2014.bhuni@analysis.urkund.com

Sources included in the report

SA	**Banaras Hindu University / ANKITA_IAS.pdf** Document ANKITA_IAS.pdf (D141071139) Submitted by: ankitasarkar@bhu.ac.in Receiver: cenlib2014.bhuni@analysis.urkund.com	10
SA	**Sher-E-Kashmir University Of ASTK Srinagar / thesis on phoma blight of soybean - Subaya Manzoor.docx** Document thesis on phoma blight of soybean - Subaya Manzoor.docx (D93709626) Submitted by: farooqbhat@skuastkashmir.ac.in Receiver: farooqbhat.skuast@analysis.urkund.com	1
SA	**Dr. Balasaheb Sawant Konkan Krishi Vidyaapeeth, / Touhid Tambe Thesis.pdf** Document Touhid Tambe Thesis.pdf (D109476412) Submitted by: potphodeparesh@gmail.com Receiver: potphodeparesh.bskkv@analysis.urkund.com	1
SA	**Sher-E-Kashmir University Of ASTK Srinagar / Ind Phytopath - edited.docx** Document Ind Phytopath - edited.docx (D104169617) Submitted by: ajaz999@gmail.com Receiver: ajaz999.skuast@analysis.urkund.com	1
SA	**Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur / THESIS FINAL_merged.docx** Document THESIS FINAL_merged.docx (D140095455) Submitted by: Govindchoudhary1644@gmail.com Receiver: hod.pl_pathology.jnkv@analysis.urkund.com	1

“Dedicated to my Teachers and Parents”

ACKNOWLEDGEMENT

With a deep sense of devotion, I bow and pray to the feet of, Lord Vishwanath, Maa Gaytri and my gurudev pandit shree Ram Sharma Aacharya who provided me the choicest, everlasting blessing to get an opportunity to study in Banaras Hindu University, the dream of Mahamana Pandit Madan Mohan Malviyaji, a man of great vision, a great patriot, nobleman and patriarch of this university, for his lifetime sacrifice and efforts in establishing such a temple of learning for the cause of millions of students like me.

With immense pleasure and a profound sense of gratitude, indeed, I take this opportunity to express my heartfelt and sincere thanks to my esteemed supervisor, Prof. Rk Singh ,Co-Advisor Dr. Ankita Sarkar Department of Mycology and Plant Pathology , Institute of Agricultural Sciences, Banaras Hindu University, for his meticulous guidance, indelible inspiration, persistent encouragement, ingenious suggestions, mellifluous nature, and indefatigable attitude. I will ever cherish the fatherly affection that he bestowed upon me throughout my tenure as a student under him which helped me to cope with many difficult situations.

I am highly obliged to Dr. Shyam Sharan Vaish Professor and Head, Department of Mycology and Pathology for providing the necessary research and academic facilities during the investigation.

I owe my sincere thanks to the members of my advisory committee, Dr. Ankita Sarkar Assistant professor, Department of Mycology and Plant Pathology, and Dr. Srinivasa N. Assistant professor Department of Entomology and Agriculture zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) for their critical suggestion, impeccable and benevolent guidance.

I extend my indebtedness to the respected teachers Dr. Ramesh Chand , Dr. BK Sarma , Dr.SP Singh, and Dr. RC Ram, Dr. DD. Bhutia of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University for their discerning comments, valuable suggestions, co-operations and helpful attitude towards me during the investigation.

I owe my sincere thanks to all the non-teaching staff Laxman Sir, Dashrath Sir, Ram Kumar Sir, Koushik sir and Krishna Sir , Rishabh sir , non-teaching staff, and my lab assistant Sadanand Bhaiya, Rajendra Bhaiya, of the Department of Mycology and Plant Pathology Institute of Agricultural Sciences, Banaras Hindu University for their keen interest taken in the work providing the necessary and timely research facilities, inspiration, and suggestion throughout the work.

I owe my sincere thanks to the Interdisciplinary school of life sciences BHU for their guidance and for providing the necessary research facilities during the investigation.

With profound regards in a more personal sense, I owe deepest debts to my father Tilok Chand Rathod and my mother Smt. Sudha Rathod . It was their zeal and enthusiasm which made it possible for me to complete my logical end of this study. Words are not enough to express my deep sense of honor, unbounded gratitude, and sincere regards to my sweet parents who set the foundation and were always with me during my ups and downs. I thank them once again because I would have never achieved this level of education without their selfless sacrifices.I will also like to acknowledge my affection towards my elder sister Mrs.Vinita Rathod and my brothers Mr. Deepak Rathod and Mr. Yogen Rathod

This thesis was impossible without the indispensable help of my respected seniors. So, heartfelt and special thanks to my senior Dr. Sandeep Gupta , Mr Saroj Belbase, Mr.lovkush Satnami , Dr. Raina Bajpai , Mr.Basavraj Teli, Mrs. Jhumishree meher , Mr. Prahalad Masurkar and Mr. vikas yadav . I am highly thankful to my friends Ms.Harshitha S. Nayak, Ms. Sadqua Fatima Mr. Deepak Malviya, Yogesh Kumar and batchmates, Ms. Nikita, Mr. Jeevan ,Mr. Suman, Ms. Ganita , Ms.Shushrutha Ms. Neha, Ms. Samiksha, Mr. Suren, Ms . Vandana, who have always been with me right from the beginning and throughout my thesis work for which I will always have gratitude and respect for them.

The graces of God are always blessed to me and give me patience and power to overcome the difficulties which came my way in the accomplishment of this endeavour. I cannot dare to say thanks but only pray to bless me always.

Last but not the least, I record my sincere thanks to all the respectable people who helped me and could not find separated mention. I still solicit their benediction to proceed at every step of a perfect destined life.

Great thanks to all

Place: Varanasi

Date: (Rakhi Rathod)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

%	**Per cent**
@	**At the rate of**
°C	**Degree Celsius**
μgml^{-1}	**micro gram per millilitre**
ANOVA	**Analysis of Variance**
ATP	**Adenosine Tri-phosphate**
b.v.	**biovar**
BLAST	**Basic Local Alignment Search Tool**
bp	**base pair**
cm	**Centi Metre**
CR	**Congo Red**
CRD	**Completely Randomised Design**
DNA	**Deoxyribose nucleic acid**
dSm^{-1}	**deci siemens per metre**
DSW	**Distilled sterilized water**
et al	**Co-workers**
f. sp.	**forma specialis**
F.W.	**Fresh Weight**
FAO	**Food and Agriculture Organisation**
Fig.	**Figure**
gm	**Gram**
hrs.	**Hours**
i.e.,	**That is**
kg	**Kilo Gram**
L	**Litre**
lbs	**unit of pressure**
M	**Molar**
mg	**Milli gram**
Mha	**Million hectare**
$mScm^{-1}$	**milli siemens per centimetre**
Mt	**Metric tonne**
N_2	**Nitrogen**
OD	**Optical density**
PCR	**Polymerase chain reaction**
PGP	**Plant growth promoting**
RNA	**Ribose nucleic acid**
SD	**Standard Deviation**
µL	**Micro Litre**
ml	**Milli Litre**
mm	**Milli Metre**
MT	**Metric Tonne**
N	**Normal**
spp.	**Species**

Chapter I

INTRODUCTION

INTRODUCTION

India is a global leader in producing and consuming the pulses . Edible seeds of cultivated legumes were considered as pulses, which serves as prominent protein source for the vegetarian people in the country. Pigeonpea is one of the major pulse crop that is a perennial legume belonging to Fabaceae family cultivated as annual crop and grown in Africa, Asia, Latin America and Caribbean regions of the world

Nutritive value of Pigeonpea

- Protein - 22.3 %
- Fat - 1.7 %
- Minerals - 3.5 %
- Fiber - 1.5 %
- Carbohydrate - 57.6 %
- Calcium - 73 mg/100 g
- Phosphorus - 304 mg/100 g
- Iron - 5.8 mg/100 g
- Moisture - 13.4%
- Calorific value - 335 Kcal/100

Table 1.1 Global Ranking : Major Countries of Pigeonpea (production- lakh tones) Source : FAO , statistics 2016

Country	production	Percent contribution
India	48.73	57.29
Myanmar	6.28	7.38
Malawi	3.71	4.36
Tanzaniya	2.72	3.20
Kenya	1.91	2.25
Haiti	1.14	1.34
Nepal	0.16	0.19
Others	20.41	23.99
World	85.06	

Source: *FAO Statistics 2016.*

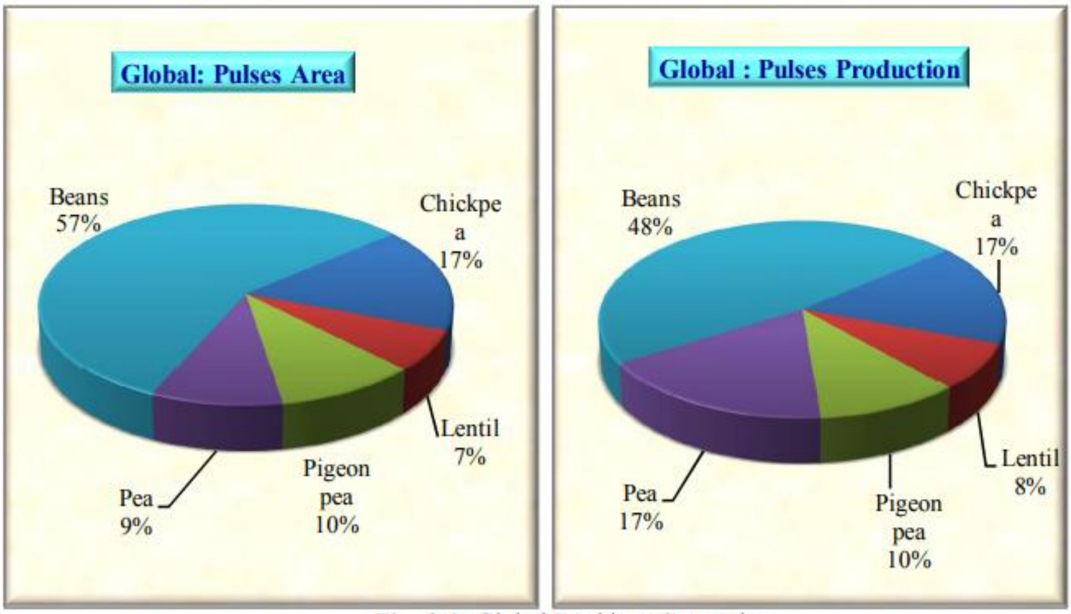


Fig.-3.1: Global Ranking: Crop-wise

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a perennial hard woody shrub with a limited life span that belongs to the fabaceae family. It has a deep tap root system and symbiotic roots with Bradyrhizobium species. The stem may reach a height of four metres and the branching style ranges from bushy to erect compact. It can be either determinate or semideterminate depending on the blooming pattern. Leaflets are lanceolate to elliptical and are pinnately trifoliate. Flowers are zygomorphic, borne on axillary or terminal racemes, and are typically yellow in colour with slight variations. The stamens are 10 in number and diadelphous. The ovary of pigeon pea is superior with a long style and attached to a thickened, incurved, and swollen stigma. Frequently cross pollinated crop, with cross pollination rates ranging from 20% to 70%. The pod of the pigeonpea displays either profound constrictions or no constriction (Kumar *et al.*, 2017).

The pigeon pea is susceptible to frost damage at all stage of growth.The optimum temperature range is between 18-29°C (64-85°F) . Bright sunlight is required for the highest seed output; heavy cloud or shadow causes spindly growth and poor seed set. In the wild, there are short day, day neutral, and intermediate types. They won't grow at elevations greater than 1800 metres. The appropriate rainfall range is 600-100mm per year. When there is adequate rainfall during the first two months of growth, followed by a dry time during flowering and harvesting, great yields are attained. Drought resistance capacity of pigeonpea is high. (source: https://www.horticulture.org.za/pigeon-pea-climatic-requirements/)

Taxonomic classification

Kingdom : Plantae

Clade : Angiosperms

Clade : Eudicots

Clade : Rosids

Order : Fabales

Family : Fabaceae

Genus : *Cajanus*

Species : *cajan*

Binomial name **:** *Cajanus cajan* (L.) Millsp.

Didymella

Taxonomic Tree

Domain: Eukaryota

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Dothideomycetes

(Source : www.Cabi.com)

It is an ascommycete fungal pathogen of plants causes asochyta blight disease in chickpea, gummy stem blight of cucurbits, in cucurbits it affects stems ,fruit, leaves and can affect entire crop in favourable condition . In chickpea it affects pod stem and leaves and make circular spots on the plant parts on which black coloured bodies are prominent. There are many species under this genus which causes disease of legumes including *D. pinodes , D. pinodella , D. arachidicola , D. rabaei* etc. *Ascochyta rabiei, Ascochyta fabae, and Ascochyta fabae f. sp. lentis,* all major seedborne pathogens, cause Ascochyta blights of chickpea, faba bean, and lentil, respectively. Infected seed is critical for the pathogen`s long-distance transmission and survival. Didymella is the genus that includes the teleomorphic (sexual) forms of the blight pathogens of chickpea, faba bean, and lentil. These fungi are heterothallic, which means that successful sexual reproduction requires the matching of two suitable mating types. Infected seed from these three crops is being moved around not only allows for the entrance of virulent pathotypes, but it may also allow for the spread of suitable mating types, allowing for the evolution of the teleomorph in nature (Kaiser 1997).

Didymella Bryoniae (Auersw.) Gummy stem blight, foliar leaf spot, and black rot of fruit are all caused by fungus , resulting in substantial cucurbit crop losses across the world (Zitter *et al* 1996). The infection is most prevalent in the southern United States, as well as in subtropical and tropical regions across the world (Sitterly and Keinath 1996). It produces black rot symptoms on the fruit of all cucurbits. Black rot was seen on all fruit developmental stages during the 1997 sticky stem blight and black rot pandemic of cantaloupe fruit in the southern Rio Grande Valley of Texas, USA, and many fields suffered 100% loss (Miller *et al* 1997). *Didymella pinodes* is the cause of ascochyta blight, one of the most common fungus diseases of pea (Pisum sativum) in the world. Losses of up to 70% are possible due to this disease (Tivoli and Banniza, 2007). Losses due to Ascochyta blight in chick pea may reach 100 per cent both in terms of yield as well as quality if environmental conditions, particularly rainfall, favourable for the development of disease persists (Porta-Puglia *et al* 1996, Pande *et al* 2005).

The Didymellaceae family is one of the most diverse in the fungal kingdom, with members living in a variety of environments. On the basis of multi-locus DNA sequence data, the taxonomy of Didymellaceae was recently updated. they looked at 108 Didymellaceae isolates from 40 host plant species from 27 plant families, as well as cave substrates like air, water, and carbonatite, from Argentina, Australia, Canada, China, Hungary, Israel, Italy, Japan, South Africa, the Netherlands, the United States, and the former Yugoslavia. Based on the multi-locus phylogeny employing LSU (Nuclear large subunit ribosomal DNA) , ITS

(The Internal Transcribed Spacer regions of fungal ribosomal DNA), rpb2 (RNA polymerase II 2nd largest subunit), and tub2 (β-tubulin) sequences, as well as morphological variations, 68 isolates representing 32 new species are recognised. Five genera of the Didymellaceae appeared to be restricted to certain habitats. (Chen *et al* 2017).

CURVULARIA

There are more than 40 species in the genus Curvularia, and they may be recognised from one another by variations in their colony morphology, number of septa, and conidial morphology. (Meng 2004, Chung, W. H., and Tsukiboshi, T. 2005). Even though the teleomorphs are distinct, certain species of Curvularia have morphological similar traits with Bipolaris and Exserohilum (Sivanesan 1987, Tsuda and Ueyama 1985) The majority of these species cause losses in agricultural produce because they are pathogenic. This genus is primarily responsible for necrotic patches on the leaves of numerous plant families, and it has been linked to a number of diseases affecting vegetables. (Dasgupta *et al.* 2005).

According to lal et al (2013). Curvularia leaf spot of black gram caused by *C. lunnata* (Wakker) Boedijn is the most serious fungal disease of blackgram crop. *C. lunata* was able to infect 24 plants species belonging to different families are Leguminaceae, Cucurbitaceae, Compositae, Solanaceae, Malvaceae and Graminae showed extensive leaf spot also. Curvularia is a hyphomycete fungus which is a facultative pathogen, or beneficial partner of many plant species and common in soil. Most Curvularia species are found in tropical regions, though a few are found in temperate zones.

Taxonomic tree

Kingdom : Fungi

Division : Ascomycota

Class : Dothideomycete

Order : Pleosporales

Family : Pleosporaceae

Genus : *Curvularia*, Boedijn, 1933

Chapter II

REVIEW

OF

LITERATURE

Review of Literature

The literature on "studies on leaf spot of pigeonpea caused by complex of *Didymella spp.*" is reviewed under following headings and sub-headings:

Objective of research

1. Isolation of pathogen
2. Pathogenicity test and detached Leaf assay of the pathogen
3. Study of Biochemical characteristics (cell wall degrading enzyme) of pathogens
4. Characterization of pathogen on different media, pH and temperature
5. Study of pathogen growth against biocontrol agents and commercial fungicides

About fungus

Coelomycetous fungi are becoming more frequent . The coelomycetous fungi constitute a large number of taxa which is characterized by the production of conidia (asexual propagules) within a cavity lined by fungal or host tissue, called conidiomata (Kirk *et al* 2008). Coelomycetous fungi are mainly saprobic and parasites of terrestrial vascular plants, but they can also infect vertebrates and other fungi (Stchigel *et al* 2013). According to Sutton *et al* (1980) Coelomycetous fungus are found all over the world and in a variety of ecological niches. Exponents of this anamorph group have been found in soil, organic debris, and water, as well as species that parasitize other fungus, lichens, insects, and vertebrates.

The Didymellaceae family is one of the most diverse in the fungal kingdom, with members living in a variety of environments. On the basis of multi-locus DNA sequence data, the taxonomy of Didymellaceae was recently updated. They looked at 108 Didymellaceae isolates from 40 host plant species from 27 plant families, as well as cave substrates like air, water, and carbonatite, from Argentina, Australia, Canada, China, Hungary, Israel, Italy, Japan, South Africa, the Netherlands, the United States, and the former Yugoslavia. Based on the multi-locus phylogeny as well as morphological variations, 68 isolates representing 32 new species are recognised. Five genera of the Didymellaceae appeared to be restricted to certain habitats. (Chen *et al* 2017).

With almost 5400 taxon names reported in MycoBank, the Didymellaceae is the biggest family in the Pleosporales (Ascomycota, Pezizomycotina, Dothideomycetes) (Crous et al., 2004). De Gruyter et al. (2009) defined the Didymellaceae family to include 3 core genera, Ascochyta, Didymella, and Phoma, as well as numerous related phoma-like genera.

Didymellaceae species are widespread and may be found in a wide variety of habitats. The majority of members of this family causes plant diseases that affect a wide range of hosts, induce mostly leaf and stem lesions; some are quarantine-importance.(Aveskamp *et al*. 2008).*Didymella lycopersici* is responsible in glasshouses for stem cankers (Didymella stem rot), located on the crown and pruning wounds. It can attack all plant organs, especially in the field and following significant rainfall or irrigation. *Cankers* often develop from de-leafing and other wounds. These lesions gradually spread and eventually surround the stem and/or petioles, thus disrupting the sap flow. Ultimately, it is not surprising to see yellowing and wilting of the leaflets and plant parts below the lesions These fungal structures produce bicellular hyaline conidia, fairly typical of the *Didymella* genus. (Blancard *et al.,* 2012)

Riesen (1985) and Sieber (1985) found that Didymella exitialis (Morini) Moiler, a fungus normally associated with leaf scorch and blotch in wheat, was the most abundant ascomycete endophyte in green leaves. *D. phleina* was reported as a pathogen of *Phleum pratense* L., *Lolium* spp., and other grasses, and caused severe disease under favourable conditions in Norway (rsvoll *et al* 1975).

Pseudothecia of *D. phleina* produced on MEA were 100-150 µm in diameter, subglobose to globose, and ostiolate. The dark brown pseudothecial wall was composed of layers of pseudoparenchymatic cells. Pycnidia of *A. phyllachoroides* f. *melicae* were 150- 290 µm in diameter, globose, and ostiolate. The yellowish-brown pycnidial wall was composed of 3-5 layers of pseudoparenchymatic cells. The inner layer of the wall and phyalides were hyaline. The conidia were oblong to broadly ellipsoid, with a rounded or slightly flattened base and a round apex. They were medianly uniseptate. (Riesen *et al.* 1987) . *Didymella pinodes* is the causal agent of ascochyta blight, which is important fungal diseases of pea (*Pisum sativum*) worldwide. Losses can be upto 70 percent . (Tivoli and Banniza, 2007) .Yield losses caused by ascochyta blight are up to 40% in lentil (Gossen and Derksen, 2003), but in severe cases losses higher than 90% have been reported in fababean as repored by Haunik *et al* .,(1980) According to Kharrat *et al.,* (2006). In Tunisia, *Ascochyta fabae* Speg. and its teleomorph *Didymella fabae* produce Ascochyta blight, which is one of the most frequent diseases affecting faba bean. During both the wet cool and the hot dry season , Ascochyta blight can result in significant grain losses. Another important disease caused by *Didymella* spp. is gummy stem blight on water and of many cucurbits, including watermelon, cantaloupe, cucumber, pumpkin, squash, muskmelon, and other melons caused by *Didymella Bryoniae.*

As shown by Newark *et al.,* (2014)., infection signs and symptoms can be present on all parts of the plant except for the roots. Chlorosis is an early symptom on the plant, and light - to dark-brown spots (necrosis) can occur on the cotyledons. These symptoms can often be visible before and after transplanting in the field. Prior to the occurrence of chlorosis or necrosis, the same tissue may appear water soaked. Wilting, followed by death of the transplants.

Chilvers *et al*.,(2009) studied that the anamorphic pycnidial fungus *Ascochyta pisi* is one member of a species complex that causes *Ascochyta* blight of pea, which is a potentially devastating disease. The teleomorphic state of the fungus was induced under laboratory conditions. Using morphological and molecular characters, they placed the teleomorph within the genus *Didymella* as *D. pisi* and describe a heterothallic mating system using a PCR-based mating type assay and *in vitro* crosses and compared *D.pisi* with other *Didymella* spp. with which it might be confused. Stem, crown, pod, and foliar diseases of pea are caused by a complex of *Ascochyta pisi* Lib. (teleom. *Didymella pisi* M.I. Chilvers, J.D. Rogers and T.L. Peever) , *Mycosphaerella pinodes* (Berk. and A. Bloxam) Vestergr. (ana. *Ascochyta pinodes* L.K. Jones) , and *Phoma pinodella* (L.K. Jones) Morgan-Jones and K.B. Burch [syns. *Ascochyta pinodella* L.K. Jones, and *Phoma medicaginis var. pinodella* (L.K. Jones) Boerema] the disease symptoms associated with all the three fungal spp. He noticed that all the above ground plant parts and all growth stages of plants were susceptible to blight disease. Symptoms mainly include purplish black to brownish black spots on lesions on leaves, stems, tendrils, and pods but the pathogens were very difficult to differentiate on the basis of symptoms On these lesions, black spore-producing entities may grow. Sunken pod lesions are possible, early signs (purple-brown irregular specks) appear on lower leaves, stems, and tendrils under the plant canopy, where circumstances are more humid. The lowest leaves get fully blighted and fall off as these specks expand and merge. Foot rot is caused by severe infections on the stem that cause girdling along the soil line (Anonymous. 2008) .

Barilli *et al*. (2016) conducted an experiment to investigate differences in susceptibility of different legume species to *D. pinodes* in comparison to other *Didymella spp*. As well as to 9haracterize different cultivars based on different responses within various legume species to several *D. pinodes* isolates. On diverse pea genotypes, *D. pinodes* pathogenicity was shown to have a very low degree of partial resistance. *P. sativum* genotypes were susceptible to all isolates, however *P. fulvum* (tawny pea) accession IFPI3260 exhibited a notable level of partial resistance. Finally, it was concluded that *D. pinodes* can cause disease in a variety of legume species, and that all isolates showed varying aggressive reactions to different legume species collected from various geoFigureical regions, and that only partial resistance was found to be present , whereas accessions belonging to *P. fulvum, P. sativum spp. Syriacum*, and *P. sativum spp*. Elatius were recently discovered to show some sources of resistance to *D. pinodes*. According to some findings, *D. pinodes* was not only exclusive to peas, but it also had a wider host range than other species such as *D. fabae, D. lentil, and D. rabiei*. (Fondevilla *et al* 2005, Carrillo *et al* 2013).

Haware *et al. (*1986) found that the pycnidia of *A. rabiei* which were visible as dark-brown, pin-head-like structures in infected plant tissues and were immersed, amphigenous, spherical to subglobose and varied in size from 65-245 μm in size. The fungus produced imperfect or asexual stage in the form of minute dot like black fruiting bodies, pycnidia arranged in concentric rings on the host. These pycnidia contain numerous hyaline spores (pycnidiospores or pycniospores or conidia) on short conidiophores. The pycnidial wall is composed of 1 to 2 layers of elongated pseudo-parenchymatous cells and the ostiole is 30-40 μm wide.

According to Nene (1982). Conidia of *A. rabiei* are hyaline, oval to oblong, slightly curved, and constricted, and are formed by the inner cells of pycnidia. The conidia are usually two-celled with blunt edges and measure 6-12 4-6 m. Pycnidia swells and becomes slimy when through the ostiole, a mass of conidia seeps out.

The mycelium of *A. rabiei* is hyaline to brownish and septate, according to Singh *et al* .(2009) The pycnidia on stems, leaves, and seed pods become erumpent, globulus, dark brown, and 140-200 m in diameter after being submerged. The wall is made up of 1-2 layers of pseudoparenchymatous cells that are elongated. The ostiole of the pycnidium is 30-50 m long and broad. Conidia are made up of hyaline ampuliform phialides found in the pycnidium’s inner cells. Conidia are hyaline, oval oblong, and hyaline. Straight or slightly curved 0-1 septate, constricted or not at the septum, rounded at the base cent of the two-celled pycnidiospores.

Sexual recombination was believed to occur routinely throughout the life cycle of *D. rabiei* in places where both mating types occur, based on heterothallic mating behaviour in the laboratory. Although it is known, the mating system of *D. rabiei* has not been determined in the field.

Based on heterothallic mating behavior in the laboratory, it is assumed that sexual recombination occurs regularly in the life cycle of *D. rabiei* in areas where both mating types are found . Although it is believed that *D. rabiei* ascospores from the sexual stage constitute recombinant progeny that might contribute to increasing genotypic variety in the *D. rabiei* population, the mating system of *D. rabiei* has not been determined in the field. This polymorphism might be adaptive, allowing for enhanced virulence in resistant chickpea cultivars and/or the development of fungicide resistance.(Peever *et al.*, 2003).

Growth of fungus depending on culture media

To distinguish *A. pinodes* and *P. pinodella*, Onfroy *et al* (1999) used malt agar, Mathur's agar, Oat meal agar, and V8 agar medium. Although the single spore colonies varied in colour depending on the medium used, they found that *A. pinodes* colonies were light to dark grey, while *P. pinodella* colonies were darker grey to black due to excess chlamydospore production, and that *P. pinodella* isolates grew at a faster rate of 4.0 ± 0.2 (4.7±5.2) mm per day, while *A. pinodes* grew at a slower rate of 3.5±0.3 (2.6±4.3) mm per day.

Devi *et al.*, (2018) used Natural agar media such as Oat Meal Agar (OMA), Malt Extract Agar (MEA), V8 Agar, and Leaf Extract Agar of the host plant, semi-synthetic media such as Potato Dextrose Agar (PDA), carrot dextrose agar (CaDA), and synthetic media such as Czapex Dox Agar (CDA), Richards Synthetic Agar (RSA), Asthana, and Hawkers Agar (AHA) in their experiment. Four mycelial development rates, cultural, and morphological features were studied. After seven days of incubation at 26°C, fungal isolates were obtained. The colony parameters including diameter, cultural properties (texture, reverse and surface coloration, zonation), and sporulation of a certain test fungi . Type of growing media has a huge impact on fungus. findings revealed the influence of culture media on the growth, colony character and sporulation of the test fungi differs based on the nature of the culture medium. They found , Out of the nine media that they studied, Oatmeal agar and Malt extract Agar were found to be suitable for heavy sporulation while PDA reproduced most visible colony morphology.

Growth of the pathogen against biocontrol agents and fungicides

Mangala and Rajkumar (2018) evaluated fungicides and culture filtrate of bio-agents to test their proficiency to control gummy stem blight disease caused by *Didymella bryoniae* in Gherkins. Amongst the tested culture filtrate of antagonistic bioagents, *Trichoderma harzianum* (THRHG1 strain) demonstrated the most mycelial growth suppression at 20 percent concentration, followed by *Penicillium purpurogenum* -1 (PPDMK1) at 5 percent concentration, which showed 3.36 percent growth inhibition. The growth inhibition of *Aspergillus terreus* (ATKGM1) and *Penicillium purpurogenum-2* (PPDMK-2) was minimal. *Didymella bryoniae* was shown to be very sensitive to fungicides such as Mancozeb 75 percent (Indofil) and Tebuconazole 50 percent (Nativo) with 100 percent growth inhibition at very low concentrations, but least susceptible to Allite, Sectin, and Kavach. When compared to the negative control, which had a disease incidence of 96.5 percent, *A. terreus* (ATKGM1) and *P. purpurogenum*-2 (PPDMK-2) significantly reduced symptoms and disease incidence by 43.8 percent and 44.6 percent, respectively, in green house trials. Furthermore, as compared to the overall control, both isolates were shown to be strong growth promoters, as evidenced by increases in shoot length (54.27.3 and 62.08.0), number leaf (13.51.0 and 11.11.8, respectively. *T. harzianum*, which was successful in vitro, was shown to be less effective in the green house. These findings suggest that *A. terreus* and *P. purpurogenium* might be useful biocontrol agents for Gherkin gummy stem blight caused by *D. bryoniae*.

Zhao *et al* (2012) investigated that under controlled settings, two Streptomyces isolates (*Streptomyces pactum* A12 and *S. globisporus subsp. globisporus* C28) previously obtained from the Qinghai-Tibet Plateau were studied for their biocontrol of gummy stem blight and melon growth promotion. In vitro, Streptomyces A12 and C28 showed clear antagonistic action against *D. bryoniae*. In vivo, both A12 and C28 significantly reduced melon gummy stem blight disease severity and AUDPC (area under the disease progress curve) (P 0.05). When compared to other therapies, ten-fold dilution of C28 culture filtrate was more efficient in the conditions, with disease reduction effects ranging from 41.0–64.2 percent. When A12 was given to both nursery and transplanted soil, mean fresh weights rose by 40.4

percent for plants, 44.2 percent for roots, and 40.3 percent for aerial parts. according to the findings Streptomyces A12 and C28 had a beneficial influence on the biocontrol of gummy stem blight and the growth promotion of *Cucumis melo* L. Shternshis *et al*. (2016). The bacterial antagonists *B. subtilis* and *P. fluorescens* were shown to be effective in suppressing the fungus *D. applanata* causes raspberry cane spur blight. The experiments *in vitro* revealed that there was higher inhibitory activity of *B. subtilis* spores compared with *P. fluorescens* living cells. These results were confirmed by the experiments on red raspberry cane inoculation by *D. applanata* where cultivar Kirzhach, susceptible and cultivar Kolokolchik relatively resistant were used. When susceptible cultivar Kirzhach was inoculated with *D. applanata*, *B. subtilis* treatment resulted in a 2-fold decrease in cane lesion area compared to *P. fluorescens* therapy. In both cultivars, *B. subtilis* had a greater effect as evidenced by the quantity of *D. applanata* fruiting bodies per cm^2.

Curvularia is a hyphomycete mold fungus , a facultative pathogen, or beneficial partner of many plant species and common in soil. Most *Curvularia* are found in tropical regions, though a few in temperate zones also. Gurung *et al.,* 2020 studied *C. eragrostidis*, caused blight disease to large cardamom (*Amomum subulatum* Roxb.) in Sikkim. At present, leaf blight is considered a major threat to cardamom cultivation in Sikkim. *Curvularia lunata* appears as shiny velvety-black, fluffy growth on the colony surface. *C.lunata* is distinguished by septate, dematiaceous hyphae producing brown, geniculate conidiophores. Sinha conducted a study to look into the link between soil physicochemical characteristics and their influence on microfloral diversity and pigeon pea wilt disease incidence in villages in the Jalaun area. The ph, EC, soil moisture, N, P, K, and organic carbon of soil samples were all measured. Microflora from the soil was isolated. Wilt frequency was calculated. A total of 20 fungal species from 12 genera were isolated, including pathogenic *Curvularia.*

According to Yashwan *et al*., (2010). *Curvularia lunata* is important leaf spot disease of okra , He has done his study to observe the growth of the fungus , dry mycelia weight and sporulation of *curvularia lunnata* . he found that maximum mycelial dry weight 830.00 mg of the pathogen was found at 28^{o}c, best temperature for its effect on amount of mycelium produced, was 30 ^{o}c. At 15 ^{o}c the fungal dry weight was minimum 144.66 mg. The pathogen sporulated well at temperatures of 28°, was good at 25°, fair at 20° and 32°C, and bad at 15° and 40°C. Excellent sporulation of the fungus was observed at the ph levels between 6.0 , 6.5; good at 5.0 , 5.5 and 7.0 and fair at 4.5 and 7.5, whereas poor sporulation was recorded at ph 4.0 and 8.0.

Osman *et al*. (2012) concluded that *Curvularia lunata* developed quickly on PDA and plant extract media from tomato, wheat flour, dukhun, and pigeon pea. *Curvularia lunata*, covered 9.0 cm plates in 7 days at 28°C, however it took 8 days on aubergine extract medium. The cultures began as white to slightly grey in colour, gradually darkening with time.

Curvularia leaf spot is caused by the fungus *Curvularia lunata*. The disease was officially reported in the U.S. for the first time in 2017. Source : https://cropprotectionnetwork.org/

Curvularia leaf spot starts as very small (1/16 to 1/8 inch) round tan lesions on leaves. Lesions often have a brown border and can be surrounded by a yellow halo symptoms , lesions can be found few in number or scattered across leaves to lesions densely covering large sections of leaves. Symptoms can be developed at any growth stage.

source : https://plantpathology.ca.uky.edu

Curvularia is also responsible for thinning out and decline of grasses, streaks and irregular patches on them. Yellowing of the leaves which will later turn into brown from the tip of leaf symptoms are severe on older leaves . A layer of mycelia covers the infected tissue . Spores , conidia are produced on mycelia or conidiophores and no fruiting bodies are found. The fungus infects the grasses through cut tips of leaves and it is favored by high temperatures This is primarily a stress pathogen that attacks low fertility and heat and drought stressed plants.

source : https://www2.ipm.ucanr.edu/agriculture/turfgrass/curvularia-blight/.

Zhu and Qiang (2003) employed a strain of *Curvularia eragrostidis*, designated as QZ-2000, isolated from a naturally-occurring diseased plant of large crabgrass (*Digitaria sanguinalis*) to biocontrol this weed . Zhao *et al* (2012) investigated that under controlled settings, two Streptomyces isolates (*Streptomyces pactum* A12 and *S. globisporus subsp. globisporus* C28) previously obtained from the Qinghai-Tibet Plateau were studied for their biocontrol of gummy stem blight and melon growth promotion. In vitro, Streptomyces A12 and C28 showed clear antagonistic action against *D. bryoniae*. In vivo, both A12 and C28 significantly reduced melon gummy stem blight disease severity and AUDPC (area under the disease progress curve) (P 0.05). When compared to other therapies, ten-fold dilution of C28 culture filtrate was more efficient in the conditions, with disease reduction effects ranging from 41.0–64.2 percent. When A12 was given to both nursery and transplanted soil, mean fresh weights rose by 40.4 percent for plants, 44.2 percent for roots, and 40.3 percent for aerial parts. According to the findings Streptomyces A12 and C28 had a beneficial influence on the biocontrol of gummy stem blight and the growth promotion of *Cucumis melo L.*

Biochemical tests

Cellulose

Plant polysaccharides primarily consist of cellulose, the β-1,4 linked homopolymer of glucose; hemicellulose, a heterogeneous, branched polysaccharide primarily made up of a β-1,4 linked polymers including xylan, glucoronxylan, xyloglucan, glucomannan and arabinoxylan backbones with heterogeneous side chains (Payne *et al* 2015)..

According to Somerville *et al.*,(2006) Cellulose serves a key structural function in plants and is synthesized by complex enzymatic machinery during cell wall synthesis .Cellulases are a group of enzymes including endoglucanase (Cx, EC 3.2.1.4, endo-β-1,4-glucanase), exoglucanase (C_1, β-1,4-cellobiohydrolase) and β-glucosidase (Dori *et al*. 1995). The role of hemicellulases and cellulases in pathogenicity and virulence of plant pathogens are largely undetermined (Novo et al. 2006). However, cellulolytic activity of *Penicillium digitatum* and *P. italicum* was reported to correlate with fungal growth and pathogenesis in citrus fruit (Barkai-Golan and Karadavid 1991). Recently, Vu *et al*. (2012) determined that cellulases were virulence factors of *Magnaporthe oryzae*. According to zhang *et al* (2014), The highly virulent isolates showed higher Cellulase activity than the moderately virulent ones in decayed fruit and in fruit tissue shake culture.

Extracts of *Ascochyta pisi* induced lesions that were limited on leaflets contained pectolytic and hemicellulolytic enzymes. Some cellulase activity was detected although there was little evidence of cellulose degradation in cell walls in infected tissue. The nature of the macerating factor remained uncertain but it was found that extracts from lesions contained inhibitors of pectic enzymes and that tissue just beyond that invaded by the fungus was resistant to maceration; this resistance is probably important in restricting the growth of the pathogen in the leaves . (Heath *et al.*, 1971).

Amylase

Amylolytic enzymes have a significant role because of the high content of carbohydrates in plants. This group comprises of α-, β-, and γ - amylases, isoamylase, and some other enzymes differing in their physicochemical properties and the mechanisms of action on starch as the substrate. (Mishra and Maheshwari 1996)

According to Morkunas *et al.,* (2013), The mobilization of defence mechanisms in the early stages of pea seed germination against *Ascochyta pisi*. Species of the genus Ascochyta and related genus Phoma are known for the presence of both α-amylase and glucoamylase. Individual species differ significantly in relative quantities, overall output, and enzyme formation rates. For example, *Ascochyta cucumeris* Fautrey and Roum. had the highest productivity, whereas Ascochyta pisi Lib. and *Ascochyta cucumeris Fautrey* and Roum. and *Phoma medicaginis* Malbr. and Roum had the lowest.

According to Bordbar *et al* .,(2005). , Amylases are starch degrading enzymes that catalyze the hydrolysis of internal glycosidic bonds in polysaccharides with the retention of anomeric configuration in the products. Most of the amylases are metalloenzymes, which require calcium ions (Ca2+) for their activity, structural integrity and stability

Meryem *et al*., (2015) studied that *A.pisi* were produced using a higher starch concentration (Czapek + 2% starch) . The amylolytic activity of the three isolates on medium Czapek + 2% starch at different pH (4, 6, 8) and at different times after incubation shows that the optimum for amylolytic activity for all of our isolates occurs between pH values of 6 and 7, and that activity declines away from either side of these limits.

Xylanase

Xylan molecules are mainly constituted by D-xylose as the monomeric unit, and traces of L-arabinose are also present. Further, some substituents (i.e. acetyl, arabinosyl, and glucuronysyl) are found on the backbone of xylan. Xylanases are hydrolases depolymerizing the plant cell component xylan, the second most abundant polysaccharide. Xylan structure is variable, ranging from linear 1,4-ß-linked polyxylose sugars other than D-xylose. Biely (1985).

According to Brück *et al.,* (2022) among eight strains isolated from soil of the paramo highlands of Ecuador, three were selected for further experimentation and identified as *Cladosporium michoacanense*, *Cladosporium* sp. (cladosporioides complex), and *Didymella* sp., the last one being reported for the first time in this area. Endoglucanase, exoglucanase, -D-glucosidase, endo-1,4-xylanase, -D-xylosidase, acid, and alkaline phosphatases secretion was studied under agitation and static environments that were suitable for the growth time and incubation temperature. *Didymella sp*. had the strongest endoglucanase, -D-glucosidase, and xylanase activation at 8 °C, indicating an excellent profile for bioremediation and wastewater treatment procedures in cold climatic circumstances.

In the study of Lübeck *et al*., (1997) *Ascochyta pisi* was cultured in media containing birchwood xylan with and without additional glucose, as well as in media containing glucose alone. Even in glucose-containing medium, the fungus expressed xylanase, as per xylanase activity test. Xylanase activity was not identified on glucose alone. The cloned gene is expressed when the fungus is cultivated in media containing xylan as the single carbon source, but not in media containing glucose as the sole carbon source, as northern blot technique indicated.

Laccase

Laccases are enzymes widely distributed in plants, fungi, bacteria, and insects. They are multicopper oxidases that catalyze the transformation of aromatic and non-aromatic compounds with reduction of molecular oxygen to water. These enzymes participate in processes such as biosynthesis and lignin degradation, morphogenesis , and pigment biosynthesis, fungal laccases are involved in sporulation, pigment production, fruit body formation, and plant pathogenesis (Alcalde *et al.* 2007).

Some laccases that exhibit a homodimeric structure have been isolated. Such is the case for those obtained from Basidiomycetes *T. villosa* (Yaver *et al.*,1996), phytopathogenic Ascomycetes , *R.solani* (Wahleithner *et al.*1996), and for the aquatic Ascomycete *PHoma* sp. UHH 5-1-03. SLB isolate of *Fusarium udum.* Butler which was evident for the production of all cellwall degrading enzymes, laccase, fusaric acid shows consistency in symptoms as well , was proved to be pathogenic isolate. (Bagale *et al* .,2020).

Enzymatic bioremediation through laccases is another area of great interest. For example, laccase can be utilized for bioremediation in compounds such as chlorophenols, dimethoxyphenols, nitrophenols, and pesticides among others (Viswanath *et al*., 2014).

The fungal isolate *Peyronellaea pinodella BL-3/4* (Synonym: *Didymella pinodella*) was discovered to be a new ascomycetes in laccase synthesis. At a ph of 6.0, temperature of 30°C, inoculum size of 5 mycelial plugs, agitation speed of 150 rpm, and 96 hours of incubation, the greatest production of laccase was achieved using a single parameter at a time method. *P. pinodella* BL-3/4 produces the most laccase after 96 hours. In contrast to basidiomycetes fungus, which can create laccase late in the incubation process, incubation shows that laccase is formed early in the process. In the future, there will be a large need for this archaic enzyme. (Alcalde *et al*., 2002 , Gayosso-Canales *et al*., 2012, Patel and Bhaskaran 2020)

Polygalactouronase

Polygalactouronase is a hydrolytic enzyme, which acts on polygalacturonic acid (PGA), hydrolyzing α-1,4 glycosidic bonds of pectic acid. The pattern of degradation proceeds in either a random (endo-polygalacturonase, EC 3.2.1.15) or terminal fashion (exo-polygalacturonase, EC 3.2.1.67). According to kubisek *et al* (2014). Since plant cell wall is a major barrier to intrusion by pathogenic microorganisms, pathogens secret an array of cell wall-degrading enzymes (CWDEs) to compromise cell wall integrity in order to gain access.

According to Amselem *et al* (2011) An important part of the CWDEs is pectin-degrading polygalacturonases (PGs) as pectin is an important component of cell wall and the middle lamella. Necrotrophic pathogens often possess multiple PGs in their genomes PGs play important roles in virulence as demonstrated using gene-deletion in *Botrytis cinerea* .

According to Samia *et al* . (2013) activity of pectin methyl esterase was baseless in the presence of glucose, but it varied in the presence of the citrus pectin and polygalacturonic acid. They recorded the weak production ranging between 0.19 and 0.55 µeg/ml/min; however activity was strong, between 2.97 and 5.88 µeg/ml/min in the presence of citrus pectin and polygalacturonic acid respectively. On the other hand, using three different carbon sources, polygalacturonase activity was found in culture filtrates. Weak activity was seen in the presence of glucose (0.28 to 2.63 mole/min), whereas high activity was observed in the presence of polygalacturonic acid (16.29 mole/min). After six days of incubation at ph 5, the maximum output of polygalacturonase was 11.7 mole/min in the presence of polygalacturonic acid and 0.9 mole/min in the presence of glucose. After 4 days of incubation at ph 8, the polygalacturonase activity ranged from 0.3 to 0.62 mole/min. The best results were found at a ph of 5.5. It should be observed that the synthesis of polygalacturonase differs significantly from that of simple sugars glucose and pectin, showing that the enzyme is produced differently.

Zhang *et al* (1999) studied relationship of developmental stage of cantaloupe fruit to black rot susceptibility and enzyme production by *Didymella bryoniae*. When environmental conditions and fruit developmental phases are ideal for infection, black rot of cantaloupe fruit caused by *Didymella bryoniae* can be severe. Black rot symptoms on cantaloupe fruit vary widely depending on the fruit's developmental stage. Only ripened fruit was found to have black rot. When cantaloupe fruit was inoculated with five *D. bryoniae* isolates at different developmental stages, the degradation was highest on 10-day-old fruit compared to 20-, 30-, 40-, and 50-day-old fruit. In decaying tissue, there was a positive association between lesion size and total fungal PG activity. Multiple PG isozymes were found in both fungal shake culture and rotting fruit using a sample *D. bryoniae* strain (OK 963096).

Proteases

Proteolytic enzymes are also generated by phytopathogenic microorganisms in addition to pectolytic and cellulolytic enzymes. The occasional observation of a direct link between the activity of extracellular microbial proteinases and the severity of plant disease demonstrates the importance of proteinases. (Kudryavtseva *et al.,* 2013).

Poisoned Food Technique

Poison food technique is used to determine the inhibition percentage of the fungi by using different fungicidal concentration and eventually determining the minimum fungicidal concentration of the EO on the test pathogen. The percentage inhibition of the mycelial growth of the test fungi by the fungicides was calculated using the formula used by Nisa *et al.* (2011)

Inhibition of mycelial growth (%)={(dc−dt) ÷ dc}×100,

where *dc* is mean diameter of colony in the control sample, and *dt i*s mean diameter of colony in the treated sample.

Mode of action of fungicides used in study

Mancozeb - It is a broad-spectrum fungicide with protective action. The product is fungitoxic when exposed to air. It is converted to an isothiocyanate, which inactivates the sulphahydral (SH) groups in enzymes of fungi. Sometimes the metals are exchanged between mancozeb and enzymes of fungi, thus causing disturbance in fungal enzyme functioning. www.biostadt.com

Folicur - It acts as a systemic fungicide. Demethylase inhibitors (DMI) - interfere in the process of building the structure of the fungal cell wall. Finally inhibit the reproduction and further growth of the fungus. www.farmersstop.com

Thifluzamide - fungicide has novel modes and sites of action against the wide range of fungi. Its unique single-site inhibition of the succinate ubiquinone reductase or succinate dehydrigenase (Sdh) complex in the respiratory chain was found so much effective. (FRAC, 2007)

Difenoconazole It is absorbed by the leaves with acropetally and shows strong translaminar translocation. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes. https://www.jaffer.com/

Dupont , It forms a protective layer on leaves and other plant parts that prevents infection through contact activity. Inhibits formation of intercellular hyphae which stops the spread of pathogen inside the plant . https://www.corteva.in/

Bahr *et al* (2016) reported the isolation and full characterization of *A. rabiei* from chickpea cultivated in Santa Fe, Argentina, its molecular identification, secreted phospholipase and proteinase activity, and its antifungal susceptibility profile for two commercial antifungals. They tested chlorothalonil and difenoconazole, two commercial antifungals commonly used to treat Ascochyta blight, on strain AR2. MIC (Minimal Inhibitor Concentration) for chlorothalonil was found to be 1–2 μg ml^{-1}, on difenoconazole, MIC was 0.06–0.03 μg ml^{-1}. When microplate wells were examined microscopically, some fungal growth was detected at concentrations greater than the difenoconazole MIC. Nevertheless, when they added MTT, no reduction to formazan was observed even after 48 h incubation, thus suggesting that no mitochondrial activity is detectable at those antifungal concentrations. Concluded that susceptibility of the Argentinean isolate is comparable with isolates from other countries.

As an experiment conducted by Cromey *et al.,* (2004) over three growing seasons, the effects of the fungicides azoxystrobin and tebuconazole on *Didymella exitialis*, as well as on wheat senescence and yield, were investigated in ten field experiments . Between flag leaf emergence and anthesis, fungicides were administered at three different growth phases. *Didymella exitialis* was often isolated from symptomless seedling and adult leaves, Pycnidia of the Ascochtya anamorph of *D. exitialis* can be seen on the dead portions of seedling leaves. Azoxystrobin decreased the rate of isolation of *D. exitialis* from symptomless flag leaves. There were no consistent impacts of crop growth stage on *D. exitialis* levels when fungicides were applied. Tebuconazole raised yield by 5.3 percent on average in nine of the trials, whereas azoxystrobin enhanced yield in all of them (average 6.1 percent). In each study, the optimal fungicide timing combination resulted in an average yield increase of 9% (range 2–27%). This research reveals that. *D. exitialis* is a minor wheat pathogen with little impact on flag leaf senescence.

Rehman *et al* (2013) studied *Cicer arietinum* L. cultivars that are frequently seeded throughout Pakistan to see if they were a source of resistance or susceptibility to the gram blight disease caused by *Didymella rabiei* (Kovatsch.) and also to assess the effect of fungicides on pathogen Five varities of gram with varying levels of resistance and susceptibility were planted individually and treated with fungicides following disease development to determine the efficiency of different fungicides. The efficiency of fungicides varied depending on the extent of disease resistance among the cultivars. Chlorothalonil, difenaconazole, mancozeb, metalxyl + Mancozeb, and thiophanate-methyl were the most efficient fungicides in suppressing the disease, in that order. Chlorothalonil was the most effective fungicide against the disease, whereas Topsin-M was the least effective.

Abdou *et al*. (1991) examined ten fungicides in vitro and discovered that at 10 ppm, benomyl (as Benlate), tridemorph (as Calixin), and thiobendazole inhibited A. rabiei. At 100 ppm, thiram inhibited fungal growth, but chlorothalonil did not (as Bravo carb) and At 1000 ppm, Zineb stopped the growth. In vitro, *A. rabiei* was shown to be particularly susceptible to propiconazole (as Tilt), penconazole (as Topas (C-50), and sulphur (as Thiovit) by Inam *et al* (1995). At 5 and 50 ppm, propiconazole and penconazole respectively suppressed the growth totally . Chlorothalonil (as Daconil) and copper were the least effective fungicides tested (in the form of Cuprocaffaro) Mancozeb + metalaxyl (as Ridomil) and propineb (as Antracol) were both shown to be effective. After 28 days of incubation at , the efficacy was moderate.

Ali *et al* (2011). The in vitro effectiveness of several fungitoxicant formulations was investigated by using the poison food approach. Carbendazim 50 WP (Bavistin), difenconazole 25 EC (Score), tebuconazole 25 EC (Folicur), hexaconazole 5 EC (Contaf), propiconazole 25 EC (Tilt), azoxystrobin 23 SC + difenoconazole 25 EC (Amistar Top 325 SC), azoxystrobin 23 SC Fungitoxicant formulations were evaluated at a various concentrations, including 1, 5, 25, 50, 75, 100, 125, 150, and 200 ppm for systemic formulations and 1, 5, 25, 50, 75, 100, 150, 200, 300, 500 ppm for contact fungicide formulations. Combination of azoxystrobin 23 SC +difenoconazole 25 EC was more effective and it resulted in cent per cent inhibition at 125 ppm. Data showed, among the contact fungicides, 60.88 per cent mean inhibition of fungal growth was achieved by chlorothalonil 75 WP. The next best fungitoxicant was captan 50 WP followed by mancozeb 75 WP with mean per cent inhibition of 51.98 and 44.18 respectively. None of the contact fungitoxicants proved to be effective as hundred per inhibition of the growth could not be achieved even at 500 ppm . In the present findings, all the systemic fungitoxicants were found to be highly effective against *Ascochyta rabiei* and cent per cent inhibition was achieved at 100 ppm concentration in all the fungitoxicants. These fungitoxicants might be responsible in hindering the metabolic pathways occurring in the cells of *Ascochyta rabiei*.

Chapter III

MATERIALS

AND

METHODS

MATERIALS AND METHODS

The present study entitled **"**studies on leaf spot of pigeonpea caused by complex of *Didymella spp.***"** was conducted during 2021 – 2022 . All the laboratory work was carried out in the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU.

General

All the glasswares used during the course of study were made up of borosilicate and of good quality. They were washed with detergent, dried and sterilized using the hot air oven at 180^{o}c for 40 min.

The metallic equipment like needle, forceps, corkborer were sterilized by dipping them in alcohol and heating to red hot over the flame of spirit lamp, and used after gentle cooling. Surface sterilisation of leaves of infected plant was done by dipping them in 0.1% HgCl2 and sodium hypochlorite for period of 30-60 sec, followed by blotter dry and then used for the inoculation. Media, blotter paper and soil were sterilised using the autoclave, by keeping them in airtight autoclavable polythene bags. Sterilisation was done at 121^{o}c, 15 lbs pressure for 15-30 minutes.

3.1 Pathogenicity testing

3.1.1. Identification and isolation

Plants were observed in month of October 2nd week 2021 when there was Daily maximum temperature of 33 ^{o}c and minimum temperature 23.1 ^{o}c and relative humidity 70 – 96 percent with 6.3 sunshine hours per day (Department of Agronomy , IAS BHU) with infected leaves showing the circular leaf spot like symptoms from the AICRP Pigeonpea Plant Pathology field I at BHU center Varanasi and were examined , two fungus were isolated *Didymella spp*. and *Curvularia spp*. namely from the infected leaves and both the fungus found to be associated in most of the isolations . Leaves were sterlized using ethanol for few seconds and then leaf sections were immersed in 1 percent sodium hypochlorite solution followed by washing the section in sterilized distilled water thrice each dip for 30 seconds. Leaf section were later dried using sterilized filter paper and then placed on petri plates containing potato dextrose agar media and incubated in BOD at 25±2 ^{o}C and through subculturing 2 fungus colonies were separated .

3.1.2. Inoculation

Fungal colonies that were of 7 days old, used for inoculation on the pots and detached leaves.

3.1.2.1 Preparation of Potato Dextrose agar

- 200g of potato was, washed well with tap water and was peeled off.
- Potato was cut in to small pieces using knife.
- Then the sliced potato was boiled in 500ml of water, until the potato pieces became soft.
- Whole content was filtered using muslene cloth and 20g of dextrose was added to the filterate.
- pH of the filterate was adjusted to 6.8+0.2 by using 0.1N HCL or 0.1N NaOH.
- Final volume of media was made up to 1 litre by the addition of distilled water.
- 20g of agar was added to the broth slowly and boiled it again till the agar is dissolved.
- Media was sterilized using autoclave at 121^{0}C, 15 lbs pressure for time period of 20-30min.
- After sterilization the media was allowed to cool down and then 15 to 20 ml media was poured in to sterilized petriplate in laminar airflow under aseptic conditions. And in same way carrot agar media and other media were also prepared .

3.1.2.2 Preparation of inoculum

Isolated fungus was sub-cultured on PDA plates and incubated for 14 days to prepare the inoculum. After that, sterile distilled water was poured over the cultures. To detach the conidia/culture, then the surface of the culture was gently scraped with a sterile glass rod. The spore suspension was then transferred to sterile distilled water flasks later strained with sterilized muslin cloth, this suspension was used for inoculation on plants .

For inoculation two methods were used

A. Pot experiment

Soil was sterilized in autoclave and mix with sand and vermicompost in 3:1:1 ratio and pots were filled with this mixure seeds of Bahar variety of pigeon pea were used for planting.

Plants with 4-5 leaf stages were inoculated using spore suspension supplemented with 0.15% of Tween 20. Plants were covered with plastic to provide required humidity and were kept in greenhouse for 7 to 8 days. During the experiment, environmental conditions were kept at 22/18°C (day/night rhythm) with 14-h photoperiod and 90% humidity as described by Farhani *et al.*, (2019).

For cuvularia, to harvest conidia, autoclaved water was added to the cultures, which were gently shaken for 10 min. The conidia were collected by centrifugation at 1000 rpm for 10 min, washed twice then diluted with distilled water Each conidia suspension was sprayed to four to five leaf seedling as described by Wang *et al.*, (2013)

B. Detached leaf assay

Sample leaves were collected from the 2 month old plants and these leaf lets were surface sterilized in 1 percent sodium hypochlorite solution and dipped in tween 0.05 percent solution.

These leaves were placed in moisture chamber made by using sterilized filter paper placed on top and bottom of the plate followed by inoculation with spore suspension and mycelial bits. Plates were kept in BOD at 27±1°C.

3.1.3 Reisolatation

In pot, after 5 to 6 days symptoms started to appear and samples were again collected and cultured on PDA plates in same condition as of step 1.

3.1.4 Reinoculation

As done in step 2, 60 days old plants were reinoculated in same manner. Plants were sprayed with inoculums and this time leaves were inoculated with *Didymella spp*. spore suspension and mixure of both the fungal spore suspension mixed in 1:1 ratio and sprayed on different branches of each plant later these branches were covered with plastic bags.

3.2 Growth of the test pathogens on different culture media

Different culture media including semi synthetic, natural media, synthetic were used for isolation, observing the growth and maintenance of the pathogen. Different agar media, viz.,

- Carrot agar media
- Malt extract media
- Potato dextrose agar media
- V8 juice agar media
- Oatmeal agar media
- Czapek dox agar media
- Corn meal agar media
- Asthana and hawker`s agar media
- Pigeon pea stem powder media

3.2.1 MEDIA PREPRATION

3.1 Media composition for Carrot agar

Components	**Amount**
Peeled and sliced carrot	200 gram
Agar	20 gram
Distilled water	1000 gram
pH	6.5±0.2

(Devi et al .2018).

3.2 Media composition for Malt extract

Components	**Amount**
Malt extract	20 gram
Agar	20 g
Distilled water	1000 ml
pH	5.4±0.2

(Devi et al .2018).

3.3 Media composition for Oatmeal agar

Components	**Amounts**
White oat **(decoction)**	40 g
Agar	20 g
Distilled water	1000 g
pH	7.2±0.2

(Devi et al .2018).

3.4 Media composition for Pigeonpea agar media

Components	**Amounts**
Stem powder	40 gram
Agar	20 g
Distilled water	1000 ml
pH	6.2±0.2

(Trapero-Casas& Kaiser 1992).

3.5 Media composition for Asthana and Hawker`s agar

Components	**Amounts**
Glucose	5 g
KNO_3	3.5 g
$MgSo_4.7h_2O$	0.75 g
KH_2PO_4	1.74 g
Distilled water	1000
Ph	6.2±0.2

(Koley& Mahapatra,2015).

3.6 . Media composition for Czapek`s dox agar

Components	**Amount**
Sucrose	30 g
Sodium nitrate	2 g
Dipotassium hydrogen phosphate	1g
Magnesium sulphate	0.5 g
Kcl	0.5g
Ferrous sulphate	0.01 g
Agar	20 g
Ph	6.8 ± 0.2

(Koley& Mahapatra,2015).

3.7 Media composition for Corn meal agar

Composition	**Amount**
cornmeal	20 g
Peptone	20 g
Glucose	20 g
Agar	20 g
pH	6.0±0.2

3.8. Media composition for Media for Qualitative assay of cellulase

S.no.	**Component**	**Amount**
1	Potato	200 g
2	Dextrose	20 g

3	Carboxy methyl cellulose	10 g
4	Agar	20 g
5	Distilled water	1000 ml
6	Ph	6.8 + 0.2

(Zakpaa *et al*., 2009)

3.9 Media composition for Qualitative assay of amylase

S. no	**Components**	**Amount**
1	Starch	20 g
2	Peptones	5 g
3	Beef extract	3 g
5	Agar	20 g
6	Distilled water	1000 ml
7	Ph	6

(Hankin & Anagnostakis, 1975)

3.10 Media composition for Qualitative assay of Xylanase

S.no	**Components**	**Amount**
1	NH_4NO_3	1.50 g
2	KH_2PO_4	2.50 g
3	NaCl	1.00 g
4	$MgSO_4$	1.50 g
5	$CaCl_2$	0.05 g
6	$MnSo_4$	0.01 g

7	Xylose	10.00 g
8	Agar	20.00 g
9	Distilled water	1000 ml
10	Ph	5

(Ramanjaneyulu *et al*., 2015)

3. 11 Media composition for Qualitative assay of Laccase

S.no	**Components**	**Amount**
1	Potato	200 g
2	Dextrose	20 g
3	Guiacol	0.2ml
4	Agar	20
5	Distilled water	1000ml

(Adiveppa *et al*., 2015)

3.12 Media composition for Qualitative assay of Polygalacturonase (Pectin Agar Medium)

S. no	**Components**	**Amount**
1	Pectin	10 g
2	$(NH_4)_2SO_4$	1.4 g
3	K_2HPO_4	6 g
4	KH_2PO_4	2.0g
5	$MgSO_4.7H_2O$	0.1 g
6	Ferrous Sulphate	Trace
7	Zinc Sulphate	Trace

8	Manganous Sulphate	Trace
9	Agar	20 g
10	Distilled water	1000 ml
11	pH	6

1. Polygalactouronase test

- ❖ Fungus were 1st screened for their polygalactouronase activity by using pectinase agar medium PAM.
- ❖ According to the table media was prepared 15-18ml of media was poured on sterile petri plates and allowed it to solidify at normal room temperature .
- ❖ A portion of mycelia was cut with cork borer (5 mm) and media plates were inoculated with inoculating hook and plates were incubated for 4- 5 days.
- ❖ After proper growth the fungus plates were flooded with with 50 mM Potassium iodide-Iodine solution.
- ❖ A clear halo zone around the colonies indicates the ability of an isolate to produce polygalacturonase.

(Hankin *et al*., 1971)

2. Cellulase test

- ❖ Prepare 1 litre potao dextrose agar and add carboxymethyl cellulose 10 g in cold water and mix it properly so there would no clumps .
- ❖ Add this solution into boiling potato dextrose agar .
- ❖ Autoclave this media at temperature 121^{o}c for 15 – 20 minutes
- ❖ Pour 15 –18 ml of media into petriplates and let it get solidify at room teprature
- ❖ Place the mycelia bits on the plates and incubate it for 5-6 days
- ❖ After incubation culture was flooded with 0.1% congo red for 30 min and destained with 5% NaCl for 5min.

- Then petridishes were observed for clear zone formation.

(Zakpaa *et al*.,2009)

3. Amyalse test

- Starch agar medium was used for screening the amylase activity of test fungi.
- Media was prepared according to composition mentioned in Table.
- The pH of the medium was adjusted to 6.0 before sterilization and then autoclaved with a temperature of 121°C for 15 minutes.
- 15-20 ml of media was poured on sterile Petri dishes and allowed to solidify at room temperature.
- 0.5 mm mycelia bits were inoculated at centre of petridish using sterilized corkborer and incubated at 25+2°C for 5-6 days.
- After incubation plates were treated with iodine and potassium iodide solution (1gm Iodine and 5gm of Potassium Iodide in 330ml distilled water).
- A clear halo formed was recorded as positive result, size of halo zones were recorded.

4. Xylanase test

- Mineral salt medium containing commercial xylose as the sole carbon source.
- Media was prepared according to composition mentioned in Table.
- The pH of the medium was adjusted to 5.0 before sterilization and then autoclaved with a temperature of 121°C for 15 minutes.
- 15-20 ml of media was poured on sterile Petri dishes and allowed to solidify at room temperature.
- 0.5 mm mycelia bits were inoculated at centre of petridish using sterilized corkborer and incubated at 25+2°C for 5-6 days.
- After 5 days of incubation, the plates were flooded with 0.01% of 10-15 ml congored solution and allowed to stand for 10 to 15 min and destained with 1%

NaCl and the zone of hydrolysis was recorded. (Teather and Wood, 1982)

5. Laccase test

- PDA was supplemented with 0.02% Guaiacol.
- Media was prepared according to composition mentioned in Table-.
- Media was autoclaved with a temperature of 121°C for 15 minutes.
- 15-20 ml of media was poured on sterile petri dishes and allowed to solidify at room temperature.
- Then the 0.5mm mycelia bits were inoculated on the media and incubated in BOD incubator for 4-5 days.
- After incubation the laccase positive fungal strains showed reddish brown color halo in plates.

(Adiveppa *et al*., 2015)

6. Lignin-degrading activity

- A qualitative assay for lignin degrading activity was performed as described by Atalla *et al* (2010).
- 2 mm mycelia discs were cut from the edge of expanding colonies of six to seven days old fungal cultures using the cork borer.
- The mycelia discs were placed on Boyd and Kohlmeyer medium (glucose: 10g, peptone:2g, yeast extract- 1g, agar:15g, distilled water:1000 ml and pH- 6) containing 4mM guaiacol.
- The petri plates were wrapped with black polythene bags and incubated at 28˚C for 2 weeks.
- Guaiacol in the media leads to the formation of reddish brown color under and around the colony which indicated the presence of lignin degrading enzyme.

Pycnidia sectioning

- Crymicrotome leica CM 1520 was used for sectioning of leaves for the better view and for observing pycnidial sectioning , arrangement of spores in pycnidia .
- Crymicrotome was turned on 5 minutes before the session and adjusted chamber temperature at -20ºC (the optimum cutting temperature for most tissues).
- Tissue freezing gel was poured on specimen disc to freez and then small sections of infected leaves were fixed on the specimen disc with tissue freezing gel to surround the leaf with gel in such way that it doesn't move later on waited for gel to get solidify
- Inserted specimen disc in opening, tighten clamping screw.
- Inserted disposable microtome blade into the blade holder. And start to trimming, after setting the required sectioning width

3.3 Effect of Hydrogen-Ion concentrations on the growth

The Effect of Hydrogen-Ion concentrations on the growth of fungus *Didymella spp*. and *Curvularia spp.* was determined by adjusting the ph of potato dextrose agar medium from 4 .0 to 8 by using 1 percent sodium hydroxide solution and 1 percent HCl solution brfore sterilization with the help of ph meter . Media was sterilized on autoclave for 20 minutes at 15 psi and allowed to cool and then was poured in the petriplates . Inoculation were made with 2 mm disc of mycelial mat obtained from 7 days old cultures of pathogens these petriplates were kept in BOD for incubation at 28 ±1 ^{o}c.

3. 4 Effect of temperature on fungal growth

For determining the growth pattern of the fungus potato dextrose agar media was used . which was prepared according to above mentioned method and then poured in sterilized petridishes , 2 mm mycelia bits were cut from the 7 days old culture and placed in middle of the plates. Which were kept at different temperatures of 18 ^{o}c, 23 ^{o}c, 28 ^{o}c, 32 ^{o}c, 38 ^{o}c in BOD for 4 to 7 days .

3.5 Antagonistic test through dual culture method

5 different biocontrol agents were used against the test pathoges viz. Sbf (*Octrobactrum spp.*), Okc (*Pseudomonas fluoroscence*), Tkf-11 (*Octrobactrum spp.*), *Bacillus spp,* T40 strain of *Trichoderma spp* were evaluated for 5 to 7 days by dual culture technique . They

were studied on Potato dextrose agar (Peeled potato 200 g, dextrose 20 g, Agar 20 g, distilled water 1000 ml). 20 ml of PDA was poured into Petri- dishes (9 cm) and were allowed to solidify. 2 days old bacterial culture and 7 days colony of *Trichoderma spp* . were screened for their antagonistic activities in media plates having 5 mm mycelial bit of *Didymella spp*. and *Curvularia spp*. PDA plates with water served as control .these plates were incubated at 25±1 °C in BOD .

3.6 Poisoned food technique

Five fungicides were used to check their activity against the fungus at different concentration of 100 ppm, 300 ppm, 500 ppm, and 700 ppm.

Fungicides used

- Mancozeb 75% WP , Hi-Thane
- Tebuconazo*le* 250 EC , (25.9% WW), folicur
- Thifluzamide 24% Sc, pulsar
- Difenoconazole 25 % Ec, score
- Cymoxanil 8% + mancozeb 64%WP, Dupont

The efficiency of five fungicides formulations was investigated using the poison food approach . In conical flasks, a calculated of the test chemical was combined with 100 ml of sterilised PDA medium, with a flask containing no test fungicides serving as a control. Under aseptic conditions , the poisoned and control media were put into Petri dishes (90 mm diameter). With the use of sterilised cork borers, round bits (2 mm) of the actively developing fungus were collected. These bits were placed aseptically in the center of each Petri dish and each concentration was replicated 3 times. The Petri dishes with PDA media alone considered as control. The Petri dishes after inoculation were incubated at 25±1°C. The growth of colony of the pathogen was recorded when the control plate showed maximum growth approximately after 3, 5, and 7 days of incubation and per cent inhibition in colony growth (Pi) was recorded by using formula devised by Vincent (1947).

$Pi\ (C - T) \div C \times 100$

Where,

C = Colony growth diameter in control

T = Colony growth diameter in treatment

Chapter IV

RESULTS

AND

DISCUSSIONS

RESULTS AND DISCUSSION

4.1 Pathogenicity testing

4.1.1 Isolation and Inoculation

From the infected leaves 2 types of colonies were isolated one *Didymella spp*.which was olivaceous green in colour having white regular margins and other *Curvularia spp*. of blackish grey colour with sectoring in colony , bearing three to four septate conidia of oval shape and septate mycelium. They were subcultured repeatedly to obtain pure colony .

4.1.1.1 Pot experiment

Plants were inoculated with cultures that were isolated from the infected leaf and found 2 types of colonies in plate both of them were subcultured as to get pure culture these colonies were of *Didymella spp* and *Curvularia spp* . Four to Five leaf stages plants were sprayed with culture suspensions of both the fungus, plants that were treated with *Didymella spp* showed circular spot on the leaves which were bearing black colored pycnidial bodies in concentric ring pattern, leaves became yellow in colour and plants were defoliated as in fig 1 . *Curvularia spp* treated plants did not show any symptoms as in fig.4 Symptoms started appearing from 5th day of inoculation on plants and thus incubation period was 4 to 6 days , During the incubation period average temperature maximum was 22.1 ^{o}c and minimum 11.8 ^{o}c , humidity 70 to 93 percent and average sunshine hours were 1.6 to 2.2 hours per day (Department of Agronomy. IAS BHU).

4.1.1.2 Detached Leaf Assay

Detached leaf assay was performed on pigeon pea leaf of 2 month old plants , leaves were sterilized using 1 percent sodium hypochlorite and inoculated with spore suspension of *Didymella spp* , *Curvularia spp*. placed in moisture chamber . And Symptoms started to appear on the 3rd day of inoculation on *Didymella spp* inoculated leaves and were bearing blighting symptoms and on some leaves circular spots were also visible but leaves with *Curvularia spp* suspension did not show any symptom . whereas leaves that were treated with suspension of both the fungi also showed the symptoms of varying type blighting , spots and upper mycelial growth of the fungi, mycelilal bits were also put on the leaf , leaves with *Didymella spp* mycelia bit and leaves with bits of both the fungus put together had symptoms, but with *Curvularia spp* mycelia bit , there was no symptom and in every case of *Didymella spp.* mycelia bits symptoms were evident.

4.1.2 Identification of the Re-isolated pathogen

The fungus *Didymella spp* varied in its colony colour , morphology and growth rates the pathogen is slow growing and takes about 10 to 14 to days to cover a standard 9 cm petriplate . Hypae of the fungus are septate measuring 6.21 µm in width. The young hypae are hyaline, sparsely septate and slender whereas the older hyphae are broader, thick, more frequently septate, irregularly branched and coloured and the imperfect state of the fungus characterised by formation of fruiting body i.e pycnidia which produce numbers of pycnidiospore fig. b This consists of numerous hyaline spores embedded in a mucilaginous matrix and when there is free moisture in environment , when pycnidia absorbs water it becomes wet and swollen which causes pycnidiospore to ooze out from the ostiole in slimy mass fig .f . Pycnidia measured 201.06 um in average width and 190.61 um in average length as in table 4.1. Pycnidiospore are oval to oblong straight but sometimes slightly bent at the end portion. Usually they are single celled with average width 2.74 um and average lenth 4.64 um as in table 4.2 . Fungus also produces chlamydospores which are unicellular, intercalary or terminal, smooth, hyaline, unicellular globose, measuring 10 – 22 µm in diameter.

Table 4.1 – Pycnidial size of *Didymella spp* in micron (µm)

Pycnidia		
S.no.	**Length**	**Width**
1	320	222.699
2	119	122.665
3	178.157	213.462
4	174.362	228.232
5	157.362	111.594
6	195.997	246.925
7	106.619	109.253

8	221.432	243.234
9	198.534	224.653
10	234.668	287.936
Average	190.613	201.065

4.2 Pycnidiospore size of *Didymella spp* in micron (µm)

Pycnidiospore		
S.no.	**Length**	**Width**
1	4.359	2.315
2	3.960	3.081
3	5.173	2.903
4	4.621	2.35
5	4.452	3.122
6	5.215	3.247
7	4.060	2.081
8	4.845	2.723
9	4.913	3.102
10	4.817	2.503
Average	4.6415	2.7427

According to the study and findings of Ahmadpour *et al.* (2017), Pycnidia of the *Didymella microchlamydospora* causing stem necrosis of Morus nigra in Iran measured 100–190 × 100–190 µm and Conidia of the fungus were hyaline to pale brown, smooth- and thin-walled, subglobose to ellipsoidal, aseptate (2.5)3–5.5(6) × (1.5)2–3.2(3.8) µm (= 4.3 × 2.4

μm, n = 70) . Diameter of hyphae was 2.5–4 μm (= 3.2 μm, n = 50) in 14-days colonies . As per the observation of Lee (1982), non-septate and microtype pycnidiospores were found in the majority of pycnidiospores collected from each isolate of fungus *Didymella bryoniae* cultured on PDA. All isolates had non-septate pycnidiospores , Chlamydospores mostly unicellular, solitary or in chain, intercalary or terminal, smooth, brown, globose to subglobose, (3)4–7.5(10) × (2.5)3–7.5 μm (= 5.9 × 4.6 μm, n = 50). Chlamydospores were, brown, intercalary, sparse and solitary, smooth. and varied in their size and shape These morphological characteristics are almost similar to measurements and morphological understandings of the test fungus *Didymella spp.* Colonies of *Curvularia spp.* were grayish black in colour with septate mycelium , and conidia were 3 to 4 septate and straight measured 8.89 μm in width and 22.37 μm in length , mycelium measured 4.57 μm in diameter.

4.1.3 Re-inoculation

Fungus that was isolated from inoculum sprayed plants was resisolated and cultured and again and inoculated on standing plants. *Didymella spp* sprayed plants showed same symptoms of leaf spot with pycnidial bodies on to it, this time we tried to compare the symptoms production by *Didymella spp* and one with both the fungus together *Didymella spp* and *Curvularia spp* and it was observed that leaves with *Didymella spp* inoculum had less number of spot per leaf around 1 to 2 and these spots were prominent at the outer side of leaves fungus. infected developing buds as as well . Chlorosis also observed on the leaves. Whereas plants treated with both the fungus together developed chlorotic areas on the leaf and similar leaf spot symptoms and there was areas with fungal outgrowth as mycelium on lower sides of the leaf .

4.2 Growth of fungi on different media

This experiment was performed to screen the growth of the test pathogens on different culture medium namely Malt extract, Oatmeal agar, Carrot agar , Asthana and hawker`s , Pigeon pea agar media , Potato dextrose agar , Czapek dox agar , Corn meal agar . Mycelial growth was found to be best on oatmeal agar , malt extract ,carrot agar and potato dextrose agar as in the result of experiment by Devi *et al* ., (2018) and for pycnidial formation pigeon pea agar whereas it was least when grown on Asthana and hawker`s , V8 juice agar and czapek`s dox.

Sectoring refers to the pattern of hyphae in fungal colony on a petriplate , sectors of homokaryotic hyphae are formed like the sectors of pie – chart . if the homokaryon and heterokaryon differ morphologically , then these sectors are visible to the naked eye . The sectors arise when a hyphal tip receive nuclei of only one type and can continue to grow as a homokaryon . Moore *et al* ., (2020). This type of sectoring was found in *Cuvularia spp.* colony.

Highest growth of *Didymella spp.* was found on oatmeal agar media with 66.66±0.88 mm as in table 4.3, on Oatmeal agar fungus produced dense mycelium of the fungus with margins having dull white colour and regular with growth rate 9.4 mm/ day . whereas growth rate of *Cuvularia spp* was 10.19 mm/day .

On carrot and malt extract growth of *Didymella spp.* was uniform and margins were regular on carrot agar colony`s colour was lighter than that is of malt agar media, on malt agar growth was 63.33±0.333 mm . *Curvularia spp.* colonies were having dark margins and filametous colony .

On Asthana and Hawker`s media, *Didymella spp.* least 41.33±0.3 with growth rate 5.8mm/day and fungus imparted no colour initially. *Curvularia spp.* showed white coloured colony with black sectoring and the fungus growth on media was 68.66±0.881mm.

Highest formation of chlamydospore by the fungus *Didymella spp.* was found on V8 juice agar medium, on this media fungal colony was flat and no mycelium portion was aerial , imparting pale brownish yellow colour and growth of the fungus was 54.33±0.33 mm. and growth rate 7.7 mm / day *curvularia spp.* grew more (76.66±0.8) than *Didymella spp*. On V8 juice agar media and possessed fluffy aerial mycelia number of spores were also profuse with high growth rate 10.95 mm/day.

On Czapek dox agar there was moderate growth of the fungus and colony colour was light watery light green and middle portion showed more darker area as compared to the edges, which was white and sticked to the media or flat . In *curvularia spp* sporulation was good when grown on czapek dox agar but mycelia was flat and did not produce characteristic greyish colour it was duller with no sectoring.and grew least on the media with 61.33±0.6 mm colony and growth rate 8.76.

On Cornmeal agar *Didymella spp.* Produced 62.66±0.3 mm colony with very thin growth of mycelium it was flat except at some areas in between, no green colour was seen upto the

5 days of growth, there was sectoring in *Curvularia spp*. upon growing on cornmeal agar produced 71±0.5 mm colony with high growth rate but spore formation was quite less, mycelium was black in colour with flat growth whereas area having grey colour were fluffier.

On pigeonpea agar media *Didymella spp*, whole petriplate got covered in 8 to 10 days and pycnidia formation started within 5 to 6 days, hard black coloured pycnidia were quite visible and which were stuck in the media. Growth rate was 9.2 mm/ day with 64.66±0.3mm colony on 7^{th} day as in table 4.3

On PDA growth rate of the fungus was high with growth rate 9.4 mm/day , colonies were homogeneous as in accordance with results of Kaiser *et al*. (1973) and pycnidia started to form in immersed conditions not visible on aerial portion of the colony but on reversing the plate it could be seen within 3 days *Didymella spp.* begin to get differentiated into green middle portion and white coloured edges. Whereas *curvularia spp.* on PDA grew well with 88.3±0.3mm diameter and highest growth rate 12.57 mm / day as in table 4.4 and profuse sporing, sectoring on the fungus was also visible.

Colony of *D. bryoniae* ranged from 8.7 to 7.2 cm i. e. 87 to 72 mm in diameter on malt extract (Keinath *et al*., 1995). Studies of Bhat *et al.,* (2009) to determine the best medium, temperature, and ph for optimum growth and fructification of *Didymella bryoniae* causing ridge gourd blight revealed maximum growth and fructification on potato dextrose agar and corn meal agar media maintained at ph 7.0 showed maximum growth when incubated at 24±1°C. The growth on potato dextrose agar and oat meal agar medium, fungal mycelial development was at its peak (90 mm) and these results were in conformity of our result in which fungus attained maximum growth in oatmeal agar and PDA. Growth of *D. bryoniae* on the malt extract agar (86.6 mm) and corn meal agar (81.0 mm) media were followed by the Czapek-Dox agar (69.0 mm) with fruit extract agar media (69.3 mm) . Minimum growth (66.6) that was observed on ridge gourd leaf extract agar medium, which was statistically equivalent to ridge-gourd leaf extract agar media. The findings show that pycnidial formation is supported by all the media but they did not support pseudothecial formation . The findings are also in conformity with the observations of Chiu and Walker (1949) and Current (1969) who found these media suitable for the growth and sporulation of *D. bryoniae.*

Table – 4. 3 Growth of the fungus *Didymella spp.* on different media (in mm)

Media	Days					
Days	2	3	4	5	6	7
Malt agar	12.66±0.8	23.33±0.3	31.66±0.3	39.66±0.8	54±0.5	63.33±0.3
Carrot agar	11.33±0.8	21.66±0.8	31.333±0.3	41.33±0.6	55±0.5	62.66±0.6
Asthana and Hawkers	3.33±0.3	7.666±0.3	12.66±0.8	22±0.5	31.66±0.3	41.33±0.3
V8 juice agar	14±1	25.33±0.3	34±0.5	43.66±0.3	49.33±0.3	54.33±0.3
Oatmeal agar	20.66±0.3	31±0.5	41.66±0.3	52.33±0.8	60.66±1.2	66.66±0.8
Cornmeal agar	18.66±0.3	27.33±1.2	36.66±0.8	46.66±0.3	55.33±0.8	62.66±0.3
czapek dox agar	10.33±0.3	21±0.5	27.66±1.3	35±0.5	41.66±0.3	46.33±0.3
PDA	14.66±0.3	23.66±0.3	32.66±0.6	42±0.5	53.33±0.6	66.33±0.8
Pigeonpea agar	22±0.5	32.33±0.3	42.66±0.3	51±0.5	60.66±0.3	64.66±0.3

CD(5%) = 1.87

Table – 4.4 Growth of the *Curvularia spp.* on different media (in mm)

Media	Days					
	2	3	4	5	6	7
Malt agar	22.33±0.8	33.33±0.6	48±0.5	56.33±0.3	61.6±0.3	73±0.5
Carrot agar	25.33±0.3	37±0.5	42.66±0.3	54±0.5	65±0.5	77±0.5
Asthana and hawkers agar	26±0.5	35.33±0.3	42.66±0.3	52.33±0.3	61±0.5	68.66±0.8

PDA	28±0.5	37.33±0.3	66.33±0.3	56.33±0.6	65±0.5	88.33±0.3
V8 juice agar	24.66±0.3	36.66±0.8	45.66±0.3	55±0.5	67.33±0.6	76.66±0.8
Oatmeal agar	25.66±0.8	33.66±0.3	40.66±0.9	53.33±0.8	62.33±0.8	71.33±0.6
Czapek dox	21.66±0.8	29±0.5	32±0.5	44.66±0.3	56.33±0.8	61.33±0.6
Corn meal agar	23.33±0.8	32.33±0.8	40.66±0.3	50±0.5	60.66±0.8	71±0.5

CD (5%) = 1.37

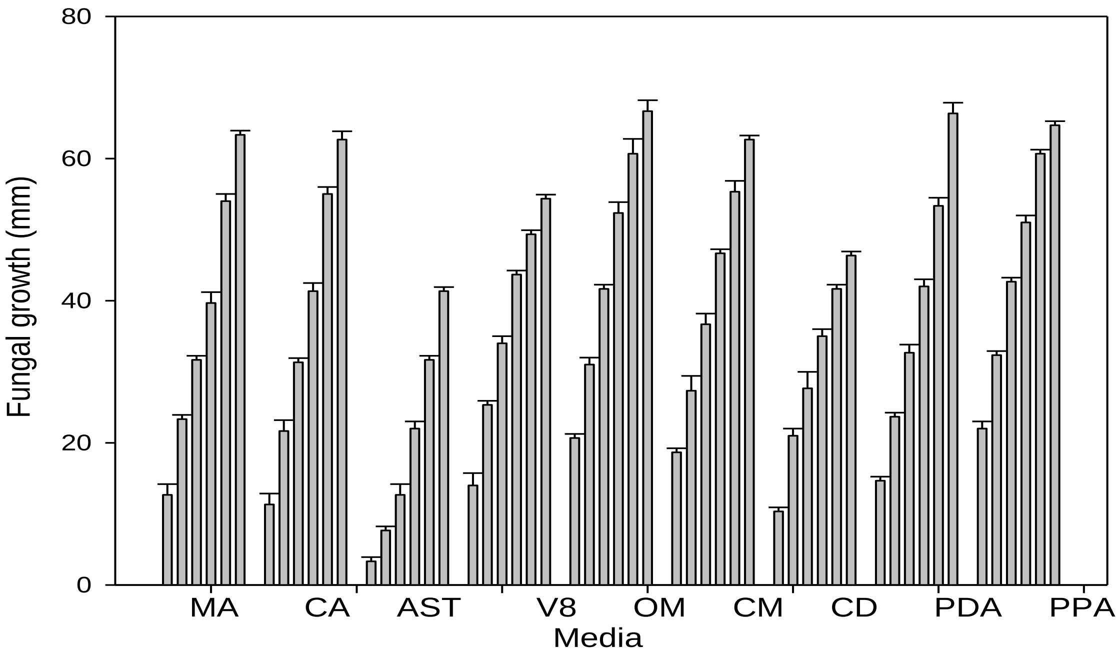


Figure 4.1 – Mean diameter of fungus *Didymella spp.* on different culture media from 2^{nd} to 7^{th} day of growth

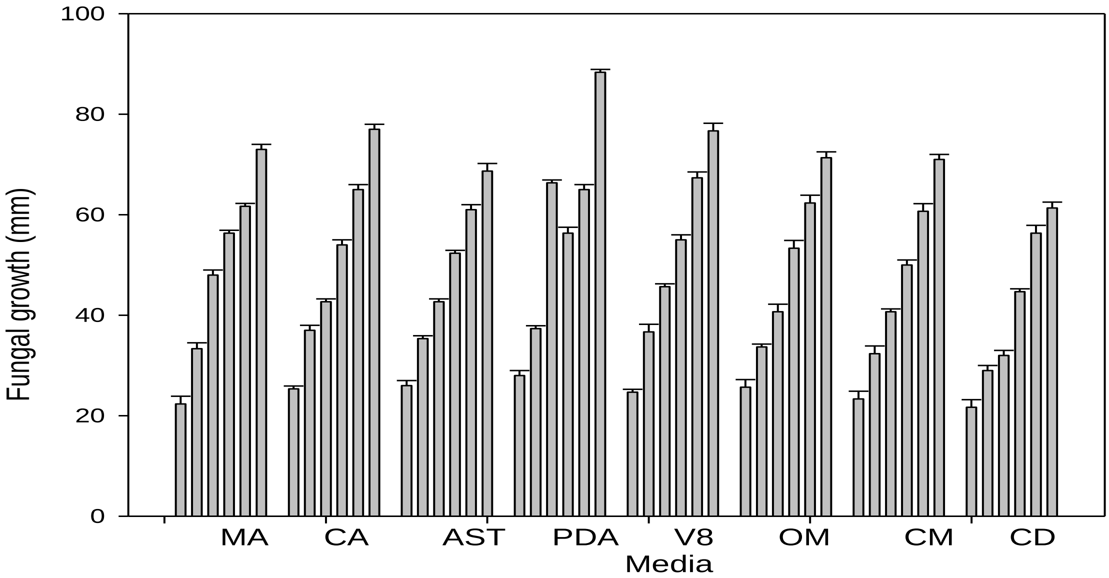


Figure 4.2 – Mean diameter of fungus *Curvularia spp.* on different culture media 2nd to 7th day of growth .

4.3 Antagonistic test

Didymella spp and *Curvularia spp* colonies that were tested against various Biocontrol agents namely Sbf (*Octrobactrum spp.*), Okc (*Pseudomonas fluoroscence*), Tkf-11 (*Octrobactrum spp.*), *Bacillus spp,* T40 strain of *Trichoderma spp* were evaluated for 5 days and later inferences were drawn based on the growth of the fungus and sporulation . The maximum percentage of inhibition was found in case of *Bacillus spp*. i.e 60.24 and in case of *Trichoderma* 59.628 % and 31.67 %, 15.52 %, 20.49% in case of Okc, Sbf , Tkf 11 respectively. *Curvularia spp*. was inhibited mostly by *Bacillus spp*. with percentage of inhibition 60.24 % and least by Tkf-11 with inhibition percent 20.49 %

T. harzianum at 10^6 conidia ml^{-1} showed high percent inhibition against *Ascochyta rabiei* .*T. harzianum* at spore concentrations 10^5, 10^6 and 10^7 conidia ml^{-1} inhibited colony growth to 17%, 28% and 41%, respectively (Ahmad *et al* 2021). Disease index decreased significantly in PGPR primed plants that were challenge inoculated with *B. sorokiniana*, by around 50% in the *Bacillus safensis* treatment and 40% in the *O. pseudogrignonense* treatment Sarkar *et al.* (2018). The antagonistic fungus Trichoderma influences the growth and survival of *A. rabiei*, according to Wang et al. *T. harzianum* generates enzymes such beta 1,3 glucanase and chitinase, which hydrolize parasitic fungi's cellular walls Elad *et al.*(1999) Highly significant effects of antagonism of *T. harzianum* on mycelial growth of

A. rabiei isolates was observed. (Benzohra *et al* 2011). The bacterial antagonists *B. subtilis* and *P. fluorescens* were shown to be effective in suppressing the fungus *D. applanata* causes raspberry cane spur blight. The experiments *in vitro* revealed that there was higher inhibitory activity of *B.subtilis* spores compared with *P. fluorescens* living cells (Shternshis *et al* ., 2016).

Table – 4.5 Mean diameter of test pathogens when tested against biocontrol agents.

Fungus	**Biocontrol agents**					
	Control	**SBF**	**Bacillus**	**TKF 11**	**OKC**	***Tricho derma***
Didymella spp.	44.66	36.3	27.3	41.33	41.12	19.33
Curvularia spp.	53.66	22.66	10.66	21.33	18.33	21.66

Table – 4.6 Inhibition percentage of pathogens when tested against biocontrol agents.

Fungus	**Biocontrol agents**					
	Control	**SBF**	**Bacillus**	**TKF 11**	**OKC**	***Trichoderma***
Didymella spp.	-	15.52	60.24	20.49	31.67	59.62
Curvularia spp.	-	18.719	38.87	7.45	7.92	56.717

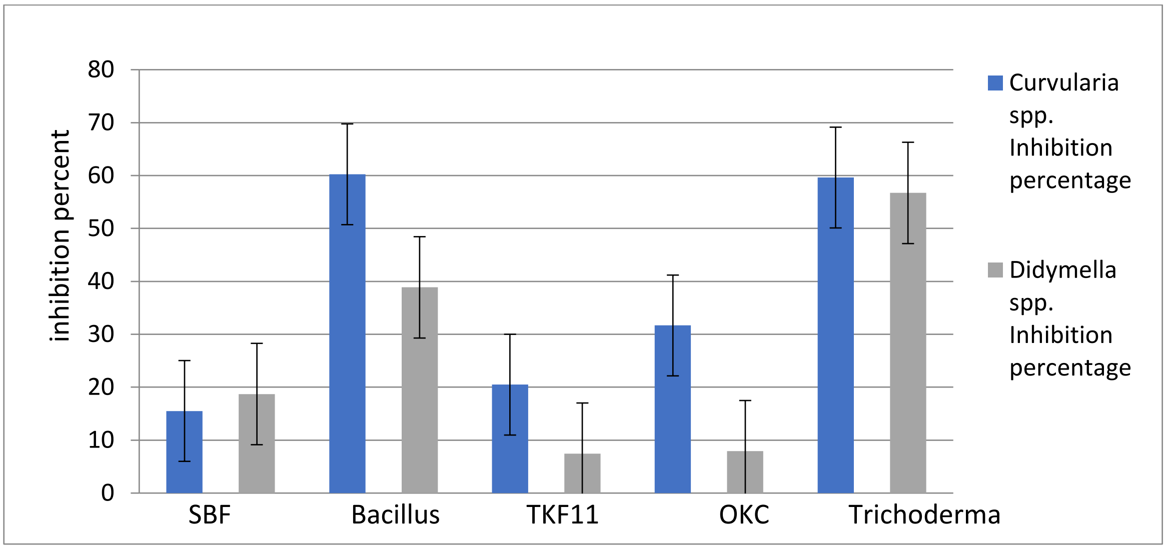


Figure 4.3 – Percentage of inhibition by different Biocontrol agents

4.4 Temperature assay

Fungus, *Didymella spp* was subjected to the temperatures 18 °C , 23 °C , 28 °C , 33 °C and 38 °C to observe the growth pattern of the fungus and there significant change in the colony diameter at . The maximum growth of the fungus was at temperature 28 °C with 69.75±0.47 mm in seven days followed by growth at 23 °c , 56±0.70 mm , at 33 °C , 54±0.7, initially at 33 °C growth rate was high but later decreased with number of days and no growth of the fungus was observed at 38 °C . Average growth rate of the fungus was 8.4 mm per day on 28 °C on which initially it grew with lower rate but after 4 th day rate increased. .Thus optimum range of growth was within the range 18 to 28 degree centigrade after which growth inhibited .

As per the findings of Kosiada *et al* ., 2012 there were significant changes in fungal growth across all of the temperatures studied, with radial growth reducing as temperature increased: 3.2 mm/day (20°C), 2.9 mm/day (15 °C), 2.4 mm/day (25 °C), 1.3 mm/day (10 °C), and 1.1 mm/day (5 °C). An interaction was found between the components studied, allowing the optimal temperature-medium combination to be determined . Growth of *Ascochyta pisi* at 4°C was retarded and was completely inhibited at 37 ° C . The favorable temperature of mycelia development was 25° C, the intermediate values are observed at 15 and 25°C. Germination of conidia of *Didymella rabiei* on cover glasses which were coated with water agar began after 2 h , with maximum germination (more than 95 percent) occurring in 6 h at 20°C and no germination at 0 and 35°C (Trapero-Casas, and Kaiser 2007).

Table – 4.7 Growth of *Didymella spp*. at different temperatures (mm).

Temper- -ature	Number of days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
18 ºc	4.25±0.2	8.75±0.4	16.24±0.4	23.25±0.7	31.63±0.4	42.75±0.7	50.25±0 .4
23 ºc	5.75±0.4	12.13±1. 08	21.34±0.4	32.45±0.6	43.53±0.8	50.54±0.6	56.23±0 .7
28 ºc	6.51±0.2	14.45±0. 7	24.21±0.4	36.25±0.7	46.75±0.6	59.23±0.4	69.75±0 .4
33 ºc	7.75±0.7	19.32±0. 4	22.52±0.6	30.25±0.4	37.75±0.6	45.25±0.2	54±0.7
38 ºc	No growth	-	-	-	-	-	-

CD (5%) = 2.66

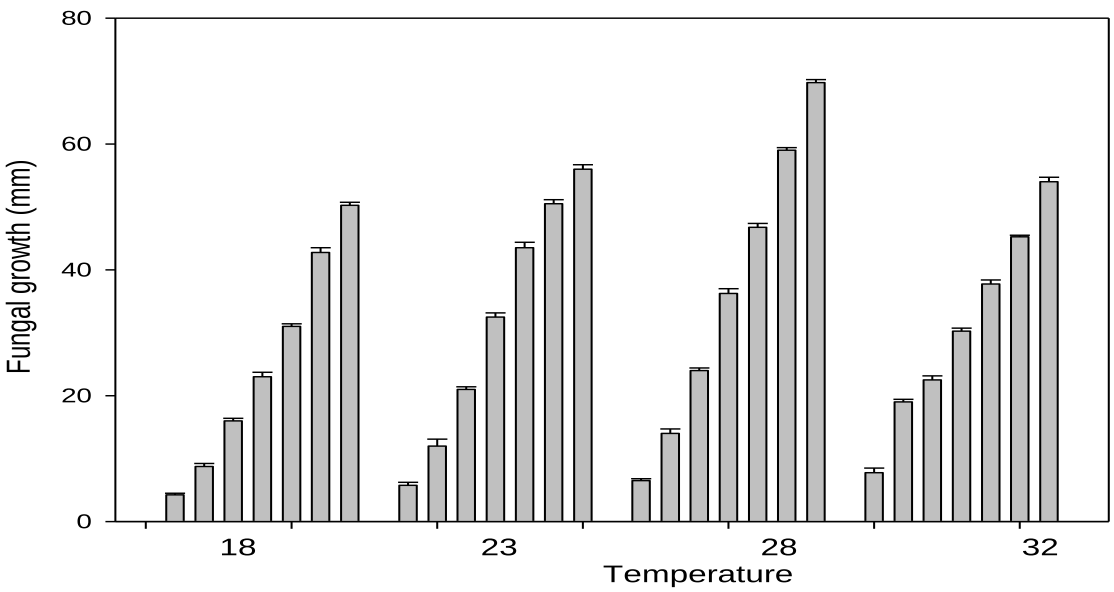


Figure – 4.4 Growth of Didymella at different temperature from 1st to 7th day of growth

4. 6 Qualitative assays for different CWDE (cell wall degrading enzymes)

Fungus *Didymella spp* and *Curvularia spp* were tested for the activity of extracellular cell wall degrading enzymes and their activity was estimated by the measurement of zone of hydrolysis. Enzyme activity (ratio) = clear zone diameter/colony diameter. (Teather, and Wood1982). Qualitative assay of different cell wall degrading enzymes are represented in form of average relative zone of hydrolysis (ratio) produced by the test pathogens which are presented in the Table: 3.6 zone of hydrolysis was higher in case of amylase which was 0.14 in *Didymella spp* and was 0.10 in case of *Curvularia spp* , cellulase was higher in case *Curvularia spp* with activity ratio 0.14 and in case of *Didymella spp* 0.09 , xylanase activity was higher in the case of *Curvularia spp* with 0.4 and in *Didymella spp* 0.12, pectic enzyme i.e polygalactouranase test showed higher activity of enzyme in fungus *curvularia spp* i.e 1.25 and in *Didymella spp* 0.18. and *Curvularia spp* did not show any protease activity but *Didymella spp* showed 0.33 protease enzymatic activity ratio. Both the fungus showed red colour formation on plate containg media for laccase degrading acitivity showed redish brown colour which indicate positive result . Cellulases and Hemicellulase plays important role in degradation of plant cellwall thereby making the pathogen feasible to enter into the host cell, in the present study cellulase (complex) and xylanase (hemicellulase) were present in test pathogens whose activity was evident in form of halo zone after staining with congo red followed by destaining with NaCl. This shows ability of isolates of test pathogen in cellwall degradation. Amylases are the group of enzymes which are involved in degradation of starch and glycogen, thereby helps the pathogen in extracellular digestion of plant and plant debris (Mishra and Maheshwari, 1996). Activity was evident in both the fungi showing that the test pathogen were able to degrade the starch and glycogen inturn extracellular digestion of host plant. PG activities produced by the highly virulent isolates in shake cultures and in decayed fruit were greater than those of the moderately virulent isolates (Zhang *et al* 2014).

Table – 4.8 Colony diameter, Halo zone and Activity ratio of test pathogens upon biochemical tests (A. Diameter of colony (cm) B. Diameter of zone (cm) C. Activity ratio

Fungus	Amylase			Cellulose			Xylanase			Polygalactour-nase			Protease		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Didymella spp	4.2	0.6	0.14	3.2	0.3	0.09	3.2	0.4	0.12	2.2	0.4	0.18	1.2	0.4	0.33
Curvularia spp	4.7	0.5	0.10	2.8	0.4	0.14	2.5	1	0.4	2	2.5	1.25	Nil	nil	-

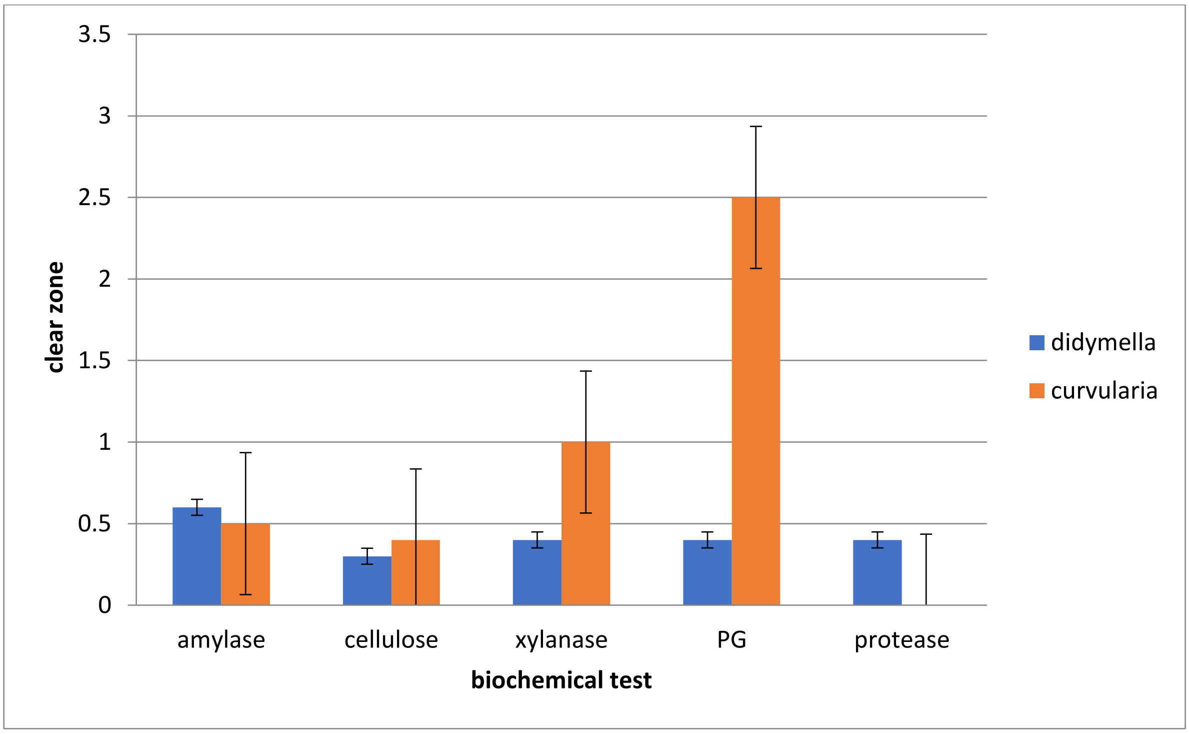


Figure -4.5 Clear zone formation in biochemical tests

3.7 Fungal growth on different pH

Fungus *Didymella spp.* was tested on plates containing potato dextrose agar with different pH mainly pH 4 , pH 5 , pH 6 , pH 7 , pH 8 . There was significant difference in growth of the pathogen at different pH, at pH 4 , maximum growth of the fungus *Curvularia spp.* (81±0.57 mm). *Didymella spp.* exhibited maximum growth on pH 6 on 7 th day i. e. 81±0.57 mm with growth rate 11.57 mm/ day and minimum on pH 8 i.e 44.33±0.88 with growth rate 6.33 mm/ day. Pycnidial formation started on 5 to 6 th day on media having pH 6, 7 and 8 but on acidic pH pycnidial formation was started after 6th day whereas at ph 8 and 7, it started earlier. The pathogen *D. bryoniae* was found to grow within wide ph range of 5.0 to 8.0, but maximum mycelial growth, spore germination was observed at pH 7.0 (Bhat *et al.*,2009) and these findings were in agreement with the findings of Chen *et al*. (1993) who also reported the maximum growth, fructification and spore germination at pH 7.0 . The impact of pH on *D. bryoniae* conidial germination, growth, and sporulation was investigated on a major pathogen of watermelons (*Citrullus vulgaris* [*C. lanatus*]),. Growth was enhanced by pH levels of 4-6, whereas conidial germination was stimulated by pH levels of 5-6. Sporulation was highest at pH 6 compared to any other pH. (Pérez *et al*, 2000)

Table: 4.9 Growth of *Didymella spp.* on different hydrogen ion concentration

pH	**Number of days**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
4	5.33±0.6	11.66±1.2	22±1	31.33±0.8	41±0.5	48.66±0.3	57±0.5
5	7.33±0.3	14.33±0.8	27.66±0.3	42.33±0.3	50.66±0.3	59.3±0.3	73.33±0.3
6	6.33±0.8	20±0.5	30.66±0.3	45±1	54.33±0.6	68.33±0.3	81±0.5
7	6.66±0.6	18±0.5	29.66±0.3	41.66±0.3	51.66±1.4	65.33±0.3	76.33±0.8
8	6.66±1.2	12.83±1.0	18.33±0.3	23.33±0.3	30±0.5	36±0.5	44.33±0.8

CD (5 %) =2.24

Table 4.10 Growth of *curvularia spp* on different hydrogen ion concentration .

PH	Numer of days						
	1	**2**	**3**	**4**	**5**	**6**	**7**
4	9.33±0.3	22.66±1.2	35±1.1	44.33±1.2	52±0.5	67.33±1.2	78.33±1.2
5	8.33±0.3	20.66±0.8	34.66±0.8	42.66±0.8	53.33±0.3	66±1	75.66±0.3
6	7.33±0.3	18.66±0.6	36.33±1.6	42.33±0.8	47.33±0.3	64.66±0.3	76.33±0.8
7	6.66±0.3	16.66±0.3	33±0.5	40.33±0.8	48±0.5	58±0.5	71.33±1.8
8	4.66±0.3	14±0.5	29.66±0.6	33.33±0.8	41.66±0.3	45.66±1.7	52.33±0.3

CD (5%) = 3.96

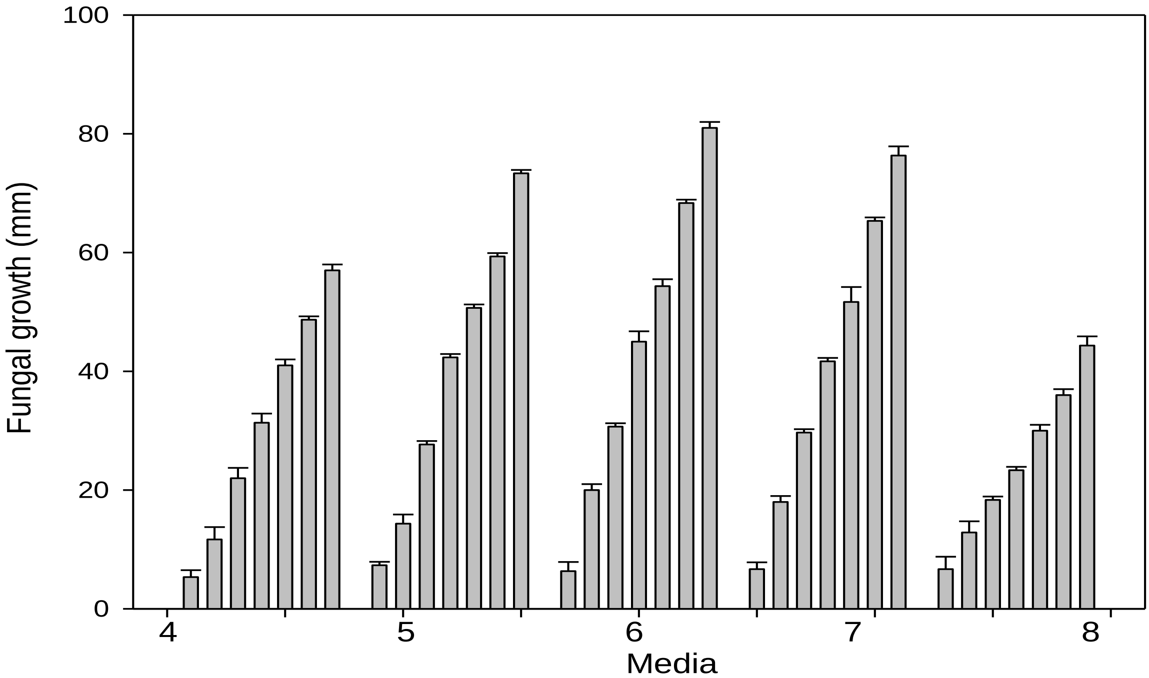


Figure – 4.6 Mycelilal growth of the fungus *Didymella spp.* From 1 st to 7 th day at different pH

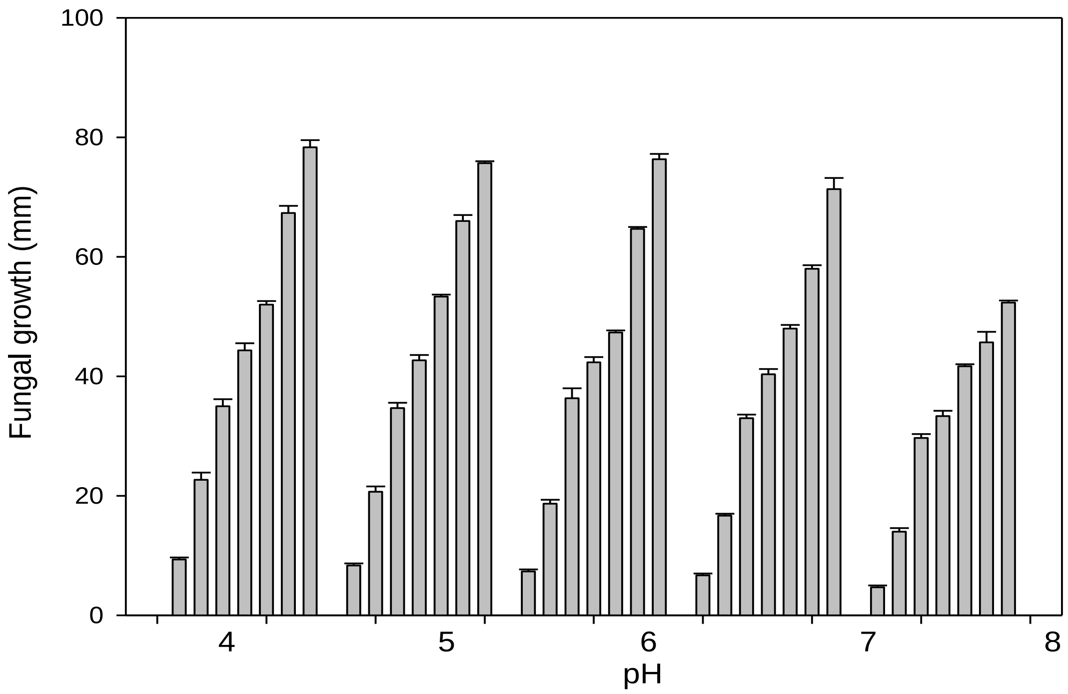


Figure - 4.7 Mycelial growth of the fungus *Curvularia spp.* From 1st to 7th day at different pH.

4.8 Poisoned Food Technique

The efficacy of five commercial namely,

- Mancozeb 75% WP , Hi-Thane
- Tebuconazole 250 EC , (25.9% WW), folicur
- Thifluzamide 24% Sc, pulsar
- Difenoconazole 25 % Ec, score
- Cymoxanil 8% + mancozeb 64%WP, Dupont

These fungicides were assayed *in vitro* against *Didymella spp and Curvularia spp* at different concentration . These fungicides were tested against 4 different concentrations 100 ppm, 300 ppm , 500 ppm and 700 ppm. The efficacy of the fungicides was expressed as percent inhibition of mycelial growth over control. On mancozeb , and pulsor upto 500 ppm *Didymella spp* showed the growth but on dupont , score and folicur fungus did not show any sign of growth and was completely inhibited by above mentioned fungicidal concentrations whereas *Curvularia spp* grew on mancozeb upto 500 ppm concentration , on dupont upto

500 ppm, on pulsor upto all the concentrations till 700 ppm but score and folicur showed 100 percent growth inhibition .

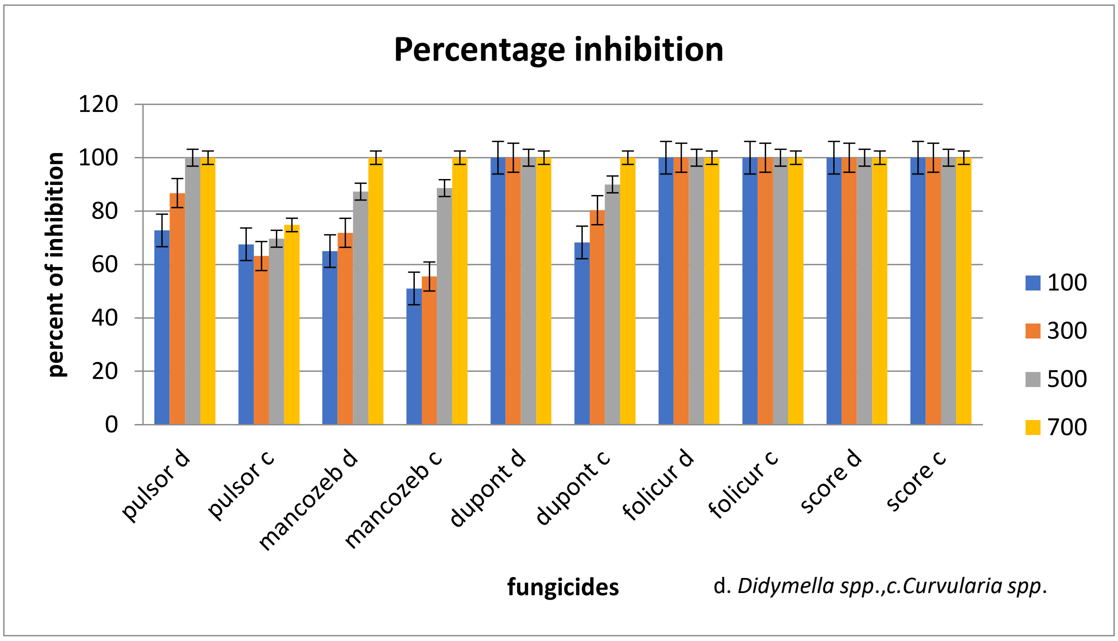


Figure – 4.8 Percent of inhibition by different fungicides on 100ppm, 300ppm, 500 ppm and 700ppm concentrations against test pathogens

Didymella spp showed growth of 1.27 mm on 100 ppm, on 300 ppm 0.77 mm , 0.47 mm on 500 ppm 7 th day of growth for fungicide mancozeb.on pulsor, growth of the *Didymella spp* was 1.79 mm on concentration 100 ppm , 0.87 mm on 300 ppm and for other fungicides at all the concentrations there was no growth .

Didymella bryoniae was found to be highly sensitive to fungicides viz. Mancozeb 75% (Indofil) and Tebuconazole 50% (Nativo) with 100% growth inhibition at even very low concentration. (Mangala *et al* 2018). Mancozeb, and thiophanate-methyl were the most efficient fungicides in suppressing the gram blight disease caused by *Didymella rabiei* (Kovatsch) in *Cicer arietinum* L. cultivars that are frequently seeded throughout Pakistan (Rehman *et al*.,2013). Combination of azoxystrobin 23 SC +difenoconazole 25 EC was more effective and it resulted in cent per cent inhibition at 125 ppm. best fungitoxicant was captan 50 WP followed by mancozeb 75 WP with mean per cent inhibition of 51.98 and 44.18 respectively In their findings, all the systemic fungitoxicants were found to be highly effective against *Ascochyta rabiei* and cent per cent inhibition was achieved at 100 ppm concentration in all the fungitoxicants. These fungitoxicants might be responsible in hindering the metabolic pathways occurring in the cells of *Ascochyta rabiei.* (Ali *et al* .,

2011) in the above findings Mancozeb, Difenoconazole and other systemic fungicides were inhibiting growth of *Didymella spp.* and other related genera .

Table 4.11 – Average mean growth of *Didymella spp*.on different concentration of fungicides (in cm)

Fungicide	**Number of days**	**100 ppm**	**300 ppm**	**500 ppm**	**700 ppm**
Pulsor	3	0.81	0.35	0	0
	5	1.37	0.62	0	0
	7	1.79	0.87	0	0
Mancozeb	3	1.27	0.77	0.47	0
	5	2	1.42	0.62	0
	7	2.32	1.85	0.83	0
Dupont	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
Score	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
Folicur	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
No. of days	**3**	**5**	**7**	-	-
control	2.36	4.21	6.63	-	-

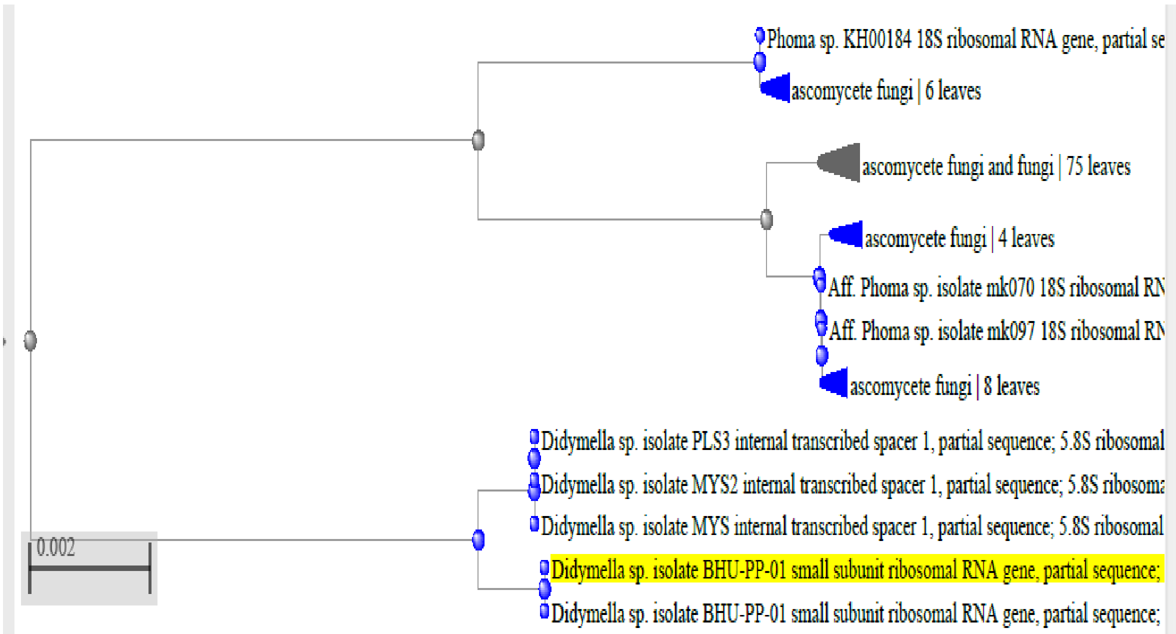
Table 4.12 – Average mean growth of *curvularia spp*.on different concentration of fungicides (in cm)

Fungicide	**Number of days Days**	**100 ppm**	**300 ppm**	**500 ppm**	**700 ppm**
Pulsor	3	1.92	1.23	0.77	0.59
	5	3.07	2.25	1.44	0.95
	7	3.57	2.67	2.27	1.82
Mancozeb	3	2.12	1.87	0.52	0
	5	2.62	2.15	0.75	0
	7	3.52	3.22	0.82	0
Dupont	3	0.92	0.65	0.51	0
	5	1.62	1	0.52	0
	7	2.3	1.42	0.72	0
Score	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
Folicur	3	0	0	0	0
	5	0	0	0	0
	7	0	0	0	0
No. of days	**3**	**5**	**7**		
control	3.73	5.63	8.83		

DNA sequencing Result

DNA was isolated from the given sample The DNA was used in PCR to amplify ITS1 F and ITS4 R Primers. The amplicon was gel eluted and the product is sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled and compared with NCBI data base and got ON506244.1 Didymella sp. isolate BHU-PP-01 small subunit ribosomal RNA gene, partial sequence;internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, completesequence; and large subunit ribosomal RNA gene, partial sequence

CCTTCCGTAGGTGAACCTGCGGAAGGATCATTACCTAGAGTTTGTGGGCTTTG
CCTGCTGTCTCTTACCCATGTCTTTTGAGTACCTTCGTTTCCTCGGCGGGTCCGC
CCGCCGATCGGACAACCATAAACCATTTGCAGTTGCAATCAGCGTCTGAAAAA
CTTTTAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAA
GAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCG
AGCGTCATTTGTACCTTCAAGCTCTGCTTGGTGTTGGGCGTTTGTCTCGCCTCC
GCGCGTAGACTCGCCTCAAAACGATTGGCAGCCGGCGTATTGATTTCGGAGCG
CAGTACATCTCGCGCTTTGCACTCACGACKACGACGTCCAAAAGTACATTTTT
ACACGCTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA
TAAGCGGAGGAA



In phylogenetic tree, Accession number ON506244.1 is the yellow highlighted sequence that has been submitted in NCBI Database . The submitted sequence showed 97.59 % similarity with the sequence of *Didymella* spp. GenBank Accession No. MN912323.1 and the submitted sequence of *Didymella* spp. also came in the same clade of phylogenetic tree along with *Didymella* spp with 99.4% similarity with PLS3 - OM52211.1, MYS2 – OM952210.1, MYS – OM948732.1 .(Tree was developed by fast minimum evolution method).

Chapter V

SUMMARY

AND

CONCLUSION

Summary and Conclusion

In this study test pathogens were isolated from the infected plant leaves which were collected from the field of AICRP Pigeonpea Plant Pathology field at BHU center Varanasi. Mainly two fungi were found in most of isolations from the infected leaves and later they were subcultured and pathogenicity test was performed to confirm disease causing fungi among two of the fungus obtained in the isolation earlier. *Curvularia spp* had greyish black colony with 3 to 4 septa conidia and septate mycelium, *Didymella spp* had olivaceous green colony initially with white margins this fungus produced pycnidia in culture plate which were stuck in the media but after 7 to 9 days pycnidial bodies can be seen on the aerial portion of the colony. These pycnidia produced numerous microscopic spores . Both the fungus were used for pathogenicity test and as a result *Didymella spp* inoculated plants showed prominent round spots on the leaves of pigeonpea plant , these spots were bearing black pycnidial bodies in concentric ring manner same as initial symptoms of isolation whereas *Curvularia spp* treated plants possessed no symptoms later on these fungus were subjected to many testing viz . Re-inoculation , Detached leaf assay and Biochemical tests . Effect of culture media , effect of ph and effect of temperature were also determined on the growth of the symptom producing fungus i.e. *Didymella spp* . Whose identity was confirmed using ITS 1 anf ITS 4 primers The ITS sequence was submitted in NCBI with GenBank Accession No.ON506244.1. The submitted sequence showed 97.59 percent similarity with the sequence of *Didymella* spp GenBank Accession No. MN912323.1. When *Didymella spp* and *Curvularia spp* were examined for mycelial growth on 7 to 9 different culture media , *Didymella spp* was found to grow fastest on oatmeal agar with 66.66±0.88mm growth, on potato dextrose agar with growth 66.33±0.88 mm with different colony characteristics ,*Curvularia spp* grew well potato dextrose agar media on which its colony diameter was 88.33±0.33 mm . These fungus were also tested for their extracellular enzymatic activities in which *Didymella spp* showed 0.14 acitivity ratio of amylase enzyme and 0.09, 0.12 , 0.18 and 0.33 activity ratio of cellulose , xylanase , polygalactournase and protease respectively. Fungus also showed laccase activity by imparting reddish brown colour when grown in media supplemented with guaicol . *Curvularia spp* also gave positive test result when studied for its extracellular enzymatic activities in which enzyme activity ratio were 0.1 , 0.14, 0.4 , 1.25, in case of amylase , cellulose, xylanase, and polygalactouranase tests respectively , *Curvularia spp* showed lignin degrading and laccase activity also but protease test was negative , In the study of effect of hydrogen ions on the growth of these two fungus , 5 different pH were investigated the fungus *Didymella spp* . showed highest growth on ph 6 which was 81±0.57 mm and on pH 7, 76.33±0.88 mm, these results clearly depicts that most suitable ph for the *Didymella spp* growth is 6 , and *Curvularia spp* showed highest growth on ph 4, 78.33±1.20 on ph 6, 76.33±0.88 when kept at temperature 27±2 ^{o}c . Further *Didymella spp* was also studied for the growth pattern at different temperatures 18 ^{o}c , 23 ^{o}c , 28 ^{o}c , 33 ^{o}c and 38 ^{o}c , highest growth was recorded at 38 ^{o}c with 69.75±0.4 mm and growth rate average growth rate of 7 days was 8.4mm/ day and no growth was observed at 38 ^{o}c. Different Biocontrol agents were also used against both the fungus viz. Sbf (*Octrobactrum spp.*), Okc (*pseudomonas fluoroscence*), Tkf-11 (*Octrobactrum spp.*), *Bacillus spp ,* T40 strain of *Trichoderma spp* which were evaluated for 5 days and later inferences were drawn based on the growth of the fungus the maximum percentage of inhibition was found in case *Bacillus spp.* i.e. 60.24 % followed by *Trichoderma spp.*59.62 % and 31.67 %, 15.52 %, 20.49 % in case of Okc, Sbf , Tkf 11 respectively. *Curvularia spp* showed least growth when grown against *Trichoderma spp* with highest inhibition percentage 56.71 followed by *Bacillus spp*. which inihibited 38.87 percent of fungal growth. Through poison food technique effect of four different concentration of five fungicides namely Mancozeb 75% WP ,Tebuconazole 250 EC (25.9% WW),Thifluzamide 24% Sc,Difenoconazole 25 % Ec,Cymoxanil 8% + mancozeb 64%WP were checked .Above xperiment, pathogenicity test and various enzymatic tests performed on the *Didymella spp.* shows that this isolated pathogen could be a potential pathogen of pigeon pea by causing leaf spots and and blight like symptoms on leaves as *Didymella* is a Teleomorph of *Asocochyta spp* and *phoma spp* and *Asocochyta spp* is reported to be a dangerous pathogen for pulses.

Chapter VI

REFERENCES

Reference

Ahmad, S., Khan, M. A., Ahmad, I., Iqbal, Z., Ashraf, E., Atiq, M.,and Naseer, S. (2021). Efficacy of fungicides, plant extracts and biocontrol agents against Ascochyta blight (*Ascochyta rabiei*) of chickpea (*Cicer arietinum* L.) under field conditions. *Plant Science Today*, *8*(2), 255-262.

Amselem, J., Cuomo, C. A., van Kan, J. A., Viaud, M., Benito, E. P., Couloux, A., ... and Dickman, M. (2011). Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. *PLoS genetics*, *7*(8), e1002230.

Alcalde, M., Bulter, T., and Arnold, F. H. (2002). Colorimetric assays for biodegradation of polycyclic aromatic hydrocarbons by fungal laccases. *Journal of biomolecular screening*, *7*(6), 547-553.

Alcalde, M. (2007). Laccases: biological functions, molecular structure and industrial applications. In *Industrial enzymes*, 461-476.

Anonymous. 2008. Ascochyta blights of field peas. *Online Ministry of Agriculture Goverment of Saskatchewan, Regina, SK, Canada.*

Ahmadpour, S. A., Farokhinejad, R., and Mehrabi-Koushki, M. (2017). Further characterization and pathogenicity of Didymella microchlamydospora causing stem necrosis of Morus nigra in Iran. *Mycosphere*, *8*, 835-852.

Atalla, M. M., Zeinab, H. K., Eman, R. H., Amani, A. Y., and Abeer, A. A. E. A. (2010). Screening of some marine-derived fungal isolates for lignin degrading enzymes (LDEs) production. *Agriculture and Biology Journal of North America*, *1*(4), 591-9.

Aveskamp, M. M., de Gruyter, J., Woudenberg, J. H. C., Verkley, G. J. M., and Crous, P. W. (2010). Highlights of the Didymellaceae: A polyphasic approach to characterize Phoma and related pleosporalean genera. *Study Mycology 6*(5)l, 1-60.

Bagale, P. A. A. S. K., and Kumar, B. T. G. (2020). Indian Journal of Hill Farming. *Indian Journal of Hill Farming*, *33*(1), 141-156.

Bahr, L., Castelli, M. V., Barolo, M. I., Mostacero, N. R., Tosello, M. E., and López, S. N. (2016). Ascochyta blight: isolation, characterization, and development of a rapid method to detect inhibitors of the chickpea fungal pathogen Ascochyta rabiei. *Fungal biology, 120*(3), 424-432.

Barilli E, Cobos M J and Rubiales D (2016) Clarification on host range of Didymella pinodes the causal agent of pea Ascochyta blight. *Frontier in Plant Science, 7*(2), 592-612.

Benzohra, I. E., Labdi, B. B. M., and Bnekada, M. Y. (2011). In vitro biocontrol using the antagonist *Trichoderma harzianum* against the algerian isolates of *Ascochyta rabiei* (Pass.) Labr., the agent of Ascochyta blight in chickpea (*Cicer arietinum* L.). *International Journal of Microbiology Research, 2* (1), 124-128.

Bhat, Z. A., Dar, G. H., Bhat, M. A., and Anhanger, M. A. (2009). Effect of Media, Temperature and ph on growth and fructification of Didymella bryoniae (Auresw.) Rehm. causing blight in ridge gourd. *Applied Biological Research*, *11*(2), 70-72.

Biely, P. (1985). Microbial xylanolytic systems. *Trends in biotechnology*, *3*(11), 286-290.

Brück, S. A., Contato, A. G., Gamboa-Trujillo, P., de Oliveira, T. B., Cereia, M., and de Moraes Polizeli, M. D. L. T. (2022). Prospection of Psychrotrophic Filamentous Fungi Isolated from the High Andean Paramo Region of Northern Ecuador: Enzymatic Activity and Molecular Identification. *Microorganisms*, *10*(2), 282.

Bordbar, A.K.,Omidiyan. K. R., and Hosseinzadeh. (2005). Study on interaction of α-amylase from *Bacillus subtilis* with cetyltrimethylammoniumbromide. *Colloids Surf. B: Biointerfaces, 40*(2), 67-71.

Blancard, D. (2012). Tomato diseases. *San Diego, CA, USA:: Academic Press* 67-74.

Carrillo E, Rubiales D, Pérez-de-Luque A and Fondevilla S (2013) Characterization of mechanisms of resistance against Didymella pinodes in Pisum spp. Eur J Plant Pathol 135: 761–69.

Chen, Q., Hou, L. W., Duan, W. J., Crous, P. W., and Cai, L. (2017). Didymellaceae revisited. *Studies in mycology*, *87*(1), 77-103.

Chiu, W. F., and Walker, J. C. (1949). Physiology and pathogenicity of the cucurbit black-rot fungus *Journal of Agricultural Research*, *78*(12), 590-615.

Chilvers, M. I., Rogers, J. D., Dugan, F. M., Stewart, J. E., Chen, W., and Peever, T. L. (2009). *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*. *Mycology Research 11*(3), 391-400.

Chung, W. H., and Tsukiboshi, T. (2005). A new species of *Curvularia* from Japan. *Mycotaxon*, *91*, 49-54.

Yashwant, C. K., Singh, R., and Singh, S. K. (2010). Effect of temperature and ph on growth and sporulation of *Curvularia lunata* causing leaf spot of okra. *Annals of Plant Protection Sciences*, *18*(2), 549-551.

Cromey, M. G., Butler, R. C., Mace, M. A., and Cole, A. L. J. (2004). Effects of the fungicides azoxystrobin and tebuconazole on *Didymella exitialis,* leaf senescence and grain yield in wheat. *Crop Protection*, *23*(11), 1019-1030.

Curren, T. (1969). Pectic and cellulolytic enzymes produced by *Mycosphaerella citrullina* and their relation to black rot of squash. *Canadian Journal of Botany*, *47*(5), 791-794.

De Gruyter, J., Aveskamp, M. M., Woudenberg, J. H., Verkley, G. J., Groenewald, J. Z., and Crous, P. W. (2009). Molecular phylogeny of Phoma and allied anamorph genera: towards a reclassification of the Phoma complex. *Mycological research*, *113*(4), 508-519.

Dasgupta, S., Saha, D., and Saha, A. (2005). Levels of common antigens in determining pathogenicity of *Curvularia eragrostidis* in different tea varieties. *Journal of Applied Microbiology*, *98*(5), 1084-1092.

Devi, K. S., Misra, D. K., Saha, J., Devi, P. S., and Sinha, B. (2018). Screening of suitable culture media for growth, cultural and morphological characters of Pycnidia forming fungi. *International Journal of Current Microbiology and Applied Sciences*, *7*, 4207-4214.

Elad, Y., and Kapat, A. (1999). The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. *European Journal of plant pathology*, *105*(2), 177-189.

Farahani, S., Talebi, R., Maleki, M., Mehrabi, R., and Kanouni, H. (2019). Pathogenic diversity of *Ascochyta rabiei* isolates and identification of resistance sources in core collection of chickpea germplasm. *The plant pathology journal*, *35*(4), 321.

Fondevilla, S., Avila, C.M., Cubero, J. L., and Rubiales, D. (2005) Response to *Mycosphaerella pinodes* in a germplasm collection of *Pisum spp. Plant Breeding 12*(4) 313–15.

FRAC (Fungicide Resistance Action Committee) (2007) FRAC code list: fungicides sorted by modes of action. URL www.frac.info.

Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology. *The Quarterly Review of Biology*, *11*(1), 345-357.

Haware, M. P., Nene, Y. L., and Mathur, S. B. (1986). Seed- borne diseases of chickpea. *Tech Bull 1. Danish Government Institute of Seed Pathology for Developing Countries ICRISAT.*

Kaiser, W. J. (1997). Inter-and intranational spread of Ascochyta pathogens of chickpea, faba bean, and lentil. *Canadian Journal of Plant Pathology*, *19*(2), 215-224.

Kaiser, W. J. (1973). Factors affecting growth, sporulation, pathogenicity, and survival of Ascochyta rabiei. *Mycologia*, *65*(2), 444-457.

Keinath, A. P., Farnham, M. W., and Zitter, T. A. (1995). Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma spp.* isolated from cucurbits. *Phytopathology*, *85*(3), 364-369.

Kim, W. (2015). Functional characterization of polyketide-derived secondary metabolites solanapyrones produced by the chickpea blight pathogen, *Ascochyta rabiei*: Genetics and chemical ecology. *Washington State University.*

Kirk, P.M., Cannon, P.F., Stalpers, J.A., Minter, D.W. (2008). Ainsworth and Bisby's dictionary of the fungi. *Centre for Agriculture and Bioscience International*, *7*(2), 347-365.

Kosiada, T. (2012). In vitro growth of some species of Ascochyta Lib. *Open Life Sciences*, *7*(6), 1076-1083.

Koley, S., and Mahapatra, S. S. (2015). Evaluation of culture media for growth characteristics of Alternaria solani, causing early blight of tomato. *Journal of Plant Pathology and Microbiology 8*(5), 569-578.

Kubicek, C. P., Starr, T. L., and Glass, N. L. (2014). Plant cell wall-degrading enzymes and their secretion in plant-pathogenic fungi. *Annual Reviewof Phytopathology, 5*(2), 427- 451.

Kumar, C. S., Naik, S. S., Mohan, N., Saxena, R. K., and Varshney, R. K. (2017). Botanical Description of Pigeonpea [*Cajanus cajan* (L.) Millsp.]. In The Pigeonpea Genome. *Springer, 8*(1), 17-29.

Kumar, P., Kumar, A., and Kumar, K. (2000). Bio-control of seed-borne fungal pathogens of pigeonpea [Cajanus cajan (l.) millsp.]. *Annals of Plant Protection Sciences, 16*(2), 124-144.

Kudryavtseva, N. N., Sofyin, A. V., Revina, T. A., Gvozdeva, E. L., Ievleva, E. V., and Valueva, T. A. (2013). Secretion of proteolytic enzymes by three phytopathogenic microorganisms. *Applied biochemistry and microbiology*, *49*(5), 514-520.

Kharrat M., Le Guen J. and B. Tivoli, (2006). Genetics of resist- ance to 3 isolates of *Ascochyta fabae* on faba bean (*Vicia faba* L.) in controlled conditions. *Euphytica*,*15*(1), 49-6.

Lal, M., Kumar, S., Ali, M., Khan, A., Singh, V., and Murti, S. (2013). Host range, susceptibility period of *Curvularia lunata* causing leaf spot of black gram and germplasm screening. *Agriways*, *1*(2), 142-146.

Lee, D. H. (1982). Morphological and cultural characters of *Didymella bryoniae* on seeds and culture media. *The Korean Journal of Mycology*, *10*(1), 7-13.

Masurkar, P., Pandey, S. K., and Singh, R. (2019). Bio efficacy of Thifluzamide 24% SC formulation of paddy with special reference to sheath blight disease. *Annals of Plant and Soil Research*, *21*(1), 71-75.

Mangala, K. G., and Rajkumar, H. G. (2018). Efficacy of Fungicides and Culture Filtrate of Native Bioagents against Didymella bryoniae Causing Gummy Stem Blight Disease in Gherkin. *International Journal of Pure and Applied Biosci*ence, *6*(1), 1122-1132.

Meryem, Y., Lalia, B. S., Rym, T., Nisserine, H. K., Mebrouk, K., and Jamel-Eddine, H. (2015). Thermophilic Fungi Causing Deterioration of Amylase Activities of *Ascochyta Pisi* Causing Anthracnose of Peas (*Pisum sativum*). *Advances in Environmental Biology*, *9*(8), 203-209.

Meng, Z., Tianyu, Z., and Yueming, W. (2004). A new name and a new variety in Curvularia. *Mycosystema*, *23*(2), 177-178.

Miller, M. E., Isakeit, T., Zhang, J. X., and Bruton, B. D. (1997). Gummy stem blight and black rot of melons. Melon production system in South Texas, *Annual Research report Texas Aand M University, Weslaco.*

Mishra, R. S., and Maheshwari, R. (1996). Amylases of the thermophilic fungus *Thermomyces lanuginosus*: Their purification, properties, action on starch and response to heat. *Journal of Biosciences*, *21*(5), 653-672.

Morkunas, I., Formela, M., Marczak, Ł., Stobiecki, M., and Bednarski, W. (2013). The mobilization of defence mechanisms in the early stages of pea seed germination against Ascochyta pisi. *Protoplasma*, *250*(1), 63-75.

Moore, D., Robson, G. D., and Trinci, A. P. (2020). 21st century guidebook to fungi. *Cambridge University Press.*

Nene, Y. L. (1982) A review of Ascochyta blight of chickpea . *Tropical Pest Management* *2*(8**),** 61-70.

Newark, M., Paret, M. L., Dufault, N. S., and Freeman, J. H. (2014). Management of gummy stem blight (black rot) on cucurbits in Florida. *UF/IFAS Extension, University of Florida, Gainesville*.

Nisa, T. U., Wani, A. H., Bhat, M. Y., Pala, S. A., and Mir, R. A. (2011). In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of Fusarium oxysporum. *Journal of Biopesticides*, *4*(1), 53.

Onfroy C, Tivoli B, Corbière R and Bouznad Z (1999) Cultural, molecular and pathogenic variability of *Mycospherella pinodes* and *Phoma medicaginis* var. pinodella isolates from dried pea in France. *Plant Pathology*, *4*(8) 218-29.

Osman, Z. A., Elsanousi, S. M., and Elsheikh, E. A. E. (2012). Plant materials as probable growth promoters for certain fungi. *Euro Journal of Experimental Biology, 2,* 1785-1791.

Pande, S., Siddique, K. H. M., Kishore, G. K., Bayaa, B., Gaur, P. M., Gowda, C. L. L., et al. (2005). Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research*, 5(6), 317–332.

Patel, R. J., and Bhaskaran, L. (2020). Orange peel as an inducer for Laccase production in a novel fungal strain peyronellaea pinodella BL-3/4 and optimization of its cultural parameters by single parameter approach. *Indian Journal of Science and Technology, 13*(16), 1656-1667.

Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., and Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and Applied Biochemistry, 8*(3), 560-578.

Payne, C. M., Knott, B. C., Mayes, H. B., Hansson, H., Himmel, M. E., Sandgren, M., ... and Beckham, G. T. (2015). Fungal cellulases. *Chemical reviews*, *115*(3), 1308-1448.

Porta-Puglia A, Crino P and Mosconi C (1996) Variability in virulence to chickpea of an Italian population of *Ascochyta rabiei. Plant Diseases, 8*(1), 39-41.

Philippe, S., Souaïbou, F., and Guy, A. (2012) Chemical Composition and Antifungal activity of essential oil of fresh leaves of *Ocimum gratissimum* from Benin against six mycotoxigenic fungi isolated from traditional cheese *wagashi*. *Research Journal of Biological Sciences,* 4(1), 22–27.

Pérez, J., Martínez, B., Rivas, E., and Díaz, M. E. (2000). pH Effect on conidial germination, growth and sporulation of Didymella bryoniae. *Revista de Protección Vegetal*, *15*(3), 185-187.

Rehman, A., Mehboob, S., Islam, W., and Khan, N. A. (2013). Reaction of gram (*Cicer arietinum* L.) varieties against gram blight disease (*Didymella rabiei* (Kovatsch.) Arx) and its management through foliar fungicides in rain fed areas of Pakistan. *Pakistan Journal of Phytopathology*, *25*(1), 07-14.

Gurung, K., Dasila, K., Pandey, A., and Bag, N. (2020). *Curvularia eragrostidis*, a new threat to large cardamom (*Amomum subulatum* Roxb.) causing leaf blight in Sikkim. *Journal of Biosciences*, *45*(1), 1-8.

Gayosso-Canales, M., Rodríguez-Vázquez, R., Esparza-García, F. J., and Bermúdez-Cruz, R. M. (2012). PCBs stimulate laccase production and activity in Pleurotus ostreatus thus promoting their removal. *Folia microbiologica*, *57*(2), 149-158.

Hanounik S.B., (1980). Effect of chemical treatments and host genotypes on disease severity /yield relationships of Ascochyta blight in faba beans. *FABIS Newslett*

Heath, M. C., and Wood, R. K. S. (1971). Role of cell-wall-degrading enzymes in the development of leaf spots caused by Ascochyta pisi and Mycosphaerella pinodes. *Annals of Botany*, *35*(2), 451-474.

Range, H. Management of Gummy Stem Blight (Black Rot) on Cucurbits in Florida. *American Phytological Society 4(*1), 458-472.

Roger, C., Tivoli, B., and Huber, L. (1999). Effects of interrupted wet periods and different temperatures on the development of *Ascochyta blight* caused by *Mycosphaerella pinodes* on pea (*Pisum sativum*) seedlings. *Plant Pathology 4*(8), 10–18.

Samia, G., Noureddine, K., Mebrouk, K., and Eddine, H. J. (2013). Carbon sources and ph effect on pectinolytic activity production by *Ascochyta rabiei* isolated from chickpea (*Cicer arietinum* L.) in West Algeria. *African Journal of Microbiology Research*, *7*(27), 3483-3488.

Sarkar, J., Chakraborty, U., and Chakraborty, B. N. (2018). Induced defense response in wheat plants against *Bipolaris sorokiniana* following application of *Bacillus safensis* and *Ochrobactrum pseudogrignonense*. *Indian PHytopathology*, *71*(1), 49-58.

Sambrook, J., Russell, D.W. (2001). Molecular Cloning: A Laboratory Manual, thirdrded., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 518–525.

Santos, P. R. R. D., Leão, E. U., Aguiar, R. W. D. S., Melo, M. P. D., and Santos, G. R. D. (2018). Morphological and molecular characterization of *Curvularia lunata* pathogenic to Andropogon grass. *Bragantia*, *77*, 326-332.

Shternshis, M., Shpatova, T., and Belyaev, A. (2016). Effect of two biological formulations based on Bacillus subtilis and Pseudomonas fluorescens on control of Didymella applanata, the causal agent of red raspberry cane spur blight. *International Journal of Agronomy*,

Singh R S (2009) Plant diseases (9th ed). *Oxford and I B H Publishing Company Pvt. Ltd. New Delhi*, *India.* 205

Sivanesan, A. (1987). *Graminicolous* species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. *CAB International*.

Sinha, P., Rizvi, G., and Singh, D. (2017). Correlation between physicochemical characters and rhizospheric mycoflora from soil of pigeon pea field in jalaun district. *Journal of Animal and Plant Sciences*, 27(4), 1256-1263.

Somerville, C. (2006). Cellulose synthesis in higher plants. *The Annual Review of Cell and Developmental Biology*, *22*(3), 53-78.

Sutton, B. C. (1977). Coelomycetes. VI. Nomenclature of generic names proposed for Coelomycetes. *Mycological papers, Commonwealth Mycological Institute*, *14*(1) 197-217.

Stchigel, A.M., and Sutton, D.A. (2013). Coelomycete fungi in the clinical lab. *Current Fungal Infection Repication*, *7*, 171–191.

Teather, R. M., and Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied and environmental microbiology*, *43*(4), 777-780..

Riesen T. K. (1987) Didymella phleina Punith. and Årsvoll endophytic in barley leaves in New Zealand. *New Zealand Journal of Botany*, *25*(2), 271-274

Terbeche, R., Karkachi, N. E., Gharbi, S., Kihal, M., and Henni, J. E. (2015). Isolation and physicochemical test studies of *Ascochyta pisi*. *International Journal of Biosciences*, *6*(1), 9-19.

Tivoli, B., and Banniza, S. (2007). Comparison of the epidemiology of *Ascochyta blights* on grain legumes. *European Journal of Plant Pathology 11*(9), 59–76.

Trapero-Casas, A., and Kaiser, W. J. (1992). Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathology*, *82*(11), 1261-1266.

Trapero-Casas, A., and Kaiser, W. J. (2007). Differences between ascospores and conidia of Didymella rabiei in spore germination and infection of chickpea. *Phytopathology*, *97*(12), 1600-1607.

Virtuoso, M. C. S., Silva, E. H. C., Silva, E. M., Valente, T. S., Vargas, P. F., Braz, L. T., and Panizzi, R. C. (2021). Providing inoculum for *Didymella bryoniae* studies: the effect of light spectrum and storing at low temperature. *Brazilian Journal of Biology*, *8*(4), 416-428.

Tsuda, M., and Ueyama, A. (1985). Two new Pseudocochliobolus and a new species of Curvularia. *Transactions of the Mycological Society of Japan*, *26*(3), 321-330.

Wang, H., Hwang, S. F., Chang, K. F., Turnbull, G. D., and Howard, R. J. (2003). Suppression of important pea diseases by bacterial antagonists. *BioControl*, *48*(4), 447-460.

Wang, J., Wang, X., Yuan, B., and Qiang, S. (2013). Differential gene expression for *Curvularia eragrostidis* pathogenic incidence in Crabgrass (*Digitaria sanguinalis*) revealed by cDNA-AFLP analysis. *PLoS One*, *8*(10), e75430.

Wahleithner, J. A., Xu, F., Brown, K. M., Brown, S. H., Golightly, E. J., Halkier, T., and Schneider, P. (1996). The identification and characterization of four laccases from the plant pathogenic fungusRhizoctonia solani. *Current genetics*, *29*(4), 395-403.

Yahia, E. M., and Carrillo-Lopez, A. (Eds.). (2018). Postharvest physiology and biochemistry of fruits and vegetables. *Woodhead publishing.*

Zhu Y, Qiang S (2003) The biological characteistics of strain QZ-2000 of *curvularia* sp. for controlling *Digitaria sanguinalis*. *Acta Phytopathologica Sinica*, *3*(3), 524–529.

Zhao, J., Xue, Q. H., Shen, G. H., Xue, L., Duan, J. L., and Wang, D. S. (2012). Evaluation of Streptomyces spp. for biocontrol of gummy stem blight (*Didymella bryoniae*) and growth promotion of *Cucumis melo* L. *Biocontrol Science and Technology*, *22*(1), 23-37.

Zhang, J. X., Bruton, B. D., Miller, M. E., and Isakeit, T. (1999). Relationship of developmental stage of cantaloupe fruit to black rot susceptibility and enzyme production by *Didymella bryoniae*. *Plant Disease*, *83*(11), 1025-1032.

Zhang, J., Bruton, B. D., and Biles, C. L. (2014). Cell wall-degrading enzymes of *Didymella bryoniae* in relation to fungal growth and virulence in cantaloupe fruit. *European journal of plant pathology*, *139*(4), 749–761.

https://cropprotectionnetwork.org/encyclopedia/curvularia-leaf-spot-of-corn#:~:text=Curvularia%20leaf%20spot%20is%20caused,surrounded%20by%20a%20yellowish%20halo.

https://plantpathology.ca.uky.edu/files/ppfs-ag-c-09.pdf

https://www2.ipm.ucanr.edu/agriculture/turfgrass/curvularia-blight/

https://www.jaffer.com/jaspl/product/19/difenoconazole/#:~:text=Mode%20of%20Action%3A,of%20sterols%20in%20cell%20membranes.

https://www.corteva.in/products-and-solutions/crop-protection/curzate-m8.html#anchor_4

https://www.farmersstop.com/products/folicur-tebuconazole-250-ec-25-9-w-w-bayer-india#:~:text=MODE%20OF%20ACTION,further%20growth%20of%20the%20fungus

https://www.biostadt.com/crop-protection/sparsh.aspx