Studies on fungal leaf blight of ridge gourd (*Luffa acutangula* (L.) Roxb.) in Kashmir valley

ZAHOOR AHMED BHAT
(2003-112-D)

DIVISION OF PLANT PATHOLOGY
FACULTY OF POSTGRADUATE STUDIES
SHER-E-KASHMIR UNIVERSITY OF AGRICULTURAL SCIENCES & TECHNOLOGY OF KASHMIR

2007

Studies on fungal leaf blight of ridge gourd (*Luffa acutangula* (L.) Roxb.) in Kashmir valley

ZAHOOR AHMED BHAT
(2003-112-D)

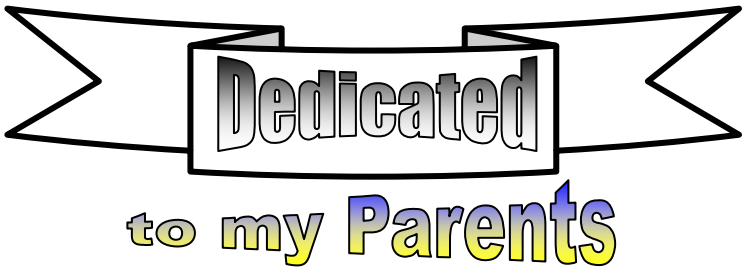
THESIS

Submitted to

The Faculty of Postgraduate Studies
Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir in partial fulfilment of requirement for the award of the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(Plant Pathology)

2007


Dedicated
to my Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology
Shalimar Campus Sriangar – 191 121
-::o::-

Certificate – I

This is to certify that the thesis entitled, **"Studies on fungal leaf blight of ridge gourd (*Luffa acutangula* (L.) Roxb.) in Kashmir valley"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)**, to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Zahoor Ahmad Bhat (Regd. No. 2003-112-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

(Dr. G.M. Dar)
Chairman
Advisory Committee

Endorsed

Prof. & Head,
Division of Plant Pathology

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology
Shalimar Campus Sriangar – 191 121
-::o::-

Certificate – II

We, the members of the Advisory Committee of **Zahoor Ahmad Bhat (Regd. No. 2003-112-D)**, a candidate for the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)** have gone through the manuscript of the thesis entitled, **"Studies on fungal leaf blight of ridge gourd [*Luffa acutangula* (L.) Roxb.] in Kashmir valley"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

Advisory Committee

Chairman

Dr. G.M. Dar,
Professor
Division of Plant Pathology

Members

Dr. G.H. Dar,
Professor
Division of Plant Pathology

Dr. Vinay Sagar,
Assistant Professor
Division of Plant Pathology

Dr. Gulam-u- Din,
Associate Professor,
Division of Olericulture

Dean PG Nominee

Dr. G.M. Beigh
Prof. & Head
Division of PHT

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Shalimar Campus Sriangar – 191 121
-::o::-

Certificate – III

This is to certify that the thesis entitled, **"Studies on fungal leaf blight of ridge gourd [*Luffa acutangula* (L.) Roxb.] in Kashmir valley"** submitted by **Mr.Zahoor Ahmad Bhat (Regd. No. 2003-112-D)** to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)** was examined and approved by the Advisory Committee and External Examiner on …….

Chairman
Advisory Committee

External Examiner

Prof. & Head,
Division of Plant Pathology

Director Resident Instruction-cum-Dean
Postgraduate Studies, SKUAST-K

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Shalimar – 191 121

-::o::-

Name of the student	:	Zahoor Ahmed Bhat
Registration No.	:	2003-112-D
Major subject	:	Plant Pathology
Minor subject	:	Olericulture/ Entomology
Major advisor	:	Dr. G.M. Dar, Professor Division of Plant Pathology, SKUAST-K, Shalimar
Title of the Thesis	:	**Studies on fungal leaf blight of ridge gourd [*Luffa acutangula* (L.) Roxb.] in Kashmir valley**

ABSTRACT

An extensive survey conducted during 2004 and 2005 revealed that Didymella blight was prevalent in all ridge gourd (*Luffa acutangula*) growing areas of Kashmir valley with varied levels of incidence and intensity. The incidence and intensity on leaves ranged from 22.0 to 64.0 and 7.7 to 34.8 per cent, respectively. The incidence on fruits was 6.8 to 18.8 per cent. The fungus inciting the disease was identified as *Didymella bryoniae* (Aureswarld) Rehm (Anamorph *Phoma cucurbitacearum* (Foutrey) Sacardo) on the basis of its morphological and pathological characteristics. Disease infection on leaves and fruits resulted in leaf blight and fruit rot, respectively. Disease initially appeared on lower leaves as small circular, light brown spots with chlorotic halo ranging in size from 0.5 to 1.0 mm. With the passage of time spots enlarged (up to 83 mm size) , developed concentric rings at the centre and turned dark brown with greyish white centre. The spots then coalesced, causing yellowing and blighting of the entire leaf. Symptoms on fruit appeared as dark green, irregularly circular water-soaked spots which enlarged rapidly and caused black rot. Black fructifications *i.e*, pycnidia and pseudothecia of the causal pathogen, were observed on infected tissue of both leaf and fruit which imparted black colour to the latter. The disease in the field appeared in June and reached its maximum by the end of September.

Maximum apparent infection rate of 0.1858 and 0.1826 unit/day in the field were observed during fifth week of July in 2004 and during second week of July in 2005, respectively. Weather factors *viz*., temperature, RH and rainfall were found positively correlated with the disease development in terms of infection rate (unit/day) with 69 per cent contribution by rainfall alone. Single spored cultures from ascospore and pycnidiospore on potato dextrose agar (PDA) produced pycnidia after 10 to 12 days of incubation at 24 ± 1^{0}C under 12/12 hour alternate cycles of light and darkness, however, pseudothecia were altogether absent. The fungus required incubation period of 4 days to cause infection in the inoculated host plants, whereas, on detached leaf and fruit it took 2 to 3 days. The fungus was found pathogenic to all the eight cucurbit plant species tested *viz*., *Cucumis sativus* (cucumber), *Lagenaria siceraria* (bottle gourd), *Cucumis melo* (muskmelon), *Citrullus lanatus* (watermelon), *Mamordicha charantia* (bitter gourd), *Cucurbita pepo* (squash), *Luffa cylindrica* (sponge gourd) and *Cucurbita pepo* (pumpkin) besides its natural host *Luffa acutangula* (ridge gourd) both under natural and artificial inoculation conditions. The best growth and fructification of the fungus was observed on potato dextrose agar and corn meal agar media with pH 7.0 when incubated at $24 \pm 1\ ^{0}$C. Spore germination was also best at this temperature and pH level. The pathogen perpetuated in the form of spores on seeds and plant debris stored indoors under ambient laboratory conditions as these produced viable spores through out the observation period of twelve months. It also perpetuated as a dormant mycelium on plant debris left on the soil surface in open, however, pathogen was unable to survive on plant debris buried 7.5 cm deep in soil. Carbendazim and chlorothalonil from systemic and non-systemic fungitoxicants, respectively, as well as *Allium sativum* from botanicals tested under *in vitro* conditions exhibited maximum inhibition in mycelial growth and spore germination of the test fungus. The treatments found most promising under *in vitro* studies, when tested as seed treatment and foliar spray, treatment combination carbendazim seed treatment together with foliar sprays of carbendazim proved superior in controlling the disease and also increasing the fruit yield.

KEY WORDS : *Didymella bryoniae*, Didymella blight, Perpetuation, Management, Ridge gourd

Signature of Student
Dated …….

Signature of Major Advisor
Dated……..

Acknowledgement

The words at my command are not adequate, either in form or spirit, to express the depth of my humility and humbleness before Almighty Allah without whose endless benevolence and blessings this tedious task could not have been accomplished.

With great reverence, I express my overwhelming gratitude to the chairman of my advisory committee Dr. G.M. Dar, Professor, Division of Plant Pathology, for excellent guidance, steadfast inspirations, perceptive enthusiasm which enabled me to accomplish the hard task of completing the present study. Inspite of his busy schedules he was always ready to provide regular encouragement, timely advice whenever required for enriching me with productive scientific discussions and above all for being at my support during the most trying times in this tenure of research work and thesis writing.

I extend my heartiest thanks to the members of my advisory committee, Dr. G. M. Dar, Professor, Division of Plant Pathology, Dr. Vinay Sagar, Assistant Professor, Division of Plant Pathology, Dr. Gulam-u-din, Associate Professor, Division of Olericulture, Dr. G. M. Beigh, Professor & Head Division of PHT for their elicit efficacy, warm and sustained guidance from time to time during the course of investigation and writing of this manuscript.

Indeed the words at my command are inadequate to express my heartfelt thanks to Dr. Mushtaq Ahmad, Professor and Head, Division of Plant Pathology, SKUAST-K for his valuable suggestions and constant encouragement from initiation upto the completion of this research project.

I am highly thankful to Dr. Shahzad Ahmad, Dr. N. A. Munshi, Dr. Qazi Nissar Ahmad, Dr. Anwar Ali, Mr. Hakim Khursheed, Mr. Tariq Ahmad, Dr. Nazir Ahmad, Dr. M. Y. Ghani, Mr. Nisar Ahmad Khan, Dr. M. A. Beigh, Dr. Abdul Gani Najar, Dr. M. Azam Wani and other faculty members for their guidance, encouragement and sympathetic attitude during the course of my study and investigation.

I also extend my thanks to non-teaching staff members, laboratory and field staff of Division of Plant Pathology in general and Mr. Habibullah, Mr. Ghulam Mohammad and Miss Feroza in particular.

I feel pleasure in extending my grateful thanks to the staff of Central Library, SKUAST-K, Shalimar especially Mr. Bashir Ahmad (Chief Librarian),

Mr. Jan Mohammad and Mr. Manzoor Ahmad for their help in collection of literature.

I extend my heartiest thanks to my friends for their appreciation and nice company and cooperation, Mr. Mushtaq Ahmad, Mr. Muneer Ahmad, Mr. Mohammad Ashraf, Mr. Zaffar Afroz, Mr. Zahoor Ahmad, Mr. Nissar Ahmad Mr. Mehraj-u-din, Mr. Najeeb ahmad Mr. Ansar-ul-Haq, Mr. Mohd. Shafi Bhat, Ms. Sabiya Bashir, Ms. Vaseema.

Above all I wish to pay my special appreciation and regards to my parents and brothers (Bilal Ahmad, Nissar Ahmad, Baseer Ahmad, Altaf and Musharaf) and acknowledge most respectfully the love, tolerance, encouragement that has been rendered by them during the study period and this proved of immense value.

Last but not least I wish to record my thanks with gratitude to my well-wishers who rendered their helping hand knowingly or unknowingly.

Zahoor Ahmed Bhat

Place : Shalimar, Srinagar

Dated: .....

CONTENTS

List of Tables

List of Figures

List of Plates

Chapter – 1

INTRODUCTION

Ridge gourd [*Luffa acutangula* (L.) Roxb.], also known as angled luffa, is an important cucurbit crop which originated in sub-tropical Asian region including India (More, 2001). From nutritional point of view, ridge gourd has low calorific value but is rich in vitamin A and C, and iron than that of most other cucurbit crops (Seshadri, 1993). The edible fresh fruit of ridge gourd per 100 g contains 0.5g protein, 3.7 g carbohydrate, 0.04 g calcium, 0.04 g phosphorus, 1.6 mg iron, 37 mg vitamin A and 18 mg vitamin C (More, 2001).

Ridge gourd although is a vegetable crop, has attained greater significance due to its industrial and medicinal use. From its dried fruits, a derived product "Loofah" is used in scrubber pads, doormats, pillows, mattresses and as filter in steam engines (More, 2001). Leaf juice of ridge gourd is used in ayurvedic medicine for treating a range of ailments including sores, inflamed spleen, ringworms, piles, leprosy, bites of insects and snakes and as a substitute for ipecacuanaha in dysentery (Kapoor, 2001). The oil extracted from ridge gourd seeds is effective against skin diseases while, the roots of the plant are used in dropsy (Aswal *et al*., 1984).

In India, ridge gourd is cultivated over an area of 10,040 hectares with an annual production of 1, 28,310 metric tones (Sidhu, 2000). The crop has recently been introduced to Kashmir and is now cultivated on a

limited scale as a main season crop in many major vegetable growing areas of the valley. However, the information on area and production of ridge gourd in Jammu and Kashmir is not documented.

Cucurbits are subjected to many fungal, bacterial and viral diseases viz., downy mildew [*Pseudoperonospora cubensis* (Berkley and Curtis) Rostowzew]; powdery mildew [*Erysiphe cichoracearum* Decandolla/*Sphaerotheca fuliginea* (Schlechtendal) Pollaci]; anthracnose [*Colletotrichum lagenarium* (Pass) Ellis and Halsted]; Didymella blight [*Didymella bryoniae* (Auersw.) Rehm]; Fusarium wilt [*Fusarium oxysporum* f. sp. *melonis* (Leach and Currence) Synder and Hansen]; angular leaf spot [*Pseudomonas syringae* pv. *lachrymans* (Smith and Bryan) Young and Wikie]; bacterial soft rot (*Erwinia carotovora*); mosaic disease etc. which affect their yield potential and considerably limit their profitable production (Singh, 1987). Among these Didymella blight incited by *D. bryoniae* (anamorph: *Phoma cucurbitacearum* (Foutry) Sacardo), is a highly destructive disease of all the cucurbitaceous crops worldwide, reducing their yield as a result of defoliation of vines, fruit infection and subsequent decay (Schenck, 1968). The disease was first reported from France in 1891 on a Chinease variety of cucumber (Roumeguere, 1891) and since then it has been reported from many countries namely, USA, Britain, Germany, Japan, New Zealand, Brunei, Mexico, Netherlands, Republic of Ireland, Salvadar (Puninthalingam and Holliday, 1972). In India, the disease was first reported from Mysore on leaves of *Scheum edule* (Sohi and Prakash, 1972) and then subsequently on *Cucumis sativus* (Kumar and khan, 1984), *Mamordicha charanta* (Kulwant and Shetty, 1996), *Benincasa hispida* (Pandey and Pandey, 2003) and C*ucumis melo* (Sudisha *et al*., 2004) Yield losses due to this disease on watermelon have been estimated

up 100 per cent in Trindad (Bala and Hosein, 1986), 43 per cent in USA (Kienath and Duthie, 1998), 30 per cent on muskmelon in Australia (Mc Grath *et al*., 1993) and 35 per cent on cucumber in Polland (Leski, 1984).

Didymella blight is characterized by the development of symptoms on all aerial parts. Characteristic symptoms appear on the leaf as small circular, light brown spots with chlorotic halo, developing irregular concentric zonations at centre and enlarge rapidly until the entire leaf is blighted (Koike, 1997; Pandey and Pandey, 2003). On stem dark spots are produced near the crown with sunken streaks or cracks, accompanied by gummy ooze (Bala and Hosein, 1986). Symptoms on fruits begin as dark green, water soaked spots, which enlarge to indefinite size and exude gummy material (Scheneck, 1962). Black fructifications (pycnidia and pseudothecia) of the causal fungus develop on the infected tissue of fruit, stem and leaf (Sudisha *et al*., 2004).

The disease has been noticed on ridge gourd in Kashmir regularly for the last few years inflicting heavy economic losses thereby warrants immediate action of plant pathologists. The practice of monoculture and prevailing disease favorable environmental conditions seems to have given fillip to the spread and development of this disease in the valley. Various aspects of this disease on cucurbitaceous crops other than ridge gourd have been studied in India or elsewhere in the world. Therefore, it was considered imperative to study various aspects of the disease in ridge gourd crop under climatic conditions of Kashmir with the following objectives:

Objectives

- To know the status of the disease in valley
- To study etiology of the disease and host range of the pathogen

- To establish role of meteorological factors on disease development
- To devise the chemical management of the disease.

CHAPTER - 2

REVIEW OF LITERATURE

The perusal of literature revealed that no work has been done on Didymella blight of ridge gourd incited by *Didymella bryoniae* in India or elsewhere in the world. Therefore, relevant literature available on the related aspects of allied hosts has been reviewed as under:

2.1 Distribution and Status of Disease

The Didymella blight is widely distributed in tropical and sub-tropical countries and also reported frequently in areas having temperate climate (Sitterly, 1969). The disease affects all the cucurbitaceous crops and reduces their yield as a result of defoliation of vines, fruit infection and subsequent decay (Schenck, 1968). The earliest evidence of the occurrence of this disease dates back to 1891 from France on a Chinese variety of cucumber (Roumeguere, 1891). Since then it has been reported from U.S.A, Britain, Germany, Japan, New Zealand Brunei, Mexico, Netherlands, Pitcairn Islands, Republic of Ireland and Salvador (Puninthalingam and Holliday, 1972). In India the disease was first reported from Mysore on the leaves of *Scheum edule* (Sohi and Prakash, 1972) and then subsequently on *Cucumis sativus* (Kumar and Khan, 1984), *Mamordicha charanta* (Kulwant and Shetty, 1996), *Benincasa hispida* (Pandey and Pandey, 2003) and *Cucumis melo* (Sudisha *et al*., 2004). Didymella blight caused by *D. bryoniae* is the most destructive disease on watermelon in the southern United States (Everts, 1999), where losses up to 43 per cent of the yield have been reported (Kienath and Duthie, 1998). Miller *et al.* (1999)

reported fruit losses up to 68.4 per cent in cantaloupe crop, amounting to 15 million U.S. dollar for South Texas producers. It is the most significant disease affecting muskmelon in all areas of extensive muskmelon production in Australia, causing annual production losses up to 30 per cent of early season muskmelons (Mc Grath *et al*., 1993). Bala and Farzan (1985) reported 30-100 per cent losses from Trindad due to this disease in muskmelon, whereas, Bala and Hosein (1986) estimated 10-100 per cent losses in watermelon and 16-100 per cent in pumpkin yield. Yield losses of about 10 per cent on cucumber in Holland (Van Steekeleburg, 1985) and 35 per cent in Poland (Leski, 1984) have been reported. In India the disease is serious on bitter gourd, causing severe fruit loss and drastically reduces the seed setting (Kulwant and Shetty, 1996). The disease generally causes severe losses during the month of October and November on seedlings of muskmelon (Sudisha *et al*., 2004).

2.2 Casual Organism

The Didymella blight pathogen of ridge gourd was first described by Fautrey and Roumeguere on a Chinese variety of cucumber in France as *Ascochyta Cucumis* (Roumeguere, 1891). Later in the same year Passerini described the fungus as *Didymella melonis* on *Cucumis melo* L. in northern Italy without knowledge of its association with *Ascochyta Cucumis* (Saccardo, 1891). Chester (1891) described the same fungus on *Citrullus vulgaris* Schard. in Delaware under the name *Phyllostica citrullina* on account of its aseptate spores without recognizing the perfect stage. Initially Smith (1905) named the causal fungus *Ascochyta citrullina* but later on after discovery of its perfect stage, he described it as *Sphaerella citrullina*. Grossenbacher (1909) designated the imperfect stage of the fungus a member of the genus *Diplodina* and referred the perfect stage to the genus

Mycosphaerella. Potebina (1910) reported a fungus on *Cucumis meio* L. under the name *Ascochyta melonis* which was considered to be closely associated with *Didymella melonis* Pass. Further, he was of the opinion that *A. citrullina* (Chester) Smith on watermelon was a form intermediate between *A. Cucumis* Fautr. and Roum. and *A. melonis* Poteb. As a result of further studies on Fautrey's collection, Keissler (1923) considered the three names to be synonymous and recognized *Ascochyta c*ucumis Faut. and Roum. as the first valid name for the imperfect stage. Ferraris and Massa (1912) published a species on *Cucumis melo* L. in Italy under the name of *Sphaerella melonis* and claimed that the asci were non-paraphysate. The descriptions of Feraris's *Sphaerella melonis* showed that it is the same fungus as Passerni's *Didymella melonis*. Chiu and Walker (1949a) considerd the teleomorph as a species of *Mycosphaerella* and in view of the priority of *Ascochyta cucumis* Faut and Roum, named the perfect stage of the fungus as *Mycospharella Cucumis* (Faut and Roum) Chiu and Walker. Muller and Von Arx (1962), however, considered *Didymella melonis* synonymous with *Sphaerella bryoniae*, described by Auerswald, therefore because specific epithet bryoniae had priority over melonis as it was described earlier, they considered *Didymella bryoniae* (Auersw.) Rehm the correct name for the fungus.

Chiu and Walker (1949a) studied the anamorph *Phoma cucurbitacerum* on various cucurbits and reported dark, globose to irregular, 120-180 µm in dia pycnidia on the leaf, stem and fruit lesions, containing hyaline, cylindrical with rounded ends, non or mono septate and 6.0-13.0 x 2.5-5.0 µm in size pycnidiospores. Pandey and Pandey (2003) also gave more or less similar type of description of the anamorph. Grossenbacher (1909) studied the pseudothecia from diseased muskmelon

and recorded their diameter as ranging from 100 µm to 165 µm. Later Wiant (1945) observed the pseudothecia from several cucurbit species and found them to vary in diameter from 71.0 µm to 224.0 µm. The dark brown to black colour and the globose shape of pseudothecia have been recorded in all descriptions since Grossenbacher (Grossenbacher, 1909; Wiant, 1945; Chiu and Walker, 1949a; Puninthalingam and Holliday, 1972; Pandey and Pandey, 2003). Puninthalingam and Holliday (1972) and Corlett *et al*. (1986) described the asci as cylindrical to subclavate, short stipitate or sessile, 60-90 x 10-15 µm, filled with 13-18 x 4-7 µm , hyaline, mono septate, constricted at septum and rounded at the ends with upper cell usually wider than the lower one, ascospores. Chiu and Walker (1949a) found pseudoparaphysis like structures always associated with the pseudothecium but they considered them as immature or abortive asci and not as true pseudoparaphysis. However, presence of pseudoparaphyses was confirmed by many workers (Puninthalingam and Holliday, 1972; Skarshaug, 1981).

Keinath *et al*. (1995) observed the colonies of *D. bryoniae* with olive to dark green or black substrate mycelium, white hairy aerial mycelium and abundant pycnidial and pseudothecial formation. Similar colony characters were observed by Sudisha *et al*. (2004) but he did not observe pseudothecial formation. Pandey and Pandey (2003) observed white to gray colonies which gradually became dark gray and finally black and reported no fructification of the fungus. Pycnidia in culture were described by Corlett *et al*. (1986) as globose to irregular, 80-380 µm in dia, filled with subglobose to ellipsoidal, usually aseptate, 4-8 x 2-3 µm pycnidiospores. Skarshaug (1981) observed erumpent to submerged pseudothecia in culture

which ranged in size from 375- 705 µm with 4.5 x 12.2 µm hyaline ascospores.

2.3 Symptomatology

The manifestation of *D. bryoniae* infection on cucurbits has been described by a number of researchers. The characteristic symptoms may be observed on leaves, stems and fruits which may vary considerably based on the specific cucurbit infected (Maynard and Hopkins, 1999). Symptoms on leaf were described by Koike (1997) and Pandey and Pandey (2003) as circular, dark brown with chlorotic halo spots which enlarge rapidly until the entire leaf becomes blighted. They also observed the black fructifications of the causal fungus arranged in circles at the centre of the spots. According to Fletcher and Preece (1966) symptoms on leaves appeared as water-soaked spots surrounded by a yellow halo which gradually enlarged, became light brow and papery in the centre. Bala and Hosein (1986) besides leaf, found symptoms on stem as dark spots near the crown with sunken streaks or cracks, accompanied by gummy ooze. Earlier, Chiu and Walker (1949b) described the symptoms on fruits as water-soaked spots which enlarge to indefinite size and exude gummy material. Schenck (1962) observed that the lesions on watermelon fruits begin as a small circular, dark green, water-soaked spots which enlarge and develop various shades of brown colour at its centre with a 1 mm water-soaked peripheral band. Whereas, Van Steekelenburg (1981) described the symptoms on fruit as yellow to light brown, somewhat irregularly circular spots which occurred allover the fruit and turned black due to the abundant pycnidial and pseudothecial formation of the causal fungus. According to Kulwant

and Shetty (1996) the disease on fruits started with small, circular water-soaked lesions on fruits which enlarged rapidly and turned into blackish spot due to black fructifications of the causal fungus.

2.4 Pathogenicity

Chiu and Walker (1949b) inoculated watermelon seedlings with pycnidiospore suspension of *D. bryoniae* and observed circular or irregularly shaped spots which were greyish green at first and changed eventually to dark brown followed by blighting of the whole leaf. They further repeated the inoculation of watermelon seedlings with separate suspensions of ascospores and pycnidiospores of *D. bryoniae* obtained from a profusely sporulating culture secured from mono-ascosporic isolation and noticed that both types of spores produced similar symptoms on leaves. Olsen and Slanghellini (1981) while proving the pathogenicity of *D. bryoniae* observed development of water-soaked lesions on leaves of cucumber, 3 days after inoculation when plants were kept in the moist chamber whileas, pycnidia and pseudothecia development was observed, 5 and 10 days after inoculation, respectively. Bala and Hosein (1986) proved the pathogenicity of *D. bryoniae* on watermelon by inoculating spore suspension ($1x10^7$ ml^{-1}) on punctured leaves of potted plants and considered the fungus as highly pathogenic to watermelon as they obtained 100 per cent infection of punctures after 48 hours of incubation in polythene tents at 26-28^oC and higher relative humidity. Pandey and Pandey (2003) reported that inoculation of wax gourd (*Benincasa hispida*) with a mycelial concentration of 542 cfu ml^{-1} by leaf dip technique resulted in symptom development only 8 days after inoculation, whereas it took 20 days when

foliar spray inoculation method was adopted. Sudisha *et al*. (2004) inoculated 30 day old muskmelon plants with a spore suspension (12 x 10^5 spores ml^{-1}) of *D. bryoniae* and observed symptoms on stem and leaves 7 days after incubation at 23^oC temperature with 90 per cent relative humidity and 12 hour photoperiod. Sudisha *et al*. (2006) found papery lesions and pycnidial development on leaves of muskmelon seedlings seven days after inoculation with varied concentrations of spore suspension and found positive correlation with the spore concentration and severity of infection.

2.5 Host Range

Puninthalingam and Holliday (1972) reported no physiological specialization of *D. bryoniae* for any host within cucurbitaceous crops. However, Lee *et al*. (1984) noted that isolates of pumpkin and cucumber although were pathogenic to cucumber, oriental melon, pumpkin and watermelon but produced more severe symptoms on some of the hosts than the others which suggested that physiological specialization may occur. Similarly, St Amand and Wehner (1995) reported that some degree of host preference may exist in isolates of *D. bryoniae* after finding the least virulence of a muskmelon isolate inoculated on cucumber. Bala and Hosein (1986), conducted a test to determine the pathogenicity of watermelon isolate of *D. bryoniae* on different cucurbit crops and observed that the isolate of the fungus from watermelon proved to be highly pathogenic on all the cucurbits tested. He recorded 90-100 per cent infection of the punctures, made before inoculation on cotyledons and leaves, in watermelon, muskmelon, pumpkin, cucumber, squash, christophine, bitter gourd and smooth gourd, whileas angled luffa and cucumber had 72.0 and 64.2 per cent infected punctures, respectively.

2.6 Physiology

Different media have been tested by various workers for their selectivity and efficacy to support the growth and fructification of *D. bryoniae*. Wiant (1945) observed best growth of *D. bryoniae* on oat meal agar. However, Chiu and Walker (1949b) found corn meal agar best medium for growth and reported excellent fructification on oat paste peptone dextrose agar medium. Curren (1969) found best fructification on potato dextrose agar and peptone dextrose salt medium by constant or intermediate illumination and least fructification in darkness.

The growth and spore germination of *D. bryoniae* is known to be governed by temperature and pH. Optimum temperature for the growth of *D. bryoniae* on solid media was found 24 to 30^{o}C, while no growth was observed at 4^{o}C and only slight growth recorded at 7^{o}C (Wiant, 1945; Luepschen, 1961). Contrarily, Chiu and Walker (1949b) reported optimum growth at 20^{o}C to 24^{o}C. Chen *et al*. (1993) stated that the optimum temperature for mycelial growth and spore germination was 27^{o}C and 25-30^{o}C, respectively, and optimum pH was 7.0.

2.7 Weather Factors

According to Chiu and Walker (1949b), the *Didymella bryoniae* is influenced greatly by environmental factors and found 20^{o}C as optimum temperature for infection in muskmelon and 24-25^{o}C in watermelon and cucumber. They further noted that moisture was more important for disease development than temperature and recorded peak ascospore dispersal after rains and during dew periods at night. Luepschen (1961) recorded no fruit rot of watermelon held at 4^{o}C, little decay at 7^{o}C and very severe rot at 24^{o}C after artificial inoculation of the fruit. Svedellus and. Unestam (1978)

found film of water necessary for the infection of leaves by conidia of *D. bryoniae* and observed expansion of the developed spots only under higher relative humidity conditions. Uspenskaya *et al.* (1979) studied the correlation between incidence of *D. bryoniae* and the glass house micro-climatic conditions and found positive and significant correlation between the disease incidence and relative humidity. Similarly, Van Steekeleburg (1983), besides moisture and a minimum temperature of 5-10^{o}C, found high relative humidity pre requisite for fructification and subsequent dispersal of ascospores. Later, Keinath *et al* (1995) reported free moisture on leaves for at least one hour essential for infection and further continuous leaf wetness as a requirement for lesion expansion.

2.8 Perpetuation of Pathogen

The perpetuation of a pathogen in a crop eco-system forms an important component for recurrence of any endemic or epidemic disease. Like other pathogens of economic importance, the survival of *D. bryoniae* in the crop-less off-season is likely to provide a potential source of primary inoculum for the disease to recur.

2.8.1 Perpetuation through seed

There are conflicting reports about the seed transmissibility of *D. bryoniae* in cucurbits. Brown and Preece (1968) reported invasion of cucurbit seeds on artificial inoculation, but did not find any trace of natural infection of seeds extracted from the infected fruits. Similarly, in artificially infected watermelon seeds, Rankin (1954) found the invasion of *D. bryoniae* in the epidermis and sclerenchyma layers and isolated the fungus from cotyledons and embryos. Lee *et al.* (1983) isolated the pathogen from seed coat, perisperm and tissue of the cotyledons from the seeds of

naturally infected fruits and confirmed both external and internal seed borne nature of the pathogen. Chen and Bao (1990) noted the survival of the fungus both in and on the seeds of infected fruits up to 21 months, whereas no pathogen was traced from the seeds of symptomless fruits. Sudisha *et al*. (2004) recorded 41 per cent natural transmission of disease from the infected seeds sown in the field. Sudisha *et al*. (2006) evaluated different components of infected seeds and indicated that the mean incidence of *D. bryoniae* pathogen was 31 per cent in seed coat, 11 per cent in cotyledon and 4 per cent in embryo.

2.8.2 Perpetuation through plant debris

Survival of *D. bryoniae* on crop debris during off season has been reported by many workers (Chiu and Walker 1949b; Schenck 1968; Uspenskaya *et al*., 1976; Van Steekelenburg, 1983; Keinath 2002). Schenck (1968) observed that the pathogen persisted through adverse weather as pseudothecia on the crop debris which produce inoculum for primary infection. However, Chiu and Walker (1949b) found empty pycnidia and pseudothecia in the overwintered crop debris by spring but could isolate the fungus readily, therefore, suggested that the fungus overwintered as dormant mycelium. Similarly, Van Steekelenburg (1983) also observed the survival *D. bryoniae* as dormant mycelium in the dry and undecomposed crop residues for more than one year. Uspenskaya *et al*. (1976) reported the longer survival of *D. bryoniae* in the sterilized soil than in the unsterilised soil and reported that the formation of dormant chlamydospores enabled the fungus to survive in soil. Kienath (2002) reported the survival of *D. bryoniae* only for 6 to 7 months on infected watermelon vines buried at 25 cm depth and attributed the short period survival of the fungus to

antagonistic activity of saprophytic soil microorganisms which seemed to have eliminated the pathogen resident in watermelon debris.

2.9 Management

2.9.1 Fungicides

For the management of Didymella blight of various cucurbit crops, several fungicides have been tested. Earlier Bordeaux mixture and copper lime either as seed treatment or foliar spray were used for the control of this disease (Webber, 1929; Walker and Webber, 1931; Wiant, 1945). Later on, Rankin (1954) tested the efficacy of different fungicides against the disease but found least number of infected watermelon seedlings by using mercury chloride as seed treatment. Hopkin (1973) compared several fungicides against Didymella blight of watermelon and found most effective the application of carbendazim, captafol, mancozeb and maneb. However, Sumner *et al*. (1981) and Kienath *et al*. (1995) found chlorothalonil the most effective. Van Steekelenburg (1981) reported lower disease index by the application of carbendazim, chlorothalonil and zineb in young cucumber plants. Kienath (2000), besides chlorothalonil, found the application of mancozeb effective in reducing the severity of the disease. Likewise, Sumner and Phatak (1987) observed approximately 50 per cent reduction in the disease severity by weekly sprays of chlorothalonil and mancozeb when sprayed alone or in combination with metalaxyl. Vawdrey (1994) evaluated ten fungicides against the Didymella blight of roackmelon and found tubuconazole followed by carbendazim, myclobutanil and chlorothalonil superior to all other fungicides in reducing the disease

severity and increasing the yield..

In *in vitro* studies on *D. bryoniae*, Chiu and Walker (1949b) tested toxicity of some fungicides on germination of pycnidiospores and found 98.6 per cent inhibition by $CuSO_4$ and 87.5 per cent by $HgCl_2$. Van Steekelenburg, 1978) reported complete inhibition of mycelial growth of *D. bryoniae* by carbendazim (100µg/ml). Later, Urbanszki *et al*. (2002) while evaluating the efficacy of different fungicides on *D. bryoniae* culture on solid nutrient medium, found fenarimol and penconazole most effective in inhibiting the growth.

2.9.2 Botanicals

Looking into the deleterious effects of synthetic pesticides on life supporting systems and other associated problems such as pest resistance and detrimental effects on non-target organisms, there is an urgent need to use alternative agents for pest control using the sources that are harmless. With the growing awareness of harmful effects of synthetic pesticides worldover, the use of plant extracts have assumed special significance in the present day strategy of developing ecofriendly products for plant diseases management (Singh, 1997). Recently, plant extracts drawn from the various parts of certain plant species have been successfully tried to demonstrate their antifungal activities (Karade and Sawant, 1999; Sindhan *et al.,* 1999; Singh and Navi, 2000; Bowers and Locke, 2000). The antifungal activities of different plant extracts against many plant pathogenic fungi have been well documented (Dubey and Dwivedi, 1991; Biswas *et al.,* 1995). Flori *et al*. (2000) suggested that leaf extracts and essential oils of certain plants could be used to suppress the growth of *D. bryoniae* after finding the crude extracts of *Ageratum conyzoides* effective

in inhibiting the mycelial growth and spore germination of *D. bryoniae*. They further noted 100 per cent inhibition of the mycelial growth and germination of spores of *D. bryoniae* by essential oils of *Cymbopogan citratus*, *A. conyzoides* and *E. citriodora*. Favaron *et al*., (1993) reported inhibition to various extents of polygalacturonases produced in *in vitro* by *D. bryoniae* by the extracts of onion and leek.. Kaushal and Paul (1989) evaluated plant extracts prepared from *Cannabis sativa, Pinus lingifolia, Eupatorium sp.* and *Lantana indica* against the *Ascochyta phaseolorum* isolated from leaves of *Vigna mungo,* all the extracts except *C. sativa* inhibited the growth of the test fungus. Tasleem *et al*. (1998) while evaluating the aqueous extracts of *Allium cepa, Calotropsis procera, Chenopodium album, Chenopodium musala, Azadirachta indica* and *Cannabis sativa* against the *Ascochyta rabiae,* found maximum reduction of colony growth by *A. cepa* extracts. Crude bulb extract of *Euconis autummalis* inhibited mycelial growth of *Mycosphaerella pinodes*, the cause of blight in pea, as well as, prevented leaf infection by *M. pinodes* spores when the leaves were inoculated with spores, both before and after the treatment with the extract (Pretorius *et al*., 2002).

Chapter – 3

MATERIALS AND METHODS

The present study was conducted during 2003-2005 in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. The materials used and the methodology adopted in achieving the set objectives of the study are described as under:

3.1 Disease survey

Survey for prevalence and severity of Didymella blight of ridge gourd was conducted in eleven major ridge gourd growing areas from two districts viz. Srinagar and Budgam during last week of August in the year 2004 and 2005. Three fields from each area and five plants per field were randomly selected. From each plant four vines selected randomly were tagged. From each vine a random sample of five leaves was drawn. In all hundred leaves from each field were brought to the laboratory for recording the incidence and intensity of disease. For recording disease incidence on fruits, all the fruits growing on the randomly selected five plants were thoroughly examined. The per cent disease incidence on leaves and fruits was calculated by using the following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves or fruits}}{\text{Total number of leaves or fruits assessed}} \times 100$$

The disease intensity on leaves was recorded on 0-5 scale (Plate-1) as per the method of Singh *et al*. (2003); where

Numerical Category	**Leaf area affected (%)**
0	Nil
1	1-10
2	11-25
3	26-50
4.	51-75
5	76-100

Per cent disease intensity (PDI) was calculated as per the formula :

$$\text{Per cent disease intensity} = \frac{\text{Sum of the all numerical ratings}}{\text{Total number of leaves assessed x highest value of rating}} \times 100$$

3.2 Symptomatology

The study on symptom development of Didymella blight was undertaken on naturally infected ridge gourd plants grown under field

conditions. These plants were not given any plant protection treatment throughout the season. The plants were observed daily for the appearance of typical disease symptoms. The date of first appearance of disease was recorded and diseased parts tagged for further observations regarding disease progression. The symptom development with respect to the size, shape, colour and fructification was recorded continuously at weekly interval. The disease development was also studied in artificially inoculated ridge gourd plants grown in laboratory.

3.3 Isolation of causal pathogen

3.3.1 Culture from pycnidiospore

The pure culture of fungus was obtained by single spore transfer method (Johnston and Booth, 1983). For this purpose a spore suspension was prepared in sterile distilled water from sporulating lesions of ridge gourd leaves. Three drops of spore suspension were evenly spread on the surface of plain agar medium in Petri plates for germination. The plates were incubated at 24±1^{o}C and regularly observed under compound microscope for spore germination. The germinating spores along with agar disc were lifted with the help of sterilized scalpel, transferred to PDA medium plates under aseptic conditions and incubated at 24±1^{o}C to maintain its growth. The pure culture so obtained was maintained for further study in different experiments by subculturing regularly after every 30 days.

3.3.2 Culture from ascospore

Single spore culture from ascospore formed in pseudothecia, was obtained following the technique adopted by Trapero and Kaiser (1992). The diseased leaves were moistened with sterilized distilled water for about

half an hour and extra water removed with the help of blotting paper. The leaves were examined under stereoscopic microscope for the presence of pseudothecia. One cm^2 leaf disc containing mature pseudothecia was cut and placed on a water agar block that was attached to the inner surface of the cover of a Petri plate. The plate cover with adhering tissue was placed over the bottom plate containing two per cent water agar. Petri plates were incubated in the dark at 20±1^oC for two hours for ascospore discharge onto water agar. After 24 hours, the lids were replaced with fresh lids. These plates were examined for the presence of ascospores by examining the inverted plates under compound microscope. The plates were then incubated at 24±1^oC. After 48 hours of incubation the plates were again examined for ascospore germination. Distantly placed germinating ascospores were located and marked. These germinating ascospores along with the agar disc were then transferred aseptically to potato dextrose agar medium and incubated at 24±1^oC. The pure culture thus obtained was maintained by regular sub culturing.

3.4. Pathogenicity

The pathogenicity of the causal fungus was established on ridge gourd plants by confirming postulates of Koch on potted plants as well as on the detached leaves and fruits.

3.4.1 On potted plants

The soil was sterilized by autoclaving at 1.05 kg cm^{-2} for three consecutive days and 2 kg of the sterilized soil was put in each pot of 18 cm diameter. The seeds of ridge gourd, sterilized by immersing in mercuric chloride solution (0.1%) for 1 minute and subsequently rinsed with sterilized distilled water, were sown in these pots. The test plants so raised

were kept in humid chamber for a period of 15 days prior to inoculation to rule out any latent infection. For plant inoculation, spore suspension was prepared by flooding the fungal culture grown on potato dextrose agar medium with sterilized distilled water (Arny and Randall, 1991) and subsequently concentration of the suspension adjusted to 1 x 10^6 spores per milliliter by haemocytometer. The spore suspension was atomized on to the test plants to run-off while as, an equal volume of sterile distilled water was used in case of check. After inoculation plants were incubated under moist chamber for 24 hours, then shifted to green house where temperature was 18±5^oC and examined critically for expression of symptoms for a period of 30 days. After recording the typical symptoms on test plants, the causal pathogen was reisolated and compared with the already isolated and inoculated one.

3.4.2 Detached plant parts

Pathogenicity of the fungus was also carried out on detached leaf and fruit of ridge gourd plants. Apparently healthy leaves and fruits were detached and brought to the laboratory, surface sterilized with 0.1 per cent $HgCl_2$ for 30 seconds and serially rinsed thrice in sterilized distilled water. These plant parts were then separately placed on sterilized glass slides, kept in sterilized Petri plates lined with blotting paper. The petioles of the leaves and peduncles of the fruit were inserted in moist cotton. For preparation of spore suspension similar method as described in section 3.4.1 was adopted. Nine drops (10 µl) of this suspension were placed at nine equidistant places on the tested plant parts. The control set was also maintained and inoculated with sterilized distilled water. The Petri plates were then placed in diffused sun light on laboratory benches at a room temperature and observed daily for the appearance of symptoms.

3.5 Morphological and cultural characteristics

Morphological and cultural characters of the causal fungus, isolated from diseased spots, on its host as well as in its pure culture were studied for its identification.

3.5.1 In culture

Colony characters such as colour and status of the mycelium were studied from the medium visually. For morphology of the fungus, mycelium from marginal portion of actively growing colonies, hundred pycnidia and two hundred pycnidiospores from 15 day old culture were examined under a microscope and the observations with respect to shape, size and colour as well as septation of mycelium and pycnidiospores were recorded.

3.5.2 On host

The leaf and fruit samples exhibiting typical symptoms of Didymella blight were observed under microscope for the presence of mycelium, pycnidia and pseudothecia. The pycnidia and pseudothecia thus located were picked up with the help of a teasing needle, placed separately in a drop of water in cavity slides and studied for shape and size. The picked pseudothecia and pycnidia were also placed in a drop of lactophenol, pressed gently under cover slip to rupture and examined under microscope. Observations for overall morphology of the fungus were recorded with respect to the following characters:

Mycelium	:	Colour, septation and branching pattern
Pycnidia	:	Size, shape and colour

Pycnidiospores	:	Size, shape, colour and septation
Pseudothecia	:	Size, shape and colour
Asci	:	Size, shape, colour
Ascospores	:	Size, shape, colour and septation
Pseudoparaphysis	:	Size, shape, colour and septation

3.6 Identification of the pathogen

Morphological characteristics of the causal organism studied were compared with standard description given by Puninthalingam and Holliday (1972) for identification of the pathogen. The identification was also confirmed by Dr. P.N. Chowdhary, Ex-Principal Scientist, Division of Plant Pathology IARI, during his visit to the Division in the year 2004-05.

3.7 Host range

Nine cucurbit plant species viz., cucumber (*Cucumis sativus* L.), muskmelon *(Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thumb.) Stendle], bottle gourd (*Lagenaria siceraria* (Molina) Stendle), bitter gourd (*Mamordicha charantia* Poir), sponge gourd (*Luffa cylindrica* L.), pumpkin (*Cucurbita pepo* Poir), squash (*Cucurbita maxima* Dusch) and ridge gourd (*Luffa acutangula*) were tested for the host range of the pathogen isolated from ridge gourd under green house conditions. The plants were grown in 25 cm dia pots and inoculated at 3-4 leaf stage. The procedures adopted for preparation of spore suspension, inoculation and incubation of test plants and recording the observations on symptom development were same as described earlier in section 3.4.1. Days taken to express first symptoms were recorded in each case. These plant species were also regularly monitored during the course of survey, wherever available to record

occurrence of the disease under natural conditions.

3.8 Physiological studies

To ascertain the best medium, optimum temperature and hydrogen ion concentration for the growth and fructification of causal fungus, following studies were undertaken.

3.8.1 Effect of media on growth and fructification of pathogen

Seven different solid media *viz*., corn meal agar, czapek-Dox agar, malt extract agar, oat meal agar, potato dextrose agar (PDA), ridge gourd fruit extract agar, ridge gourd leaf extract agar (Appendix-I) were evaluated with respect to their support for growth and fructification of the causal fungus. The culture discs of 5 mm dia were lifted with a sterilized cork borer from a 10 days old culture. Each disc was aseptically transferred to a Petri plate (90 mm dia) containing test medium and incubated at 24±1^{o}C. The experiment was laid out in a completely randomized design (CRD) with three replications. The observations for colony diameter and fructification were recorded after eight and fifteen days of incubation respectively. For recording relative fructification, 5 mm disc from 15 days old colony was taken and observed under stereoscopic microscope for number fruiting bodies. The intensity of fructification was graded as under

Category	No. of pycnidia/ 5 mm culture disc	Rating
-	0	Absent
+	1-3	Scanty
++	4-6	Moderate

+++	7-9	Good
++++	>15	Abundant

3.8.2 Effect of temperature on growth and fructification of pathogen

The Petri plates each containing a 20 ml of sterilized basal medium (PDA) was inoculated with a 5 mm culture disc of test fungus and then incubated at seven different temperatures viz., 12, 16, 20, 24, 28, 32 and 36 ($\pm 1^{o}$C). Three replications were maintained for each treatment in CRD. Methods adopted for recording observations on colony diameter and fructification and the scale adopted for grading fructification were same as described in section 3.4.1.

To study the effect of temperature on spore germination, a spore suspension of 50,000 spores per ml was prepared from 15 days old culture of test fungus. One drop of spore suspension was kept in each cavity slide and incubated in a moist chamber of Petri plates at each test level of temperature. After 24 hour of incubation, hundred spores were observed under microscope and per cent spore germination calculated by using the following formula:

$$\text{Per cent spore germination} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores examined}} \times 100$$

3.8.3 Effect of pH on growth and fructification of pathogen

In order to determine the optimum pH level for better growth and fructification of the test fungus, the pH of basal medium was adjusted at desired levels viz., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 with the help of pH meter using N/10 hydrochloric acid (HCl) and N/10 sodium hydroxide (NaOH). Inoculation and observations were made in similar manner as described in section 4.3.1.

To determine the optimum hydrogen ion concentration for spore germination of causal fungus, spore suspension (250 ml) was taken from the stock volume (section 3.8.2) and equally distribute in seven sterilized test tubes. The pH of spore suspension was adjusted separately at each test level with the help of pH meter using N/10 HCl and N/10 NaOH. The drops of spore suspension were then placed in cavity slides contained in moist chamber of Petri plates and incubated at 24±1^{o}C. Each treatment was replicated three times in CRD and observation on per cent spore germination recorded after 24 hours by using the formula as given in section 4.8.2.

3.9 Perpetuation of pathogen

3.9.1 Perpetuation in/on seed

The seeds collected at harvest from severely infected ridge gourd plants were stored in cotton cloth bags in laboratory and assessed for presence of the pathogen through blotter and agar plate methods at monthly intervals.

3.9.1.1 Spore viability

Twenty ridge gourd seeds were taken randomly at monthly intervals from harvest and 1 cm^2 hilum portion from each seed carved out and crushed in a pestle and mortar in 40 ml of distilled water. The crushed material was strained through a double layer of cheese cloth. Twenty milliliter of the filtrate was centrifuged at 6000 rpm for 15 minutes. After centrifugation, supernatant was discarded and pellet was made 5 milliliter by adding sterilized water (Fi1ajdic and Sutton, 1995). Number of pycnidiospores/ ascospores were counted with the help of haemocytometer and the average spore load of three replications was estimated. To estimate the pycnidiospore/ ascospore germination one drop of 50 µl from processed sample was placed on a glass slide and incubated in a moist chamber at 24±1^{o}C. After 24 hour incubation, the slide was viewed under binocular

microscope to record spore germination index of spore viability. The spore viability was recorded using the formula :

$$\text{Spore viability} = \frac{\text{Number of spores germinated}}{\text{Total number of spores viewed}} \times 100$$

3.9.1.2.Seed infection

a) Blotter method

Random sample of sixty seeds were taken at monthly intervals from harvest and surface sterilized by immersing in 0.1% mercuric chloride for one minute followed by three subsequent rinses with sterilized distilled water. The seeds were placed in sterilized Petri dishes on three folds of sterilized blotter papers moistened with sterilized distilled water. Twenty seeds were placed aseptically in each Petri dish maintaining three replications for each plate. The plates were incubated at 24±1^{o}C. The blotter paper in Petri dishes was kept moist by carefully pouring a few drops of sterilized distilled water in the plates as and when required. The plates were examined from 4th up to 15th day after incubation for the appearance of fungal colonies. The number of seeds showing *D. bryoniae* growth were recorded and per cent infected seeds estimated by using the formula:

$$\text{Per cent infected seeds} = \frac{\text{Number of seed infected}}{\text{Total number of seeds examined}} \times 100$$

b) Agar plate method

Sixty seeds were taken from the seed lot and surface sterilized. Twenty surface sterilized seeds were aseptically placed on 2 per cent water agar in Petri dishes and incubated at 24±1^{o}C. The plates were examined from 4th to 10th day after incubation for recording the percentage of seeds showing *D. bryoniae* growth.

3.9.2 Perpetuation through plant debris

The infected plant debris including fruit husk and leaves were collected at harvest from the diseased crop and kept separately in nylon mesh bags. The bags were divided into three sets of 12 bags each. One set, each of leaves and fruit husk, was placed on the soil surface in vacated ridge gourd fields. The other set was buried 7.5 cm deep in such soil and third set was stored under ambient laboratory conditions. Each set was replicated three times. One month after placement of the bags at appropriate positions, twenty bits of 1 cm^2 area were randomly taken from one randomly selected bag of each leaf and fruit husk and examined for the presence of pycnidia/pseudothecia. These leaf and fruit discs were then crushed separately in 40 ml of sterilized distilled water and strained through a double layered cheese cloth. Twenty milliliter of the filtrate was centrifuged at 6000 rpm for 15 minute. After discarding the supernatant, sterilized distilled water was added to the pellet to make 5 ml of spore suspension in each sample and the number of pycnidiospores/ascospores were counted with the help of a haemocytometer. To estimate the per cent viability of pycnidiospores/ascospores in over-wintering leaf and fruit husk debris, spore germination method was used. Two drops of 50 µl from each processed sample were placed on glass slides and incubated in a moist chamber at 24±1^oC. The number of spores observed and the number that germinated were recorded after 24 hour incubation for calculating the per cent viability of pycnidiospores/ascospores.

3.9.3 Effect of meteorological factors on development of Didymella blight of ridge gourd

The role of various meteorological factors on disease intensity and infection rate (unit/day) of Didymella blight of ridge gourd was assessed during the year 2004 and 2005 at Shalimar campus. The weather variables

were temperature, relative humidity (RH) and precipitation. The field experiments were conducted in randomized block design with three replications. Three plots (3 x 3 m) of ridge gourd were maintained unsprayed to record the disease development under natural conditions. Development of the disease in terms of intensity was recorded periodically at seven days interval starting from the first appearance of the disease. Disease intensity was recorded on hundred leaves randomly selected from each replication using 0-5 rating scale as described in section 3.1.1. Weekly means of the temperature, RH, and precipitation that prevailed prior to each disease scoring date were recorded and correlated with disease development. Stepwise multiple regression analysis was conducted to determine the effect of individual as well as combined weather factors on disease development. Growth of disease development in terms of apparent infection rate (unit/day) was calculated as per the formula given by Vanderplank (1963):

$$r = \frac{2.3}{t_2 - t_1} \text{ X } \log x \frac{x_2 (1-x_1)}{x_1 (1-x_2)}$$

Where;

r = apparent infection rate (unit/day)

x_1 and x_2 = disease intensity at time t_1 and t_2, respectively

3.11 Management of the disease

3.11.1 *In vitro* management of the disease

3.11.1.1 *In vitro* screening of fungitoxicants

Ten fungitoxicants (Appendix-II), both systemic and non systemic,

at four concentrations viz., 10, 50, 100 and 200 µg ml^{-1} for systemic and 50, 250, 500, and 1000 µg ml^{-1} for non systemic were evaluated against the test fungus by poisoned food (Grower and Moore, 1962) and spore germination (Montgomery and Moore, 1938) techniques.

a) Poisoned food technique

Appropriate quantity of each fungicide was separately dispensed in molten sterilized PDA medium, contained in 100 ml Erlenmeyer flasks, under aseptic conditions to make a desired concentration. After thorough mixing, 20 ml of poisoned food, thus prepared, was poured in 90 mm diameter Petri plates. The mycelial discs of 5 mm dia, taken from 10 days old monosporic culture of the test fungus with the help of sterilized cork borer, were aseptically placed in the centre of solidified poisoned PDA. Three replications were maintained for each concentration. The Petri plates were incubated at 24±1^{o}C and observation on mycelial growth of test fungus recorded after seven days of incubation. The growth of the test fungus on non-poisoned PDA served as check. The per cent inhibition in growth due to various fungitoxicant treatments at different concentrations was computed by the following formula of Vincent (1947).

$$\text{Percent inhibition} = \frac{\text{Germination in check} - \text{Germination in treatment}}{\text{Germination in check}} \times 100$$

b) Spore germination technique

One drop of the fungitoxicant solution with double the required concentration was placed in a cavity slide to which one drop of spore suspension (q.v. section 3.4.1) was added. In case of control a drop of sterilized distilled water was put in a cavity slide instead of fungicidal solution. The cavity slides were incubated in moist Petri plate chamber at 24 ± 1 ^{o}C, maintaining three replications for each treatment. After 24 hours

of incubation, one hundred spores were observed for each replication and the average percentage germination inhibition of the spores was calculated by adopting the following formula of Vincent (1947).

$$\text{Percent growth inhibition} = \frac{\text{Growth in check} - \text{Growth in treatment}}{\text{Growth in check}} \times 100$$

3.11.1.2*In vitro* screening of botanicals

Efficacy of aqueous leaf extracts of five botanicals (Appendix-III) viz., *Allium sativum* L. (Rohan), *A. cepa* L. (Ganda), *Artemisia annua* (Tethwan), *Urtica dioica* (Soi) and *Datura stramonium* L. (Datur) were evaluated at 1, 5, 10 and 15 per cent concentration against the test fungus by poisoned food and spore germination method.

a) Poisoned food technique

The plant leaf extracts were prepared following the method given by (Jadeja, 2003). Leaves of the plants were first washed with sterilized distilled water and then shade dried. One hundred g of each plant material was crushed in 100 ml of sterilized distilled water for crude extract preparation. The extract was homogenized, filtered and centrifuged at 5000 rpm for 15 minutes. The supernatant was filtered through Whatman No. 1 filter paper. The clear extract so obtained, referred to as 100 per cent stock solution, was used for experiment. Desired concentrations viz., 1, 5, 10 and 15 per cent were prepared by adding appropriate amount of standard solution of plant extracts to PDA medium. Inoculation and observations were made in similar manner as described earlier in section 3.11.1.1.

b) Spore germination technique

One drop of double the desired concentration of botanical extract prepared by adding appropriate amount of sterilized distilled water to the

standard stock solution was thoroughly mixed with one drop of spore suspension (q.v. section 4.3.1) in a cavity slide. After 24 hour of incubation at 24±1^{o}C in a moist chamber of Petri plates, observations on spore germination were taken and percent germination inhibition calculated by using the formula given in section 4.6.1.1.

3.11.2 *In vivo* management of the disease

The treatments that proved most effective in *in vitro* studies were evaluated as seed treatments and foliar sprays under field conditions for their efficacy in controlling the disease. The experiment was conducted at Shalimar campus for two years during kharif 2004 and 2005. A factorial experiment of seed treatments × foliar sprays was laid in a randomized block design' consisting of 16 treatments in all, each replicated thrice. Naturally infected seeds of ridge gourd were used for sowing in three blocks (replications). Each block consisted of a plot of 48 × 3 m size divided into 16 plots of 3 × 3 m size each. Infected ridge gourd seeds were soaked separately in fungicidal solution viz., carbendazim (0.1%), mancozeb (0.3%) and leaf extract of *Allium sativum* (20%). The seeds soaked in sterilized distilled water served as check. Treated ridge gourd seeds were sown at a spacing of 150 × 100 cm @ five seeds per hole, which after germination were thinned out to one seedling per hill. Three foliar sprays with the same fungicidal solutions as used for seed treatment were made at interval of 15 days starting from the initial disease appearance. Sprays with sterilized distilled water served as check. All the sprays were conducted in late afternoon hours and care was taken to prevent drift of chemicals from one treatment to another by erecting polythene sheets around the individual plots while conducting the sprays. The crop was maintained by following all the recommended agronomic practices except

plant protection measures. After twenty days of last spray, 100 leaves were randomly selected from five randomly selected plants from each plot for recording observations on disease incidence and intensity as per the method given in section 3.1.1. The crop was harvested in four pickings after every 15 days. The observations with respect to yield, percent infected fruits were recorded at the time of each picking and were represented by the cumulative data of all pickings.

3.12 Statistical analysis

The data of various experiments were subjected to appropriate statistical analysis and wherever needed subjected to appropriate transformation, as suggested by Panse and Sukhtme (1978).

Chapter – 4

EXPERIMENTAL FINDINGS

The results of the present study on Didymella blight of ridge gourd in Kashmir, carried out both in the field and laboratory of the Division of Plant Pathology, SKUAST-K, Shalimar are as under:

4.1 Disease status

Survey of the major ridge gourd growing areas of two districts viz., Srinagar and Budgam of Kashmir valley, was conducted during 2004 and 2005 to assess the status of Didymella blight (*Didymella bryoniae*) of ridge gourd (*Luffa acutangula*).

4.1.1 Disease incidence on leaves

The disease on leaves was prevalent during both the years (2004 and 2005). On an overall basis, the disease incidence was more (36.4%) in 2005 as compared to that of (32.6%) in 2004 (Table 1, Fig.1a). The disease incidence over the years was higher in district Srinagar (38.5%) than district Budgam (30.4%). During the crop season 2004, the Didymella blight incidence on ridge gourd leaves ranged from 16.6 to 60.6 per cent in district Srinagar and from 23.0 to 34.0 per cent in district Budgam. During crop season 2005, the disease incidence ranged from 29.0 to 67.3 per cent in district Srinagar, whereas it ranged from 21.0 to 39.0 per cent in district Budgam. On an overall comparison amongst different locations surveyed, the highest disease incidence of 64.0 per cent was recorded in

Dal followed by Habak (55.8%), Shalimar (40.1%) and Narkura (36.5%). In location Gangbug, 32.3 per cent disease incidence was recorded which was statistically at par with that of Ganderbal (31.5%), Bugam (31.0%) and Brain (29.3%). The least disease incidence of 22.00 per cent was recorded in Panzan which was statistically at par with Noorbagh.(22.8%).

4.1.2 Disease intensity on leaves

During the year 2004 and 2005, the disease intensity on leaves varied at all the surveyed locations. On an overall basis, the disease intensity on leaves was more (18.8%) in 2005 compared to that of 14.8 per cent in 2004 (Table 1, Fig. 1b). During the crop season 2004, the Didymella blight intensity on ridge gourd leaves ranged from 10.2 to 32.2 per cent in district Srinagar and from 8.2 to 16.0 per cent in district Budgam, whereas, in 2005, the disease intensity ranged from 14.5 to 37.5 per cent in district Srinagar and from 7.3 to 19.8 per cent in district Budgam. The disease intensity on leaves over the years was higher in district Srinagar (20.3%) than district Budgam (13.3%). On an overall comparison amongst different locations surveyed, the maximum disease intensity of 34.8 per cent on leaves was recorded from Dal, followed by Habak, Shalimar and Narkura with 29.0, 20.9 and 17.9 per cent intensity, respectively. In location Noorbagh, 15.7 per cent disease intensity was recorded which was statistically at par with that of Ganderbal (15.2%), Brain (14.6%), Bugam (13.9%) and Gangbug (13.6%). The least disease intensity of 7.7 per cent was recorded in Panzan.

4.1.3 Disease incidence on fruits

The prevalence of Didymella blight on ridge gourd fruit was also recorded during the years 2004 and 2005. On an overall basis, the disease incidence on fruits was more (11.2%) in 2005 as compared to that (9.6%) in 2004 (Table 2, Fig. 2). The incidence of Didymella blight on ridge gourd fruits over the years was higher in district Srinagar (11.7%) than district Budgam (9.2%). During the crop season 2004, the disease incidence on fruits ranged from 7.2 to 17.8 per cent in

district Srinagar and from 7.7 to 11.7 per cent in district Budgam, whereas in 2005 it ranged from 8.8 to 19.8 per cent in district Srinagar and from 6.0 to 13.5 per cent in district Budgam. On an overall basis, among different locations surveyed, the maximum disease incidence of 18.8 per cent on fruits was recorded at Dal followed by 15.4 per cent at Habak. Disease incidence of 12.6, 11.0, 10.4 and 9.8 per cent was recorded at Narkura, Shalimar, Brain and Gangbug, respectively, with no significant difference among them. The least disease incidence was recorded at Panzan (6.8%) which was statistically at par with Bugam (7.5%), Darbagh (8.0%) and Ganderbal (9.1%).

4.2 Symptomatology

The ridge gourd plants, both naturally infected and artificially inoculated, were examined from third week of June, 2004 for appearance of the characteristic symptoms of Didymella blight. After recording the first appearance of disease, weekly observations on symptomatology were recorded and are presented in Table. 3 and Plates 2 & 3.

The disease in the field was first noticed on the lower most leaves of ridge gourd plants in the third week of June 2004. The symptoms initiated as small circular, light brown spots with chlorotic halo, ranging from 0.5 to 1 mm in size (Plate 2a). With the passage of time the spots enlarged, developed concentric rings at the centre (Plate 2b) and turned dark brown with greyish white centre (Plate 2c). The spots enlarged up to 83 mm in diameter and coalesced (Plate 2d) caused yellowing and blighting of the whole leaf (Plate 2e). Black pin head fructifications (pycnidia and pseudothecia), arranged in concentric circles, were developed within the greyish white centre of the spots. During the first week of August, dark green, water soaked, irregularly circular spots were observed on the fruits. These spots enlarged rapidly and caused black rot of the whole fruit (Plate 3a). The black colour of the affected fruit was due to the abundant pycnidial and pseudothecial formation of the causal fungus on the surface of infected tissue. The young affected fruits were shrivelled and deformed (Plate 3b).

4.3 Isolation of causal pathogen

Single spore cultures were obtained from pycnidiospores as well as ascospores on potato dextrose agar medium incubated at 24±1^{o}C under 12/12 hour alternate cycles of light and darkness. Observations were recorded on the development of cultures from both the sources and are presented as follows:

4.3.1 Isolation from pycnidiospores

Observations recorded on the development of culture from pycnidiospores revealed that the maximum number of pycnidiospores germinated within 24 hours of incubation. One to two germ tubes were produced by each spore (Plate 5d). The colony developed faster from the germinating spores and covered the whole plate (90 mm) within 8 days. Pycnidia were the only fruiting bodies observed after 10-12 days of incubation period.

4.3.2 Isolation from ascospores

The cultures were also developed from ascospores. The ascospores germinated after 24 to 36 hours of incubation and produced one germ tube from upper as well as basal cell (Plate 5h). The germinated ascospores, transferred to potato dextrose agar (PDA) medium, developed fungal colonies which covered the whole plate within 8 to 9 days of incubation whereas, pycnidia developed after 10 to 12 days of incubation.

4.4 Pathogenicity

The pathogenicity of the causal fungus was established by confirming postulates of Koch on 35 days old potted ridge gourd plants as well as on the detached leaves and fruits of the plant.

4.4.1 On potted plants

Observations regarding the pathogenicity of the test fungus on potted ridge gourd plants revealed the initiation of typical symptoms of the disease

after 4 days of inoculation (Plate 4a). Pycnidia followed by pseudothecia were observed after 12 and 15 days after symptom expression, respectively. The fungus on reisolation from artificially inoculated plants resembled with initially isolated and inoculated pathogen and hence pathogenicity of the isolate was proved by satisfying postulates of Koch (1884).

4.4.2 On detached leaf and fruit

The pathogenicity of the isolated fungus was also proved on the detached leaf and fruit of ridge gourd plant. It was observed that symptoms developed after 2 days of incubation on injured leaf whereas, it took 3 days for symptom development on uninjured one (Plate 4b). Similarly, on injured fruit, symptoms developed after 2 days of incubation (Plate 4c), however, symptoms were not observed at all on inoculated uninjured fruits. Pycnidia were the only fruiting bodies observed, 7 days after inoculation.

4.5 Morphological characters of the fungus

The morphological characters of the isolated fungal pathogen were studied both on host as well as in its pure culture and are presented in Table 4 and Plates 5 & 6.

4.5.1 On host

Hyphae were branched, septate, smooth and hyaline, measuring 3.2-5.3 µm (Av. 4.5 µm) in width. Black pycnidia (Plate 5a) of the anamorph (*P. cucurbitacearum*), 120.4 -172.0 (Av. 142.7 µm) µm in diameter, formed on the surface of infected tissue of leaf and fruit were globose in shape, subepidermal, solitary, arranged in circles and contained large number of pycnidiospores (Plate 5b). These pycnidiospores were hyaline, ellipsoidal to allantoid, one septate (rarely 2 septate), constricted at septa, with rounded ends and measured 8.6-12.9 x 3.2-4.3 µm (Av. 11.5 x 3.6 µm) in size (Plate

5c). Pseudothecia of the teleomorph, observed together with pycnidia on the infected tissue of both leaf and fruit, were dark, globose to subglobuse, subepidermal, solitary and measured 146.2-206.4 µm (Av. 186.3 µm) in diameter (Plate 5e). The asci were hyaline, bitunicate, cylindrical to subclavate, short stipitate or sessile, biseriate, eight spored and measured 55.9- 64.5 x 10.7-12.9 µm (Av. 62.2 x 11.1 µm) in size (Plate 5f). Ascospores were 13.9-17.2 x 4.3- 5.4 µm (Av. 16.4 x 5.1 µm) in size, hyaline, mono septate, constricted at the septum, multiguttulate and rounded at ends with upper cell usually wider than the lower one (Plate 5f). Pseudoparaphyses (Plate 5g) were hyaline, branched, septate and measured 2.1-2.6 µm (Av. 2.2 µm).

4.5.2 In culture

4.5.2.1 Macroscopic characters

The cultural characters of the fungus (Table. 4, Plate 6a) isolated from diseased ridge gourd leaf and fruit were studied on potato dextrose agar medium. The fungus produced submerged hyphae with sparse aerial mycelium. The colonies were circular with cottony growth, initially white to dirty white, turned greyish and finally black. Pycnidia formed after 10 to12 days were solitary to confluent.

4.5.2.2 Microscopic characters

The pathogen in culture produced hyaline to light brown septate mycelium measuring 3.2 to 4.3 µm (Av. 3.6 µm) in width (Plate 6b). The mycelium was irregularly branched and hyphae slightly bulged at septa. Black pycnidia formed after 10 to 12 days of incubation were globose to irregular in shape, solitary to confluent, glabrous or with mycelial outgrowths and measured 167.7–326.8 µm (Av. 236.5 µm) in diameter

(Plate 6c). Oozed pycnidiospores (Plate 6d) from the pycnidia were variable in shape, subglobose to ellipsoidal or allantoid with several small guttules, aseptate rarely one septate, 6.4–9.6 x 2.1-4.3 µm (Av. 8.3 x 3.7 µm) in size (Plate 6e). Pseudothecia were not observed throughout the study period.

4.6 Identification of the pathogen

On the basis of morphological and pathological characteristics as well as comparison with authentic descriptions, the fungus was identified as *Didymella bryoniae* (Aureswarld) Rehm [Anamorph *Phoma cucurbitacearum* (Foutrey) Sacardo]. The identification was also confirmed through Dr. P.N. Chowdhary, Ex Principal Scientist, Division of Plant Pathology IARI, during his visit to the Division in the year 2004-05.

4.7 Physiological studies

4.7.1 Effect of media on growth and fructification

In order to ascertain the best media for achieving the optimum growth and fructification of the fungus, seven solid media were tested. Average colony diameter and fructification (per 5 mm culture disc) were recorded in each medium after 8 and 15 days of incubation at 24±1^{o}C, respectively. The results obtained are presented in Table 5; Fig.3; Plate 7a.

Perusal of Table 5 revealed that the mycelial growth of the fungus was best achieved (90 mm) on potato dextrose agar and oat meal agar media followed by malt extract agar (86.6 mm) and corn meal agar (81.0 mm) media. Minimum growth (66.6) was observed on ridge gourd leaf extract agar medium which was statistically at par with that of ridge gourd fruit extract agar (69.3 mm) and Czapek-Dox agar (69.0 mm) media. The results further indicated that all the media supported the pycnidial formation, whereas, no media favoured the pseudothecial formation.

Abundant pycnidial formation was recorded on potato dextrose agar, malt extract agar, oat meal agar and corn meal agar and moderate on Czapek-dox agar and ridge gourd fruit extract agar media. The pycnidial formation on ridge gourd leaf extract agar was scanty.

4.7.2 Effect of temperature on growth, fructification and spore germination

The observations regarding the growth response of test fungus to various temperatures after 8 days of incubation are presented in Table 6; Fig 4a; Plate 7b. It is evident from the data that the fungus could grow and fructificate at a wide range of temperatures (4-32^{o}C), however, no growth and as such fructifications was observed at 36^{o}C. The growth (90 mm) was significantly superior at 24^{o}C, followed by 20^{o}C (81.6 mm) and 28^{o}C (74.3 mm) with abundant pycnidia formation achieved at all these temperatures. The 16.3 mm and 38.0 mm growth with scanty pycnidial formation was recorded at 12 and 16^{o}C, respectively.

Observations on per cent spore germination (after 24 hours of incubation) indicated that all the temperatures tested differed significantly in their effect on spore germination. Maximum spore germination (95.6%) was recorded at 24^{o}C followed by 28^{o}C (75.6%). The spore germination at 20^{o}C was 68.0 per cent and was statistically at par with that of 28^{o}C. Minimum spore germination of 24.6 per cent was recorded at 32^{o}C, however, spores could not germinate at 12 and 36^{o}C.

4.7.3 Effect of pH on growth, fructification and spore germination

It is obvious from the data (Table 7; Fig. 4b; Plate 7c) that the fungus grew and fructificated throughout the pH range of 5.0 to 8.0 (data recorded after 8 days of incubation at 24 ±1^{o}C). However, maximum mycelial growth of 90 mm and abundant pycnidial formation was recorded

at pH 7.0 which was statistically at par with pH levels of 6.5 (88.6 mm) and 6.0 (81.0 mm). Increase or decrease in optimum pH (7.0) resulted in decrease of mycelial growth and fructification of the test fungus. The least mycelial growth (14.6 mm) was recorded at pH 8.0 followed by that of 7.5 (35.3 mm) and 5.0 (59.3 mm) with scanty pycnidial formation at all these temperatures.

Per cent spore germination, after 24 hours of incubation at 24±1^{o}C was best achieved at pH 7.0 (95.6%) and the least (32.0%) at pH 8.0. Spore germination of 85.3 per cent was recorded at pH 6.5 which was statistically at par with that of pH 6.0 (84.0%).

4.8 Host range of *Didymella bryoniae*

The host range studies were carried out using nine cultivated plant species belonging to family Cucurbitaceae under natural and artificial inoculation conditions, and the results are summarized in Table 8.

4.8.1 Under natural conditions

Symptoms of Didymella blight under natural conditions during survey were noticed on all the nine plant species *viz*., cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thumb) Stendle), pumpkin (*Cucurbita pepo* Poir), squash (*Cucurbita maxima* Dusch), bottle gourd (*Lagenaria siceraria* (Molina) stendle), bitter gourd (*Mamordicha charantia* Poir), sponge gourd (*Luffa cylindrica* L.) and ridge gourd (*Luffa acutangula* (L.) Roxb.

4.8.2 Under artificial inoculation conditions

The artificial inoculation of above mentioned plants with *Didymella bryoniae*, isolated from ridge gourd (*Luffa acutangula*), revealed that all the

plant species tested were successfully infected by the pathogen. The incubation period for symptom expression was minimum 4 days for ridge gourd followed by 6 days for sponge gourd, 7 days for cucumber, bottle gourd and bitter gourd, 9 days for muskmelon and watermelon and 10 days for pumpkin and squash.

4.9 Perpetuation of pathogen

The possibilities of perpetuation of the pathogen (*D. bryoniae*), during crop less months after harvest, on ridge gourd seeds and diseased plant debris were explored during the year 2004 and 2005.

4.9.1 Perpetuation in/on ridge gourd seeds

4.9.1.1 Spore viability

Random seed sample collected from heavily infected crop in October 2004 and drawn at monthly intervals from November 2004 to October 2005 for examination of spore viability indicated 93.9 per cent spore viability immediately after one month of harvest in November 2004 (Table 9). The viability showed a gradual decrease as the storage period advanced such that after eight months of storage in June 2005, the viability was reduced to more than half (29.8%). The viability of 18.6 per cent was, however, observed even after the storage period of twelve months in October 2005.

The samples collected in October 2005 and drawn at monthly intervals from November 2005 to October 2006 for examination of spore viability indicated 93.7 per cent spore viability immediately after harvest in October 2005 (Table 10). The viability showed a gradual decrease as the storage period advanced, such that after eight months of storage in June 2004, the viability was reduced to 46.6 per cent. The viability was reduced to 20.3 per cent as the storage period further advanced to October 2006.

4.9.1.2 Seed infection

The seeds showing natural infection with *D. bryoniae* were examined by using blotter and agar plate method:

a) Blotter method

The seeds, collected from *D. bryoniae* infected ridge gourd crop in October 2004 and 2005, were incubated on wet blotter paper at 24±1^{o}C at monthly intervals starting from November for both the years and observed for *D. bryoniae* growth.

The data recorded for the seeds collected in 2004 revealed that 45.0 per cent seeds showed *D. bryoniae* growth in November 2004 (Table 9; Plate 8b). The percentage of seeds exhibiting *D. bryoniae* growth showed a gradual decrease as the storage period advanced, such that the percentage of these seeds was 26.6 per cent after eight months of storage in June 2005. In October 2005, after twelve months of storage percentage of such seeds reduced to only 16.66.

The data recorded for the seeds collected in November 2005 revealed that 48.3 per cent seeds showed *D. bryoniae* growth in November 2005 (Table 10). The percentage of seeds exhibiting *D. bryoniae* growth again showed a gradual decline as the storage period advanced, such that the percentage of these seeds was 26.6 after eight months of storage in June 2006. The percentage of 18.3 of such seeds retained even after twelve months of storage in October 2006.

b) Agar plate method

The collected seeds incubated aseptically in petriplates containing 2 per cent agar agar at 24±1^{o}C and examined for *D. bryoniae* growth (Table 9; Plate 8a), revealed that 41.6 per cent seeds showed *D. bryoniae* growth in November 2004. The percentage of seeds exhibiting *D. bryoniae* growth showed a gradual decrease as the storage period advanced. The percentage of seeds exhibiting *D. bryoniae* growth was only 21.6 after eight months of storage in June 2003. In October 2005, after twelve months of storage, percentage of seeds depicting *D. bryoniae* growth was only 8.3.

The data recorded for the seeds collected in October 2005 revealed that 45.0 per cent seeds had *D. bryoniae* growth in November 2005 (Table 10). The percentage of seeds exhibiting *D. bryoniae* growth showed a gradual decline as the storage period advanced. The percentage of seeds exhibiting *D. bryoniae* growth was only 21.6 after eight months of storage in June 2006. However, after twelve months of storage only 11.6 per cent seeds showed *D. bryoniae* growth in October 2006.

4.9.2 Perpetuation through plant debris

The infected leaf and fruit husk bits, kept under different conditions after harvest were examined for the presence of fruiting bodies (pycnidia/pseudothecia), spores (pycnidiospores/ascospores) and viability of spores at monthly intervals starting from November for both 2004 and 2005 crop seasons. The monthly observations recorded for the leaf and fruit husk bits of year 2004 and 2005 are presented in Tables 11-14.

4.9.2.1 Perpetuation through leaves

The monthly observations recorded for the leaf bits of year 2004, presented in Table 11, revealed that leaf bits stored indoors under laboratory conditions exhibited the presence of fruiting bodies and viable spores throughout the storage period. However, the average number of fruiting bodies and spores cm^{-2} leaf area as well as the per cent viability of spores reduced from 47.6, 5166 and 90.4 in November 2004 to 6.5, 416 and 8.8 after twelve months of storage in October 2005, respectively. The number of fruiting bodies and spores cm^{-2} leaf area lasted only up to June 2005 (eight months after storage) until the leaf sample got decomposed, besides two months of January and February (2005) when only empty fruiting bodies were found. Although the number of fruiting bodies, spores and per cent viability of spores cm^{-2} leaf bits stored on soil surface reduced from 45.6, 4100 and 92.2 in November 2004 to 19.5, 1226 and 78.6 in June 2005, however, the maximum per cent spore viability (94.4%) was recorded in April On

leaf bits stored at 7.5 cm deep in soil fruiting bodies and thus spores were altogether absent through out the observation period, although few empty fruiting bodies were observed in the month of November 2004 (one month after harvest).

The data recorded for the leaf bits of the year 2005 presented in Table 12 again revealed the presence of fruiting bodies and spores on the leaf bits stored indoors under ambient laboratory conditions throughout the storage period. However, the average number of fruiting bodies and spores cm^{-2} leaf area decreased with the increase in sampling time as it reduced from 49.8 and 4643 in November 2005 to 8.3 and 343 after twelve months of storage in October 2006, respectively. The per cent viability of spores produced on the leaf bits stored indoors under laboratory conditions also decreased from 87.6 in November 2005 to 7.2 per cent after twelve months of storage in October 2006. The leaf bits placed on soil surface exhibited fruiting bodies and viable spores up to June 2006 (eight months after storage), after that leaf sample was decomposed. The number of fruiting bodies, spores and per cent viability of spores on leaf bits stored on soil surface reduced from 49.8, 3956 and 90.1 in November 2005 to 11.6, 1640 and 73.4 in October 2006 with two months of January and February when only empty fruiting bodies were observed. On leaf bits stored at 7.5 cm deep in soil fruiting bodies and thus spores were absent through out the observation period, although few empty fruiting bodies were observed in the month of November 2005 (one month after harvest).

4.9.2.2 Perpetuation through fruit husk

Perusal of the data obtained for the year 2004 presented in Table 13 revealed that the fruit husk bits kept indoors under laboratory conditions exhibited the presence of fruiting bodies and viable spores for a comparatively longer period from November 2004 to October 2005, than when kept at soil surface or buried 7.5 cm deep in soil. The fruit husk bits placed on soil surface in open exhibited fruiting bodies and viable spores only up to July 2005 (nine months after storage), after that fruit husk sample got decomposed. On fruit husk bits buried 7.5 cm deep

in the soil, fruiting bodies and spores were absent throughout the observation period, although few empty fruiting bodies were observed in the month of November 2004 (one month after harvest). The number of fruiting bodies, number of spores and per cent viability of spores cm^{-2} fruit husk area decreased with the increase in storage period and also varied considerably among different storage conditions. On fruit husk bits kept under ambient laboratory conditions, the average number of fruiting bodies, spores and per cent viable spores cm^{-2} fruit husk area decreased from 78.1, 6926 and 94.2 per cent in November 2004 to 8.2, 983 and 13.2 after twelve months of storage in October 2005, respectively. However, on fruit husk kept on soil surface in open number of fruiting bodies, spores and per cent viability of spores cm^{-2} fruit husk area were 76.6, 6126 and 92.0 per cent in November 2004 and 13.23, 1096 and 88.2 in July 2005, respectively.

The data recorded for the fruit husk bits of the year 2005 presented in Table 14 again revealed the presence of fruiting bodies and spores throughout the storage periods on fruit husk bits kept indoors under laboratory condition. The fruit husk bits placed on soil surface in open exhibited fruiting bodies and viable spores up to July 2006 (nine months after storage), although, absent in the months of January and February. Thereafter the leaf sample was decomposed. However, when the fruit husk bits were buried 7.5 cm deep in the soil, except some empty fruiting bodies observed in November 2006, neither fruiting bodies nor spores were observed throughout the observation. The number of fruiting bodies, number of spores and per cent viability of spores cm^{-2} fruit husk area decreased with the increase in storage period and also varied considerably among different storage conditions. On fruit husk bits kept under ambient laboratory conditions, the average number of fruiting bodies, spores and per cent viable spores cm^{-2} fruit husk area decreased from 71.8, 6576 and 93.2 in November 2005 to 10.5, 780 and 11.8 per cent after twelve months of storage in October 2006, respectively. Fruit husk kept on soil surface in open exhibited 71.8 fruiting bodies, 4936 spores and

91.1 per cent viable spores cm^{-2} fruit husk area in November 2005, which reduced to 9.2, 813 and 75.1 per cent up to the last observation in July 2006, respectively.

4.10 Role of Meteorological factors on Didymella blight development

In order to ascertain the role of various meteorological factors on disease development, an attempt was made to correlate the periodic disease intensity and apparent infection rate with prevailing temperature, relative humidity (RH), and precipitation during 2004 and 2005. Weekly data on mean temperature, mean RH, mean precipitation and per cent disease intensity as well as apparent infection rate (unit/day) recorded are presented in Table 15 and Fig. 8.

It is evident from the data that the disease appeared during third week of June in 2004 and during first week of June in 2005 and became conspicuous during the month of August (Table 15 and Fig. 5). The disease reached its peak at the end of September and no disease severity was recorded beyond September because of leaf defoliation due to low temperature in the field. Maximum apparent infection rate of 0.1858 (unit/day) was observed during fifth week of July in 2004 and 0.1826 (unit/day) during second week of July in 2005. The temperature 21.95 and 22.42 ^{o}C, relative humidity 80 and 76 per cent and precipitation 7.54 and 15.77 mm apparently favoured the maximum disease development during these periods.

The data was subjected to statistical analysis for finding out correlation between various meteorological factors and infection rate. The correlation matrix (Table 16) indicated that temperature showed non significant but positive correlation with infection rate whereas, RH and precipitation showed significant and positive correlation.

Stepwise multiple regression analysis was also worked out to know the extent of correlation which is presented in Table 17 and 18. Results of stepwise multiple regression analysis revealed that weather factors accounted for 70.5 per

cent variation. Of these, 70.4 per cent variation was due to RH and precipitation. Precipitation alone contributed to 69.4 per cent variation.

4.10 Disease management

4.10.1 *In vitro* management of the disease

4.10.1.1 *In vitro* screening of systemic fungitoxicants

a) Mycelial growth

The data on *in vitro* efficacy of systemic fungitoxicants tested at 10, 50, 100 and 200 µg ml^{-1} concentration against mycelial growth of *D. bryoniae* is presented in Table 19; Fig. 6a; Plate 9. The data revealed that all the fungitoxicants significantly inhibited mycelial growth of *D. bryoniae* at all test concentrations. Carbendazim proved significantly superior to all other fungitoxicants exhibiting 87.5 per cent mycelial growth inhibition followed by thiophanate methyl (76.1%), myclobutanil (63.0%) and diniconazole (48.7%). Bitertanol proved least effective fungitoxicant with mycelial growth inhibition of 41.6 per cent. On an overall basis the extent of mycelial growth inhibition of the test fungitoxicant increased with increase in their concentration. A minimum inhibition of 28.3 per cent was achieved at 10 µg ml^{-1} which increased gradually to 56.2, 78.5 and 90.5 per cent at 50, 100 and 200 µg ml^{-1} concentration. A significant interaction existed between fungitoxicant and concentration. At lowest concentration of 10 µg ml^{-1} carbendazim showed maximum inhibition in mycelial growth (55.9%) followed by thiophanate methyl (34.8%) and myclobutanil (22.5%), whereas, least inhibition of 11.1 per cent was exhibited by bitertanol. A similar, trend in inhibition of mycelial growth of the test fungus was exhibited by all the treatments, when assayed at 50, 100 and 200 µg ml^{-1} concentrations.

b) Spore germination

Perusal of the data (Table 20, Fig.6b) further revealed that all the test systemic fungitoxicants significantly differed in inhibiting the spore germination of *D. bryoniae*. Carbendazim, irrespective of concentration, proved significantly superior to all other fungitoxicants exhibiting 74.6 per cent inhibition of spore germination followed by thiophanate methyl (69.5%) and myclobutanil (59.9%) and bitertanol (54.8%). Diniconazole with 45.6 per cent of spore germination inhibition was the least effective fungitoxicant. On an overall basis the inhibitory effect of test fungitoxicants on per cent spore germination increased significantly with the increase in concentration. A minimum inhibition of 31.6 per cent was achieved at 10 µg ml^{-1} and the maximum inhibition of 95.1 per cent at 200 µg ml^{-1} concentration. A significant interaction existed between fungitoxicant and concentration. At lowest concentration of 10 µg ml^{-1}, carbendazim showed maximum (49.2%) inhibition in spore germination and least inhibition (16.4%) was observed by diniconazole. A similar, trend in inhibition of spore germination was exhibited by other treatments assayed at 50, 100 and 200 µg ml^{-1} concentrations.

4.10.1.2 *In vitro* screening of non systemic fungitoxicants

a) Mycelial growth

The data on effect of non-systemic fungitoxicants at 100, 250, 500 and 1000 µg ml^{-1} concentrations on the mycelial growth of *d. Bryoniae* is presented in table 21, fig 7a, plate 10. Perusal of data revealed that all the fungitoxicants at all the concentrations significantly inhibited the mycelial growth of *d. Bryoniae*. On an average, maximum inhibition in mycelial growth (67.4%) was exhibited by chlorothalonil followed by mancozeb (63.0%), zineb (50.5%) and captan (47.2%). A minimum inhibition of 36.9 per cent in mycelial growth of the test pathogen was exhibited by copper

oxychloride. The fungitoxicant concentrations differed significantly from each other, irrespective of treatments. The per cent inhibition in mycelial growth was least (13.9%) at 100 µg/ml concentration, which increased to 34.0, 69.9 and 94.1 per cent at 250 and 500 and 1000 µg/ml concentrations, respectively. A significant interaction existed between treatments and their concentrations. At 100 µg/ml concentration, the chlorothalonil showed maximum (22.9%) inhibition in mycelial growth followed by mancozeb (19.6%) and zineb (15.1%). At 250 µg/ml concentration, chlorothalonil again exhibited maximum inhibition of mycelial growth (46.6%) followed by mancozeb (45.1%) and zineb (31.4%). Similar, trend in inhibition of mycelial growth was observed, when 500 and 1000 µg/ml concentrations were assayed.

the data (table 22, fig.7b) on *in vitro* efficacy of non-systemic fungitoxicants at above tested concentrations on the spore germination of *d. Bryoniae* revealed that on an average, maximum inhibition in spore germination of the test pathogen was exhibited by chlorothalonil (74.3%) followed by mancozeb (65.1%) and zineb (58.9%). A minimum inhibition of 45.4 per cent in spore germination of the test pathogen was exhibited by copper oxychloride. The fungitoxicant concentrations significantly varied from each other, irrespective of treatments. The per cent inhibition in spore germination was least (22.6%) at 100 µg ml^{-1} concentration, which increased to 47.1, 69.1 and 98.7 at 250, 500 and 1000 µg ml^{-1} concentrations, respectively. There also existed a significant interaction between treatments and their concentrations. At 10 per cent concentration, the chlorothalonil showed maximum (42.8%) inhibition in spore germination followed by mancozeb (30.8%) and zineb (22.6%). A similar,

trend in inhibition of spore germination was exhibited by all the treatments assayed at 250, 500 and 1000 µg ml^{-1} concentrations.

4.10.1.3 *In vitro* screening of botanicals

a) Mycelial growth

The results on effect of aqueous plant extracts tested at 1, 5, 10 and 15 per cent concentration on the mycelial growth of *D. bryoniae* are presented in Table 23; Fig. 8a; Plate 11. The results revealed that on an average, maximum inhibition in mycelial growth of the test pathogen was exhibited by *Allium sativum* (53.5%) followed by *Allium cepa* (39.7%) and *Artemesia annua* (27.6%). The least inhibition of 17.0 per cent in mycelial growth of the test pathogen was exhibited by *Urtica dioica.* The inhibitory effect of extract concentrations increased with the increase in concentration. On an average basis, the per cent inhibition in mycelial growth was least (5.0%) at 1 per cent concentration, which increased to 20.1, 39.0 and 60.8 at 5, 10 and 15 per cent concentrations, respectively. A significant interaction existed between treatments and their concentrations. At 1 per cent concentration *A. sativum* exhibited maximum (9.6%) inhibition in mycelial growth followed by *A. capa* (6.3%) and *A. annua* (5.1%). A similar, trend in inhibition of mycelial growth was exhibited by all the treatments, when assayed at 5, 10 and 15 per cent concentrations.

b) Spore germination

The perusal of Table 24, Fig. 7b indicated the supremacy of *A. sativum* over other plant extracts in inhibiting the spore germination of test fungus. On an overall mean basis, *A. sativum* exhibiting maximum inhibition (47.6%) and was followed by *A. cepa* (41.4%t) and *A. annua*

(34.0 %). The extract of *U. dioica* (22.4%) exhibited the least inhibition of spore germination of the test pathogen. On an overall basis the extent of spore germination inhibition increased with increase in extract concentration. The per cent inhibition in spore germination was least (8.4%) at 1 per cent concentration, which increased to 25.1, 43.1 and 59.9 per cent at 5, 10 and 15 per cent concentrations, respectively. A significant interaction existed between treatments and their concentrations. At 1 per cent concentration, the aqueous plant extract of *A. sativum* showed maximum (14.7%) inhibition in spore germination followed by *A. cepa* (10.1%), whereas, least inhibition (3.5%) was exhibited by *U. dioica.* A similar, trend in inhibition of spore germination was exhibited by all the treatments, when assayed at 5, 10 and 15 per cent concentrations.

4.10.2 *In vivo* management of the disease

The experiments were conducted during 2004 and 2005 to study the effect of most promising fungicides and botanical found under *in vitro* studies as seed treatment and foliar sprays against Didymella blight of ridge gourd. The results on disease incidence, disease intensity, infected fruits and yield of ridge gourd fruits are presented in Tables 25-32.

4.10.2.1 Disease incidence on leaves

The data on the effect of various seed treatments and foliar sprays on incidence of Didymella blight of ridge gourd on leaves during 2004 revealed that all the seed treatments, foliar sprays and their combinations significantly reduced the disease incidence as compared to control (Table 25). Carbendazim seed treatment plus carbendazim foliar sprays proved superior over all other treatments as it produced 13.00 per cent disease incidence as against 68.66 per cent observed in control. The above treatment combination was statistically at par with chlorothalonil seed

treatment plus carbendazim foliar sprays, *A. sativum* seed treatment plus carbendazim foliar sprays which showed 14.00 and 17.33 per cent disease incidence, respectively. The next effective treatment combinations were seed treatment with chlorothalonil or carbendazim in combination with chlorothalonil foliar sprays which exhibited 23.66 and 26.66 per cent disease incidence, respectively, with no significant difference between them. The least effective treatment combinations were seed treatment with carbendazim plus *A. sativum* foliar sprays (33.33%), seed treatment with chlorothalonil plus *A. sativum* foliar sprays (36.33%) and seed treatment with *A. sativum* plus *A. sativum* foliar sprays (38.33%).

The individual effect of all the seed treatments proved significantly superior over the control. The least average disease incidence of 30.08 per cent was recorded in case of carbendazim followed by chlorothalonil (32.16%) and *A. sativum* (35.99%).

The data on individual effect of foliar sprays reveals that all the foliar sprays tested differed significantly from one another and were statistically superior over control in reducing the disease incidence. Carbendazim foliar sprays recorded least disease incidence of 15.58 per cent, followed by chlorothalonil and *Allium sativum* with disease incidence of 28.08 and 36.16 per cent, respectively.

Data pertaining to year 2005 revealed that all the treatment combinations of seed treatment and foliar sprays significantly reduced the disease incidence on leaves as compared to control, which recorded highest disease incidence of 75.00 per cent (Table 26). Seed treatment with carbendazim, *A. sativum* or chlorothalonil in combination with carbendazim foliar sprays recorded the least disease incidence of 14.33, 16.66 and 17.00 per cent respectively. These treatment combinations were followed by

carbendazim seed treatment plus chlorothalonil foliar sprays (26.00%), chlorothalonil seed treatment plus chlorothalonil foliar sprays (26.66%) and *A. sativum* seed treatment plus chlorothalonil foliar sprays (28.00%) with no significant difference among all of them. Seed treatment with carbendazim, chlorothalonil or *A. sativum*, when foliar sprays were given by *A. sativum* were the least effective treatment combinations which exhibited the disease incidence of 40.00, 41.66 and 43.66 per cent respectively with statistically no significant difference among them.

Amongst seed treatments, all the treatments recorded significantly lesser disease incidence as against 41.83 per cent observed in control. Carbendazim recorded the least disease incidence of 34.50 per cent followed by chlorothalonil (37.25%) and *A. sativum* (37.75%) with no significant difference between them.

The data on effect of foliar sprays revealed that all the foliar sprays were significantly superior over the control, which recorded 64.75 per cent disease incidence on leaves. Carbendazim proved best with 16.33 per cent disease incidence followed by chlorothalonil (27.58%) and *A. sativum* (42.66%).

4.10.2.2 Disease intensity on leaves

The data on efficacy of seed treatments and foliar sprays alone and in combinations, against Didymella blight intensity on leaves is presented in Table 27. It indicates that all the treatments recorded significantly lower disease intensity compared to control. Seed treatment with carbendazim (Plate 12a), chlorothalonil or *A. sativum* in combination with carbendazim foliar sprays were most effective exhibiting 2.93, 3.93 and 4.06 per cent

disease intensity, respectively, as against 40.06 per cent in control (Plate 12b), with no significant difference between all of them. Seed treatment with carbendazim, chlorothalonil or *A.* sativum, when foliar sprays were given with chlorothalonil recorded disease intensity of 7.73, 8.26 and 8.60 per cent, respectively, which were all at par with each other. Seed treatment with *A. sativum* in combination with *A. sativum* foliar sprays was the least effective treatment combinations which recorded 14.46 per cent disease intensity.

The individual effect of seed treatments on the intensity of Didymella blight on leaves indicated that all the treatments significantly reduced the disease intensity compared to control. On an average, carbendazim was the most effective recording 11.23 per cent disease intensity (Plate 12c), as against 17.16 per cent in control. Seed treatment with *A. sativum* was least effective with 14.93 per cent disease intensity.

The data on individual effect of foliar sprays revealed that all the foliar sprays tested recorded significantly lower disease intensity as against 31.05 per cent in control. On an average carbendazim as foliar sprays proved best with 3.80 per cent disease intensity (Plate 12d) followed by chlorothalonil (8.38%) and *A. sativum* (13.26%).

The data presented in Table 28 reveals that during 2005, carbendazim seed treatment plus foliar sprays with the same fungicide (3.73%), chlorothalonil seed treatment plus carbendazim foliar sprays (4.00%) and *A. sativum* seed treatment plus carbendazim foliar sprays (4.26%) were the most effective in reducing the disease intensity as against the control (40.46%). These treatment combinations were followed by seed treatment with carbendazim, *A. sativum* or chlorothalonil in combination

with chlorothalonil foliar sprays which recorded 8.00, 8.80 and 8.93 per cent disease intensity, respectively, with no significant difference among them. Seed treatment with carbendazim plus foliar sprays with *A. sativum*, seed treatment with *A. sativum* plus *A. sativum* foliar sprays and seed treatment with chlorothalonil plus foliar sprays with *A. sativum* were the least effective exhibited 13.86, 14.40 and 14.46 per cent disease intensity with no significant difference between them.

Out of seed treatments, carbendazim was the most effective which exhibited 14.30 per cent disease intensity as against 17.28 per cent in control followed by chlorothalonil (16.06%) and *A. sativum* (16.18%).

All the foliar sprays differed significantly from each other and were superior over the control, which recorded average disease intensity of 36.55 per cent. Carbendazim proved best foliar spray recording an average disease intensity of 4.18 per cent, which was significantly less than that of chlorothalonil and *A. sativum* which exhibited 8.71 and 14.38 per cent disease intensity, respectively.

4.10.2.3 Per cent Infected fruits

Data on effect of various seed treatments and foliar sprays on Didymella blight incidence of ridge gourd fruits during 2004 and 2005 is presented in Tables 29-30.

Data pertaining to year 2004 revealed that all the treatment combinations of seed treatment and foliar sprays recorded significantly lesser number of infected fruits as compared to control, which recorded 14.50 per cent infected fruits (Table 29). Carbendazim seed treatment plus carbendazim foliar sprays recorded least number of infected fruits (1.41%) and was statistically at par with chlorothalonil seed treatment plus

carbendazim foliar sprays (1.44%), *A. sativum* seed treatment plus carbendazim foliar sprays (1.50%), carbendazim seed treatment plus chlorothalonil foliar sprays (2.76%), and chlorothalonil seed treatment plus chlorothalonil foliar sprays (2.83%). Seed treatment with carbendazim, chlorothalonil or *A. sativum* in combination with *A. sativum* foliar sprays were all statistically at par and least effective treatment combinations recording 5.57, 6.75 and 7.28 per cent infected fruits, respectively.

Amongst seed treatments, only carbendazim recorded significantly lesser number of infected fruits (5.13%) as compared to control which recorded 6.92 per cent infected fruits. Chlorothalonil (6.02%) and *A. sativum* (6.47%) could not significantly reduce the number of infected fruits as compared to control.

Data on individual effect of foliar sprays revealed that all the foliar sprays differed significantly from each other and were superior over the control, which recorded 12.98 per cent infected fruits. Carbendazim proved best chemical for foliar sprays recording 1.60 per cent infected fruits followed by chlorothalonil (3.17%) and *A. sativum* (6.79%).

Data pertaining to experimental year 2005 (Table 30) revealed that all the treatment combinations recorded significantly lesser number of infected fruits as compared to control, which recorded highest number of infected fruits (18.11%). Carbendazim seed treatment plus carbendazim foliar sprays recorded the least number of infected fruits (1.44%) and was statistically at par with chlorothalonil or *A. sativum* seed treatment in combination with carbendazim foliar sprays which exhibited 1.46 and 1.58 per cent infected fruits, respectively. The above treatment combinations were followed by carbendazim seed treatment plus chlorothalonil foliar

sprays (2.30%), chlorothalonil seed treatment plus chlorothalonil foliar sprays (2.95%) and *A. sativum* seed treatment plus chlorothalonil foliar sprays (3.41%) with no significant difference between them. The least effective treatment combination with 6.51 per cent infected fruits was *A. sativum* seed treatment plus *A. sativum* foliar sprays.

Amongst seed treatments, all the treatments except *A. sativum* (7.08%) recorded significantly lesser number of infected fruits as compared to control which recorded 8.00 per cent infected fruits. Carbendazim and chlorothalonil were at par recording 5.29 and 5.69 per cent infected fruits, respectively.

All the foliar sprays tested recorded significantly lesser number of infected fruits as compared to control, which recorded 14.52 per cent infected fruits. Carbendazim proved superior to all other foliar sprays recording 1.65 per cent infected fruits followed by chlorothalonil (3.20%) and *A. sativum* (6.68%)..

4.10.2.4 Number of fruits

Data on effect of various seed treatments and foliar sprays on number of fruits plot^{-1} of ridge gourd crop during 2004 and 2005 is presented in tables 31-32.

Perusal of data (Table 31) revealed that during the experimental year 2004, all the treatments combinations increased the number of fruits plot^{-1} significantly over the control, which recorded least 23.6 fruits/plot. Carbendazim seed treatment plus carbendazim foliar sprays, chlorothalonil seed treatment plus carbendazim foliar sprays and *A. sativum* seed treatment plus carbendazim foliar sprays were the most effective treatment combinations with 69.6, 69.3 and 65.3 fruits plot^{-1}, respectively and were

statistically at par with one another. These treatment combinations were followed by seed treatment either with carbendazim, chlorothalonil or *A. sativum* in combination with chlorothalonil foliar sprays with 59.3, 58.3 and 55.0 fruits plot^{-1}, respectively. Carbendazim seed treatment plus *A. sativum* foliar sprays (39.6 fruits/ plot), chlorothalonil seed treatment plus *A. sativum* foliar sprays and *A. sativum* seed treatment plus *A. sativum* foliar sprays, with 39.6, 38.0 and 35.0 fruits plot^{-1} were the least effective and statistically at par with each other.

The data on individual effect of seed treatments revealed that all the treatments except *A. sativum* increased the number of fruits plot^{-1}. Highest average number of 49.0 and 48.1 fruits plot^{-1} was obtained in case of carbendazim and chlorothalonil, respectively, which were at par with each other but superior over *A. sativum* which recorded an average yield of 45.2 fruits plot^{-1}. Least fruit yield of 44.1 fruits plot^{-1} was obtained in case of control.

All the foliar sprays differed significantly from each other and were superior over the control, which recorded least average number of fruits (25.9 plot^{-1}). Highest average number of fruits (67.2 plot^{-1}) was recorded in case of carbendazim followed by chlorothalonil (56.6 plot^{-1}) and *A. sativum* (36.7 plot^{-1}).

It is evident from the data (Table 32) that during the experimental year 2005, all the treatment combinations increased the number of fruits significantly as compared to control (22.0 fruits plot^{-1}).. Carbendazim seed treatment plus carbendazim foliar sprays (68.0), chlorothalonil seed treatment plus carbendazim foliar sprays (67.3) and *A. sativum* seed treatment plus carbendazim foliar sprays (62.0) recorded highest number of

fruits plot^{-1} and were statistically at par with one another. Seed treatment with carbendazim, chlorothalonil or *A. sativum* in combination with chlorothalonil foliar sprays recorded 58.3, 56.6 and 57.3 fruits plot^{-1}, respectively, with all of these statistically at par with each other. *A. sativum* seed treatment plus *A. sativum* foliar sprays was the least effective treatment combination with 38.0 fruits plot^{-1}.

Data on individual effect of seed treatments revealed that only carbendazim (48.0 fruits plot^{-1}) and chlorothalonil (47.3 fruits plot^{-1}) seed treatment significantly increased the number of fruits plot^{-1} as against the 42.9 fruits in case of control. *A. sativum* with 45.6 fruits plot^{-1} was statistically non significant as compared to control.

All the foliar sprays differed significantly from each other and were superior over the control, which recorded least average number of 25.4 fruits plot^{-1}. Highest average yield of 64.7 fruits plot^{-1} was recorded in case of carbendazim followed by chlorothalonil (56.2 fruits plot^{-1}) and *A. sativum* (37.5 fruits plot^{-1}), respectively.

4.10.2.5 Yield

Data on effect of various seed treatments and foliar sprays on yield of ridge gourd is presented in Tables 31-32.

Perusal of data pertaining to experimental year 2004 (Table 31) revealed that all the treatment combinations of seed treatment and foliar sprays significantly increased fruit yield compared to 34.8 q ha^{-1} observed in control. Carbendazim seed treatment when foliar sprays were given with carbendazim produced the highest yield of 130 q ha^{-1} and was statistically at par with chlorothalonil seed treatment plus carbendazim foliar sprays (129.8 q ha^{-1}). Carbendazim seed treatment plus chlorothalonil foliar

sprays, chlorothalonil seed treatment plus chlorothalonil foliar sprays and *A. sativum* seed treatment plus chlorothalonil foliar sprays were the next best treatments with fruit yield of 107.7, 105.1 and 98.3 q ha-1, respectively. *A. sativum* seed treatment plus *A. sativum* foliar sprays proved to be the least effective treatment combination with yield of 58.8 q ha^{-1}.

Analysis of data for individual effect of seed treatments revealed that all the seed treatments significantly increased the fruit yield as against 76.2 q ha^{-1} in case of control. Carbendazim, chlorothalonil and *A. sativum* recorded average yields of 87.1, 85.6 and 79.3 q ha^{-1}, respectively, with no significant difference between carbendazim and chlorothalonil.

All the foliar sprays tested were highly effective in increasing the fruit yield as compared to control, which recorded average yield of 40.8 q ha^{1}. Highest average fruit yield of 123.7 q ha^{-1}, among foliar sprays was recorded in case of carbendazim followed by 101.6 and 62.2 q ha^{-1} in chlorothalonil and *A. sativum*, respectively.

The data presented in Table 32 depicted that all the treatments enhanced the yield significantly over the control, which recorded least fruit yield of 33.3 q ha^{-1} during the experimental year 2005. Seed treatment with carbendazim along with carbendazim foliar sprays and chlorothalonil seed treatment plus carbendazim foliar sprays proved best recording highest yield of 126.3 and 121.0 q ha^{-1} followed by *A. sativum* seed treatment plus carbendazim foliar sprays (113.0 q ha-1). The next best treatment combinations were carbendazim, chlorothalonil or *A. sativum* seed treatment in combination with chlorothalonil foliar sprays. *A. sativum* seed treatment plus *A. sativum* foliar sprays proved least effective treatment combination with fruit yield of 63.1 q ha-1.

Further analysis of the data for individual effect of seed treatments revealed that highest average fruit yield of 85.2 q ha^{-1} was recorded in case of carbendazim followed by chlorothalonil (82.1 q ha^{-1}) and *A. sativum* (78.4 q ha^{-1}). The least fruit yield of 73.8 q ha^{-1} was recorded in control.

All the foliar sprays differed significantly from each other and were superior over the control, which recorded least average yield of 39.9 q ha^{-1}. Highest average yield of 118.1 q ha^{-1} was recorded in case of carbendazim followed by chlorothalonil (98.8 q ha^{-1}) and *A. sativum* (62.7 q ha^{-1}).

Chapter – 5

DISCUSSION

Ridge gourd (*Luffa acutangula* (L.) Roxb.) is an important cucurbitaceous vegetable crop. The crop is prone to a number of biotic stresses which inflict considerable yield losses under natural epiphytotic conditions. Amongst them Didymella blight caused by *Didymella bryoniae* (Auersw.) Rehm has emerged in the recent past as one of the major constraints in the successful cultivation of the crop in Kashmir valley and has been responsible for causing a marked reduction in both quality and quantity of the fruit yield.

The preliminary survey has revealed the occurrence of Didymella blight in most of the commercial ridge gourd growing areas in Kashmir valley which necessitated exploring various aspects of the disease to have an effective management. Didymella blight was found prevalent in all the eleven major ridge gourd growing areas in districts Srinagar and Budgam of the valley, though in varying proportions. Occurrence of this disease on cucurbit crops has been reported from all over the world (Puninthalingam and Holliday, 1972) including India (Sohi and Prakash, 1972; Kumar and Khan, 1984; Kulwant and Shetty, 1996; Sudisha *et al*., 2004).

In surveyed areas of the valley, the average disease incidence of 32.6 and 36.4 per cent and intensity of 14.8 and 18.8 per cent on leaves, besides disease incidence of 9.6 and 11.2 per cent on fruits was recorded during the year 2004 and 2005, respectively. The higher level of disease was observed in 2005 as compared to 2004, which may be attributed to the higher amount of precipitation received during 2005. Besides precipitation,

higher relative humidity and congenial temperatures favoured pathogen growth and proliferation and thus predisposed the host to the infection. Svedellus and Unestam (1978) found a free film of water necessary for the dispersal and subsequent infection of leaves by the conidia of *D. bryoniae* and after development of spots noted further expansion of spots only in presence of higher relative humidity.

The highest incidence and intensity of the disease as well as fruit infection amongst the surveyed areas was recorded from Dal area followed by Habak and Shalimar. This could be attributed to the very humid conditions prevailing on mounds erected in swamps or on floating gardens of water bodies. Besides this, farmers in Dal, Habak and Shalimar follow monoculture of cucurbits over large contiguous areas. The impact of monoculture (Sitterly, 1969) and humidity on the occurrence and subsequent development of Didymella blight has been earlier reported (Chiu and Walker, 1949b; Svedellus ann Unestam, 1978; Wehner *et al*., 2000). The least disease incidence and intensity recorded at Panzan and Noorbagh seems possibly due to proper field sanitary measures as well as crop rotation adopted by the farmers, however, the use of infected seeds seems to have favoured the disease development at these locations. Breaking the sequence of continuous cucumber growing was found effective in reducing the severity of Didymella blight (Sitterly, 1969). Lee *et al*. (1984) have reported the introduction of the inoculum to previously uninfected areas and subsequent development of the disease by the use of infected seeds.

Symptoms of the disease were observed on leaves and fruits of ridge gourd. On leaves symptoms appeared as small circular, light brown with chlorotic halo spots, which with the passage of time enlarged, developed

concentric rings at the centre and turned dark brown with greyish white centre. The spots enlarged up to 83 mm, coalesced and caused blighting and yellowing of the whole leaf. Black pin head fructifications (pycnidia and pseudothecia) arranged in circles were observed at the centre of the spots. Symptoms on fruits appeared during second week of August as dark green water soaked spots which enlarged rapidly and caused black rot of the whole fruit. Black colour of fruit was due to the abundant pycnidia and pseudothecia of the causal fungus. The present observations on symptomatology agree with those reported by Chiu and Walker (1949b) and Koike (1997), however, they also observed stem infection but in the present studies no such symptoms were observed. Pandey and Pandey (2003) gave similar account of leaf infection but in their reports there is no mention of fruit infection.

The fungal pathogen isolated from the naturally infected ridge gourd plants, when artificially inoculated on potted ridge gourd plants produced typical disease symptoms, 4 days after inoculation. Whereas, on uninjured and injured detached leaf, symptoms appeared 2 and 3 days after inoculation, respectively. Similarly, symptoms on injured fruits appeared 2 days after inoculation, however, symptoms were not observed on inoculated uninjured fruits. Koch's postulates were confirmed by reisolating the pathogen from the artificially inoculated and infected plants and detached parts. Van Steekelenburg (1981) obtained symptoms 3 to 4 days after inoculation of young cucumber plants and found wounding essential for fruit rot. A $1x10^7$ spore suspension of *D. bryoniae* when applied on seedlings of different cucurbitaceous crops developed typical disease symptoms in all the test plants after 48 hours of incubation in polythene tents at 26-28^oC temperature and high relative humidity (Bala and Hosein,

1986). Svedellus and Unestam (1978) observed more severe symptoms three days after inoculation on detached injured leaves of cucumber than on uninjured leaves.

Host range studies indicated that the pathogen, *D. bryoniae*, was not specific to ridge gourd (*Luffa acutangula*) but also produced typical disease symptoms both under natural and artificial inoculation conditions on all the tested cucurbitaceous plant species *viz*., bitter gourd, bottle gourd, cucumber, muskmelon, pumpkin, sponge gourd, squash and watermelon. These findings are in agreement with the observations of Bala and Hosein (1986) who found watermelon isolate of *D. bryoniae* highly pathogenic to watermelon, muskmelon, pumpkin, cucumber, squash, bitter gourd, sponge gourd and ridge gourd. Similarly, Lee *et al*. (1984) found *D. bryoniae* isolated from cucumber pathogenic to pumpkin and watermelon as well, however, observed more severe symptoms on same hosts than the others.

The morphological characters of the pathogen were studied both on host as well as on potato dextrose agar medium. On host the pathogen formed irregularly branched, septate, smooth and hyaline mycelium measuring 3.2-5.3 µm (Av. 4.5 µm) in width, however, in culture it produced hyaline to light brown septate mycelium, slightly bulged at septa which measured 3.2-4.3 µm (Av. 3.6 µm) in width. Black pycnidia of the anamorph (*Phoma cucurbitacearum*) on the host, 120.4 -172.0 µm (Av. 142.7 µm) in diameter were globose in shape, glabrous and contained pycnidiospores which were hyaline, ellipsoidal to allantoid, constricted at septa, with rounded ends and 8.6-12.9 x 3.2-4.3 µm (Av. 11.5 x 3.6 µm) in size, whereas, pycnidia in culture were black, globose to irregular in shape, solitary to confluent, glabrous or with mycelial outgrowths and measured 167.7 – 326.8 µm (Av. 236.5 µm) in diameter, filled with pycnidiospores

which were variable in shape, subglobose to ellipsoidal or allantoid with several small guttules, aseptate rarely one septate, 6.4–9.6 x 2.1-4.3 µm (Av. 8.3 x 3.7 µm) in size. Chiu and Walker (1949a) and Puninthalingam and Holliday (1972) collectively corroborate our identification of the anomorph, *Phoma cucurbitacearum.* Pseudothecia of the teleomarph (*D. bryoniae*) found together with pycnidia on infected tissue which, however, were not observer in culture, were dark, globose to subglobose and measured 146.2-206.4 µm (Av. 186.3 µm) in diameter. The asci were hyaline, bitunicate, cylindrical to subclavate, short stipitate or sessile, biseriate, eight spored and measured 55.9- 64.5 x 10.7-12.9 µm (Av. 62.2 x 11.1 µm) in size. Ascospores were 13.9-17.2 x 4.3- 5.4 µm (Av. 16.4 x 5.1 µm), hyaline, mono-septate, constricted at the septum, multiguttulate and rounded at ends with upper cell usually wider than the lower one. Pseudoparaphyses also observed were hyaline, branched, septate and 2.1-2.6 µm (Av. 2.2 µm) in width. Similar descriptions of the teleomarph *D. bryoniae* have been given by Puninthalingam and Holiday (1972), Corlett *et al*. (1981), Kienath *et al*. (1995) and Pandey and Pandey (2003). Chiu and Walker (1949a), however, observed pseudoparaphyses like structures in young fruiting bodies but they considered them as immature or abortive asci and not as true pseudoparaphyses. However, Skarshaug (1981), Puninthalingam and Holliday (1972) and Corlett (1981) have clearly reported the presence of pseudoparaphyses. Our results on development of only pycnidia as fruiting body are supported by the findings of Arny and Randall (1991) and Urbanszki *et al*. (2002) who observed only pycnidia as fruiting structure in culture.

On the basis of morphological and pathological characteristics and comparison to the authentic description, the pathogen was identified as

Didymella bryoniae (Auersw.) Rehm (anamorph *Phoma cucurbitacearum* (Foutrey) Sacardo) and the identity was confirmed by Dr. P.N. Chowdhary, Ex Principal Scientist, Division of Plant Pathology IARI, during his visit to the Division in the year 2004-05.

Studies on comparative growth and fructification on different agar media revealed that potato dextrose agar and oat meal agar were best media for both mycelial growth and fructification of *D. bryoniae*. Present findings on the suitability of potato dextrose agar and oat meal agar media for supporting maximum growth and fructification of *D. bryoniae* are in conformity to the observations of Chiu and Walker (1949a) and Current (1969) who found these media suitable for the growth and fructification of *D. bryoniae*.

The pathogen *D. bryoniae* was found to grow within a wide pH range of 5.0 to 8.0, however maximum mycelial growth, fructification and spore germination was observed at pH 7.0. The above findings are 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 mycelial growth, fructification and spore germination of *D. bryoniae* varied significantly among different temperatures tested (4-36 $\pm 1^{o}$C). Maximum growth, fructification and spore germination was observed at 24 ± 1^{o}C, however, no growth and as such fructification was observed at 36 ± 1^{o}C. These results on effect of temperature on growth, fructification and spore germination are in conformity with the findings of Chiu and Walker (1949b).

The seeds were found to harbour viable spores of *D. bryoniae* throughout the observation period of twelve months starting from one

month after harvest (in November during both the crop seasons of 2004 and 2005). The extent of viability constantly decreased with the advancement of storage period such that after 12 months of storage seeds harboured 18.6 and 20.3 per cent viable conidia in 2004 and 2005, respectively. Similarly using blotter and agar plate methods, the seeds were found infected with *D. bryoniae* and the infection was found to persist from harvest up to 12 months of storage under ambient room conditions. These findings provide sufficient grounds to infer that the pathogen *D. bryoniae* perpetuates in/on the seeds as spores and the pathogen inoculum reaches the cropping fields along with seeds. Similar observations have also been made by several workers. Rankin (1954) found the invasion of *D. bryoniae* in the epidermis and sclerenchyma layers and isolated the fungus from cotyledons and embryo.Chen and Bao (1990) noted the survival of the fungus both in and on the seeds of infected fruits up to 21 months of storage at room temperature. Sudisha *et al*. (2006) also reported the seed borne nature of *D. bryoniae* and noted mean incidence of 31 per cent in seed coat, 11 per cent in cotyledons and 4 per cent in embryo while evaluating the different components of infected seeds. Similarly,

The other possibility of the pathogen survival during cropless winter could be through infected crop debris which includes infected leaves and infected fruit husk. The periodic examination of the infected debris placed indoors, on soil surface and buried under soil indicated the presence of fruiting bodies as well as viable spores throughout the observation period of twelve months when placed indoors under roof protection and only up to four to five months when left on soil surface as debris perished beyond this period. However, only empty fruiting bodies were noticed during January and February when left on the ground surface, suggesting the survival of *D.*

bryoniae as dormant mycelium. The survival of the pathogen lasted for only four to five months when buried in soil, beyond this period debris was decomposed. The lower recovery rate of *D. bryoniae* from the infected plant debris buried in soil or placed on soil surface could be because of the fact that the hibernating pathogen was exposed to chilly winters, and had to compete with other soil inhabiting microorganisms for food and niche, a situation quite different than the one when the trash is stored under roof protection. Kienath (2002) could not recover *D. bryoniae* beyond 6 to 7 months of storage in buried infected watermelon debris which he attributed to the antagonistic activities of saprophytic soil microorganisms that seemed to have eliminated the pathogen resident in the watermelon debris. Van Steekelenburg (1983) observed old pseudothecia with ascospores and pycnidia with some pycnidiospores, even after storage of diseased plant debris for 18 months, whereas on debris kept on soil surface in open only empty fruiting bodies were observed during winter months when average monthly temperature was below 5^{o}C. Similarly, Chiu and Walker (1949a) found empty pycnidia and pseudothecia in the overwinterd crop debris but could isolate the fungus readily and suggested the overwintering of fungus in winter as dormant mycelium.

The correlation studies of disease development with meteorological factors revealed that the period from fifth week of July to first week of August and second to third week of July during 2004 and 2005, respectively, favoured the maximum disease development in terms of infection rate (unit/ day) as during these periods maximum infection rate of 0.1858 and 0.1826, respectively was observed. A temperature of 21.95 and 22.42^{o}C, 80 and 76 per cent relative humidity and 7.54 and 15.77 mm precipitation apparently favoured the maximum disease development

during these periods, respectively. These studies indicated the maximum disease development during the periods of highest rainfall and humidity of both 2004 and 2005 cropping seasons and thus suggest the relationship between environmental factors and disease development. The disease development in terms of infection rate (unit/ day) was found positively correlated with temperature, relative humidity and rainfall, though non-significantly with temperature. The step-wise multiple regression analysis showed that the weather factors accounted for 70.5 per cent variation of disease development. Of these precipitation alone contributed to 69.4 per cent variation. These observations derive support from the findings of other scientists who studied the effect of meteorological factors on Didymella blight of various cucurbit crops. Chiu and Walker (1949b) found moisture more important for disease development than temperature and recorded peak ascospore dispersal after rain and during dew periods at night. Similarly, Svedellus and Unestam (1978) and Kienath *et al*. (1995) noted free moisture on leaves necessary for infection and further lesion expansion. Van Steekelenburg (1983) found minimum temperature of 5 to 10^{o}C along with moisture important for fructification of *D. bryoniae* and found ascospore release dependent on high relative humidity. Disease development was also found dependent on humid conditions by Vawdrey (1994) and Wehner and Shetty (2000).

Studies on *in vitro* evaluation of five systemic and fine non-systemic fungitoxicants as well as five botanical extracts at different concentrations against the mycelial growth and spore germination of *D. bryoniae* were conducted to get a preliminary idea about the fungitoxicants and botanical to be used under field conditions against the disease. The studies revealed that among systemic fungitoxicants carbendazim exhibited maximum

inhibition of 87.5 and 74.6 per cent of the mycelial growth and spore germination, respectively, followed by thiophanate methyl (76.1 and 69.5%) and myclobutanil (63.0 and 59.9%), while as among non-systemic fungitoxicants chlorothalonil proved most effective exhibiting 67.4 and 74.3 per cent inhibition of mycelial growth and spore germination, respectively, which was followed by mancozeb causing an inhibition of 63.0 and 65.1 per cent, respectively. The present findings on the supremacy of carbendazim and chlorothalonil among systemic and non-systemic fungitoxicants, respectively, are in agreement with the results reported by Van Steekelenburg (1978), who found 100 and 55.4 per cent mycelial growth inhibition by carbendazim and chlorothalonil, respectively, at 100 µg ml^{-1} concentration. The present results on effect of fungitoxicants on spore germination could not be compared as no such type of studies have earlier been carried out. *A. sativum* among botanicals tested against *D. bryoniae* was found superior with 53.5 and 47.6 per cent inhibition of mycelial growth and spore germination, respectively. The effectiveness of various botanical against *D. bryoniae* has previously been reported by Flori *et al*. (2000) who reported the 100 per cent inhibition of mycelial growth and spore germination of *D. bryoniae* by essential oils of *Cymbopogan citrates, Ageratum conyzoides* and *Eucalyptus citriodora*. Similarly, Favaron et el. (1993) reported inhibitory effect of onion and garlic extracts against the polygalacturonases produced *in vitro* by *D. bryoniae*.

Studies were made to control Didymella blight of ridge gourd under field conditions with seed treatments and foliar sprays individually and in combinations by the most effective treatments found under *in vitro* conditions. It was observed that all the treatments, individually or in combinations, restrained the disease incidence, intensity and percentage of

infected fruits to a level significantly lower than that of control and appreciably enhanced the fruit yields in both the experimental years. The trend followed by various treatments with respect to their efficacy during the two years was also similar. Results revealed that the treatment combination of seed treatment with carbendazim along with foliar spray of carbendazim proved best, which gave least disease incidence of 13.00 and 14.33 per cent, intensity 2.93 and 3.73 per cent, infected fruits 1.41 and 1.44 per cent as against the control which recorded highest incidence of 68.66 and 75.00 per cent, intensity 40.06 and 40.46 per cent and infected fruits 14.50 and 18.11 per cent, during 2004 and 2005, respectively. Carbendazim application as seed treatment and foliar sprays was also found most effective against Didymella blight of cucumber (Van Steekelenburg, 1978) and watermelon (Hopkin, 1973). Highest number of fruits 69.66 and 68.00 as well as fruit yield of 130.0 and 126.3 q ha^{-1} was also recorded in case of treatment combination, carbendazim seed treatment together with carbendazim foliar sprays and lowest number of fruits 23.66 and 22.00 $plot^{-1}$ and yield of 34.8 and 33.3 q ha^{-1} were recorded in control during 2004 and 2005, respectively. Other treatment combinations, which proved very effective, were chlorothalonil or *A. sativum* seed treatment in combination with carbendazim foliar sprays. Effectiveness of carbendazim in reducing the didymella blight severity of roackmelon as well as in increasing the yield has been reported by Vawdrey (1994).

CHAPTER – 6

SUMMARY AND CONCLUSION

Didymella blight of ridge gourd caused by *Didymella bryoniae* is a serious disease and a limiting factor in successful cultivation of ridge gourd in Kashmir valley. The survey conducted in major ridge gourd growing areas of the valley in 2004 and 2005 revealed that the disease was prevalent in all the locations surveyed, though in varying proportions. The average disease incidence of 32.6 and 36.4 per cent and intensity of 14.8 and 18.8 per cent on leaves, besides the disease incidence of 9.6 and 11.2 per cent on fruits was recorded during the year 2004 and 2005, respectively. Disease in ridge gourd fields was first noticed during third week of June, progressed steadily and reached its peak by the end of September. The causal fungus was found to infect leaves and fruits of the plant. Symptoms on leaves appeared as small circular, light brown spots with chlorotic halo. The spots with passage of time enlarged, developed concentric rings at the centre and turned dark brown with grayish white centre. The spots enlarged up to 83 mm, coalesced and caused blighting and yellowing of whole leaf. Black pycnidia followed by pseudothecia, arranged in circles, were observed at the centre of the spots. Symptoms on fruits appeared during second week of August as dark green circular, water soaked spots which enlarged rapidly and caused black rot of the whole fruit. Black colour of the affected fruits was due to the abundant pycnidial and pseudothecial formation of the causal fungus.

The causal fungus was obtained from diseased leaf samples in pure culture and its pathogenicity proved. Pathogen exhibited characteristic

symptoms within 4 days after inoculation and produced pycnidia as well as pseudothecia, 12 and 15 days after symptom expression, respectively.

Morphological studies of the causal pathogen were studied both on the host as well as in its pure culture. The fungus in culture produced submerged hyphae with sparse aerial mycelium. The colonies were circular with cottony growth, initially white to dirty white, turned greyish and finally black. The pathogen produced septate mycelium which was irregularly branched, slightly bulged at septa, hyaline to light brown in culture and measured 3.2 to 4.3 µm. (Av. 3.6 µm). Black pycnidia formed after 10 to 12 days of incubation were globose to irregular in shape, solitary to confluent, glabrous or with mycelial outgrowths and measured 167.7 – 326.8 µm (Av. 236.5 µm) in dia. Pycnidiospores were variable in shape, subglobose to ellipsoidal or allantoid with several small guttules, aseptate rarely one septate, 6.4–9.6 x 2.1-4.3 µm (Av. 8.3 x 3.7 µm) in size. On infected tissue of leaf and fruit black pycnidia of the anamorph (*P. cucurbitacearum*), 120.4 -172.0 µm (Av. 142.7 µm) in dia, were globose in shape, subepidermal, solitary, arranged in circles and filled with large number of pycnidiospores. The pycnidiospores were hyaline, ellipsoidal to allantoid, one septate rarely two septate, constricted at septa, with rounded ends and 8.6-12.9 x 3.2-4.3 µm (Av. 11.5 x 3.6 µm) in size. Pseudothecia, found together with pycnidia on infected tissue were dark, globose to subglobuse, subepidermal, solitary and measured 146.2-206.4 µm (Av. 186.3 µm) in dia. The asci were hyaline, bitunicate, cylindrical to subclavate, short stipitate or sessile, biseriate, eight spored and measured 55.9- 64.5 x 10.7-12.9 µm (Av. 62.2 x 11.1 µm) in size. Ascospores were 13.9-17.2 x 4.3- 5.4 µm (Av. 16.4 x 5.1 µm), hyaline, mono septate, constricted at the septum, multiguttulate and rounded at ends with upper

cell usually wider than the lower one. Pseudoparaphyses were observed hyaline, branched, septate and 2.1-2.6 µm (Av. 2.2 µm).

On the basis of source of isolation and morphological characters of isolate in pure culture as well as on host, the fungus inciting the disease was identified as *Didymella bryoniae* (Auerswarld) Rehm [Anamorph *Phoma cucurbitacearum* (Foutr.) Sacardo].

D. bryoniae, isolated from ridge gourd was pathogenic to all the eight cucurbitaceous plant species tested *viz*., bottle gourd, bitter gourd, cucumber, muskmelon, pumpkin, sponge gourd, squash and watermelon both under natural and artificially inoculated conditions. However, the incubation period varied among different species which was 6 days for sponge gourd, 7 days for bottle gourd, bitter gourd and cucumber, 9 days for muskmelon and watermelon and 10 days for pumpkin and squash.

Potato dextrose agar and corn meal agar media favoured maximum mycelial growth and fructification of the fungus. Mycelial growth and spore germination was highest at temperature of 24 ± 1^0C and pH of 7.0 with no spore germination recorded at 12^oC and least at 32^oC.

The correlation of various meteorological factors with disease development in terms of infection rate (unit/ day) indicated that temperature, humidity and rainfall were positively correlated with infection rate and rainfall alone had an impact of 69.4 per cent on it.

The perpetuation studies of the pathogen in/on seed indicated the survival of pathogen through seeds as spores. The viability of spores however, decreased from 93.9 in November 2004 to 18.6 in October 2005 and from 93.7 in November 2005 to 20.3 per cent in October 2006, after harvest in 2004 and 2005, respectively. The percentage of seeds showing *D.*

bryoniae growth also declined with the advancement in storage period. In blotter method, 45.0 and 48.3 per cent seeds showed *D. bryoniae* growth immediately after harvest in November 2004 and 2005, respectively, which gradually decreased to 16.6 and 18.3 per cent after twelve months of storage in October 2005 and 2006, respectively. However, in agar plate method, 41.6 and 45.0 per cent seeds showed *D. bryoniae* growth after one month of harvest in November 2004 and 2005, respectively, which declined gradually to 8.3 and 11.6 per cent after twelve months of storage in October 2004 and 2005, respectively.

The studies on perpetuation through plant debris indicated that the leaf and fruit husk bits, harvested in November 2004 and 2005 and stored indoors, exhibited the presence of fruiting bodies and viable spores throughout the observation period of twelve months. However, fruiting bodies and viable spores were found only up to June and July on leaf and fruit husk bits, respectively, besides two months of January and February when only empty fruiting bodies were noticed. The fruiting bodies and as such spores were altogether absent throughout the observation period on leaf and fruit husk bits buried at 7.5 cm deep in soil, although, some empty fruiting bodies were observed during November of both the years of experimentation.

The *in vitro* evaluation of fungitoxicants and aqueous extracts of different botanicals against *Didymella bryoniae* indicated that all the fungitoxicants and botanical extracts significantly inhibited the spore germination and mycelial growth of the test pathogen. Among the systemic fungitoxicants, carbendazim proved most effective with inhibition of 87.5 and 74.6 per cent of the mycelial growth and spore germination, respectively whereas among the non-systemic fungitoxicants, chlorothalonil

proved most effective causing 67.4 and 74.3 per cent inhibition of mycelial growth and spore germination, respectively. Among botanical extracts *Allium sativum* gave highest inhibition of 53.5 and 47.62 per cent of mycelial growth and spore germination, respectively, of the test pathogen.

Studies were made to control Didymella blight of ridge gourd under field conditions with seed treatments and foliar sprays individually and in combinations by the most promising treatments found under *in vitro* conditions. It was observed that all the treatments, restrained the disease incidence, intensity, percentage of infected fruits level significantly lower than that of control and enhanced the fruit yields appreciably in both the experimental years. Carbendazim seed treatment in combination with three foliar sprays of carbendazim at an interval of 15 days proved best, which gave least disease incidence (13.00 and 14.33%), disease intensity (2.93 and 3.73%) and infected fruits (1.41 and 1.44%) and highest number of fruits (69.66 and 68.00 per $plot^{-1}$) and yield (130 and 126.3 q ha^{-1}), during 2004 and 2005, respectively. The next effective treatment combinations were chlorothalonil seed treatment plus carbendazim foliar sprays, *A. sativum* seed treatment plus carbendazim foliar sprays and carbendazim seed treatment plus chlorothalonil foliar sprays. *A. sativum* seed treatment and foliar sprats though least effective, managed the disease significantly and increased the yield appreciably as compared to control.

In light of present investigations, it is deduced that:-

- Didymella blight is prevalent in all ridge gourd growing areas of the valley in moderate to severe form.
- The disease is a potential threat to ridge gourd and also to other cucurbits grown in valley.

- Symptoms appear on leaves and fruits of the plant during the month of June and August, respectively
- The causal pathogen of the disease was identified as *Didymella bryoniae* (Auerswarld) Rehm [Anamorph *Phoma cucurbitacearum* (Foutr.) Sacardo] and is the first report from the valley.
- Temperature, relative humidity and rainfall positively favours the disease development.
- The fungus perpetuates both on seed as well as on crop debris left on the soil surface during crop less season and produces a primary inoculum for the next cropping season.
- Infected seed is the source of primary inoculum for the next cropping season therefore; healthy seeds or seeds treated with carbendazim @ 0.1 per cent or chlorothalonil @ 0.3 per cent should be used.
- Pathogen survives on crop debris beyond the next sowing time. Therefore, all the crop debris should be collected from the field and destroyed soon after harvesting. Pathogen could not survive up to the next cropping season on debris buried under soil therefore, ploughing the ridge gourd fields after harvesting can prove useful in controlling the disease.
- After the appearance of the disease in the field, foliar sprays of carbendazim @ 0.05 per cent or chlorothalonil @ 0.3 per cent should be given at 15 days interval.

Table 1. Incidence and intensity of Didymella blight (*Didymella bryoniae*) of ridge gourd on leaves at various locations of Kashmir during 2004 and 2005

Location	Disease incidence (%)			Disease intensity (%)		
	2004	2005	Mean	2004	2005	Mean
District Srinagar						
Brain	28.6 (32.3)	30.0 (33.1)	29.3 (32.7)	11.9 (20.1)	17.4 (24.6)	14.6 (22.5)
Dal	60.6 (51.1)	67.3 (55.1)	64.0 (53.1)	32.2 (34.5)	37.5 (37.7)	34.8 (36.1)
Darbag	24.0 (29.2)	29.0 (32.7)	26.6 (31.0)	10.2 (18.5)	14.5 (22.3)	12.4 (20.5)
Ganderbal	30.3 (33.4)	32.6 (34.7)	31.5 (34.1)	12.0 (20.2)	18.5 (25.4)	15.2 (22.9)
Habak	54.3 (47.4)	57.3 (49.2)	55.8 (48.3)	26.3 (31.0)	31.3 (33.9)	29.0 (32.5)
Noorbagh	16.6 (24.0)	29.0 (32.5)	22.8 (28.5)	14.2 (22.0)	17.2 (24.4)	15.7 (23.3)
Shalimar	39.3 (38.8)	41.0 (39.7)	40.1 (39.3)	17.2 (24.5)	24.6 (29.7)	20.9 (27.2)
Mean	**36.2**	**40.9**	**38.5**	**17.7**	**23.0**	**20.3**
District Budgam						
Bugam	27.6 (31.5)	34.3 (35.8)	31.0 (33.7)	10.9 (19.1)	16.8 (24.2)	13.9 (21.8)
Gangbug	31.3 (33.9)	33.3 (35.2)	32.3 (34.6)	12.9 (21.0)	14.4 (22.2)	13.6 (21.6)
Narkura	34.0 (35.6)	39.0 (38.5)	36.5 (37.1)	16.0 (23.5)	19.8 (26.4)	17.9 (25.0)
Panzan	23.0 (28.5)	21.0 (27.2)	22.0 (27.9)	8.2 (16.5)	7.3 (15.6)	7.7 (16.1)
Mean	**28.9**	**31.9**	**30.4**	**12.0**	**14.5**	**13.3**
Overall mean	**32.6 (34.8)**	**36.4 (37.1)**	**34.5 (35.9)**	**14.8 (22.6)**	**18.8 (25.6)**	**16.8 (24.2)**
CD (P=0.05)	(4.84)	(4.79)	(3.76)	(3.45)	(0.59)	(2.28)

Figures in parentheses arc sine transformed values

Table 2. Incidence of Didymella blight (*Didymella bryoniae*) of ridge gourd on fruits at various locations of Kashmir during 2004 and 2005

Location	Disease incidence (%)		
	2004	**2005**	**Mean**
District Srinagar			
Brain	9.9 (18.3)	10.9 (19.3)	10.4 (18.8)
Dal	17.8 (24.9)	19.8 (26.4)	18.8 (25.7)
Darbag	7.2 (15.5)	8.8 (17.2)	8.0 (16.3)
Ganderbal	8.3 (16.6)	9.9 (18.3)	9.1 (17.5)
Habak	13.5 (21.5)	17.2 (24.5)	15.4 (23.0)
Noorbagh	8.0 (16.4)	10.4 (18.7)	9.2 (17.6)
Shalimar	9.0 (17.4)	13.0 (21.1)	11.0 (19.3)
Mean	**10.5**	**12.9**	**11.7**
District Budgam			
Bugam	6.6 (14.8)	8.4 (16.8)	7.5 (15.8)
Gangbug	9.2 (17.6)	10.4 (18.8)	9.8 (18.2)
Narkura	11.7 (19.9)	13.5 21.5)	12.6 (20.7)
Panzan	7.7 (16.1)	6.0 (14.1)	6.8 (15.1)
Mean	**8.8**	**9.5**	**9.2**
Overall mean	**9.6 (18.1)**	**11.2 (19.6)**	**10.4 (18.8)**
CD(P=0.05)	(2.85)	(2.80)	(2.95)

Figures in parentheses are arc sine transformed values

Table 5. Effect of media on growth and fructification of *Didymella bryoniae*

Agar media	*Colony diameter (mm)	**Fructification	
		Pycnidial formation	Pseudothecial formation
Corn meal	81.0	++++	-
Czapek-Dox	69.0	++	-
Ridge gourd leaf extract	66.6	+	-
Ridge gourd fruit extract	69.3	++	-
Malt extract	86.6	++++	-
Oat meal	90.0	++++	-
Potato dextrose	90.0	++++	-
CD (P=0.05)	4.99		

* Observations recorded after 8 days of incubation at 24±1^{o}C

** Observations recorded after 15 days of incubation 24±1^{o}C

-	=	Absent	(absent)
+	=	Scanty	(1-3)
++	=	Moderate	(4-6)
+++	=	Good	(7-9)
++++	=	Abundant	(>9)

Table 3. Symptomatology of Didymella blight (*Didymella bryoniae*) of ridge gourd

Date of observation (Year 2004)		Leaf blight				Fruit rot			
		Size (mm)	Shape	Colour	Other characters	Size (mm)	Shape	Colour	Other characters
Month	Weak								
June	III	0.5-1.0	Circular spots	Light brown	Spots on lower most leaves, chlorotic halo	-	-	-	-
	IV	12.0-21.5	-do-	-do-	Developed concentric rings at the centre	-	-	-	-
July	I	34.5-42.0	Irregularly Circular spots	Dark brown with greyish white centre	Pycnidia formed at centre in concentric rings	-	-	-	-
	II	51.5-64.5	-do-	-do-	Pseudothecia followed pycnidia, appearance of fresh spots on the upper leaves	-	-	-	-
	III	74.5-83.0	-	-do-	Spots coalesced	-	-	-	-
	IV	-do-	-	-do-	Coalescing caused blight and yellowing of affected leaves	-	-	-	-
	V	-do-	-	Greyish	-	-	-	-	-
August	I	-do-	-	-do-	-	5-10	Irregularly circular	Dark green	-
	II	-do-	-	-do-	-	Covered whole fruit	-	Black	Pycnidia formed
	III	-do-	-	-d0-	-	-	-	Black	Pseudothecia followed pycnidia
	IV	-do-	-	-do-	-	-	-	-do-	Affected newly formed fruits became shrivelled and misshaped
September	I	-do-	-	-do-	-	-	-	-do-	-

Table 4. Morphological characters of *Didymella bryoniae* (Auersw.) Rehm

Structure	Shape*	Colour*	Size (µm)*		Septation*
On host					
Mycelium	Irregularly branched	Hyaline	3.2-5.3	4.5	Septate
Pycnidium	Globose, subepidermal,solitary	Dark	120.4-172.0	142.7	-
Pycnidiospore	Ellipsoidal or allantoid, constricted at septa, rounded ends	Hyaline	8.6-12.9 x 3.2-4.3	11.5 x 3.6	1 septate, rarely 2 septate
Pseudothecium	Globose to subglobose, subepidermal, solitary	Dark	146.2-206.4	186.3	-
Ascus	Cylindrical to subclavate, short stipitate or sessile, biseriate	Hyaline	55.9-64.5 x 10.7-12.9	62.2 x 11.1	-
Pseudoparaphyses	Branched	Hyaline	2.1-2.6	2.2	Present
Ascospore	Ellipsoidal, ends mostly rounded, slightly constricted at septum, upper cell usually wider than lower cell	Hyaline	13.9-17.2 x 4.3-5.4	16.4 x 5.1	1 septate
In culture					
Colony	Hyphae submerged with sparse aerial mycelium, circular, velvety or cottony growth	Initially white to grey, gradually turns dark grey and finally blackish	-		-
Mycelium	Irregularly branched, bulged at septa	Hyaline to light brown	3.2-4.3	3.6	Present
Pycnidium	Globose to irregular, glabrous or with mycelial outgrowths, solitary to confluent	Dark	167.7-326.8	236.5	-
Pycnidiospore	Ellipsoidal or allantoid with small guttules	Hyaline	6.4-9.6 x 2.1-4.3	8.3 x 3.7	Aseptate, rarely I septate

***** Average of 100 observations

Table 8. Host range of *Didymella bryoniae* in nature and under artificial inoculation conditions

Host tested	Natural infection in field	Artificial inoculation	
		Reaction	Incubation period (days)
Bottle gourd (*Lagenaria siceraria* (Molina) Stendle)	+	+	7
Bitter gourd (*Mamordica charantia* Poir)	+	+	7
Cucumber (*Cucumis sativus* L.)	+	+	7
Muskmelon *(Cucumis melo* L.)	+	+	9
Pumpkin (*Cucurbita pepo* Poir)	+	+	10
Ridge gourd (*Luffa acutangula* (L.) Roxb.)	+	+	4
Sponge gourd (*Luffa cylindrica* L.)	+	+	6
Squash (Cucurbita maxima Dusch)	+	+	10
Watermelon [*Citrullus lanatus* (Thumb.) Stendle]	+	+	9

\+ Infected

Table 5. Effect of media on growth and fructification of *Didymella bryoniae*

Agar media	Colony diameter* (mm)	Fructification	
		Pycnidial formation**	Pseudothecial formation
Corn meal	81.0	++++	-
Czapek-Dox	69.0	++	-
Ridge gourd leaf extract	66.6	+	-
Ridge gourd fruit extract	69.3	++	-
Malt extract	86.6	++++	-
Oat meal	90.0	++++	-
Potato dextrose	90.0	++++	-
CD (P=0.05)	4.99		

Table 6. Effect of temperature on growth, fructification and spore germination of *Didymella bryoniae*

Temperature (±1°c)	Colony diameter* (mm)	Spore germination*** (%)	Pycnidial formation**
12	16.3	0.0 (4.0)	+
16	38.0	29.0 (32.8)	+
20	81.6	68.0 (55.9)	++++
24	90.0	95.6 (79.1)	++++
28	74.3	75.6 (61.1)	++++
32	45.3	24.6 (29.9)	++
36	0.0	0.0 (4.0)	-
C.D (P=0.05)	6.38	(7.22)	

Table 7. Effect of pH on growth, fructification and spore germination of *Didymella bryoniae*

pH	Colony diameter (mm)*	Spore germination*** (%)	Pycnidial formation **
5.0	59.3	64.3 (53.3)	+
5.5	78.6	70.6 (57.3)	++
6.0	81.0	84.0 (66.5)	++++
6.5	88.6	85.3 (67.6)	++++
7.0	90.0	95.6 (78.0)	++++
7.5	35.3	49.3 (44.6)	+
8.0	14.6	32.0 (34.4)	+
C.D (P= 0.05)	10.96	(6.53)	

Figures in parenthesis are arc sine transformed values

* Observations recorded after 8 days of incubation

** Observations recorded after 15 days of incubation

*** Observations recorded after 24 hours of incubation

- = 0 (absent); + = Scanty (1-3); ++ = Moderate (4-6); +++ = Good (7-9); ++++ = Abundant (>9)

Table 9. Survival of *Didymella bryoniae* in/on ridge gourd seeds observed at monthly intervals after harvest in 2004

Month	Spore viability (%)	Seeds showing *D. bryoniae* growth (%)	
		Blotter method	Agar plate method
November 2004	**93.9 (76.0)**	**45.0 (42.1)**	**41.6 (40.1)**
December	**95.4 (77.8)**	**41.6 (40.1)**	**38.3 (38.2)**
January 2005	**81.2 (64.3)**	**38.3 (38.2)**	**36.6 (37.2)**
February	**72.0 (58.0)**	**33.3 (35.2)**	**33.3 (35.1)**
March	**61.3 (51.5)**	**31.6 (34.2)**	**31.6(34.2)**
April	**55.9 (48.4)**	**30.0 (33.1)**	**28.3 (32.0)**
May	**32.5 (34.7)**	**28.3 (32.1)**	**25.0 (29.9)**
June	**29.8 (33.0)**	**26.6 (30.9)**	**21.6 (27.6)**
July	**28.0 (31.9)**	**23.3 (28.8)**	**20.0(26.4)**
August	**28.35 (32.0)**	**20.0 (26.4)**	**16.6 (24.0)**
September	**23.3 (28.8)**	**20.0 (26.5)**	**11.6 (19.8)**
October	**18.6 (25.5)**	**16.6 (24.0)**	**8.3 (16.5)**
CD (P=0.05)	(4.69)	(4.32)	(4.92)

Figures in parentheses are arc sine transformed values

Table 10. Survival of *Didymella bryoniae* in/on ridge gourd seeds observed at monthly intervals after harvest in 2005

Month	Spore viability (%)	Seeds showing *D. bryoniae* growth (%)	
		Blotter method	Agar plate method
November 2005	**93.7 (75.9)**	**48.3 (44.0)**	**45.0 (42.1)**
December	**92.9 (74.7)**	**45.0 (42.1)**	**43.3(41.1)**
January 2006	**86.9 (68.9)**	**41.6(40.1)**	**38.3 (38.2)**
February	**80.8 (64.0)**	**38.3 (38.2)**	**35.0 (36.2)**
March	**78.0 (62.0)**	**35.0 (36.2)**	**28.3 (32.1)**
April	**73.2 (58.8)**	**31.6 (34.2)**	**25.0 (29.9)**
May	**60.8 (51.2)**	**30.0 (33.1)**	**23.3 (28.8)**
June	**46.6 (43.0)**	**26.6 (30.9)**	**21.6 (27.5)**
July	**45.9 (42.6)**	**25.0 (29.9)**	**21.6 (27.5)**
August	**32.6 (34.8)**	**21.6 (27.5)**	**20.0 (26.5)**
September	**24.1 (29.4)**	**20.0 (26.4)**	**15.0 (22.5)**
October	**20.3 (26.7)**	**18.3 (25.2)**	**11.6 (19.8)**
CD (P=0.05)	(3.88)	(4.43)	(4.58)

Figures in parentheses are arc sine transformed values

Table 11. Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd leaves kept under different conditions after harvest in 2004

Month	*Number of pycnidia/ pseudothecia cm^{-2} leaf area			*No. of pycnidiospores/ ascospores cm^{-2} leaf area			*Pycnidiospore/ ascospore viability (%)		
	Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)	
		0	7.5		0	7.5		0	7.5
November 2004	**47.6**	**45.6**	**17.2**	**5166.0**	**4100.0**	**0.0**	**90.4 (72.1)**	**92.2**	**NA**
December	**40.1**	**34.2**	**0.0**	**3646.0**	**3453.0**	**0.0**	**76.5 (61.0)**	**81.2**	**NA**
January 2005	**38.0**	**21.2**	**0.0**	**3166.0**	**0.0**	**0.0**	**64.0 (53.1)**	**NA**	**NA**
February	**31.2**	**13.2**	**0.0**	**2643.0**	**0.0**	**0.0**	**48.4(44.1)**	**NA**	**NA**
March	**23.5**	**19.4**	**-**	**1833.0**	**1846.0**	**-**	**30.3 (33.5)**	**85.2**	**NA**
April	**20.6**	**29.6**	**-**	**1413.0**	**2533.0**	**-**	**23.3 (28.8)**	**94.4**	**-**
May	**18.5**	**24.5**	**-**	**1046.0**	**2013.0**	**-**	**20.3 (26.7)**	**84.8**	**-**
June	**12.1**	**19.5**	**-**	**1026.0**	**1226.0**	**-**	**20.0 (26.5)**	**78.6**	**-**
July	**10.9**	**-**	**-**	**956.0**	**-**	**-**	**16.4 (23.9)**	**-**	**-**
August	**12.5**	**-**	**-**	**646.0**	**-**	**-**	**10.4 (18.8)**	**-**	**-**
September	**8.5**	**-**	**-**	**576.0**	**-**	**-**	**9.6 (18.0)**	**-**	**-**
October	**6.5**	**-**	**-**	**416.0**	**-**	**-**	**8.8(17.2)**	**-**	**-**
CD(P=0.05)	**2.90**	**3.28**		**2.95**	**2.59**		**(2.60)**		

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

Table 13. Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd fruit husk kept under different conditions after harvest in 2004

Month	*No. of pycnidia/ pseudothecia cm^{-2} fruit husk area			*No. of pycnidiospores/ ascospores cm^{-2} fruit husk area			*Pycnidiospore/ ascospore viability (%)		
	Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)	
		0	7.5		0	7.5		0	7.5
November 2004	**78.1**	**76.6**	**13.4**	**6926.0**	**6126.0**	**0.0**	**94.2 (66.5)**	**92.05**	**NA**
December	**70.8**	**63.2**	**0.00**	**6200.0**	**4533.0**	**0.0**	**84.6 (59.7)**	**87.33**	**NA**
January 2005	**62.4**	**36.7**	**0.00**	**5036.0**	**0.00**	**0.0**	**72.5 (52.2)**	**NA**	**NA**
February	**54.4**	**22.2**	**0.00**	**4520.0**	**0.00**	**0.0**	**51.2 (45.7)**	**NA**	**NA**
March	**43.2**	**24.2**	**0.00**	**3865.0**	**2946.0**	**-**	**43.2 (41.1)**	**89.55**	**-**
April	**37.1**	**41.0**	**-**	**3353.0**	**3756.0**	**-**	**41.5 (40.1)**	**88.05**	
May	**30.2**	**25.1**	**-**	**2850.0**	**2253.0**	**-**	**38.4 (38.2)**	**85.16**	**-**
June	**24.1**	**18.4**	**-**	**2630.0**	**1676.0**	**-**	**32.0 (34.4)**	**80.38**	**-**
July	**20.2**	**13.2**	**-**	**1746.0**	**1240.0**	**-**	**28.4 (32.2)**	**78.25**	**-**
August	**14.1**	**-**	**-**	**1573.0**	**-**	**-**	**22.5 (28.3)**	**-**	**-**
September	**10.1**	**-**	**-**	**1256.0**	**-**	**-**	**18.5 (25.5)**	**-**	**-**
October	**8.2**	**-**	**-**	**983.0**	**-**	**-**	**13.2 (21.3)**	**-**	**-**
CD(P=0.05)	**2.27**	**3.25**		**3.20**	**3.64**		**(0.91)**		

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

Table 14. Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd fruit husk kept under different conditions after harvest in 2005

Year/ Month	No. of pycnidia/ pseudothecia cm^{-2} fruit husk area			No. of pycnidiospores / ascospores cm^{-2} fruit husk area			Pycnidiospore/ ascospore viability (%)		
	Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)	
		0	7.5		0	7.5		0	7.5
November 2005	**71.8**	**71.8**	**24.6**	**6576.0**	**4936**	**0.0**	**93.2 (74.9)**	**91.1**	**NA**
December	**69.2**	**53.6**	**0.0**	**5963.0**	**3863.0**	**0.0**	**89.4 (71.0)**	**88.7**	**NA**
January 2006	**65.7**	**30.6**	**0.0**	**5376.0**	**0.0**	**0.0**	**77.1 (61.4)**	**NA**	**NA**
February	**57.8**	**10.5**	**0.0**	**4656.0**	**0.0**	**0.0**	**53.2 (46.8)**	**NA**	**NA**
March	**52.1**	**19.4**	**-**	**4226.0**	**1453.0**	**-**	**45.2 (42.2**	**90.6**	**-**
April	**47.1**	**37.2**	**-**	**3623.0**	**2100.0**	**-**	**33.2 (35.2)**	**82.4**	**-**
May	**37.2**	**18.2**	**-**	**3150.0**	**1353.0**	**-**	**29.8 (33.0)**	**80.2**	**-**
June	**31.4**	**12.5**	**-**	**2413.0**	**1000.0**	**-**	**23.4 (28.9)**	**75.1**	**-**
July	**21.5**	**9.2**	**-**	**1853.0**	**813.0**	**-**	**24.2 (29.4)**	**-**	**-**
August	**19.6**	**-**	**-**	**1356.0**	**-**	**-**	**21.0 (27.3)**	**-**	**-**
September	**13.5**	**-**	**-**	**923.0**	**-**	**-**	**16.2 (23.7)**	**-**	**-**
October	**10.5**			**780.0**			**11.8 (20.1)**	**-**	**-**
CD(P=0.05)	**2.83**	**3.10**		**3.29**	**2.41**		**(1.57)**		

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

Table 12. Fructification and pycnidiospore/ ascospore production and viability on infected ridge gourd leaves kept under different conditions after harvest in 2005

Month	No. of pycnidia/ pseudothecia cm^{-2} leaf area			No. of pycnidiospores/ ascospores cm^{-2} leaf area			Pycnidiospore/ascospore viability (%)		
	Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)		Ambient conditions	Leaf burial at soil depth (cm)	
		0	7.5		0	7.5		0	7.5
November 2005	**49.8**	**49.8**	**23.6**	**4643.0**	**3956.0**	**0.0**	**87.6(69.3)**	**90.1**	**NA**
December	**46.9**	**39.5**	**0.0**	**3916.0**	**3126.0**	**0.0**	**73.2(58.8)**	**86.9**	**NA.**
January 2006	**44.9**	**18.0**	**0.0**	**2926.0**	**0.0**	**0.0**	**58.6(49.9)**	**NA**	**NA**
February	**39.9**	**10.2**	**0.0**	**2336.0**	**0.0**	**0.0**	**49.0(44.4)**	**NA**	**NA**
March	**33.0**	**20.7**	**-**	**2173.0**	**2100.0**	**-**	**38.4(38.3)**	**82.6**	**-**
April	**29.0**	**20.0**	**-**	**1856.0**	**2836.0**	**-**	**35.0(36.2)**	**80.2**	**-**
May	**21.3**	**17.4**	**-**	**1643.0**	**2126.0**	**-**	**32.7(34.9)**	**80.0**	**-**
June	**16.4**	**11.6**	**-**	**1393.0**	**1640.0**	**-**	**28.9(32.5)**	**73.4**	**-**
July	**15.1**	**-**	**-**	**876.0**	**-**	**-**	**24.2(29.4)**	**-**	**-**
August	**12.6**	**-**	**-**	**630.0**	**-**	**-**	**19.0(25.9)**	**-**	**-**
September	**9.5**	**-**	**-**	**646.0**	**-**	**-**	**13.7(21.7)**	**-**	**-**
October	**8.3**	**-**	**-**	**343.0**	**-**	**-**	**7.2(15.6)**	**-**	
CD(P=0.05)	**3.07**	**2.54**		**3.23**	**3.18**		**(3.29)**		

Figures in parentheses are arc sine transformed values

- Material perished; NA Spores not available

Table 19. ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the mycelial growth and spore germination of** ***Didymella bryoniae***

Fungitoxicant	Per cent radial growth inhibition at concentration (*µg/ml)					Percent spore germination inhibition at Concentration (*µg/ml)				
	10	**50**	**100**	**200**	**Mean**	**10**	**50**	**100**	**200**	**Mean**
Carbendazim 50 WP	55.9 (48.3)	94.4 (81.9)	100.0 (89.9)	100.0 (89.9)	**87.5 (77.5)**	49.2 (44.5)	63.8 (53.0)	85.4 (67.7)	100.0 (89.9)	**74.6 (63.8)**
Thiophanate Methyl 70 WP	34.8 (36.1)	69.6 (56.5)	100.0 (89.9)	100.0 (89.9)	**76.1 (68.1)**	38.6 (38.3)	55.8 (48.3)	83.6 (66.2)	100.0 (89.9)	**69.5 (60.7)**
Myclobutanil 10 WP	22.5 (28.3)	52.5 (46.4)	77.0 (61.3)	100.0 (89.9)	**63.0 (56.5)**	31.7 (34.2)	43.8 (41.4)	66.8 (54.9)	97.4 (82.4)	**59.9 (53.2)**
Diniconazole 25 WP	17.4 (24.6)	31.4 (34.1)	64.8 (53.6)	100.0 (89.9)	**48.7 (44.2)**	16.4 (23.7)	28.1 (31.9)	52.6 (46.5)	85.4 (67.6)	**45.6 (42.4)**
Bitertanol 25 WP	11.1 (19.3)	33.3 (35.2)	51.1 (45.6)	81.4 (64.5)	**41.6 (39.4)**	22.3 (28.0)	38.0 (38.0)	66.1 (54.5)	93.0 (74.7)	**54.8 (48.8)**
Mean	**28.3 (31.3)**	**56.2 (50.8)**	**78.5 (68.1)**	**90.5 (78.3)**		**31.6 (33.7)**	**45.9 (42.5)**	**70.9 (58.0)**	**95.1 (80.9)**	
CD (P=0.05) Fungitoxicant		(2.83)					(3.27)			
Concentration		(2.53)					(2.92)			
Fungitoxicant x Concentration		(5.66)					(6.55)			

Figures within parentheses are arc sine transformed values

** Active ingredient basis

Table 20. ***In vitro*** **efficacy of various non-systemic fungitoxicants in inhibiting the mycelial growth and spore germination of** ***Didymella bryoniae***

Fungitoxicant	Per cent radial growth inhibition at concentration (*µg ml^{-1})					Percent spore germination inhibition at concentration (*µg ml^{-1})				
	100	**250**	**500**	**1000**	**Mean**	**100**	**250**	**500**	**1000**	**Mean**
Chlorothalonil 75 WP	22.9 (28.6)	46.6 (43.0)	100.0 (89.9)	100.0 (89.9)	**67.4 (62.9)**	42.8 (40.7)	66.3 (54.5)	88.4 (70.2)	100.0 (89.9)	**74.3 (63.8)**
Zineb 75 WP	15.1 (22.8)	31.4 (34.1)	55.5 (48.1)	100.0 (89.9)	**50.5 (48.7)**	22.6 (28.2)	47.7 (43.6)	65.2 (53.8)	100.0 (89.9)	**58.9 (53.9)**
Captan 50 WP	6.6 (14.5)	27.0 (31.2)	58.8 (50.1)	96.3 (83.4)	**47.2 (44.8)**	11.1 (19.2)	40.2 (39.3)	61.4 (51.7)	100.0 (89.9)	**53.2 (50.0)**
Copper oxychloride 50 WP	5.1 (12.8)	20.0 (26.2)	48.1 (43.9)	74.4 (59.6)	**36.9 (35.6)**	5.7 (13.3)	30.1 (33.2)	52.3 (46.3)	93.5 (75.2)	**45.4 (42.0)**
Mancozeb 75 WP	19.6 (26.2)	45.1 (42.2)	87.4 (69.2)	100.0 (89.9)	**63.0 (56.9)**	30.8 (33.6)	51.4 (45.7)	78.3 (62.2)	100.0 (89.9)	**65.1 (57.9)**
Mean	**13.9 (21.0)**	**34.0 (35.3)**	**69.9 (60.2)**	**94.1 (82.5)**		**22.62 (27.06)**	**47.1 (43.3)**	**69.1 (56.8)**	**98.7 (87.0)**	
CD (P=0.05) Fungitoxicant		(2.94)					(3.13)			
Concentration		(2.62)					(2.79)			
Fungitoxicant x Concentration		(5.88)					(6.26)			

Figures in parentheses are arc sine transformed values

**Active ingredient basis

Table 20. *In vitro* efficacy of various botanicals in inhibiting the mycelial growth and spore germination of *Didymella bryoniae*

Botanical	Local name	Percent radial growth inhibition at concentration (%)					Percent spore germination inhibition at concentration (%)				
		1	**5**	**10**	**15**	**Mean**	**1**	**5**	**10**	**15**	**Mean**
Urtica dioica	Soi	1.4 (6.8)	7.7 (16.0)	22.2 (28.0)	36.6 (37.2)	**17.0 (22.0)**	3.5 (10.8)	17.5 (24.6)	27.3 (31.4)	41.3 (40.0)	**22.4 (26.7)**
Artemesia annua	Tethwan	5.1 (12.9)	19.2 (25.9)	34.4 (35.9)	51.8 (46.0)	**27.6 (30.2)**	10.8 (18.9)	27.7 (31.7)	39.6 (38.9)	57.9 (49.5)	**34.0 (34.8)**
Datura stramonium	Datur	2.5 (9.2)	8.5 (16.9)	21.8 (27.8)	40.7 (39.6)	**18.4 (23.4)**	3.1 (9.5)	14.3 (22.1)	36.1 (36.9)	47.7 (43.6)	**25.3 (28.0)**
Allium cepa	Rohan	6.3 (14.3)	24.0 (29.3)	53.3 (46.8)	75.1 (60.1)	**39.7 (37.6)**	10.1 (18.4)	30.8 (33.7)	50.8 (45.4)	73.7 (59.2)	**41.4 (39.2)**
Allium sativum	Ganda	9.6 (18.0)	41.1 (39.8)	63.3 (52.7)	100.0 (89.9)	**53.5 (50.1)**	14.7 (22.5)	35.0 (36.2)	61.7 (51.7)	78.9 (62.7)	**47.6 (43.3)**
Mean		**5.0 (12.2)**	**20.1 (25.6)**	**39.0 (38.2)**	**60.8 (54.6)**		**8.4 (16.0)**	**25.1 (29.7)**	**43.1 (40.9)**	**59.9 (51.0)**	
CD (P=0.05) Botanical			(1.27)					(2.18)			
Concentration			(1.42)					(1.94)			
Botanical x Concentration			(2.85)					(4.36)			

Figures in parentheses are arc sine transformed values

Table 25. Effect of seed treatment and foliar spray on incidence of Didymella blight (*Didymella bryoniae*) on leaves of ridge gourd during 2004

Seed treatments	Disease incidence (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05%)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20%)	Check (Water spray)	**Mean**
Carbendazim 50 WP (0.1 %)	13.00 (21.05)	26.66 (30.95)	33.33 (35.21)	47.33 (43.44)	**30.08 (32.66)**
Chlorothalonil 75 WP (0.3 %)	14.00 (21.86)	23.66 (29.07)	36.33 (37.04)	54.66 (47.66)	**32.16 (33.90)**
Allium sativum (20 %)	17.33 (24.56)	28.66 (32.34)	38.33 (38.23)	59.66 (50.55)	**35.99 (36.42)**
Check (Water Spray)	18.00 (25.02)	30.00 (33.17)	39.66 (39.01)	68.66 (55.98)	**39.08 (38.29)**
Mean	**15.58 (23.12)**	**28.08 (31.18)**	**36.16 (37.37)**	**57.57 (49.40)**	

CD (P=0.05)	Seed treatment	(2.26)
	Foliar sprays	(2.26)
	Seed treatments X Foliar sprays	(4.52)

Figures in parentheses are arc sine transformed values

Table 26. Effect of seed treatment and foliar spray on incidence of Didymella blight (*Didymella bryoniae*) on leaves of ridge gourd during 2005

Seed treatments	Disease incidence (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05%)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20%)	Check (Water Spray)	**Mean**
Carbendazim 50 WP (0.1%)	14.33 (22.03)	26.00 (30.57)	40.00 (39.16)	57.66 (49.39)	**34.50 (35.29)**
Chlorothalonil 75 WP (0.3%)	17.00 (24.28)	26.66 (31.01)	41.66 (40.16)	63.66 (52.91)	**37.25 (37.09)**
Allium sativum (20%)	16.66 (23.91)	28.00 (31.84)	43.66 (41.33)	62.66 (52.32)	**37.75 (37.35)**
Check (Water Spray)	17.33 (24.56)	29.66 (32.97)	45.33 (42.29)	75.00 (60.00)	**41.83 (39.95)**
Mean	**16.33 (23.70)**	**27.58 (31.60)**	**42.66 (40.73)**	**64.75 (58.23)**	
CD (P=0.05) Seed treatment		(2.31)			
Foliar sprays		(2.31)			
Seed treatments X Foliar sprays		(4.63)			

Figures in parentheses are arc sine transformed values

Table 27. Effect of seed treatment and foliar spray on intensity of Didymella blight (*Didymella bryoniae*) on leaves of ridge gourd during 2004

Seed treatments	Disease intensity (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05%)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20%)	Check (Water Spray)	**Mean**
Carbendazim 50 WP (0.1%)	2.93 (9.81)	7.73 (16.11)	11.00 (19.34)	23.26 (28.75)	**11.23 (18.50)**
Chlorothalonil 75 WP (0.3 %)	3.93 (11.39)	8.26 (16.70)	12.20 (20.42)	28.26 (32.07)	**13.16 (20.15)**
Allium sativum (20 %)	4.06 (11.62)	8.60 (17.04)	14.46 (22.34)	32.60 (34.80)	**14.93 (21.45)**
Check (Water Spray)	4.26 (11.88)	8.93 (17.38)	15.40 (23.09)	40.06 (39.25)	**17.16 (22.90)**
Mean	**3.80 (11.17)**	**8.38 (16.80)**	**13.26 (21.30)**	**31.05 (33.72)**	

CD (P=0.05) Seed treatment	(1.03)
Foliar sprays	(1.03)
Seed treatments X Foliar sprays	(2.06)

Figures in parentheses are arc sine transformed values

Table 28. Effect of seed treatment and foliar spray on intensity of Didymella blight (*Didymella bryoniae*) on leaves of ridge gourd during 2005

Seed treatments	Disease intensity (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05%)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20 %)	Check (Water Spray)	**Mean**
Carbendazim 50 WP (0.1 %)	3.73 (11.03)	8.00 (16.40)	13.86 (21.84)	31.60 (34.18)	**14.30 (20.86)**
Chlorothalonil 75 WP (0.3 %)	4.00 (11.44)	8.93 (17.38)	14.46 (22.33)	36.86 (37.36)	**16.06 (22.13)**
Allium sativum (20 %)	4.26 (11.78)	8.80 (17.23)	14.40 (22.28)	37.26 (37.60)	**16.18 (22.22)**
Check (Water Spray)	4.73 (12.54)	9.13 (17.57)	14.80 (22.60)	40.46 (39.48)	**17.28 (23.05)**
Mean	**4.18 (11.70)**	**8.71 (17.15)**	**14.38 (22.26)**	**36.55 (37.16)**	

CD (P=0.05)	Seed treatment	(0.87)
	Foliar sprays	(0.87)
	Seed treatments X Foliar sprays	(1.74)

Figures in parentheses are arcsine transformed values

Table 31. Effect of seed treatment and foliar spray on number of fruits (plot^{-1}) and yield (q ha^{-1}) of ridge gourd during 2004

Seed treatments	Number of fruits and yield (q ha^{-1})				
	Foliar sprays				
	Carbendazim 50 WP (.05%)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20 %)	Check (Water pray)	**Mean**
Carbendazim 50 WP (0.1 %)	69.6 (130.0)	59.3 (107.7)	39.6 (67.3)	27.6 (43.7)	**49.0 (87.1)**
Chlorothalonil 75 WP (0.3 %)	69.3 (129.8)	58.3 (105.1)	38.0 (64.6)	26.6 (43.0)	**48.1 (85.6)**
Allium sativum (20 %)	65.3 (118.7)	55.0 (98.3)	35.0 (58.8)	25.6 (41.5)	**45.2 (79.3)**
Check (Water Spray)	64.6 (116.4)	54.0 (95.4)	34.3 (58.2)	23.6 (34.8)	**44.1 (76.2)**
Mean	**67.2 (123.7)**	**56.6 (101.6)**	**36.7 (62.2)**	**25.9 (40.8)**	

CD (P=0.05)	Seed treatment	3.65	(2.91)
	Foliar sprays	3.65	(2.91)
	Seed treatments X Foliar sprays	7.30	(5.82)

Figures in parentheses are yield (q ha^{-1})

Table 32. Effect of seed treatment and foliar spray on number of fruits (plot^{-1}) and yield (q ha^{-1}) of ridge gourd during 2005

Seed treatments	Number of fruits and yield (q ha^{-1})				
	Foliar sprays				
	Carbendazim 50 WP (.05 %)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20 %)	Check (Water spray)	**Mean**
Carbendazim 50 WP (0.1 %)	68.0 (126.3)	58.3 (107.0)	38.0 (63.8)	27.6 (43.7)	**48.0 (85.2)**
Chlorothalonil 75 WP (0.3 %)	67.3 (121.0)	56.6 (99.2)	38.6 (65.7)	26.6 (42.4)	**47.3 (82.1)**
Allium sativum (20 %)	62.0 (113.0)	57.3 (96.9)	38.0 (63.1)	25.3 (40.2)	**45.6 (78.4)**
Check (Water Spray)	61.6 (111.7)	52.6 (92.2)	35.3 (58.1)	22.0 (33.3)	**42.9 (73.8)**
Mean	**64.7 (118.1)**	**56.2 (98.8)**	**37.5 (62.7)**	**25.4 (39.9)**	
CD (P=0.05) Seed treatment		4.13 (3.42)			
Foliar sprays		4.13 (3.42)			
Seed treatments X Foliar sprays		8.26 (6.85)			

Figures in parentheses are yield (q ha^{-1})

Table 29. Effect of seed treatment and foliar spray on per cent infected fruits of ridge gourd during 2004

Seed treatments	Infected fruits (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05 %)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20%)	Check (Water Spray)	**Mean**
Carbendazim 50 WP (0.1 %)	1.41 (6.82)	2.76 (9.43)	5.57 (13.57)	10.75 (19.09)	**5.13 (12.24)**
Chlorothalonil 75 WP (0.3 %)	1.44 (6.89)	2.83 (9.53)	6.75 (14.91)	13.08 (21.18)	**6.02 (13.11)**
Allium sativum (20 %)	1.50 (7.04)	3.51 (10.79)	7.28 (15.50)	13.59 (21.60)	**6.47 (13.73)**
Check (Water Spray)	2.06 (8.08)	3.58 (10.89)	7.56 (15.76)	14.50 (22.37)	**6.92 (14.27)**
Mean	**1.60 (7.21)**	**3.17 (10.16)**	**6.79 (14.94)**	**12.98 (21.06)**	**6.13 (13.34)**

CD (P=0.05)	Seed treatment	(1.41)
	Foliar sprays	(1.41)
	Seed treatments X Foliar sprays	(2.73)

Figures in parentheses are arc sine transformed values

Table 30. Effect of seed treatment and foliar spray on per cent infected fruits of ridge gourd during 2005

Seed treatments	Infected fruits (%)				
	Foliar sprays				
	Carbendazim 50 WP (.05 %)	Chlorothalonil 75 WP (0.3%)	*Allium sativum* (20 %)	Check (Water Spray)	**Mean**
Carbendazim 50 WP (0.1 %)	1.44 (6.89)	2.80 (9.50)	6.07 (14.05)	10.87 (19.16)	**5.29 (12.40)**
Chlorothalonil 75 WP (0.3 %)	1.46 (6.93)	2.95 (9.71)	6.06 (14.01)	12.29 (20.41)	**5.69 (12.77)**
Allium sativum (20 %)	1.58 (7.23)	3.41 (10.63)	6.51 (14.64)	16.82 (24.04)	**7.08 (14.14)**
Check (Water Spray)	2.14 (8.26)	3.66 (11.02)	8.10 (16.32)	18.11 (25.08)	**8.00 (15.17)**
Mean	**1.65 (7.33)**	**3.20 (10.21)**	**6.68 (14.76)**	**14.52 (22.17)**	**6.51 (13.61)**

CD (P=0.05)	Seed treatment	(1.84)
	Foliar sprays	(1.84)
	Seed treatments X Foliar sprays	(3.69)

Figures in parentheses are arc sine transformed values

Table 15. Influence of weather factors on development of leaf blight (*Didymella bryoniae*) of ridge gourd during 2004 and 2005

Date of observation		Disease intensity	Infection rate (unit/day)	Mean temperature* (^{o}c)	Mean relative humidity* (%)	Mean rainfall* (mm)
Year 2004						
Month	**Week**					
June	III	0.20	-	21.55	71	0.42
June	IV	0.33	0.0716	21.25	67	0.85
July	I	0.47	0.0507	24.15	66	0.00
July	II	1.20	0.1348	21.40	72	6.42
July	III	1.53	0.0351	20.50	67	0.51
July	IV	2.27	0.0574	23.65	69	0.71
July	V	7.87	0.1858	21.95	80	7.54
August	I	15.47	0.1087	23.85	70	2.14
August	II	17.47	0.0208	23.80	73	0.11
August	III	20.33	0.0267	21.00	69	1.20
August	IV	21.67	0.0115	20.85	64	0.00
September	I	22.40	0.0061	20.85	61	0.00
September	II	24.07	0.0134	22.95	69	0.00
September	III	30.00	0.0430	18.30	70	0.31
September	IV	32.07	0.0138	17.40	64	0.00
Year 2005						
June	I	0.20	-	18.50	62	1.07
June	II	0.20	0.0000	18.70	56	0.00
June	III	0.27	0.0429	22.20	57	0.00
June	IV	0.47	0.0794	19.93	71	2.72
July	I	1.67	0.1826	22.42	76	15.77
July	II	2.00	0.0262	23.35	75	0.45
July	III	3.00	0.0593	25.15	72	0.88
July	IV	3.73	0.0321	25.10	69	0.37
July	V	4.60	0.0312	21.90	68	0.54
August	I	5.13	0.0164	24.75	64	0.20
August	II	15.33	0.1724	22.35	66	4.62
August	III	19.13	0.0381	20.90	68	0.31
August	IV	19.40	0.0025	24.05	65	0.00
September	I	24.27	0.0408	20.70	73	0.28
September	II	45.73	0.1379	18.40	68	3.00
September	III	48.46	0.0156	18.20	68	0.00
September	IV	50.13	0.0095	17.40	66	0.00

* Mean of seven days

Table 16. Coefficients of simple correlation of meteorological factors with disease intensity and infection rate

Weather factors	Disease intensity	Infection rate
Temperature	-0.535*	0.094
Relative humidity	0.062	0.519*
Rainfall	-0.201	0.833*

* Significant at 5%

Table 17. Step wise multiple regression equations indicating the relationship of meteorological factors with disease intensity

Stepwise prediction equations
$Y = 42.4 – 3.77\ X_1 + 0.810\ X_2 – 1.46\ X_3$; $R^2 = 38.6\%$
$Y = 89.4 – 3.46\ X_1 – 0.777\ X_3$; $R^2 = 31.4\%$
$Y = 89.8 – 3.54\ X_1$; $R^2 = 28.7\%$

Table 18. Step wise multiple regression equations indicating the relationship of meteorological factors with infection rate

Stepwise prediction equations
$Y = -0.0605 + 0.00042\ X_1 + 0.00126\ X_2 + 0.0129\ X_3$; $R^2 = 70.5\%$
$Y = -0.0549 + 0.00131\ X_2 + 0.0129\ X_3$; $R^2 = 70.4\%$
$Y = 0.0328 + 0.0139\ X_3$; $R^2 = 69.4\%$

X1, X2 and X3 represent temperature, relative humidity and rainfall, respectively

Table 19. I*n* vitro efficacy of various systemic fungitoxicants in inhibiting the mycelial growth of *Didymella bryoniae*

Fungitoxicant	Per cent mycelial growth inhibition at concentration (*µg ml^{-1})				
	10	**50**	**100**	**200**	**Mean**
Carbendazim 50 WP	55.9 (48.3)	94.4 (81.9)	100.0 (89.9)	100.0 (89.9)	**87.5 (77.5)**
Thiophanate Methyl 70 WP	34.8 (36.1)	69.6 (56.5)	100.0 (89.9)	100.0 (89.9)	**76.1** (**68.1)**
Myclobutanil 10 WP	22.5 (28.3)	52.5 (46.4)	77.0 (61.3)	100.0 (89.9)	**63.0 (56.5)**
Diniconazole 25 WP	17.4 (24.6)	31.4 (34.1)	64.8 (53.6)	100.0 (89.9)	**48.7 (44.2)**
Bitertanol 25 WP	11.1 (19.3)	33.3 (35.2)	51.1 (45.6)	81.4 (64.5)	**41.6** (**39.4)**
Mean	**28.3** (**31.3)**	**56.2** (**50.8)**	**78.5** (**68.1)**	**90.5 (78.3)**	
CD (P=0.05) Fungitoxicant	(2.83)				
Concentration	(2.53)				
Fungitoxicant x Concentration	(5.66)				

Figures in parentheses are arc sine transformed values
* Active ingredient basis

Table 20. I*n* vitro efficacy of various systemic fungitoxicants in inhibiting spore germination of *Didymella bryoniae*

Fungitoxicant	Percent spore germination inhibition at concentration (*µg ml^{-1})				
	10	**50**	**100**	**200**	**Mean**
Carbendazim 50 WP	49.2 (44.5)	63.8 (53.0)	85.4 (67.7)	100.0 (89.9)	**74.6 (63.8)**
Thiophanate Methyl 70 WP	38.6 (38.3)	55.8 (48.3)	83.6 (66.2)	100.0 (89.9)	**69.5 (60.7)**
Myclobutanil 10 WP	31.7 (34.2)	43.8 (41.4)	66.8 (54.9)	97.4 (82.4)	**59.9 (53.2)**
Diniconazole 25 WP	16.4 (23.7)	28.1 (31.9)	52.6 (46.5)	85.4 (67.6)	**45.6 (42.4)**
Bitertanol 25 WP	22.3 (28.0)	38.0 (38.0)	66.1 (54.5)	93.0 (74.7)	**54.8 (48.8)**
Mean	**31.6 (33.7)**	**45.9 (42.5)**	**70.9 (58.0)**	**95.1 (80.9)**	
CD (P=0.05) Fungitoxicant	(3.27)				
Concentration	(2.92)				
Fungitoxicant x Concentration	(6.55)				

Figures in parentheses are arc sine transformed values

* Active ingredient basis

Table 21. **I*n* vitro efficacy of various non-systemic fungitoxicants in inhibiting the mycelial growth of *Didymella bryoniae***

Fungitoxicant	Per cent mycelial growth inhibition at concentration (*µg ml^{-1})				
	100	**250**	**500**	**1000**	**Mean**
Chlorothalonil 75 WP	22.9 (28.6)	46.6 (43.0)	100.0 (89.9)	100.0 (89.9)	**67.4 (62.9)**
Zineb 75 WP	15.1 (22.8)	31.4 (34.1)	55.5 (48.1)	100.0 (89.9)	**50.5 (48.7)**
Captan 50 WP	6.6 (14.5)	27.0 (31.2)	58.8 (50.1)	96.3 (83.4)	**47.2 (44.8)**
Copper oxychloride 50 WP	5.1 (12.8)	20.0 (26.2)	48.1(43.9)	74.4 (59.6)	**36.9 (35.6)**
Mancozeb 75 WP	19.6 (26.2)	45.1 (42.2)	87.4 (69.2)	100.0 (89.9)	**63.0 (56.9)**
Mean	**13.9 (21.0)**	**34.0 (35.3)**	**69.9 (60.2)**	**94.1 (82.5)**	
CD (P=0.05) Fungitoxicant		(2.94)			
Concentration		(2.62)			
Fungitoxicant x Concentration		(5.88)			

Figures in parentheses are arc sine transformed values
* Active ingredient basis

Table 22. **I*n* vitro efficacy of various non-systemic fungitoxicants in inhibiting the spore germination of *Didymella bryoniae***

Fungitoxicant	Percent spore germination inhibition at concentration (*µg ml^{-1})				
	100	**250**	**500**	**1000**	**Mean**
Chlorothalonil 75 WP	42.8 (40.7)	66.3 (54.5)	88.4 (70.2)	100.0 (89.9)	**74.3 (63.8)**
Zineb 75 WP	22.6 (28.2)	47.7 (43.6)	65.2 (53.8)	100.0 (89.9)	**58.9 (53.9)**
Captan 50 WP	11.1 (19.2)	40.2 (39.3)	61.4 (51.7)	100.0 (89.9)	**53.2 (50.0)**
Copper oxychloride 50 WP	5.7 (13.3)	30.1 (33.2)	52.3 (46.3)	93.5 (75.2)	**45.4 (42.0)**
Mancozeb 75 WP	30.8 (33.6)	51.4 (45.7)	78.3 (62.2)	100.0 (89.9)	**65.1 (57.9)**
Mean	**22.6 (27.0)**	**47.1 (43.3)**	**69.1 (56.8)**	**98.7 (87.0)**	

CD (P=0.05) Fungitoxicant	(3.13)
Concentration	(2.79)
Fungitoxicant x Concentration	(6.26)

Figures in parentheses are arc sine transformed values
* Active ingredient basis

Table 23. I*n* vitro efficacy of various botanicals in inhibiting the mycelial growth of *Didymella bryoniae*

Botanical	Local name	Per cent mycelial growth inhibition at concentration (%)				
		1	**5**	**10**	**15**	**Mean**
Urtica dioica	Soi	1.4 (6.8)	7.7 (16.0)	22.2 (28.0)	36.6 (37.2)	**17.0 (22.0)**
Artemisia annua	Tethwan	5.1 (12.9)	19.2 (25.9)	34.4 (35.9)	51.8 (46.0)	**27.6 (30.2)**
Datura stramonium	Datur	2.5 (9.2)	8.5 (16.9)	21.8 (27.8)	40.7 (39.6)	**18.4 (23.4)**
Allium cepa	Rohan	6.3 (14.3)	24.0 (29.3)	53.3 (46.8)	75.1 (60.1)	**39.7 (37.6)**
Allium sativum	Ganda	9.6 (18.0)	41.1 (39.8)	63.3 (52.7)	100.0 (89.9)	**53.5 (50.1)**
Mean		**5.0 (12.2)**	**20.1 (25.6)**	**39.0 (38.2)**	**60.8 (54.6)**	
CD (P=0.05) Botanical		(1.27)				
Concentration		(1.42)				
Botanical x Concentration		(2.85)				

Figures in parentheses are arc sine transformed values

Table 24. I*n* vitro efficacy of various botanicals in inhibiting the spore germination of *Didymella bryoniae*

Botanical	Local name	Percent spore germination inhibition at concentration (%)				
		1	**5**	**10**	**15**	**Mean**
Urtica dioica	Soi	3.5 (10.8)	17.5 (24.6)	27.3 (31.4)	41.3 (40.0)	**22.4 (26.7)**
Artemisia annua	Tethwan	10.8 (18.9)	27.7 (31.7)	39.6 (38.9)	57.9 (49.5)	**34.0 (34.8)**
Datura stramonium	Datur	3.1 (9.5)	14.3 (22.1)	36.1 (36.9)	47.7 (43.6)	**25.3 (28.0)**
Allium cepa	Rohan	10.1 (18.4)	30.8 (33.7)	50.8 (45.4)	73.7 (59.2)	**41.4 (39.2)**
Allium sativum	Ganda	14.7 (22.5)	35.0 (36.2)	61.7 (51.7)	78.9 (62.7)	**47.6 (43.3)**
Mean		**8.4 (16.0)**	**25.1 (29.7)**	**43.1 (40.9)**	**59.9 (51.0)**	
CD (P=0.05) Fungitoxicant		(2.18)				
Concentration		(1.94)				
Botanical x Concentration		(4.36)				

Figures in parentheses are arc sine transformed values

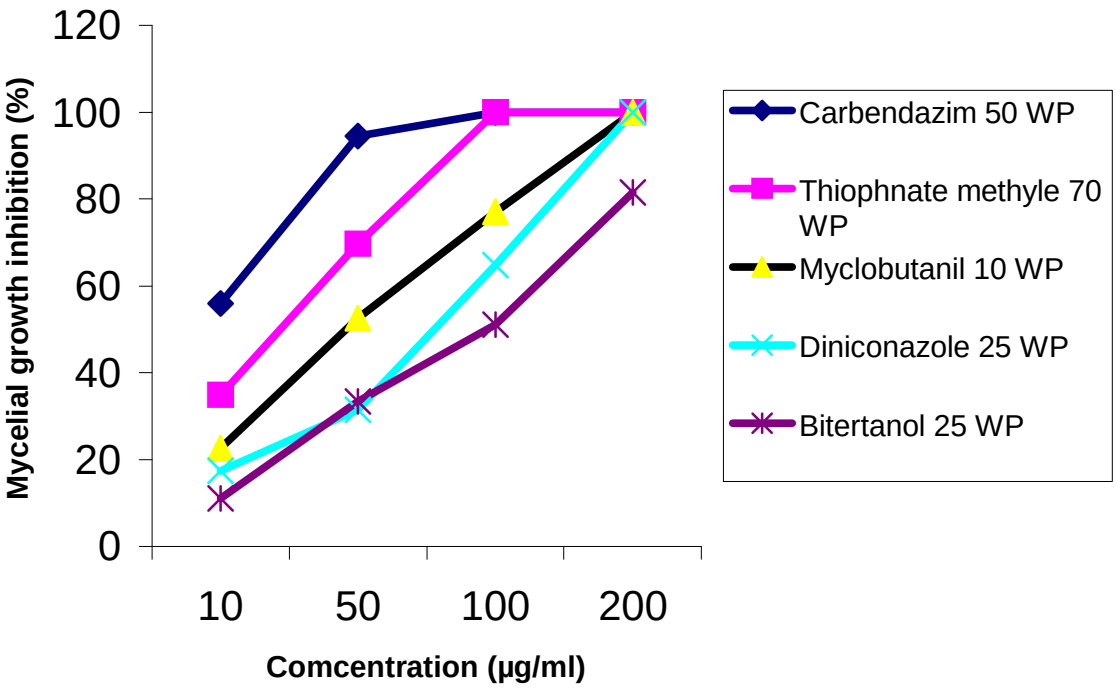


Fig. : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the mycelial growth of** ***Didymella bryonie***

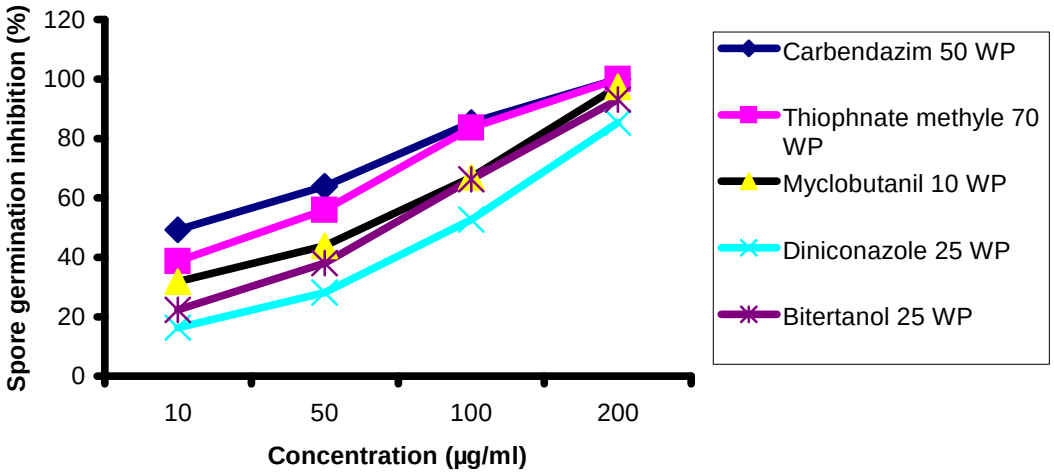


Fig. : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the spore germination of** ***Didymella bryonie***

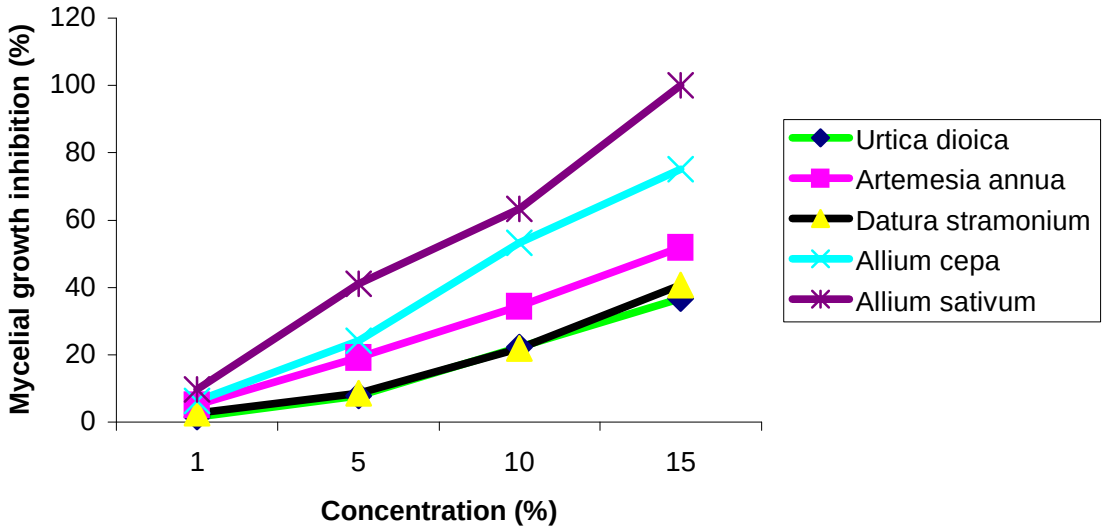


Fig. : ***In vitro*** **efficacy of various botanicals in inhibiting the mycelial growth of** ***Didymella bryonie***

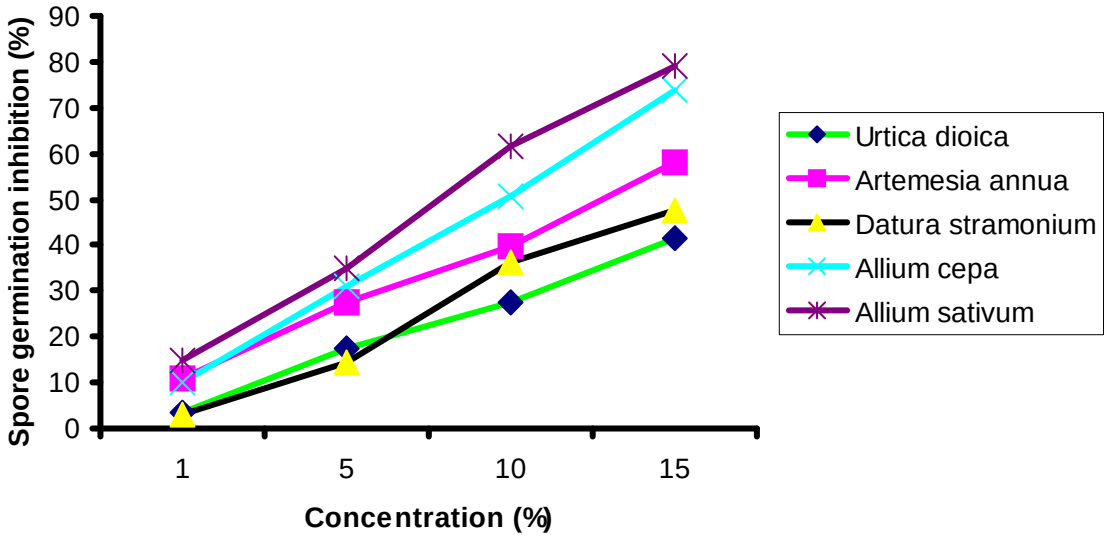


Fig. : ***In vitro*** **efficacy of various botanicals in inhibiting the spore germination of** ***Didymella bryonie***

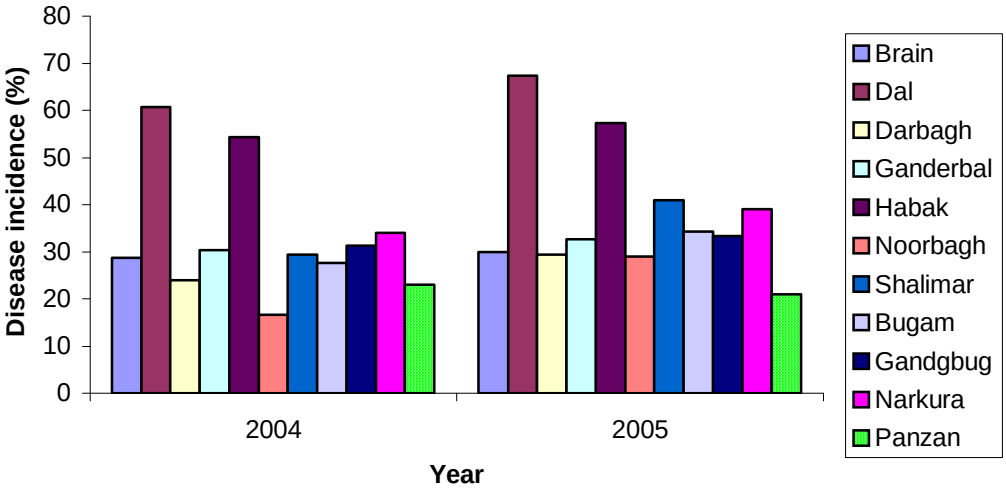


Fig : 1. **Incidence of Didymella blight (*Didymella bryoniae*) of ridge guard on leaves at various locations of Kashmir during 2004 and 2005**

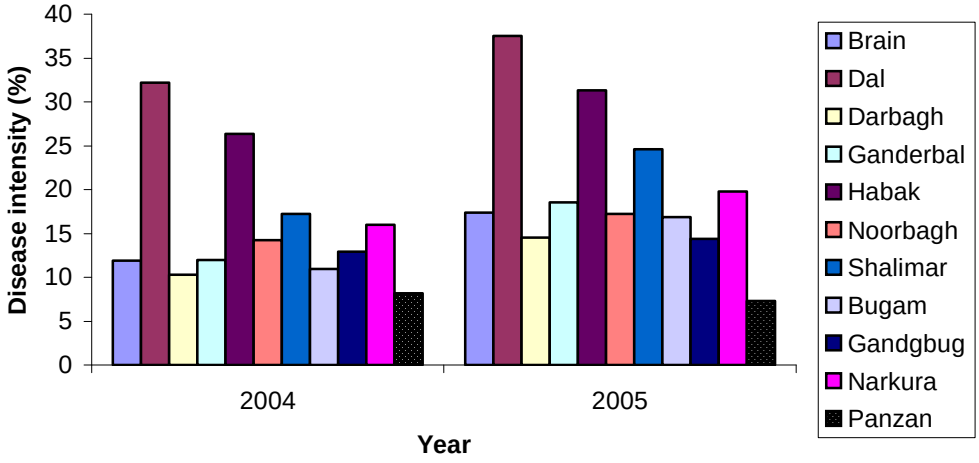


Fig : 2. **Intensity of Didymella blight (*Didymella bryoniae* of ridge guard on leaves at various locations of Kashmir during 2004 and 2005**

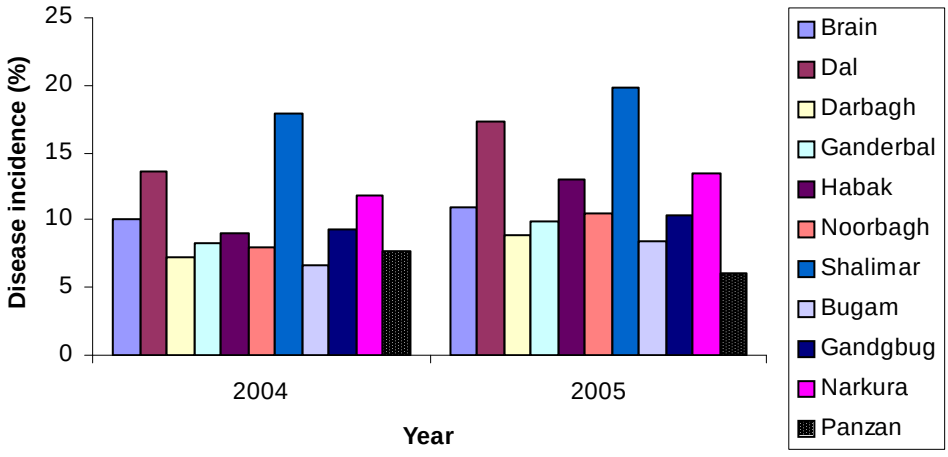


Fig : 3. **Incidence of Didymella blight (*Didymella bryoniae* of ridge guard on fruits at various locations of Kashmir during 2004 and 2005**

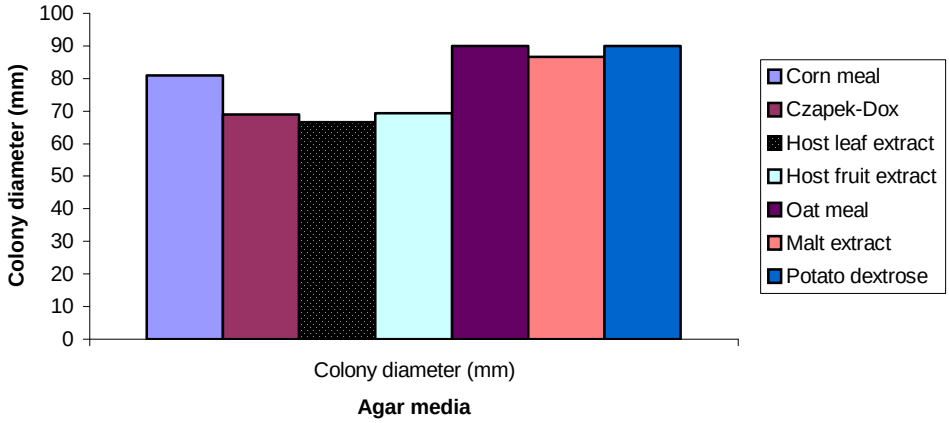


Fig : 4. **Effect of media on growth and fructification of *Didymella bryoniae***

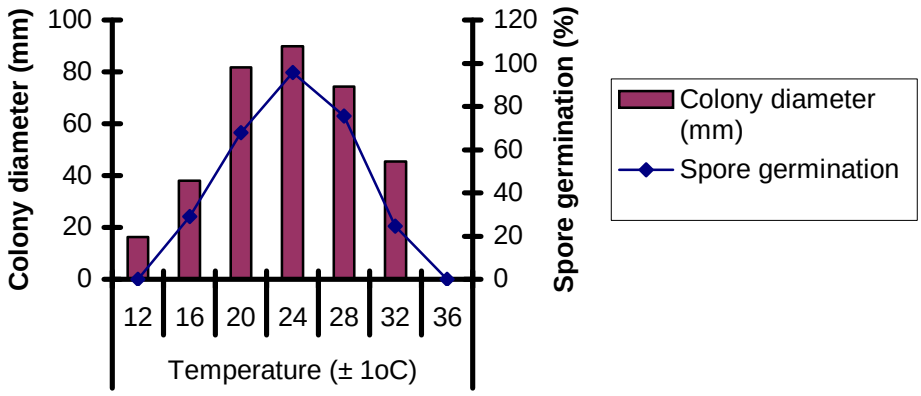


Fig : 5. **Effect of temperature on growth, fructification and spore germination of *Didymella bryoniae***

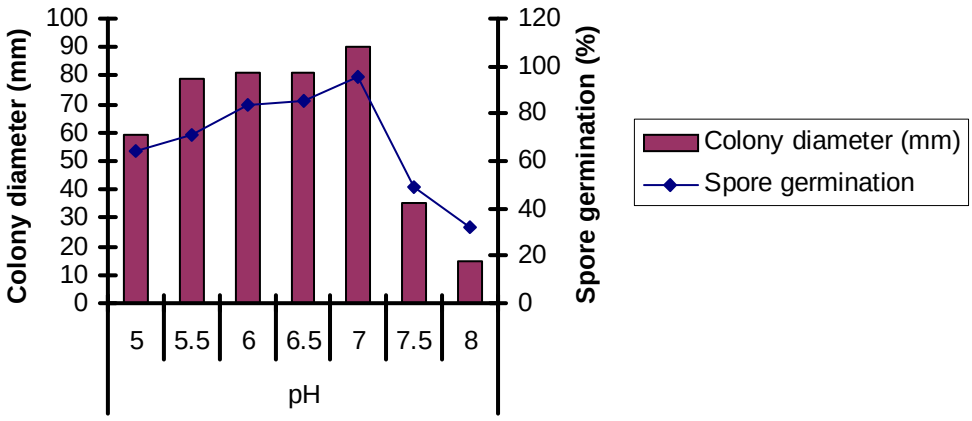


Fig : 6. **Effect of pH on growth, fructification and spore germination of *Didymella bryoniae***

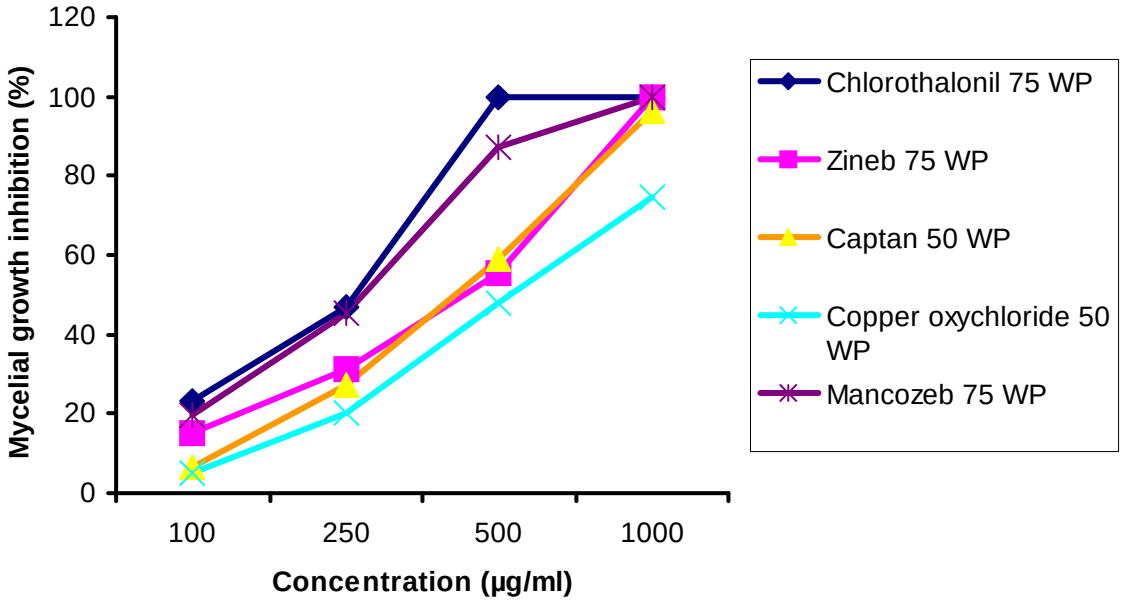


Fig. : ***In vitro*** **efficacy of various non-systemic fungitoxicants in inhibiting the mycelial growth of** ***Didymella bryonie***

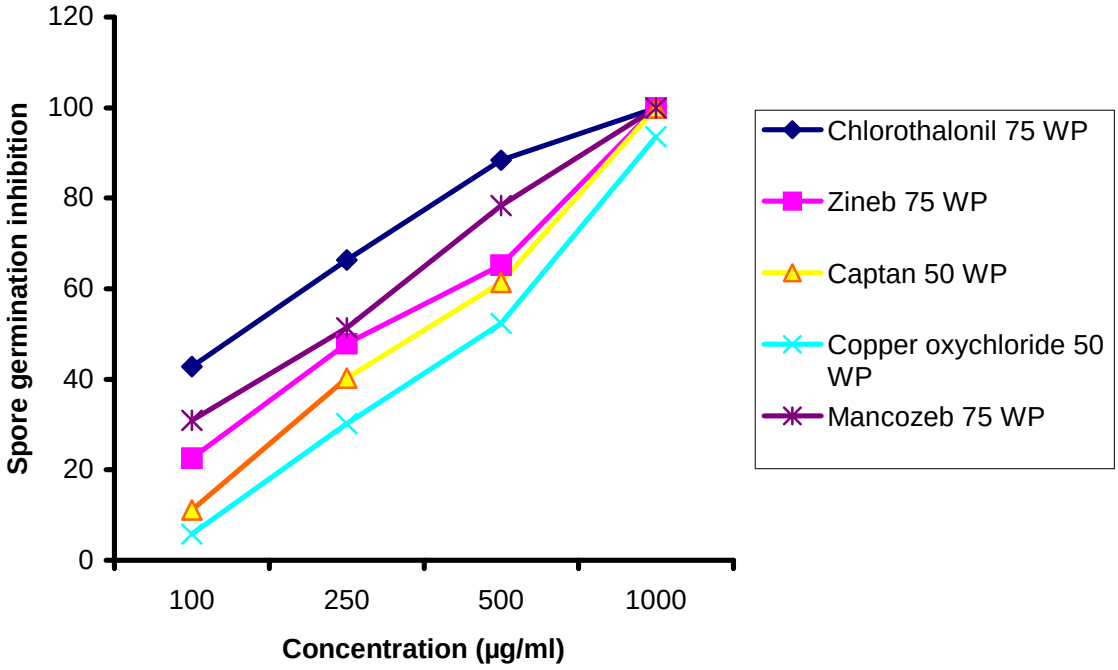


Fig. : ***In vitro*** **efficacy of various non-systemic fungitoxicants in inhibiting the spore germination of** ***Didymella bryonie***

LITERATURE CITED

Arny, C.J. and Randall, R.C. 1991. Effect of temperature and duration of surface wetness on spore production and infection of cucumber by *Didymella bryoniae*. *Phytopathology* **81**: 206-209.

Aswal, B.S., Bhakuni, D.S., Goel, A.K., Kar, K., Mehrotra, B.N. and Mukherjee, K.C. 1984. Screening of Indian plants for biological activity. *Indian Journal of Experimental Biology* **22**: 312.

Bala, G. and Farzan, H. 1985. Gummy stem blight of watermelon and its control. *Journal of the Agricultural Society of Trinidad and Tobago* **85**: 17-23.

Bala, G. and Hosein, F. 1986. Studies on gummy stem blight disease of cucurbits in Trinidad. *Tropical Agriculture* **63**: 195-197.

BISWAS, S., DAS, N. K., QADRI, S. M. H. AND CHANDRA, B. S. 1995. EVALUATING DIFFERENT PLANT EXTRACTS AGAINST THREE MAJOR DISEASES OF MULBERRY. *INDIAN PHYTOPATHOLOG*.**48**: 342-346.

BOWERS, J. H. AND LOCKE, J. C. 2000. EFFECT OF BOTANICAL EXTRACTS ON THE POPULATION DENSITY OF *FUSARIUM OXYSPORUM* IN SOIL AND CONTROL OF FUSARIUM WILT IN THE GREENHOUSE. *PLANT DISEASE* **84**: 300-305.

Brown, M.E. and Preece, T.F. 1968. Examination of cucumber seeds for *Mycosphaerella melonis*. *Plant Pathology* **17**: 116-118.

*Chen, X. and Bao, J.R. 1990. Studies on gummy stem blight of

watermelon, seed borne pathogen and its infectivity *Acta Agriculture Universitatis Zhejiangensis* **16:** 258-262. [cf: CAB Abstracts, CD Rom 1993-1994]

*Chen, X.R., Wei, Y.L. and Zhang, J.W. 1993. Studies on the muskmelon black rot fungus and its biological characters. *Gansu Nongye Daxue Xuebao* **28**: 56-61. [cf: CAB Abstracts, CD Rom 1995]

Chester, F.D. 1891. Notes on three new or noteworthy diseases of plants. *Torrey Botanical Club. Bulletin* **18**: 371-374.

Chiu, W.F. and Walker, J.C. 1949a. Morphology and variability of the cucurbit black rot fungus. *Journal of Agricultural Research* **78**: 81-102.

Chiu, W.F. and Walker, J.C. 1949b. Physiology and pathogenicity of the cucurbit black rot fungus. *Journal of Agricultural Research* **78**: 589-615.

Corlett, M. 1981. A taxonomic survey of some species of *Didymella* and *Didymella* like species. *Canadian Journal of Botany* **59**: 2116-2042.

Corlett, M., Jarvis, W.R. and Maclatchy, I.A. (1986). *Didymella bryoniae*. *Fungi Canadenses* 303.

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

DUBEY, R. C. AND DWIVEDI, R. S. 1991. FUNGITOXIC PROPERTIES OF SOME PLANT EXTRACTS AGAINST VEGETATIVE GROWTH AND SCLEROTIAL VIABILITY OF *MACROPHOMINA PHASEOLINA*. *INDIAN PHYTOPATHOLOGY*

44: 411-413.

Everts, K.L. 1999. First report of benomyl resistance of *Didymella bryoniae* in Delaware and Maryland. *Plant Disease* **83**: 304.

Favoron, F., Castiglioni, C., Lenna, P.D. and Lenna, P. 1993. Inhibition of some rot fungi polygalacturonases by *Allium cepa* L. and *Allium porrum* L. extracts. *Journal of Phytopathology* **139**: 201-206.

Ferraris, T. and Massa, C. 1912. Micromiceti nuovi o rari per la flora micrologia Italiana. *Annales Mycologici* **10**: 285-302.

Filajdic, N. and Sutton, T.B. 1995. Overwintering of *Alternaria mali*, the casual agent of Alternaria blotch of apple. *Plant Disease* **79**: 695-698.

Fletcher, J.T. and Preece, T.F. 1966. Mycospharella stem rot of cucumbers in the Lea Valley. *Annals of Applied Biology* **58**: 423-430.

Flori, A.C.G., Schwan, E.K.R.F., Stangarlin, J.R., Vida, J.B., Scapim, C.A., Cruz, M.E.S. and Pascholati, S.F. 2000. Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae. Journal of Phytopathology* **148**: 483-487.

Grossenbacher, J.G. 1909. A mycospaerella wilt of melons. *New York Agriculture Experimental Station Technical Buleitin* **9**: 193-229.

Grower, R.K. and Moore, J.D. 1962. Toximetric studies of fungicides against brown rot organism *Sclerotinia fruticola* and *Sclerotinia laxa*. *Indian Phytopathology* **15**: 876-880.

Hopkin, D.L. 1973. Fungicidal control of downy mildew and gummy stem blight of watermelon. **In***: Proceedings of the 85th Annual Meeting of the Florida State Horticultural Society* **85**: 108-110. Agricultural Research centre, Lesenburg/ Miami Beach, U.S.A.

Jadeja, K.B. 2003. Evaluation of different herbicides, fungicides, phytoextracts and bioagents against *Phomopsis vexans* causing stem and branch blight in brinjal. *Journal of Mycology and Plant Pathology* **33**: 446-450.

Johnston, A. and Booth, C. 1983. *Plant Pathologist's Pocket Book.* Commonwealth Mycological Institute Kew. Surrey England. 439 p.

Kapoor, L.D. 2001. *Handbook of Ayurvedic Medicinal Plants*. CRC Press, New YorK, Washington DC, 416 p.

KARADE, V. M. AND SAWANT, D. M. 1999. EFFECT OF SOME PLANT EXTRACTS ON THE SPORE GERMINATION OF *ALTERNARIA ALTERNATA. PLANT DISEASE RESEARCH* **14**: 75-77.

Kaushal, R.P. and Paul, Y.S. 1989. Inhibitory effects of some plant extracts on some legume pathogens. *Legume Research* **12**: 131-132.

Keinath, A.P. 2000. Effect of protectant fungicide application schedules on gummy stem blight epidemics and marketable yield of watermelon. *Plant Disease* **84**: 254-260.

Keinath, A.P. 2002. Survival of *Didymella bryoniae* in buried watermelon vines in South Carolina. *Plant Disease* **86**: 32-38.

Keinath, A.P. and Duthie, J.A. 1998. Yield and quality reductions in watermelon due to anthracnose, gummy stem blight and black rot. *Recent Research Developments in Plant Pathology* **2**: 77-90.

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

Keissler, K. 1923. Revision einiger von Fautrey aufgestellter pilze. *Annales Mycologici* **21**: 70-83.

Koike, S.T. 1997. First report of gummy stem blight, caused by *Didymella bryoniae* on watermelon transplants in California. *Plant Disease* **81**: 1331.

Kulwant, S. and Shetty, K.P.V. 1996. Didymella black fruit rot of bittergourd. *Indian Phytopathology* **49**: 294-296.

Kumar, R. and Khan, M.T. 1984. Leaf spot disease of *Cucumis sativus.* *Indian Phytopathology* **37**: 374.

Lee, D., Mathur, S.B. and Neergaard, P. 1984. Detection and location of seed borne inoculum of *Didymella bryoniae* and its transmission in seedlings of cucumber and pumpkin. *Phytopathology Z.* **109**: 301-308.

Leski, B. 1984. Black fruit and stem rot caused by *Didymella bryoniae* an important disease of glasshouse cucumber, new to Poland. *Acta Horticulturae* **156**: 245-251.

Luepschen, N.S. 1961. The development of Mycospaerella black rot and *Pellicularia rolfsii* rot of watermelon at various temperatures. *Plant*

Disease Reporter **45**: 557-559.

Maynard, D.N. and Hopkins, D.L. 1999. Watermelon fruit disorders. *HortTechnology* **9**: 155-161.

Mc Grath, D.J., Vawdrey, L. and Walker, I.O. 1993. Resistance to gummy stem blight in muskmelon. *HortScience*. **28**:930-931.

Miller, M.E., Isakiet, T. and Hemandez, R.A. 1999. Evaluation of fungicides for gummy stem blight control on muskmelon. *Fungicide and Nematicide Tests* **53**: 159

Muller, M.E. and Von Arx, J.A. 1962. Die gattungen der didymosporen pyrenomyceten. *Beitr Kryptogamenflora Schweiz* **11**: 921-922.

Montgomery, H.B.S. and Moore, M.H. 1938. A laboratory method for testing the toxicity of protective fungicides. *Journal of Pomology and Horticulture Science* **15**: 253-266.

More, T.A. 2001. Cucurbitaceous vegetables. pp. 238-319. **In**: *Textbook of Vegetables, Tuber crops and Spices.* (eds. S. Thamburaj and N. Singh). Directorate of Information and Publications of Agriculture, ICAR, New Delhi.

Olsen, M.W. and Slanghellini, M.E. 1981. *Mycosphaerella melonis* on greenhouse cucumber. *Plant Disease* **65**: 157-159.

Pandey, K.K. and Pandey, P.K. 2003. First report of some new fungal diseases on wax gourd (*Benincasa hispida*) in the world from India. *Journal of Mycology and Plant Pathology* **33**: 439-441.

Panse, V.G. and Sukhtme, P.V. 1978. *Statistical Methods for Agricultural*

Workers. Indian Council of Agricultural Research, New Delhi, 347 p.

Potebina, A. 1910. Breitrage zur micromycetenflora mittel russlands. *Annales Mycologici* **8**: 42-93.

Pretorius, J.C., Craven, P., Watt, E.V., derWatt, E.V. and VanderWatt, E. 2002. *In vitro* control of *Mycosphaerella pinodes* on pea leaves by a crude bulb extract of *Eucomis autumnalis*. *Annals of Applied Biology* **141**: 125-131

Puninthalingam, E. and Holliday, E. 1972. *Didymella bryoniae*. *CMI Descriptions of Pathogenic Fungi and Bacteria*. No. **332**. Common Wealth Mycological Institute, Ferry Lane, Kew, U.K.

Rankin, H.W. 1954. Effectiveness of seed treatments for controlling anthracnose and gummy stem blight of watermelon. *Phytopathology* **44**: 675-680.

Roumeguere, C. 1891. Fungi exsiccate precipue gallici. *Revue Mycologia* **13**: 73-78.

Saccardo, P.A. 1891. *Sylloge Fungorum* **9**: 662.

Schenck, N.C. 1962. Mycosphaerella fruit rot of watermelon. *Phytopathology* **52**: 635-638.

Schenck, N.C. 1968. Epidemiology of gummy stem blight (*Mycosphaerella citrullina*) on watermelon: Ascospore incidence and disease development. *Phytopathology* **58**: 1420-1422.

Seshadri, V.S. 1993. Cucurbits. pp. 40-109. **In**: *Vegetable Crops* (eds. T.K. Bose, M.G. Som and J. Kabir). Naya Prakash Calcutta, India.

Sidhu, A.S. 2000. Current status of vegetable research in India. http//

www. Green-seeds.com/ind_veg_res.html

SINDHAN, G. S., HOODA, I. AND PARASHAR, R. D. 1999. EVALUATION OF PLANT EXTRACTS FOR THE CONTROL OF POWDERY MILDEW OF PEA. *JOURNAL OF MYCOLOGY AND PLANT PATHOLOGY* **29**: 257-258.

SINGH, G. 1997. PEST CONTROL THROUGH ESSENTIAL OILS PP. 555. **IN***: SUPPLEMENT TO CULTIVATION AND UTILIZATION OF AROMATIC PLANTS.* (EDS. HANDA. S. S. AND KAUL, M. K.), REGIONAL RESEARCH LABORATORY, JAMMU-TAWI..

Singh, S. D. and Navi, S. S. 2000. Garlic as a biocontrol agent for sorghum ergot. *Journal of Mycology and Plant Pathology* **30**: 350-354.

Singh, P.P., Wadhwa, A. and Sandhu, K.S. 2003. Influence of post inoculation diurnal conditions on development of Ascochyta blight of pea. *Indian Phytopathology* **56**: 446-447.

Singh, R.S. 1987. *Diseases of vegetable crops*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi pp 362.

Sitterly, W.R. 1968. A new symptom of gummy stem blight (*Mycosphaerella melonis*) on cucumber fruit. *Plant Disease Reporter* **52**: 49-51.

Sitterly, W. R. 1969. Effect of crop rotation on cucumber gummy stem blight. *Plant Disease Reporter* **53**: 417-419.

Skarshaug, A.J. 1981. Centrum development in *Didymella bryoniae*. *American Journal of Botany* **68**: 1096-1103.

Smith, C.O. 1905. The study of the diseases of some truck crops in

Delaware. *Delaware Agriculture Experimental Station Bulletin* 70, 16 pp.

Sohi, H.S. and Prakash, O. 1972. New records of fungal diseases from India. *Indian Journal of Mycology and Plant Pathology* **2**: 139-142.

St. Amand, P.C. and Wehner, T.C. 1995. Eight isolates of *Didymella bryoniae* from geographically diverse area exhibit variations in virulence but no isolate by cultivar interaction on *Cucumis sativus*. *Plant Disease* **79**: 1136-1139.

Sudisha, J., Kumar, T. V., Niranjana, S.R. and Shetty, H.S. 2004. First report of gummy stem blight caused by *Didymella bryoniae* on muskmelon (*Cucumis melo*) in India. *Plant Pathology* **53**: 533.

Sudisha, J., Niranjana, S.R., Umesha, S., Prakash, H.S. and Shetty, H.S. 2006. Transmission of seed borne infection of muskmelon by *Didymella bryoniae* and effect of seed treatments on disease incidence and fruit yield. *Biological Control* **37**: 196-205.

Sumner, D.R., Phatak, S.C. 1987. Control of foliar diseases of cucumber with resistant cultivars and fungicides. *Applied Agricultural Research* **2**: 324-329.

Sumner, D.R., Phatak, S.C., Smittle, D., Johnson, A.W. and Glaze, N.C. 1981. Control of cucumber foliar diseases, fruit rot and nematodes by chemicals applied through overhead irrigation water. *Plant disease* **65**: 401-404.

Svedellus, G. and Unestam, T. 1978. Experimental factors favoring infection of attached cucumber leaves by *Didymella bryoniae*. *Transactions of British Mycological Society* **74**: 89-97.

Tasleem, K., Nasir, M.A. and Bokhan, S.A.A. 1998. Antifungal properties of some plant extracts. *Pakistan Journal of Phytopathology* **10**: 62-65.

Trapero, C.A. and Kaiser, W.J. 1992. Development of *Didymella rabie*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathology* **82:** 1261-1266.

*Uspenskaya, G.D., Plukarova, G.A. and Orlova, G.L. 1976. Conservation of diasporas of *Ascochyta cucumeris* Foutr. and Roum. in soil. *Biologicheskie Nauki* **19**: 76-79. [cf: CAB Abstracts, CD Rom 1976-1978]

*Uspenskaya, G.D., Lebl, D.O., Ivanov, V.I. and Suchkova, L.V. 1979. The effect of glasshouse microclimate on the pathogen of Ascochytosis of cucumber; the distribution of spores of *Ascochyta cucumeris* Fautr. and Roum. (Ascus state *Didymella bryoniae* (Auers.) Rehm) in winter block glasshouse on winter spring cucumber crops. *Mikologiya Fitopathologiya* **13**: 418-421. [cf: CAB Abstracts, CD Rom 1979- 1981]

Urbenszki, K., Ulveczki, G. and Balogh, P. 2002. Effect of certain fungicides on *Phoma cucurbitacearum* (Fr.:Fr.) Sacc. culture. *Novenyvedelem* **38**: 31-35.

Vanderplank, J.E. 1963. *Plant disease : Epidemics and Control. Academic* Press, New York, p. 349.

Van Steekelenburg, N.A.M. 1978. Chemical control of *Didymella bryoniae* in cucumbers. *Netherland Journal of Plant Pathology* **84**: 27-34.

Van Steekelenburg, N.A.M. 1981. Comparison of inoculation methods with *Didymella bryoniae* on *Cucumis Sativus. Euphytica* **30**: 515-520.

Van Steekelenburg, N.A.M. 1983. Epidemiological aspects of Didymella bryoniae, the cause of stem and blight rot of cucumber. *Netherland Journal of Plant Pathology* **89**: 75-86

Van Steekelenburg, N.A.M. 1985. Influence of humidity on incidence of *Didymella bryoniae* on cucumber leaves and growing tips under controlled environmental conditions. *Netherland Journal of Plant Pathology* **91**: 277-283

Vawdrey, L.L. 1994. Evaluation of fungicides and cultivars for control of gummy stem blight of roackmelon caused by *Didymella bryoniae*. *Australian Journal of Experimental Agriculture* **34**: 1191-1195.

Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **15**: 850.

Walker, M.N. and Webber, G.F. 1931. Diseases of watermelons in Florida. *Florida Agriculture Experimental Station Bulletin.* **225**: 18-22.

Webber, G.F. 1929. Cucumber diseases in Florida. *Florida Agriculture Experimental Station Bulletin*. 208, 48 pp.

Wehner, T.C. and Shetty, N.V. 2000. Screening the cucumber germplasm collection for resistance to gummy stem blight in North Carolina field tests. *HortScience* **35**: 1132-1140.

Wiant, J.S. 1945. Mycosphaerella black rot of cucurbits. *Journal of Agricultural Research* **71**: 193-213.

---

*Original not seen

APPENDIX-II

Fungitoxicants and their chemical names

S. No.	Common name	Trade name	Chemical name
1.	Carbendazim 50 WP	Bavistin 50 WP	2-(methoxy-carbomyl) benzimedazole
2.	Thiophanate methyl 70 WP	Topsin-M 70 WP	(1,2-bis(3-methoxy-carbonyl-2-thioureido)-benzene
3.	Myclobutanil 10 WP	Systhane 10 WP	A-butyl-α(4-chlorophenyl)
4.	Diniconazole 25 WP	Sumi 25 WP	4-dimethyl-2-(1,2,4-triazol-1-yl)-1-pentene-3-ol
5.	Bitertanol 25 WP	Baycor 25 WP	1-(1,1-Biphenyl)-1H-1,2,4-Triazole-1-1 ethanol
6.	Chlorothalonil 75 WP	Kavach 75 WP	Tetrachloroisphthalonitrile
7.	Zineb 75 WP	Indofil Z-78	Zinc ethylene bisdithiocarbamate
8.	Captan 50 WP	Captaf 50 WP	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
9.	Copper oxychloride 50 WP	Fytolon 50 WP	Copper oxychloride
10.	Mancozeb 75 WP	Dithene M-45	Zinc manganese ethylene bisdithiocarbamate

APPENDIX-I

Composition of various culture media used

S. No.	Agar medium	Constituents	Quantity used
1	Ridge gourd leaf extract	Fresh ridge gourd leaves	300 g
		Agar	20 g
		Distilled water	1000 ml
2	Ridge gourd fruit extract	Fresh ridge gourd fruits	300 g
		Agar	20 g
		Distilled water	1000 ml
3	Potato dextrose	Peeled potato	200 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
4	Corn meal	Corn meal	50 g
		Agar	20 g
		Distilled water	1000 ml
5	Oat meal	Oat meal	50 g
		Agar	20 g
		Distilled water	1000 ml
6	Czapek-dox	Magnesium sulphate	0.50 g
		Potassium sulphate	1.0 g
		Potassium chloride	0.50 g
		Ferrous sulphate	0.01 g
		Sodium nitrate	2.00 g
		Sucrose	30.00 g
		Agar	20 g
		Distilled water	1000 ml
7	Malt extract	Malt extract	20 g
		Agar	20 g
		Distilled water	1000 ml

APPENDIX-III

Botanicals and their parts used

S.No.	Scientific names	Common name	Local name	Plant part used
1	*Urtica dioica*	Stinging nettle	Soi	Leaves
2	*Artemisia annua*	Worm wood	Tethwan	Leaves
3	*Datura stramonium*	Thorn apple	Datur	Leaves
4	*Allium sativum*	Garlic	Rohan	Leaves
5	*Allium cepa*	Onion	Ganda	Leaves

SHER-E-KASHMIR
UNIVERSITY OF AGRICULTURAL SCIENCES & TECHNOLOGY OF KASHMIR
DIVISION OF PLANT PATHOLOGY, SHALIMAR CAMPUS SRINAGAR
191 121

CERTIFICATE

This is to certify that all the corrections and modifications suggested by the external examiner(s) in the thesis script of **Mr.** Zahoor Ahmad Bhat **(Registration No. 2003-112-D), "Studies on fungal leaf blight of ridge gourd (Luffa acutangula (L.) Roxb.) in Kashmir valley"** have been taken care of before final binding of the same.

Dr. G. M. Dar

Chairman

Advisory Committee