“STUDIES ON MYROTHECIUM LEAF SPOT (*Myrothecium roridum*) Tode ex Fries. OF SOYABEAN”

M. Sc. (Ag.) THESIS

By
DIGANGGANA TALUKDAR

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
RAIPUR (C. G.)
2011

“STUDIES ON MYROTHECIUM LEAF SPOT (*Myrothecium roridum*) Tode ex Fries. OF SOYABEAN”

Thesis

Submitted to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

By
DIGANGGANA TALUKDAR

IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR
THE DEGREE OF

Master of Science

In

Agriculture

(Plant Pathology)

Roll No. 12471 **ID No. ID No.**
120109026

JUNE, 2011

CERTIFICATE – I

This is to certify that the thesis **"STUDIES ON MYROTHECIUM LEAF SPOT (*Myrothecium roridum*)Tode ex. Fries. of Soyabean"** submitted in partial fulfillment of the requirement for the degree of **"MASTER OF SCIENCE IN AGRICULTURE"** of the Indira Gandhi Agricultural University, Raipur, is a record of the bonafide research work carried out by **DIGANGGANA TALUKDAR** under my guidance and supervision. The subject of the thesis has been approved by Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma (certificate awarded etc.) or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date:

Chairman
Advisory Committee

THESIS APPROVED BY THE STUDENTS ADVISORY COMMITTEE

Chairman: Dr. R. K. Dantre -------------------------------

Member: Dr. K. P. Verma -------------------------------

Member: Dr. A.S. Kotasthane --------------------------------

Member: Dr. (Smt.) G. Chandrakar -------------------------------

CERTIFICATE-II

This is to certify that the thesis entitled **"STUDIES ON MYROTHECIUM LEAF SPOT (*Myrothecium roridum*)Tode ex. Fries. of Soyabean"** submitted by **DIGANGGANA TALUKDAR** to the Indira Gandhi Agricultural University, Raipur in partial fulfillment of the requirements for the degree of **M. Sc. (Ag.)** in the **Department of Plant Pathology** has been approved by the external examiner and Student's Advisory Committee after oral examination.

Date: **External Examiner**

Major Advisor ---------------------------------

Head of the Department ---------------------------------

Dean/Dean Faculty ---------------------------------

Director of Instructions ---------------------------------

ACKNOWLEDGEMENT

First of all I would like to thank and praise Almighty "God" the most beneficent and merciful, for all his love and blessing conferred upon mankind. "A journey is easier when you travel together. Interdependence is especially more valuable than independence". This is the result of two years work whereby, I have been accompanied and supported by many people. It is a pleasant aspect that I got a golden opportunity to express my gratitude for all of them.

I take this golden opportunity to express my deepest sense of gratitude to the chairman of my advisory committee Dr. R.K. Dantre, Professor, Department of Plant Pathology, for his research insight, valuable guidance, constant encouragement, unique supervision and kind sympathetic attitude, despite his heaviest schedule of work, his helpful, patience, creative and talented guidance has given touch of excellence to this manuscript.

I express my deep sense of gratitude to Dr. K.P. Verma, Senior scientist, Department of Plant Pathology and member of my advisory committee for his illuminating guidance, constant encouragement, valuable suggestions, unique supervision and I am highly indebted to his painstaking efforts taken towards my research work while devoting his precious time amidst his busy schedule.

With extreme pleasure, I extent my heartiest thanks to the members of my advisory committee Dr. A. S. Kotasthane, Professor, Department of Biotechnology, IGKV, for his illuminating guidance, constant encourengement and suggestions during the course of my work and Dr.(Smt.) G. Chandrakar, Professor, Department of Agricultural Statistics, Mathematics and Computer Science for their excellent guidance, suggestions, supervisions and regular encouragement during the course of investigation.

I express my sincere and profound gratitude to Dr. V.S. Thrimurty, Prof. and Head, Department of Plant Pathology for providing me all the necessary facilities, during my study.

I am deeply indebted to my teachers of Department of Plant Pathology Dr. G. K. Awadhiya, Pr. Scientist, Dr. N. Khare, Professor, Sr Scientist C.S.Shukla, Asso Professor Dr. C. P. Khare, Sr. Scientist, Dr. P. K. Tiwari, Sr. Scientist and Dr. S. S. Chandrawanshi, Scientist, Department of Plant Pathology, for their encouragement.

Plant Pathology, for their encouragement and constant help throughout course of my studies.

I wish to record my grateful thanks to Dr. M.P. Pandey, Hon'ble Vice Chancellor, Dr. S.K. Patil, Director Research Services, Dr. U.K. Mishra, Director of Instructions and Dr. O.P. Kashayap, Dean, College of Agriculture, IGKV, Raipur for providing necessary facilities, technical and administrative supports for conductance of my research work.

I extend my thanks to Shri M. B. Roy, Shri S. R. Tikariha, Shri B. L. Sinha , Shri. S. R. Sahu, Bhudharu, Patel, Santu, non–teaching staffs of our department who were always ready to provide help in this study.

My due regards and greatness is extended to the Chaliganjewar sir, Jitendra Meshram sir and Wagh Sir for their immense help and guidance during course of this investigation.

I will be failing in my duties if I don't convey my sincere thanks to batchmates Manali, Indu, Umesh sir, Vandana, Ritika, Janni and Shweta and juniors Minakshi, Nushrat, Rashmi, Manu, Deepmala, Deepti, Santosh and Suryakant who coordinated with me whenever I am in need.

I am deeply priviledged to express any heartfelts thanks to best friends Soumitra Tiwari and Sangeeta Sharma who provided me an inner strength and guided me during my entire academic career as to steer up my ambition in a proper way.

For the most important personalities of my life, there aren't enough words to express my gratitude to My father Dr. R.K. Talukdar, my mom Mrs. Mamoni Talukdar, My brother Jogabrata, My uncle, Dr J.D. Sarkar and Sarkar aunty for their constant encouragement, sincere prayers, expectations and blessings which have always been the most vital source of inspiration, assets and motivation in my life.

I would like to convey my cordial thanks to all those unmentioned persons who helped me directly and indirectly to fulfill my dream come true.

Department of Plant Pathology, ***(Diganggana Talukdar)***
College of Agriculture,
IGKV, Raipur (C. G.)

CONTENTS

CHAPTER	PARTICULARS	PAGE NO.
I	**INTRODUCTION**	1-2
II	**REVIEW OF LITERATURE**	4-18
2.1	The disease	4
2.2	Symptomatology	5
2.3	Characterization of causal organism	6
2.4	Isolation, purification, identification & pathogenicity	6
2.5	**Disease cycle**	8
2.5.1	Environmental factors for disease development of Myrothecium leaf spot of soyabean,	8
2.5.2	Effect of date of sowing on disease severity of Myrothecium leaf spot of soyabean under field conditions	9
2.6	**Physiological studies**	10
2.6.1	Effect of pH on radial growth and sporulation of *M. roridum*	10
2.6.2	Effect of different media (synthetic & non synthetic) on radial growth and sporulation of *M. roridum*	10
2.6.3	Effect of different Temperature on radial growth and sporulation of *M. roridum*	11
2.7	**Enzymatic and Toxin activity**	11
2.7.1	Enzymatic activity	11
2.7.2	Effect of toxins	13
2.8	Effect of culture filtrate	14
2.9	Management	15
2.9.1	Management by different medicinal plants leaf extracts.	15
2.9.2	Management by bioagent	16
2.9.3	Management by different fungicides *in vitro* condition	17
2.10	Study on host plant resistence	18
III	**MATERIALS AND METHODS**	19-35
3.1	Materials	19

3.1.1	Source of material	19
3.1.2	Experimental site	20
3.1.3	General procedure	20
3.2	**Isolation Purification & Identification**	21
3.2.1	Collection of diseased sample	21
3.2.2	Isolation & Purification of test fungus	21
3.3	**Pathogenicity of test fungus**	21
3.3.1	On leaf	21
3.3.1.1	Attached method	22
3.3.1.2	Detached methods	22
3.3.2	Testing of pathogenicity by seed inoculation method	22
3.4	**Physiological studies**	23
3.4.1	$_P$H and	23
3.4.2	Media	23
3.4.3	Temperature	25
3.5	**Determination of enzymatic activity**	26
3.5.1	Cellulase (Cx) activity	26
3.5.2	Polygalacturonase (PG) activity	27
3.5.3	Macerating enzymes	27
3.6	**Toxic effect of culture filtrate**	28
3.6.1	On leaf	28
3.6.1.1	Attached method	28
3.6.1.2	Detached method	28
3.6.2	On seed	28
3.7	Management of test fungus	29
3.7.1	Medicinal plant leaf extract	29
3.7.2	Bio-control	30
3.7.3	Fungicides	32
3.8	**Host plant resistance**	33
3.8.1	Evaluation of Soyabean Genotypes against Myrothecium leaf spot under field conditions.	33
3.8.2	Evaluation of Soyabean Genotypes against	34

	Myrothecium leaf spot under glass house conditions	
3.9	Effect of date of sowing on disease severity of Myrothecium leaf spot of soyabean under field condition	35
3.10	Disease development in relation to environmental factors	35
IV	**RESULTS AND DISCUSSION**	36-65
4.1	Symptomatology	36
4.2	Isolation, Purification and Identification of Pathogen.	37
4.3	Pathogenicity	37
4.3.1	On leaves	37
4.3.1.1	Attached method	37
4.3.1.1.1	Field condition	37
4.3.1.1.2	Laboratory condition	38
4.3.2.1	Detached method	38
4.3.2	Testing of pathogenicity by seed inoculation method	39
4.4	**Physiological studies**	41
4.4.1	Effect of different $_{P}$H on radial growth and sporulation of *M. roridum*	41
4.4.2	Effect of different media (synthetic & non synthetic) on radial growth and sporulation of *M. roridum*	42
4.4.3	Effect of different temperature on radial growth and sporulation of *M. roridum.*	43
4.5	**Determination of enzymatic activity**	44
4.5.1	Influence of culture filtrate of *M. roridum* on the activity of Carboxy methyl cellulose (CMC)	44
4.5.2	Influence of culture filtrate of *M. roridum* on the activity of Polygalacturonase (PG) at different $_{P}$H levels	45
4.5.3	Effect of culture filtrate of *M. roridum* on macerating of potato and carrot pith	47
4.5.4	Influence of culture filtrate of *M. roridum* on attached leaf of soyabean	48
4.5.5	Influence of culture filtrate of *M. roridum* on detached leaf of soyabean	49
4.5.6	Influence of culture filtrate of *M. roridum* on	50

	germination of soyabean seed tested by standard blotter method.	
4.6	**Management of test fungus**	51
4.6.1	Efficacy of different medicinal plants leaf extracts on inhibition of radial growth of *M. roridum*.	51
4.6.2	Evaluation of fungicides at different concentration on inhibition of radial growth *M. roridum* by Poisoned food technique	53
4.6.3	Screening of different isolates of *Trichoderma* spp. against *M. roridum*	58
4.7	Evaluation of host resistant of different soyabean varieties against *M. roridum*	61
4.8	Effect of date of sowing on disease severity of *Myrothecium roridum* on soyabean under field conditions	62
4.9	Disease development in relation to environmental factors	64
V	**SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH WORK**	65-68
	ABSTRACT	67
	REFERENCES	70-80

LIST OF FIGURES

FIGURE NO.	PARTICULARS	PAGES IN BETWEEN
1.	Effect of different pH on radial growth and sporulation of *Myrothecium roridum*	41-42
2.	Effect of different media on radial growth and	43-44

	sporulation of *Myrothecium roridum*	
3.	Effect of different temperature on radial growth and sporulation of *Myrothecium roridum*	44-45
4.	Influence of culture filtrate of *Myrothecium roridum* on the activity of CMC at different pH levels	45-46
5.	Influence of culture filtrate of *Myrothecium roridum* on the activity of PG at different pH levels	47-48
6.	Per cent inhibition of radial growth of *Myrothecium roridum* on different medicinal plant extract	52-53
7.	Evaluation of different fungicides and their concentration on radial growth of *Myrothecium roridum* (250ppm)	55-56
8.	Evaluation of different fungicides and their concentration on radial growth of *Myrothecium roridum* (500ppm)	56-57
9.	Evaluation of different fungicides and their concentration on radial growth of *Myrothecium roridum*(1000ppm)	57-58
10.	Effect of different *Trichoderma* spp. on radial growth of *M. roridum*	60-61

LIST OF PLATES

PLATE NO	PARTICULARS	PAGES IN BETWEEN
1.	Symptom exhibiting on leaf stem & pod of soyabean plant	36-37
2.	Twenty days old culture, sporodochia, mycelium, morphology of conidia	37-38
3.	Testing of pathogenicity of *M. roridum* under field conditions	38-39
4.	Testing of pathogenicity of *M. roridum* under laboratory condition by attached method	38-39
5.	Testing of pathogenicity of *M. roridum* under laboratory condition by detached method	39-40
6.	Testing of pathogenicity of *M. roridum* on soyabean seed	40-41
7.	Effect of different pH on radial growth and sporulation of *M. roridum*	41-42
8.	Effect of different media on radial growth and sporulation of *M. roridum*	43-44
9.	Effect of different temperature on radial growth and sporulation of *M. roridum*	44-45
10.	Effect of culture filtrate of *M. roridum* on macerating of potato and carrot pith	48-49
11.	Effect of culture filtrate of *M. roridum* on attached plant leaf of soyabean	49-50
12.	Effect of culture filtrate of *M. roridum* on detached leaves of soyabean	50-51
13.	Influence of culture filtrate of *M. roridum* on germination of soyabean seeds (seed treated)	51-52
14.	Influence of culture filtrate of *M. roridum* on germination of soyabean seeds (blotter treated)	51-52
15.	Influence of culture filtrate of *M. roridum* on germination of soyabean seed(seed + blotter treated)	51-52

16.	Efficacy of different medicinal plants extracts on growth of *Myrothecium roridum*	52-53
17.	Efficacy of different fungicides on inhibition of radial growth of *Myrothecium roridum* (250ppm)	55-56
18.	Efficacy of different fungicides on inhibition of radial growth of *Myrothecium roridum* (500ppm)	56-57
19.	Efficacy of different fungicides on inhibition of radial growth of *Myrothecium roridum* (1000ppm)	57-58
20.	Effect of different *Trichoderma* spp. on radial growth of *M. roridum*	60-61

LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
%	Per cent
^{O}C	Degree celsius
CD	Critical difference
Cm	Centimeter
DAI	Days after inoculation
et al.	And other
Fig.	Figure
G	Gram
Ha	Hectare
Hrs	Hours
i.e.	That is
Mm	Milli Meter
m ha	Million hectare
Mt	Metric tonne
No.	Number
SEm	Standard error of mean
Wt	Weight
viz.	Namely
TMT	Thousand metric tonnes

CHAPTER-I

INTRODUCTION

Soyabean (*Glycine max* (L.) Merrill) is one of the most important oil seed crops of India. It is also named as wonder crop. Soyabean is a native of eastern Asia and is the oldest crop in the area and is considered to be a vital grain crop (Zhang &Smith, 1995). Though the origin is not accurately understood, it is believed to have originated in China in around 2800 BC (Bhatnagar and Tiwari, 1996). Soyabean is a subtropical crop which grows well in temperate region. In India it is grown as kharif crop. It belongs to family leguminaceae. It is one of the most economical & valuable agricultural oil seed crop of India. It is an excellent source of protein, fat and oil. It contains about 40-42% protein and 20% edible oil on dry weight basis. It contains high level of amino acid such as lycine, leucine, lecithin and large amount of phosphorous. Soybean is used for preparation of milk, curd, backed soybean, roasted soyabean etc. It has capacity to fix nitrogen with the help of rhizobium and improve soil fertility. Soyabean is the most economical legume for protein and oil production.

The United States is the largest producer of soyabeans in the world, averaging a production of 69,682 MT of beans. The United States is also the world's largest consumer of soyabean, sitting at an average annual consumption of 45,313 MT.

India is the fifth largest producer of soyabean in the world, with an average production of 4,809 TMT of soyabean. Average consumption in India is 4,812 TMT of soybeans, giving them the rank of sixth largest consumer of beans in the world. The Majority of all the soybean in India are grown in the province of Madhya Pradesh. Alone,

this province produces 81 percent of all the soyabean grown in India. The remaining growing areas are centered around Madhya Pradesh, which lies in the center of the nation. In most of India, soyabean are planted from the first of June through the end of August. Soyabean will then begin to flower and develop pods during the time span of mid-July through mid-October. The harvest will then take place from mid-September through the end of December. In India, soyabean ranks 3rd in oil seed after Groundnut and Rapeseed. The estimated national production of soyabean during this year (2010) would be 101.283 lakh MT compared to that of 97.246 lakh MT during 2009, resulting in 4.15% growth in one year**.** In India M.P. is the leading state in soybean production followed by Maharashtra and Rajasthan. In Madhya Pradesh soybean is grown in 55.193 lakh ha with an average yield of 1105 kg/ha and a total production of 60.987 lakh MT. In Chhattishgarh soyabean is grown in 1.490 lakh ha with an average yield of 1050 kg per ha and a total production of 1.565 lakh MT.(Dey, 2010.)

The global soyabean production is projected at a record 250.25 million tonnes the year (2010), up by 18.67 % as compared to 210.87 million tons during the previous year (2009).

Soyabean crop is known to suffer from many diseases. One of such disease is Myrothecium leaf spot. It is one of the important disease which caused by *Myrothecium roridum*. Myrothecium leaf spot was reported to be in epidemic form in Madhya Pradesh in 1994-95. (Singh *et al.*,1994).

Myrothecium is a facultative parasite with a large number of plant host, including vegetable, fruits, ornamentals and crops. *Myrothecium roridum* was reported as a pathogen of more than two hundred plant species belonging to distinct botanical families.

Initial symptoms of *Myrothecium roridum* appeares as small round or oval, brown spots developed with dark brown margin on leaves in the infected plant. Under favourable conditions (high humidity) creamy layer of erumpent fungus appeared and then oval to irregular shaped sporodochia formed on the infected area of leaf spot.

The best way to manage this disease is to grow resistant varieties. (Singh *et al.,* 1994). Field sanitation by burning the crop debris will reduce the primary inoculums of the disease. (Srivastava *et al*., 1995). Although it is an important disease which occures in severely in all soyabean growning areas specially Madhya Pradesh and Chhattishgarh, it has not been studied properly. Knowledge about this disease is nill to many of us. In India itself, no proper research work has been done regarding this disease. It causes yield loss in soyabean crops to a considerable amount. So taking into account about this and keeping in view the importance of this disease, present investigations were proposed with the following objectives :

- Isolation, purification, identification of *Myrothecium roridum* & testing the pathogenicity of it.
- Physiological & Biochemical studies of *Myrothecium roridum.*
- Management practices of *Myrothecium roridum*.

CHAPTER –II

REVIEW OF LITERATURE

2.1 The disease :

Soyabean (*Glycine max (L.)* Merrill) is an important oil seed crop grown in kharif season. Soyabean is a native of eastern Asia and is the oldest crop in the area considered to be a vital grain crop (Zhang &Smith, 1995). Though the origin is not accurately understood, it is believed to have originated in China in around 2800 BC (Bhatnagar and Tiwari, 1996). The crop is known to suffer from a number of disease such as, Myrothecium leaf spot, Cercospora leaf spot, Wilt, Target spot, Rust, Rhizoctonia areal blight, Charcoal rot, Choanephora leaf blight etc.

The *Myrothecium roridum* was first reported in Britain on the roots and stem of viola and pancy plant.(Preston, 2009). It was further reported in Bangladesh, China, Cambodia, Island, Japan & India etc. and from in India is observed in Madhya Pradesh, Chhattishgarh, Assam, Bihar, Delhi, Gujarat, Karnataka, Kerala, Punjab, Rajasthan and U.P states. It is an important disease in Chhattishgarh and appears every year in moderate to severe intensity and cause substaintial losses. (Annonymous 2009).

Di Menna *et al.*(1973) reported that Myrothecium leaf spot is important disease of soyabean crop. Many spp. found in Myrothecium i.e. *M. roridum, M. verrucaria, M. gramineum, M. tongaense, Myrothecium leucotrichum* and *M. cinctum* and occurred on leaves of perennial ryegrass, white clover, kikuyu grass and mixed pasture in the North Island of New Zealand. Yam and Park (1990) reported that *Myrothecium roridum* causes leaf spot disease in soyabean plant.

2.2 Symptomatology :

Lakshminarayana and Joshi (1978) reported that in soyabean *M. roridum* cause dark brown, circular leaf spots on soybean, with a necrotic zone surrounded by a chlorotic band. There were numerous black sporodochia in the necrotic zone. Symptoms are found in leaves, flowers and pods.

Mohan *et al*.(1988) observed Myrothecium leaf spot in the form of small dirty yellow to brown coloured water soaked spots on the leaves having chlorotic halo lesion in papaya cultivar Co2 in Coimbatore orchard. It is mainly confined to leaves. Later on the spots coalace and entire leaf gets dried.

Chase (1992) observed the black and white fungal fruiting bodies in concentric rings near the outer edge of the spot or rotted areas in the ornamental flowers. The presence of these bodies is good evidence that the cause is Myrothecium.

Byrne and Raymond (2007) reported that the disease causes leaf spots. Concentric rings may develop in the lesions. Raised, black sporodochia develop on diseased tissue. In high humidity, the sporodochia are encircled by a tuft of white growth.

Seebold *et al.* (2005) observed black sporodochia with white marginal tuffs present on the lower surface of older lesions. Isolations in PDA brings white fluccose colonies with sporodochia in dark green to black concentric rings bearing viscid masses of conidia. Conidia are hyaline and cylindrical with rounded ends and measured 7.4×2.0 µm.

Leaves of soyabean showed necrotic round to oblong lesions with concentric rings of brown and dark brown coloured (Mmbaga, 2010).

2.3 Characterization of causal organism :

Nguyen *et al.* (1973) studied *Myrothecium leucotrichum* in cowpea*, M. roridum* in dahlia and nasturtium, and *M. verrucaria* in soybean and rice were found to be seed-borne for the first time. These are new records on their respective hosts, and their pathogenicity was confirmed. *M. leucotrichum* was the most virulent species, whereas *M. roridum* was moderate and *M. verrucaria* was moderate to weakly virulent.

Mohan *et al.* (1988) isolated myrothecium fungus on PDA media and described conidia as hyaline, single celled, cylindrical to elliptical, agreegated in the form of a gelatinous mass and measured 6.7-9µm×2.1-2.7µm.

Mmbaga (2010) isolated *Myrothecium roridum* from leaves of garden hydrangea Isolates developed white colonies and dark gray-to-black, spore-bearing mycelial cushions (sporodochia) that formed on older colonies (30 to 45 days old) at 25 ± 2°C. Conidia were hyaline to slightly dark, one-celled, ovoid to elongate with rounded ends, and 2.0 to 2.5 × 5.5 to 6.5 µm in size.

2.4 Isolation, purification, identification & pathogenicity :

Ponnappa (1970) recorded mortality of *Echhornia crassipes* either by spraying spore suspension of *M. roridum* or growing in the moist chamber and determined the specificity.

Kuti *et al.*(1989) inoculated 50 cultivars and breeding lines of muskmelon (*Cucumis melo* L.) with pathogenic strain of *Myrothecium roridum*. Experiment was done on detached leaf tissues from greenhouse plants and immature fruits from field-grown plants. Leaf susceptibility was characterized by chlorosis, necrosis, and extensive fungus

sporulation, whereas fruit susceptibility was evidenced by host tissue decay and fungus sporulation.

Sengupta *et al.* (1989) collected diseased leaves of mulberry plants, surface sterilized with 0.1% $HgCl_2$ and plated on Czapek's and PDA media. Plates incubated at 28°C for 7 days and isolated the *M. roridum* , purified and maintained in PDA slants.

Escalona *et al*. (1992) isolated *Myrothecium* sp. from diseased *Heliconia psittacorum* x *H. spathocircinata* cultivars Golden Torch and Tropic with dark-brown lesions on spathes of inflorescences, which resulted in 30% cut flower loss, in the Carabobo state, Venezuela, in 1991 and confirmed pathogenicity.

Horinouchi *et al.* (1999) reported occurance of stem and leaf spot of Kalanchoe in commercial grower's glasshouses in Gifu city, Japan. *Myrothecium* sp. isolated from diseased plants was demonstrated as the causal agent. Inoculation tests showed that the causal fungus was pathogenic to tomato, *Spathiphyllum* sp., *Dieffenbachia* sp. and *Vinca* sp. The causal fungus was identified as *M. roridum* on the basis of morphology, cultural characteristics and pathogenicity.

Murthy *et al.* (2004) conducted several experiments in Karnataka, India, to determine the pathogenicity of *Myrothecium roridum* on teak saplings. and reported that decreased germination percentage and seedling emergence when inoculated on teak seeds

Mangandi *et al.*(2007) confirmed pathogenicity on salvia plants inoculated by spraying with a conidial suspension of *M. roridum* (1×10^5 conidia per ml) then covered with plastic bags over the plants and incubated in a growth chamber at 28°C for 7 days.

Sultan and Ghaffar (2009) inoculated seed and soil by *M. roridum* and *M. verrucaria* and recorded seed germination and observed seed rot, damping off, root rot

and spots on aerial parts of bitter gourd. Isolates of *M. roridum* were found more pathogenic than isolates of *M. verrucaria.* This is the first report of seedling and root infection of *M. roridum* and *M. verrucaria* and also the first report of *M. verrucaria* leaf spot disease in bitter gourd.

Duvel *et al*. (2009) reported that *Myrothecium roridum* is plant pathogenic species causing foliar spots in a large number of cultivated plants like vegetable crops (sweet pepper, tomato and cucumber), ornamental plants (*Spathiphyllum wallisii*, *Solidago canadensis*, *Anthurium andreanum*, *Dieffenbachia amoena*) and a solanaceous weed plant (*Nicandra physaloides*). All the isolates were pathogenic to their original plant hosts and also to some other plants.

2.5. Disease cycle :

2.5.1 Role of environmental factors for development of Myrothecium leaf spot of soyabean :

Srivastava and Khan (1994) conducted field trials in soyabean variety cv. JS 72-44 during the rainy season of 1990 and established that yields were greater and disease index was less when younger (20-day-old) plants inoculated with *M. roridum* as compared with soyabean crops of advanced age (34-48 days old). This effect was due to the result of creation of a favourable microclimate for *M. roridum* within the more mature, dense crop canopy, rather than a physiological response in the soyabeans.

Murakami *et al.* (1998) described that the density of *Myrothecium roridum* increased in field soil from June to October and decreased from February to April in 1996 - 1998. The fluctuation in density of *M. roridum* in the surface soil was affected by the average temperature. In the greenhouse, mulberry leaves were infected by conidia that

splashed from soil artificially infested with conidia of *M. roridum*. Disease incidence on mulberry increased when soil was amended with the fallen leaves and when mulberries were planted densely in soil artificially infested with a high conidial density of *M. roridum.*

Singh *et al.*(2003) conducted an experiment during 1999 and 2000 in Kanpur, Uttar Pradesh, India, to determine the effects of environmental factors, namely atmospheric temperature, relative humidity, and rain on Myrothecium leaf spot (*M. roridum*) of pigeon pea cultivar T-21 (susceptible). Data on disease development was recorded for 10 fortnights beginning from first appearance of the disease symptoms until harvest. The disease started to appear in traces in the second fortnight of July which gradually increased. The maximum disease intensity was observed in the first fortnight of September (45.6%) when the average atmospheric temperature, relative humidity, and rain were 27 degrees C, 84.7%, and 11.4 mm. Rain coincided with the rapid increase in disease intensity.

Singh *et al.*(2004) studied the survivability of *Myrothecium roridum* in diseased plant debris stored both in laboratory conditions and field conditions and found that it caused infection in 17.1% seedlings in laboratory conditions while 26.4% seedlings under field conditions. Agar plate method showed that the pathogen was present in 22.0% of plated seeds while in standard blotter method, 23.2% seeds were found infected.

2.5.2 Effect of date of sowing on disease severity of Myrothecium leaf spot of soyabean under field conditions :

Chauhan and Suryanarayan (1970) studied the effect of different dates of sowing on the incidence of Myrothecium leaf spot disease of cotton caused by *M. roridum* Tode

Ex. Fr. in Haryana. The incidence of leaf spot disease on cotton increased and seed-cotton yields decreased progressively with delay in sowing date at 15 days interval from 1st Apr. to 1st June.

Singh (1999) conducted two field trials at the Zonal Agricultural Research Station, Ujjain, Madhya Pradesh, India, during kharif seasons of 1992 and 1993 to determine the effects of variety (MACS-75, JS 76-259, DS 76-1-29, PK-472, Bragg and JS 72-74), sowing date (June 25, July 8 and 23) and plant density (0.2, 0.4 and 0.6 million/ha) on the incidence of leaf spot of soyabean caused by *Myrothecium roridum* and *Phyllosticta sojicola* (*Pleosphaerulina sojicola*). There were significant differences in disease severity between sowing dates and varieties. The early sown soyabean crop (25 June) exhibited less severe disease than that sown on July 8 and 23. Similarly, disease severity was lowest at a planting density of 0.2 million/ha than at 0.4 and 0.6 million/ha plant population. The interactions of variety with sowing date and variety with plant density were statistically non-significant.

2.6 Physiological studies :

2.6.1 Effect of $_{p}$H levels :

Chauhan and Suryanarayan (1970) reported that *Myrothecium roridum* culture grew and sporulated best at 6 pH in 25^{0}C with alternate light and darkness.

Okunowo *et al*.(2010) determined the influence of pH on fungal growth *Myrothecium roridum* by turbidometry measurement of the fungal spores in aqueous solution. The result obtained showed that the fungus was able to grow over the pH range (5.5 to 8.6). The growth of the organism was highest at pH 5.5 and lowest at 8.6.

2.6.2 Effect of media:

Okunowo *et al*.(2010) evaluated seven culture media i.e potato dextrose agar (PDA), malt extract agar (MEA), potato sucrose agar (PSA), sabouraud agar (SA),potato carrot agar (PCA) and Czapek-Dox agar (ZA) and a semi artificial diet, which included the material from the fungal host's plant (water hyacinth i.e. WHA). Maximum growth was found in PSA media, while minimum growth was in ZA media.

2.6.3 Effect of Temperature :

Temperature is the most important environmental factor that influences the growth and sporulation of *Myrothecium roridum.*

Tsay *et al.*(1996) conducted an experiment on Myrothecium leaf spot on *Vriesea* × *poelmannii* in Taiwan. Agar disc (6-mm diam.) with mycelia of *Myrothecium* obtained from 8-day-old culture was used as inoculum for growth and sporulation. They reported that the fungus grew about 80 mm in diameter of colonies on PDA at 30^0C in 8 days. The optimum temperature for growth was from 20^0C to 25^0C. Conidia germinated slowly, about 25% on water agar for 8 hr and 75% for 14.5 hr of incubation at 25^0C. The optimum temperature for conidium germination was from 20 to 25^0C. The optimum temperature for spore production was at 30^0C.

Chauhan and Suryanarayan (1970) reported that *M. roridum* grew and sporulated best at 25^0C and at pH 6 with alternate light and darkness.

Chase (1984) investigated that *Myrothecium roridum* grows properly at 21.11 ^oC to 29.44^{o}C and to some extant up to 32.22 ^{o}C in summer time.

Worapong *et al.*(2009) observed conidial germination highest at 28^0C and minimum at 12^0C.

2.7 Enzymatic and Toxin activity :

2.7.1 Enzymatic activity:

Lucas and Sherwood (1966) reported that the brown spot of fungus *A. alternata* readily produced polygalacturonase, pectin esterase and cellulase (Cx) when cultured on modified Richard's solution with cellulose, tobacco leave or starch as carbon source. They suggested that polygalacturonase hydrolyses terminal linkages more readily than interior linkages of polygalacturonide chain. On a pectin salt's medium, the isolates produced polygalacturonase, pectinesterase and pectinlyase. The pectin lyase was most active on pectin at PH 7.2. Enzyme production was weak on a glucose sodium polypectate salt medium.

Umana and Ikotum (2000) isolated three pathotypes of *Choanephora cucurbitarum* from *Abelmoschus esculentus*, *Amaranthus hybridus* and *Vigna unguiculata* for their *in vitro* ability to produce pectolytic enzymes at different pH levels. They reported that all the three pathotypes did not vary in their ability to produce enzymes *in vitro*. The enzymes were produced exo-polygalacturonase, endo-polygalacturonase and endo-peptate lysate. The endo-polygalacturonase was the predominant enzyme at a lower pH of 4.0-6.0, with the highest activity at pH 5.0. The endo-pectate lysate which has an optimal pH of 8.0 occurred less often. The exo-polygalacturonase had a lower pH of between 3.0 and 5.0. The amount of enzymes produced varied with the age of culture. More polygalacturonase was produced from the 6th-18th days of incubation, whereas endo-pectatelyase was produced only on the 18th day.

Moreira *et al*. (2005) and Okunowo *et al*. (2010) both reported that *Myrothecium* spp. produced a range of cellulolytic enzymes.

Okunowo *et al.* (2010) investigated the cellulolytic and xylanolytic activity of a pathogenic *Myrothecium roridum* Tode (IMI 394934) isolates from water hyacinth. The mycelial plugs of each isolate was grown in submerged cultures of Czapeck Dox broth containing the appropriate carbon source carboxy methyl cellulose (CMC), sawdust and homogenized dry water hyacinth leaf) at 25°C for 16 days. The enzyme activity assay was carried out on the culture filtrates obtained. This was measured as micromole sugar released per min. The result obtained showed that the enzyme activity (U/ml) for b-1,4-exoglucanase, b-1,4-endoglucanase and xylanase was 0.12 ± 0.02, 0.13 ± 0.03 and 0.34 ± 0.01 respectively, in *M. roridum* grown on homogenized dry water hyacinth leaf. The b-glucosidase activity (U/ml) was highest, 1.74 ± 0.06 in *M. roridum* grown on sawdust. The maximum (324.00 ± 19.51 mg/ml) and minimum (130.00 ± 5.83 mg/ml) total extracellular protein was produced in *M. roridum* grown on homogenized dry water hyacinth leaf and carboxy methyl cellulose, respectively. This study showed that the phytopathogenic strain of *M. roridum* is capable of producing cellulases and xylanase enzyme in submerged cultures.

2.7.2 Effect of toxins :

Jarvis *et al*. (1985) reported that *M. roridum* and *M. verrucaria* are two species of the Fungi Imperfecti which are the principal sources of the macrocyclic trichothecenes. During the past 5 years, they examined many isolates of these *Myrothecium* spp. for the production of macrocyclic and trichoverroid trichothecenes. They observed that these fungi produce trichothecenes in submerged cultures, although some isolates produced antibiotics either in very low yields or in a very erratic manner. They also reported that the only trichothecenes produced by our *Myrothecium* isolates are the macrocyclic

trichothecenes and the biosynthetically related trichoverroids. There are two reports of the isolation of the simple trichothecenes, trichodermol and diacetylverrucarol from *Myrothecium* cultures. Trichodermol was minor metabolite accompanied by much larger amounts of the macrocyclic roridin and verrucarin trichothecenes. They examined several plant-pathogenic isolates of *M.roridum* from Florida and reported that these isolates produce only the simple trichothecenes, both in submerged liquid cultures and on solid rice media.

Murakami *et al.* (1998) both reported that M. *roridum* produces toxins in culture medium.

Khisal *et al.* (2002) isolated two new trichothecenes, 14-hydroxymytoxin B and 16-hydroxyroridin E, from a fermented extract of *M. roridum*. The structures were determined by spectral data interpretation. Both compounds showed potent cytotoxic activity against primary soft-tissue sarcoma cells.

2.8 Effect of Culture filtrate :

Siddaramaiah *et al.* (1979) obtained the culture filtrate from *A. carthami* chowdhary and reported that it was toxic to all 25 cvs inhibiting seed germination upto 50 per cent and reducing root elongation by 16.66 to 65 per cent that of the shoot's (less sensitive) up to 37.5 per cent.

Siddaramaiah *et al*. (1984) grown *Alternaria alternata* on PDA broth for 20 days filtered through filter paper and then filtrate was centrifuged at 30,000 rpm and the supernatant was tested for its phytotoxicity on detached leaves of safflower and sesamum, necrotic flecks appeared after 16 hrs of inoculation in moist chamber.

Movahedi and Heale (1990) observed a single spore isolation of *B. cinerea* obtain from carrot tissue, produced on aspartic proteinase and endopectine lyase both *in-vitro* in a liquid medium containing 0.4 per cent gelatin as protein source and in infected carrot tissue . Both enzyme caused cell death and induced resistance to *B.*

cinerea in surface cell layer of carrot slices when applied at relatively low concentration. At higher concentration, both caused extensive cell death in carrot tissue and of carrot suspension cells. The infected leaves in these host was markedly reduced when spores were pre- treated with pepstatin, a specific inhibitor of the aspartic proteinase, employer a primary role for this enzyme in pathogenesis.

Mukarami *et al.* (1995) reported that the fungus produced toxin on PSA media that produces necrosis and browning on leaf of mulberry.

Kapat *et al*. (1998) observed that liquid culture filtrate *B. cinerea* produced both conductive and inductive forms of hydrolic enzymes and also suggested that enzymes may have important role in penetrating the cutin in layer.

Murakami *et al.* (1998) reported that Mytotoxin B toxin produced by *Myrothecium roridum* causing myrothecium leaf spot of mulberry.

Lee *et al.*(2008) observed that the fungal spores of *Myrothecium roridum* inhibited the seed germination, infected the seedlings, and caused an abnormal withering and inhibition of seedling growth of different kinds of weed seeds.

Binjhare (2002) reported that higher concentration of culture filtrate of *B. cinerea* cause extensive cell death in potato tissue.

Anderson and Hallett (2004) investigated the toxins of *Myrothecium verrucarria* that acts as bioherbicide against many economically important weeds.

2.9 Management:

2.9.1 By different medicinal plant extracts:

Lakshmanan (1990) tested antifungal property of 10 plant extracts under *in-vitro* against the cotton boll rot *Corynespora cassiicola*. Among different plant extract's, garlic cloves was most effective in inhibiting mycelial growth by 95.8% and sporulation by 78.6 %.

Datar (1994) reported that the extract from Eucalyptus, Datura & *Azadirachta indica*, *Ipomea cornea* inhibited mycelial growth and spore germination of *Alterneria porri*.

Thakur *et al*. (1995) reported that the leaf extract of Datura have antifungal activity against *Alternaria alternata* and many other fungi.

Olufolaji (1999) also tested antifungal property of medicinal plant extract under *in-vitro* and *in-vivo* condition and found *Azadiracta indica* (neem) to be effective in inhibition of mycelial growth (13.85%) & sporulation (82.50%).

Manas *et al.*(2005) screened twenty one plant species *in vitro* for their fungitoxic properties against *Myrothecium roridum* by poisoned food technique. Maximum inhibition (33.33%) of colony growth of *M. roridum* was observed with amendment of 5% solvent extracts of *Datura metel* followed by *Alium sativum* (25%), *Chromoleana odorata* (20%) and *Eucalyptus citriodora* (16.66%).

2.9.2 By Bioagent :

Baker and Cook (1974) illustrated the concept of biological control as "the reduction of inoculum density or disease producing activities of pathogen or a parasite in its active or dormant state by one or more organism, accomplished naturally or through manipulation of environment, host or antagonist or by introduction of one or more antagonist".

Agrawal *et al.* (1971) reported that *Trichoderma harzianum* was found antagonistic against *Sclerotium rolfsii* causing collar rot of lentil. Filtrate of this organism also checked the growth of *Sclerotium rolfsii* on potato dextrose agar. *Trichoderma harzianum* could check the mortality of lentil caused by *Sclerotium rolfsii* under pot condition. The culture of *Trichoderma* was more effective when used with the seeds as compared to that used in soil.

Okigbo and Ikediugwu (2001) inoculated Yam cv. Iyawo tubers by spraying with conidiospore suspension of *Trichoderma viride* in potato dextrose broth showed a drastic

reduction in the range and number of mycoflora, including pathogens (*Rhizoctonia sp., Aspergillus niger, A. flavus, Penicillium oxalicum, Botryodiplodia theobromae, Fusarium oxysporum, F. solani, Neurospora sp*. and *Choanephora sp*.), on the tuber surface during five months of storage in a traditional yam barn.

Trichoderma spp. employs various mechanisms to affect disease control and improve over all plant health (Singh *et al.* 2004).

Murthy *et al.* (2004) conducted several experiments in Karnataka, India, to determine the management of *Myrothecium roridum* on teak saplings. For Management, biocontrol agent like *Trichoderma harzianum*, *Pseudomonas fluorescens* were used. *P. fluorescens* gave the lowest mean disease incidence percentage of *Myrothecium roridum.*

Siddiqui (2008) studied the potential of water extract from rice straws (RST) and empty fruit bunch of oil palm (EFB) composts fortified with *Trichoderma harzianum* for the control of *Choanephora cucurbitarum* under field condition, disease severity was lowest in plant treated with *Trichoderma* fortified (RST) extract.

2.9.3 By fungicides *in- vitro* condition:

Carter (1980) reported that a mixture of benomyl and zinc ion-maneb complex significantly controlled *Myrothecium roridum* on cantaloup leaves and stems.

Singha and Narain (1993) tested seven fungicides tested during 1990 and 1991, carbendazim gave the best control of *Myrothecium roridum* on soyabean and also increased yields.

Horinouchi *et al.* (1999) tested eight fungicides against *Myrothecium.* sp causing stem and leaf spot of Kalanchoe that occurred in commercial growers glasshouses in Gifu city, Japan.. Among which bitertanol and kresoxim-methyl controlled the disease best.

Murthy *et al.* (2004) evaluated fungicides i.e., Bavistin (carbendazin), captan, captafol, *Trichoderma harzianum*, *Pseudomonas fluorescens*, urea, diammonium

phosphate and NPK. Bavistin, *P. fluorescens* and NPK fertilizer gave the lowest mean disease incidence percentage of *Myrothecium roridum* on teak seedlings.

Tomar and Shashtri (2006) conducted an experiment to find out the efficacy of five fungicides *viz.* Carbendazim (0.1%), Carboxin (0.2%), Chlorothalonil (0.2%), Triademefon (0.2%) and Propineb (0.2%) in suppressing seed- borne *M. roridum.* All the five test fungicides increased the percent germination and reduced the recovery of *M. roridum* from cotton seed. Carbendazim was the most effective fungicide, which decreased the recovery by 100% over the control. Chlorothalonil decreased the recovery by 90.69%. All the fungicidal seed treatments increased the vigour of the cotton seed. Carbendazim exerted the maximum influence in this respect.

Duvel *et al.* (2010) tested some fungicides *in vitro* against an isolate of *M. roridum* and reported that the fungicides with quartenary ammonium, tebuconazole and copper were highly effective in inhibiting the mycelial growth.

2.10 Host Plant Resistance:

Norman *et al.*(2003) evaluated host resistant of different *Syngonium* species against *Myrothecium roridum* causing Myrothecium leaf spot. Among *Syngonium* species and cultivars, five commercial cultivars (Holly M, White Butterfly, Pink Allusion, Cream Supreme, and Regina Red) and 30 accessions, comprising 16 different *Syngonium* species (*S. macrophyllum, S. podophyllum, S. neglectum, S. yurimaguense, S. hastiferum, S. rayii, S. armigerum, S. wendlandii, S. hoffmannii, S. auritum, S. sagittatum, S. steyermarkii, S. angustatum, S. dodsonianum, S. chiapense, S. erythrophyllum*), were screened for resistance to *M. roridum*. All five commercial cultivars were susceptible to *M. roridum*. However, 6 species (*S. neglectum*, *S. wendlandii, S. erythrophyllum, S. chiapense, S. dodsonianum, and S. angustatum*) showed the highest resistance, as did two noncultivated accessions of *S. podophyllum*.

Kumar *et al.* (2003) screened mulberry germplasm for resistance to leaf spot caused by *Myrothecium roridum*. 64 genotypes were resistant (PDI=6-15), whereas 73

were moderately resistant (PDI=11-30) and 10 were susceptible. No cultivar was completely resistant or highly susceptible.

CHAPTER-III

MATERIALS AND METHODS

- **Materials :**

The present study entitled "Studies on *Myrothecium roridum* Tode ex. Fries. causing Leaf spot of Soyabean (*Glycine max (L.)* Merrill). All the laboratory work was carried out at the Department of Plant Pathology Laboratory, college of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur. During the course of study, potato dextrose agar was used for the culture of *Myrothecium roridum*.

All the glass wares prior to use were cleaned and washed with detergents followed by rinsing with tap and distilled water. The glass wares dried in oven at 60° C for half an hour. All glass wares were sterilized in hot air oven at 180° C for 3 hours.

Plastic Petri plates were surface sterilized with alcohol, where as inoculation needle, cork borer, knife, scissors, and blade were sterilized by heating over the flame after dipping in 95 per cent alcohol. Sterilization of the media was done by autoclaving at 1.02-kg/cm^2 pressure for 20 minutes.

3.1.1 Source of material:

All the glass wares, chemical, viz. streptomycin, alcohol, $HgCl_2$, different fungicides, Blotting paper, and other material's were obtained from Department of Plant Pathology, college of Agriculture, IGAU, Raipur. Soyabean seed obtained from oilseed project, Department of Plant Breeding & Genetics, IGAU, Raipur.

The following instruments were used in the present studies**:**

- Autoclave for sterilization.
- BOD incubator for incubation of pathogen.

- Compound microscope for identification of pathogen.
- Hot air oven for glassware sterilization.
- Laminar Air Flow for isolation, purification & inoculation of pathogen.
- Anamid Electronic Digital Balance for weighing.
- Forceps, needles, blades, cork borer & inoculation needle.
- Spirit lamp for sterilization.
- Microwave oven for melting of media.
- pH meter.
- Desiccators for growth of pathogen.
- Micro pipette etc.
- **Experimental site :**

The present investigation was carried out at the Department of Plant Pathology, IGAU, Raipur. All *in-vitro* studies on *Myrothecium roridum* were conducted in laboratory of the Department of Plant Pathology, IGAU, Raipur. Some soybean crop pots sowing were carried out in glass house of Plant Pathology, IGAU, Raipur.

3.1.3 General procedure followed:

For each set of treatment different replication were used in all *in- vitro* studies. In general, in each Petri dish about 15-20 ml of potato dextrose agar medium was poured, supplemented with streptomycin in order to check the unwanted bacterial contamination. Wherever growth studies were conducted five mm disc of pure culture of *Myrothecium roridum* Tode ex. Fries. by the help of cork borer, was used for inoculation of medium in Petri dish. The inoculated plates were incubated in the $25^{o}C \pm 2^{o}C$ for three days.

Observation for the growth and sporulation were recorded at 10 to 15 days after inoculation.

3.2 Isolation, Purification & Identification:

3.2.1 Collection of diseased sample:

The naturally infected leaf of soyabean crop with the *Myrothecium* leaf spot symptoms were collected from the oil seed research farm of the university. Collected samples were brought to the laboratory for critical examination of the symptoms for the identification studied under compound microscope & isolation of the pathogen.

- **Isolation & Purification of test fungus** :

The entire work of isolation & purification was done in isolation chamber and laminar air flow, which were sterilized by alcohol or formeldehyde and UV light prior to use.

The fresh infected leaves of soyabean plant samples were cut into small pieces, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution followed by three washing with sterile distilled water and placing in moist chamber than after 1 to 2 days fungal mycelium growth were seen than finally small bits of fungus kept on the previously poured and solidified potato dextrose agar medium in Petri plates for isolation of the pathogen. The plates were incubated at 25^0C ± 2^0C in BOD incubator.

The plates were observed after mycelial growth from the inoculated mycelium bits. Mycelial mates were then sub-cultured, purified by hyphal tip method and maintained culture on PDA slant & Petri plate kept on incubator at 25^0C ± 2^0C. All the growth characters were recorded and compared with the standard reports publish for confirmation.

- **Pathogenicity confirm of test fungus :**

- **On leaf**

Pathogenicity of isolated fungus was tested in field conditions by attached method while in laboratory conditions by attached method and detached method.

3.3.1.1 Attached method:

Twenty days old healthy plant of susceptible soyabean cv JS -335 was used for pathogenicity test under field condition. Selected plant was sterilized with 0.1 percent $HgCl_{2,}$ then three subsequent washing with sterilized water. *Myrothecium roridum* culture was multiplied in petriplates on potato dextrose agar medium (10 days old culture) and five disc of fungus culture dissolve in 100 ml of sterilized water After this, fungal suspension was sprayed on the soyabean plant leaf. Soyabean plant sprayed with water was kept as control. In field conditions, the inoculated and uninoculated (control) soyabean plants were covered by perforated polythene bag to maintain the humidity and the polythene bag was removed after 5 days.

In laboratory condition, 20 days old healthy potted soyabean plants of susceptible soyabean variety cv JS-335 were selected for testing the pathogenicity. The plants were inoculated by spray of fungal suspension. Plants were kept in desiccators for 5 days to maintain the humidity and were observed at frequent intervals for appearance of symptoms.

3.3.1.2 Detached method:

Twenty days old leaves of healthy plants of cvs JS-335 were sterilized with 0.1 percent mercuric chloride & three subsequent washing with sterilized water, placed in sterilized desiccators. 5 mm disc of test fungus *M. roridum* was inoculated on detached

leaves with the help of inoculation needle by three ways i.e., slight pinprick, gently scratched and normal (without pin prick or scratch).

- **Testing of pathogenicity by seed inoculation method**

In order to know the effect of culture of *M. roridum* on the germination and mortality of soyabean seed cv (JS-335) the following treatments were used:

1) Surface sterilized + inoculated seed

2) Without surface sterilized + inoculated seed

3) Control

Four hundreds seeds were used in each treatments and surface sterilized with $HgCl_2$ (1:1000) for 1 min followed by three subsequent washing with sterilized water and inoculated by spore suspension of 10 days old culture of *M. roridum.* In without surface sterilization, seeds were dipped in sterilized water and inoculated with fungal culture of test pathogen. One was dipped with sterilized water serve as control. These seeds were kept in sterilized box, and box was kept in inoculation chamber having 95 percent humidity and observation was recorded after six days of inoculation.

3.4 Physiological studies:

3.4.1 pH:

The effect of different $_p$H ranging from 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 studied on potato dextrose agar medium. The $_p$H of the medium was adjusted before autoclaving with help of digital $_p$H meter by addition of 0.1 N HCl and 0.1 N NaOH into the PDA medium after autoclaving, 20 ml of medium was poured in the sterilized Petri plates. The Petri plates were inoculated with 7 days old culture of 5 mm disc of inoculums with the help of cork borer. Petri plates were incubated in BOD incubator at $25^0C \pm 2^0C$. The three

replications were maintained for each treatment and observation for radial growth was recorded at 10th days and 15th days and sporulation after 16th days of inoculation.

3.4.2 Media:

To know the effect of different media viz. Richard's, Kerr's, Dextrose nitrate agar, Asthana & Hawker's, Malt extract, Czapex dox media, Potato sucrose agar media, Potato dextrose agar media were used to find out the best medium for the growth and sporulation of *Myrothecium roridum.* 20 ml of media was poured in Petri plate, 5 mm disc of seven days old culture of test fungus was placed in the center of medium. The three replication were maintained for early treatment and observation for radial growth were recorded at after inoculation 10th days and 15 th days and sporulation also after 16th days.

Following media with given composition were :

Media	Ingredient	Quantities (g)
Richard's medium	Potassium nitrate	10.00
	Potassium dehydrogen phosphate	5.00
	Magnesium sulphate	2.50
	Ferric chloride	0.02
	Sucrose	50.00
	Agar	20.00
	Water	1000 ml
Asthana& Hawkers Medium	Potassium dehydrogen phosphate	1.75
	Magnesium sulphate	0.75
	Potassium nitrate	3.50
	Sucrose	5.00
	Agar	20.00
	Water	1000 ml

Kerr's medium	Sodium nitrate	2.00
	Potassium chloride	0.50
	Ferrous sulphate	0.01
	Yeast extract	0.50
	Potassium dehydrogen phosphate	1.00
	Magnesium sulphate	0.50
	Sucrose	30.00
	Agar	20.00
	Water	1000 ml
Dextrose nitrate Agar medium	Dextrose	1.00
	Potassium dihydrogen phosphate	0.10
	Sodium nitrate	0.10
	Potassium chloride	0.10
	Magnesium sulphate	0.10
	Agar	20.00
	Water	1000 ml
Malt extract medium	Malt extract	20.00
	Agar	20.00
	Water	1000 ml
Czapek dox agar medium	Sodium nitrate	2.00
	Potasium dihydrogen phosphate	1.00
	Magnesium sulphate	0.50
	Potassium sulphate	0.50
	Ferrus sulphate	0.01
	Sucrose	30.00
	Agar	20.00
	Water	1000 ml
Potato sucrose agar medium	Peeled potato	200.00
	Sucrose	20.00
	Agar	20.00
	Water	1000 ml

Potato dextrose medium	Peeled potato	200.00
	Dextrose	20.00
	Agar	20.00
	Water	1000 ml

3.4.3 Temperature:

The experiment was conducted to find out, the most suitable temperature for radial growth and sporulation of *M. roridum*. The sterilized poured Petri plate with potato dextrose media were inoculated with 5 mm disc of the test pathogen of seven days old culture. The Petri plates were incubated at 0^0C, 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 35^0C & 40^0C temperature. Four replication were maintained for each treatment and observation for radial growth was recorded at 10th days and 15 th days after inoculation. For sporulation, 5 mm disc of each treatment was completely shaken in 10 ml of sterilized water. Proper dilution was made for easier counting of spores. One drop of spore suspension was kept in cavity slide and spores were counted per microscopic field under compound microscope. Spore were counted at three microscopic fields in one cavity slide and in one Petri plate of each treatment, three disc i.e. center, middle, peripheral were used for count the sporulation.

3.5 Determination of enzymatic activity :

3.5.1 Cellulase (Cx) activity:

Cellulase activity was determined by measuring the reduction in viscosity of 0.5 percent Carboxy Methyl Cellulose (CMC) solution (Muse *et al*., 1972). Viscometric measurements were made with Ostwald's viscometer at time intervals 0, 60, 120, 180, 240, 300, 360, 420 & 480 minutes. The reaction mixture consisted of the following:-

(a) 5 ml of 0.5% Carboxy Methyl Cellulose solution.

(b) 2 ml of sodium citrate buffer (at $_{P}$H level of 4, 7, & 9.2).

(c) 2 ml of enzymes preparation. (Broth fungus culture filtrate).

The enzyme preparation heated to 100^{0}C for 10 minutes served as control. 15 days old culture filtrate solution was used in the experiment.

Preparation of culture filtrate :

Potato dextrose broth was used as medium. After autoclaving, the medium was inoculated with the 7 mm disc of the test fungus (fifteen days old culture). When culture of the pathogen cover the upper layer of medium, culture filtrate was filtered with the help of funnel and Watsman filter paper and filter used for enzymatic study.

3.5.2 Polygalacturonase (PG) activity :

Polygalacturonase activity was determined by measuring loss in viscosity of 1.2 percent pectin solution (muse *et al*. 1972). Viscometric measurements were made with Ostwald's viscometer at different time interval i.e. 0, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720,780,840,900,960 & 1020 minutes. The reaction mixture consisted of the following.

(a) 5 ml of 1.2 % pectin solutions**.**

(b) 2 ml of sodium citrate buffer (at $_{P}H$ level of 4, 7, & 9.2).

(c) 2 ml of enzymes preparation. (Broth fungus culture filtrate).

For estimation of PG activity, 15 days culture filtrate solution was taken. The reduction in viscosity of CMC as well as pectin was calculated by the following formula:

% loss in viscosity = $\mathbf{T_0 - T_1 / T_0 - T_{W \times 100}}$

Where,

T_0 = flow time of reaction mixture at '0' minutes.

T_1 = flow time of reaction mixture at a particular time interval.

Tw = flow time of distilled water.

3.5.3 Macerating enzymes :

Seven mm diameter and 1 mm thick disc of potato and carrot were prepared and sterilized with $HgCl_2$ (1:1000) followed by three subsequent washing with sterilized water. Two concentrations of culture filtrate viz. pure culture filtrate (100 %) and 50% were used for estimation of macerating enzymes. Twenty ml of each concentration was taken in 90 mm sterilized Petri plates. Three discs were dipped in each concentration,

disc dipped in sterilized water served as control. These Petri plates were kept in room temperature. Three replications for each concentration were maintained and observation of rotting of potato and carrot disc was taken at every one hour interval.

- **Toxic effect of culture filtrate :**

3.6.1On leaf:

3.6.1.1 Attached method

Firstly sterilized seeds of soyabean cv JS -335 were sown in pots already filled with sterilized soil for growing of the plants. Upper and lower surface of leaf was selected in 30 days old plants for inoculation. Both the surface were sterilized with 0.1 percent $HgCl_2$ then washed with sterilized water, than cotton swab was dipped in culture filtrate and then spread by rubbing uniformly over the surface of the leaves with culture filtrate of the test pathogen. Inoculated plants were kept into desiccator (humid chamber). Observation for effect of culture filtrate was recorded at an everyday basis after inoculation.

3.6.1.2 Detached method :

Firstly both the surface 30 days old soybean leaf were sterilized with 0.1 percent $HgCl_2$ and then washed with sterilized water. These sterilized leaves were kept in moist chamber and then cotton swab dipped in culture filtrate and uniformly spread over the surface of the leaves with culture filtrate of the test pathogen. Three methods were used for inoculation of culture filtrate on leaf i.e. scratch leaf, pin-pricked leaf and plain leaf. Sterilized water sprayed on soybean leaf was used as a control. Prepared moist chamber (plate) was placed in growth chamber and observation was recorded at after 2 days interval for typical symptom of leaf.

3.6.2 On seed :

To study the effect of toxic metabolites of *M.roridum,* the fungus were grown in 100 ml PDA broth in 500 ml conical flasks. After the incubation period of 15 days at 25^{0}C, the broth culture was filtrated with watsman filter paper no. 42. The filtrate thus obtained was used to assay the toxin.

In order to know the effect of culture filtrate of *M.roridum* on the germination of soyabean seed cv (JS-335), the following treatments were used :

(a) Seed treated with culture filtrate,

(b) Blotter treated with culture filtrate,

(c) Seed and blotter both treated with in culture filtrate,

(d) Broth treated control.

Four hundred seeds were placed in each sterilized box for each treatment. Germination percent was recorded after six days, seed treated with uninoculated medium (sterilized potato dextrose broth medium) served as control.

3.7 Management of test pathogen:

3.7.1 Medicinal plant leaf extract

Antifungal activity of different medicinal plant leaf extracts were studied under *in-vitro* taking plant leaf extract, dextrose agar medium. The seven medicinal plant species viz. Pudina, Tulsi, Onion, Lemon grass, Garlic, Neem and Karanj were used for antifungal activity.

Name of medicinal plant	**Scientific name**
Japanese Pudina	*Mentha* spp.
Tulsi	*Occimum* spp.

Onion	*Alium cepa*
Lemon grass	*Cymbopogon flexiosus*
Garlic	*Allium sativum*
Neem	*Azadiracta indica*
Karanj	*Pongamia pinnata*

Twenty gm leaf of each medicinal plant was taken in 100 ml of water and boiled till the softening of the leaf and then extract was filtered. 2 g. of dextrose and 2 g. agar-agar were mixed in filtrated leaf extract, the volume was make up to 100 ml and then sterilization was done by autoclaving at 15 Ibs for 20 minutes. To avoid bacterial contamination, a little amount of streptomycin sulphate was added at the time of pouring of media. In each Petri plate 20 ml medium was poured in sterilized Petri plates and allowed to solidify. PDA without extract served as control. A five mm disc from seven days old culture of the test pathogen by the help of sterilized cork borer and was placed at the center of medium, three replications were kept in each treatment along with a control. The inoculated Petri plates were then incubated at 25 ± 2^{0}C and observation was recorded at 5 days and 15 days after inoculation.

Calculated the percent inhibition by using followed formula,

Inhibition (%) = C-T/C × 100

Whereas

C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in treated plate.

3.7.2 Bio-control :

The experiment was conducted to study the efficacy of biocontrol agent of different isolates of *Trichoderma* spp. against *M. roridum* pathogen. Seventeen isolates of *Trichoderma* spp. were used to test antagonistic performance in dual culture with a test pathogen *Myrothecium roridum.* All the seventeen isolates of *T.*spp (T93, T73, T66, T31, T29, T27, N, T132, T120, T114, T101, T110, T7, T158a, T16, T15 and T14) were obtained from Department of Biotechnology I.G.K.V. Raipur, multiplied and maintained on PDA media and kept in BOD at 25^0C. The species name and section name (whose species name is not identified) were mentioned below:

S. No.	*Trichoderma* spp.	Species name/Section
1.	T93	Section- Pachybasium
2.	T73	Section- Pachybasium
3.	T66	Section- Pachybasium
4.	T31	Section longibrachiatum
5.	T29	*Trichoderma aureoviride*
6.	T27	*Trichoderma aureoviride*
7.	N	*Trichoderma virens*
8.	T132	Section- Pachybasium
9.	T120	*Trichoderma aureoviride*
10.	T114	*Trichoderma aureoviride*
11.	T101b	*Trichoderma virens*
12.	T110	Section- Pachybasium
13.	T7	Section longibrachiatum
14.	T158a	Section- Pachybasium
15.	T16	*Trichoderma harzianum*
16.	T15	*Trichoderma harzianum*
17.	T14	*Trichoderma viride*

An amount of 20 ml sterilized melted PDA was poured in 90 mm diameter petriplates. After solidification of medium, 5mm disc of the antagonist and the test pathogen were separately cut with the help of a sharp sterilized cork borer from the edge of 4 days old culture and placed in straight line at distance of 5 mm from edge. Without antagonist serve as control. Three replications were maintained. The inoculatd petriplates were incubated at 25^0C. Observations was made on radial growth of antagonist and test pathogen when fungus in control plate reached to rim of the plate. The observation was taken after 10 days of inoculation. The per cent growth inhibition of test pathogen in presence of antagonist was calculated over control as given below:

Inhibition (%) = C-T/C × 100

Whereas

C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in dual culture plate.

3.7.3 Fungicides:

Poisoned food technique was employed for the evaluation of fungicides in the laboratory**.** Eight fungicides viz. Dhanucop (Copper oxychloride 50% WP), Dithan M-45 (Mancozeb 80% WP), Benomyl (Benlate 50%WP), Curzate M-8 (Cymoxanil 8%+ mancozeb 64%), Saaf (Carbendazim12%+Mancozeb 63%), Dhanustin-50%WP (Carbendazim 50%WP), and Vitavex power (Carboxin 37.5% WS+Thiram 37.5%WS) were evaluated against *M. roridum.* Three concentrations i.e., 250 ppm, 500 ppm, & 1000 ppm of each treatment were used. The required quantity of fungicide was mixed with PDA at the time of pouring. Three replication were maintained for each fungicide for each of it's concentration in CRD. The media was shaken well so as to enhance proper mixing of the fungicides. To avoid bacterial contamination a little

amount of streptomycin was added in each flask before plating; five mm disc was cut with the help of sterilized cork borer from seven days old culture of the test fungus and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicide served as control. The radial growth of the colony was measured when the growth in control plates reached the rim of the Petri plates. Percent growth inhibition under the influence of different fungicides was calculated on the basis of the control.

The sporulation of *M. roridum* was recorded separately for each fungicide and each concentration. Five mm diameter disc was dipped in 20 ml sterilized distilled water in a test tube and was thoroughly shaken. One drop of suspension was placed in a cavity slide and the spores were counted per microscopic field. In one cavity slide, three microscopic fields were focused for counting the spore.

Percent inhibition of radial growth were calculated by the following formula,

Inhibition % = C–T/C × 100

Whereas

C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in treated plate.

3.8 Host plant resistance:

3.8.1 Evaluation of Soyabean Genotypes against *Myrothecium* leaf spot under field conditions.

The field experiment was conducted at the research farm, IGAU Raipur, in kharif season 2009-10. 56 soybean varieties were screened which was sown on 4th July 2010. Plot size was 3×5 m and replicated thrice. All the recommended agronomic practices were adopted. The observation on natural occurrence of *M. roridum* on soyabean leaf were recorded after 45 days of sowing. The observation on incidence of disease was recorded. For recording the observation, five plant of each variety were randomly

selected and tagged. Total healthy and disease leaf in each plant were counted to observe the disease incidence. While disease severity was calculated by using 0-9 scale

Where –

0 –No lesions

1 –1% leaf area covered with lesion

3 -1.1 - 10 % leaf area cover with lesion

5 –10.1 – 25 % of the leaf area covered no defoliation, little damage

7 -25.1 – 50 % leaf area covered, some leaf drop, death of a few plant damage conspicuous.

9 -More than 50% area cover, lesion very common on all plant's , defoliation common, death of plant common, damage more than 50%. PDI was calculated by the following formula:

Percent disease index (PDI) =

And on the basis of PDI, the varieties were classified as followed-

PDI	Reaction category
0	Absolutely resistant (AR)
0.01-11.11	Highly resistant (HR)
12.22-33.33	Moderately resistant (MR)
34.44-55.55	Moderately susceptible(MS)
56.66-77.77	Susceptible (S)
78.88-100.00	Highly susceptible (HS)

3.8.2 Evaluation of soyabean genotypes against Myrothecium leaf spot under glass house conditions.

Similar set of genotypes were also grown in the black color plastic pots in glass house on 4th July 2010. Ten plants were maintained in each pot to achieve maximum development. Two replications were maintained in the glass house and pots were irrigated at weekly interval to provide favorable conditions for the establishment and development of *M. roridum* on soybean leaf. Spore suspension of *M. roridum* were spread over the plants at early 15 days old plants.

Disease severity was calculated by using 0-9 scale and PDI was calculated by above mentioned formula and categorized accordingly.

3.9 Effect of date of sowing on disease severity of Myrothecium leaf spot of soyabean under field conditions.

The experiment was conducted to find out the effect of date of sowing on severity of Myrothecium leaf spot of Soybean. Three soybean cultivars i.e., JS-9035, JS-9752, JS-335 were sown at Four dates i.e., 31st July, 8th Aug, 16th Aug, and 24th Oct in the year 2010 at ASP-2 in Kharif, season at the farm of IGAU, Raipur. Design used was Split plot design where main plots were the four dates and the sub plots were the three varieties with three replications Plot size was 6×4m. Recommended agronomic practices were adopted for cultivation of soybean crop. Visual observations were done and recorded by selecting the plants randomly. Numerical grade were assigned to the amount of disease observed applying 0-9 disease rating scale and per cent disease index (PDI) was computed applying the formula mentioned above.

3.10 Disease development in relation to environmental factors.

The effect of weather factors like temperature (maximum, minimum), RH (morning and evening) in percent) and rainfall on the incidence and development of Myrothecium leaf spot of soyabean were studied at research farm, IGAU, Raipur. The meteorological observations at Agricultural Research Stations, IGAU, Raipur was used for this experiment. This study was undertaken during Kharif season 2010-11. Healthy

cvs JS-335 soyabean seeds were shown into the field. All other cultural and pest control practices were followed as recommended in package of practices. Five plants were selected randomly in each plot and tagged and observation on incidence of disease on the foliage were recorded using 0-9 scale and percent disease index (PDI) was worked out as mentioned above.

CHAPTER-IV

RESULTS AND DISCUSSION

The experimental results and discussion of various studies conducted on *Myrothecium roridum* Tode ex.Fries of soyabean (*Glycine max.* (L.) Merrill) crop.

4.1 Symptomatology:

Myrothecium leaf spot is an important disease of soyabean crop. Characteristic symptoms observed in soybean under natural field conditions were as follows:

- Myrothecium Leaf spot disease was observed twenty days after sowing on leaf of soyabean plant.
- Initial symptoms was appeared as small round or oval, brown spots developed with dark brown margin on leaves in the infected plant (Plate 1A).
- Translucent area seen around the spot which appeared as concentric rings.
- Later on number of spots were formed get enlarged and merged to form irregular shape spot and severely infected leaves become dry (Plate 1B).
- Under favourable conditions (high humidity) creamy layer of erumpent fungus appeared and then oval to irregular shaped sporodochia formed on the infected area of leaf spot (Plate 1C).
- Later on the shot holes were formed (Plate 1D).
- The symptoms were also observed in other parts like stem (Plate 1E) and pod (Plate 1F).
- On the stems, black colour sporodochia formed around which tuff of white mycelial growth appeared (Plate 1E)

- Similarly, in case of pods sporodochia formed very distinctly over the surface in the form of rings (Plate 1F)

Similar symptoms were also reported by Singh and Pandey (1998) on soyabean, Byrne and Raymond (2007) reported it on *Salvia* spp. and Zhao *et al*. (2010) found similar symptoms on common bean plants in China.

4.2 Isolation, Purification and Identification of isolated fungus :

The disease sample of *Myrothecium roridum* were collected from the IGKV research farm. Isolation and purification of the pathogen was done in the laboratory. Pathogen was purified by single part hyphal techniques and pathogen culture was multiplied and maintained on PDA at 25 ± 2^0C in BOD incubator. The isolated fungus was compared with literature for identification. Fungus was recovered from leaf samples of variety Bragg and plated in potato dextrose agar (PDA). Isolates developed white mycelial colonies and dark green-to-black, spore-bearing mycelial cushions (Plate 2A) that is called sporodochia that formed on colonies at 25 ± 2°C (Plate 2B). Mycelium of the fungus was branched and non-septate (Plate 2C). Conidia were hyaline or olive green to slightly dark, one-celled, ovoid to elongate or rod shaped with rounded ends and measures 2.0 to 2.5 × 5.5 to 6.5 µm (Plate 2D).

There morphological characteristics were accordance with the Mohan *et al*. (1998) in papaya; Seebold *et al*. (2005) in watermelon and Mmbaga (2010) garden hydrangea.

4.3.Pathogenicity test :

The pathogenicity of isolated fungus *Myrothecium roridum* was tested on leaves and seeds of soyabean.

4.3.1On leaves:

4.3.1.1 Attached method:

4.3.1.1.1 Field condition:

The results indicate that inoculated plants produced symptoms of grayish lesions on the leaf surface after 5 days of inoculation. Numerous lesions were formed on upper surface and become dark grey to brown in colour (Plate 3). After 8 days, the lesions merged, get enlarged and leaves become dried.

4.3.1.1.2 Laboratory condition :

The results of pathogenicity presented in Table 1 indicates that the numerous small, round to oval grayish spots were formed after 2 days. The lesions become darker after 4 days and after 7 days, the lesions merged and entire leaves get dried (Plate 4).

Table 1: Testing of pathogenicity of *M. roridum* by attached method

Sl. No	Symptoms	Disease development after inoculation (in days)
1.	Formation of numerous small, round to oval grayish necrotic spots	2
2.	Darkening of lesions	4
3.	Merging of lesions and drying of leaves	7

Ponnappa (1970) confirmed pathogenicity of the *M. roridum* on seedlings of *Eichhornia crassipes* by attached methods where necrotic area developed on the leaves after 2 days which gradually extended to the stems and then dried up on 3rd day. Mangandi (2007) also incubated salvia plants in growth chamber and confirmed the pathogenicity of *M. roridum* causing leaf spot of *Salvia* spp.

4.3.2.1 Detached method:

The results of pathogenicity presented in Table 2 indicates that inoculated leaves produced symptoms of necrotic brown spots on the detached after 12 hrs in scratch method in comparision to pin-prick method (24 hrs) and normal leaf (without scratch and pinprick method) (36 hrs) (Plate 5).

Table 2: Testing of pathogenicity of *M. roridum* by detached method

Sl. No	Inoculation methods	Development of necrosis after inoculation (in hrs.)
1.	Normal(without pin-prick or scratch)	36
2.	Pinprick	24
3.	Scratch	12

By this ways the Koch postulates was proved and pathogenicity of *M. roridum* on soyabean was confirmed. This result was comprised with the available literature that shows the pathogen was identified as *M. roridum.*

Kuti *et al.* (1989) confirmed pathogenicity of the *M. roridum* by detached method on the leaves of muskmelon in which susceptibility was determined by necrosis and chlorosis symptoms. Mmbaga (2010) also confirmed pathogenicity of *M. roridum* on detached leaves of Garden Hydrangea where necrotic lesions started after 4-5 days after inoculation of 5 mm mycelia plugs.

4.3.2 Testing of pathogenicity by seed inoculation method:

The data presented in Table 3 and Table 4 indicates that the seed germination and seed mortality were affected by inoculation of *M. roridum* test pathogen. 56 percent germination of seed was recorded in surface sterilized inoculated method while 42 % recorded in without surface sterilized inoculated seeds of soyabean in comparison to control (76 %). Mortality of seeds were also recorded after germination. Data showed that percent seed mortality was recorded within 10 days in surface and without surface sterilized inoculated seeds, while 10 percent seeds were mortile in uninoculated control.

Changes in seed colour, plumule size and fungal growth were also recorded in all treatments. Data showed that 37 % seeds were black in colour in without surface sterilized seed while 28 % seeds were black in surface sterilized method, only 15 % seeds were black in uninoculated control. Reduction in plumule size was more in without surface sterilized seeds (1.0 cm) followed by surface sterilized seeds (1.5 cm) in comparision to control (2.5 cm) after 7 days of inoculation.

Duke (1980) confirmed that germination of cotton seeds was greatly reduced and emergence was delayed when treated with *Myrothecium roridum*.

Purohit and Purohit (2002) confirmed pathogenicity of the *M. roridum* on the seeds of minor millets under different storage structures.

Table 3: Effect of *Myrothecium roridum* on seed germination & mortality

S. No.	Treatment	Germination (%)	Percent mortality of seeds		
			Before germination	After germination	Total mortality
1.	Surface sterilized seed + inoculated	56	58	42	100
2.	Without Surface satirized + inoculated	42	44	56	100
3.	Control	76	24	10	34

Table 4: Effect of *Myrothecium roridum* on seed germination.

S. No.	Surface sterized + inoculated	Without surface sterilized + inoculated	Without surface sterilized + Uninoculated control
1.	56 % seeds were germinated	42 % seeds were germinated	76 % seeds are germinated
2.	28% seeds were black	37 % seeds were black	15% seeds were black

	color	color	color
3.	Pumules size (1.5 cm)	Pumules size (1 cm)	Pumules size (2.5 cm.)
4.	30% seed covered by *M. roridum.*	40% seed covered by *M. roridum* & 7% covered by other fungus	1% seed covered by *M. roridum* & 15% seed cover by different other fungus.

4.4 Physiological studies:

4.4.1 Effect of different PH on radial growth and sporulation of *Myrothecium roridum* :

The radial growth of *M. roridum* was recorded at 10 and 15 DAI to observe the effect of PH. The data presented in Table 5, Fig 1 indicates that the radial growth of *M. roridum* varied significantly at varying pH. Potato dextrose agar medium when adjusted to PH 6 recorded significantly maximum radial growth (45.7 mm, 90 mm) at 10 and 15 DAI respectively followed by PH 7 (44.68 mm, 89.19 mm.). On other hand , minimum radial growth (23.5 mm, 39.50 mm) was recorded at PH 9 and pH 4 after 10 and 15 DAI respectively. An average radial growth indicates that *Myrothecium roridum* can grow better at 6 PH (67.85 mm). An average minimum radial growth was recorded at 4 PH (35.00 mm.). The maximum sporulation was noticed at PH 6 (444.00 spores) and minimum at PH 9 (99.55).

Chauhan and Suryanarayan (1970) reported maximum growth in acidified potato dextrose agar medium. Okunowo *et al*. (2008) reported that the fungus was able to grow over the pH range (5.5 to 8.6) employed in the study. The growth of the organism was highest at pH 5.5 and lowest at 8.6.

Table 5 Effect of pH on radial growth and sporulation of *Myrothecium roridum.*

S. No	pH	Radial growth of fungus (mm)		Average growth (mm)	No of spores per microscopic field
		10 DAI	15 DAI		

1	4	30.50	39.50	35.00	205.00
2	5	32.83	43.83	38.33	303.33
3	6	45.70	90.00	67.85	444.00
4	7	44.68	89.18	66.93	400.55
5	8	29.50	44.83	37.16	111.00
6	9	23.50	55.18	39.34	99.55
	SEm± CD (5%)	0.33 1.02	0.29 0.90		1.03 0.34

Average of three replications

- **Effect of different media (synthetic & non synthetic) on radial growth and sporulation of *Myrothecium roridum* :**

The data presented in Table 6 , Fig. 2 indicates that the radial growth of *Myrothecium roridum* differed significantly with different types of media used. The maximum radial growth of fungus (55.67 mm, 90 mm) was noticed on PSA media followed by Malt extract (54.33 mm, 89 mm) and Richards media (51.23 mm, 85.30 mm) at 10 DAI &15 DAI respectively and superior to PDA, in context with mycelial growth. Malt extract was statistically at par with PSA media. Average maximum radial growth in PSA was 72.83 mm followed by Malt extract (71.66 mm) and Richards's media (68.26 mm). The average growth of PDA media was (64.94 mm). Minimum radial growth (26.90 mm, 33.60mm) was recorded on Asthana & Hawker's media at 10 DAI and 15 DAI respectively. Minimum average growth showed by Asthana & Hawker's (29.93 mm) followed by Kerr's media (47.22 mm) and then by Dextrose nitrate agar media (51.67 mm).

The number of spore per microscopic field was maximum in Malt extract medium (998.90 spores) followed by PSA (500 spores), PDA (998.00 spores) and then Richards

media (880.00 spores). Minimum sporulation was observed in Asthana & Hawker's (2.33 spores) followed by Kerr's media (7.55 spores)

The most suitable solid media identified for the radial growth was PSA (Potato Sucrose Agar), Malt extract, Richards media in comparison to PDA media. The sporulation was maximum on Malt extract, followed by Potato Sucrose Agar medium.

The present investigations were very close to the observation by Okunowo *et al.* (2008). They also observed PSA media was most favourable for the radial growth of the pathogen and Malt extract for sporulation.

Table 6 Effect of different (synthetic & non synthetic) media on radial growth and sporulation of *Myrothecium roridum*

S. No	Media	Radial growth (mm)		Average growth (mm)	No of spores per Microscopic field.
		10 DAI	15 DAI		
1.	Malt extract	54.33	89.00	71.66	998.99
2.	Potato sucrose agar	55.67	90.00	72.83	500
3.	Richards	51.23	85.30	68.26	880
4.	Czapek dox agar	45.54	79.64	62.59	95
5.	Dextrose nitrate agar	36.56	66.78	51.67	13.33
6.	Kerr's	32.44	62.00	47.22	7.55
7.	Asthana &Hawkers	26.90	33.00	29.95	2.33
8.	Control	47.88	82.01	64.945	335
	SEm ± CD (5%)	0.98 2.96	0.56 1.68		0.18 0.55

Average of three replications

- **Effect of different temperature on radial growth and sporulation of *Myrothecium roridum* :**

The data presented in Table 7, Fig. 3 indicates that the radial growth of *M. roridum* varied significantly when it was grow at different temperature. The mycelial growth was significantly higher (45.53 mm; 90 mm) at 25°C after 10 and 15 DAI,

respectively followed by 30oC (43.23 mm) at 10 DAI and 20^{0}C (87.56 mm) at 15 DA. Least growth at 40^{0}C (23.33 mm, 41.43 mm). Mycelial growth was not initiated at 0^{0}C and 10^{0}C and slowed at 15^{0}C and 40^{0}C.

Maximum sporulation was noticed at 30^{0}C (991.00 spores) and minimum sporulation at 15 ^{0}C (25.55 spores), while none of the sporulation was recorded at 0^{0}C, 5°C and 10^{0}C. The mycelial growth of *M. roridum* was found to be maximum at 25^{0}C followed by 30^{0}C & 20^{0}C. The maximum sporulation was found in 30^{0}C.

Tsay *et al.* (1996) reported that the optimum temperature for spore production was at 30^{0}C. Chauhan and Suryanarayan (1970) recorded 25°C as a best temperature for growth and sporulation of *M. roridum.*

Table 7: Effect of different temperature on radial growth and sporulation of *Myrothecium roridum*

S. No.	Temperature (^{0}C)	Growth (mm)		Average Growth (mm)	No of spores per microscopic field
		10th DAI	15th DAI		
1.	0	0.00	0.00	0.00	0.00
2.	5	0.00	0.00	0.00	0.00
3.	10	0.00	0.00	0.00	0.00
4.	15	25.06	45.44	35.25	25.55
5.	20	40.00	87.56	63.78	800.33
6.	25	45.53	90.00	67.765	990.55
7.	30	43.23	87.23	65.23	991.00
8.	35	27.45	47.00	37.225	22.22
9.	40	23.33	41.43	32.365	0.00
	SEm ±	0.17	0.22		1.07

	CD (5%)	0.53	0.65		4.22

Average of three replications

4.5 Determination of enzymatic activity:

4.5.1 Influence of culture filtrate of *Myrothecium roridum* on the activity of Carboxy Methyl Cellulase (CMC) at different $_{P}$H levels :

The data on the influence of culture filtrate of *M. roridum* on the activity of CMC at different $_{P}$H levels was given in Table 8 and Fig 4 , which clearly shows that cellulolytic activity differed with the change of $_{P}$H levels from 4 to 9.2 & time interval from 0 to 480 minutes. From the data, it is evident that per cent loss in viscosity was very high (88.57) at $_{P}$H 7.0 as compared to 85.54 and 77.16 per cent at $_{P}$H 4.0 and 9.2 respectively. The per cent loss of viscosity was maximum at 360 minutes at ranges of $_{P}$H 7.0 and 420 minutes for pH 4.0 and pH 9.2. The enzymatic activity in culture filtrate was started at 60 minutes and become maximum at 360 minutes. It is evident from the data that culture filtrate adjust at $_{P}$H 7.0 was most suitable for rapid degradation of CMC by the enzymatic activity of *M. roridum*.

. The production of cellulase enzyme by a strain of *Myrothecium* sp. was determined by Kassim (1983) and Reddy (1998). Okunowo (2010) found that the cellulase enzyme produced in *Myrothecium roridum.*

Table 8: Influence of culture filtrate of *Myrothecium roridum* on the activity of Carboxymethyl cellulase (CMC) at different $_{P}$H levels

S.No	Time interval (in minutes)	Per cent loss in viscosity at different $_{P}$H level		
		$_{P}$H 4.0	$_{P}$H 7.0	$_{P}$H 9.2
1.	0	0.00	0.00	0.00

2.	60	45.19	60.16	39.65
3.	120	56.69	65.45	50.94
4	180	60.14	71.00	68.03
5.	240	65.89	75.55	68.17
6.	300	71.19	80.33	70.74
7.	360	81.09	88.57	73.88
8.	420	85.54	88.57	77.16
9.	480	85.54	88.57	77.16

4.5.2 Influence of culture filtrate of *M. roridum* on the activity of Polygalacturonase (PG) at different $_{P}$H levels :

The data on the influence of culture filtrate of *M. roridum* on the activity of PG at different $_{P}$H levels was given in Table 9 and Fig 5. clearly showed that pectin activity differed with the change of $_{P}$H levels.

From the data, it was evident that the per cent loss in viscosity was very high (90.67%) at $_{P}$H 7.0 as compared to 80.01 and 79.65 per cent at $_{P}$H 9.2 and $_{P}$H 4.0 respectively. The per cent loss of viscosity was maximum at 780 minutes at PH 7.0 followed by $_{P}$H 9.0 and $_{P}$H 4.0 at 900 and 720 minutes respectively. The PG enzymatic activity was started at 60 minutes and become maximum at 780 minutes. It is evident from the data that culture filtrate adjusted at $_{P}$H 7.0 was most suitable for rapid degradation of pectin by the enzymatic activity of *M. roridum.*

The similar work done by Lucas and Sherwood (1966) reported that the brown spot of fungus *A. alternata* readily produces Polygalacturonase, Pectin esterase and Cellulase (Cx) when culture developed on modified Richard's solution with cellulose, starch as carbon source. On pectin salts medium, the isolates produced

Polygalacturonase, Pectin esterase and Pectinlyase. The pectin lyase was most active on pectin at pH 9.2

Umana and Ikotum (2000) investigated *in-vitro* the three pathotypes of *Choanephora cucurbitarum* from *Abelmoschus esculentus, Amarenthus hybridus* and *vigna unguiculata* for production of enzyme. The endo-polygalacturonase was the predominant enzyme at a lower pH of 4.0-6.0, with the highest activity at pH 5.0. The endo-Pectate lysate which has an optimum pH of 8.0 occurred less often. The exo-polygalacturonase had a lower pH of between 3.0 and 5.0. The amount of enzymes produced varied with the age of culture.

Table 9: Influence of culture filtrate of *Myrothecium roridum* on the activity of Polygalacturonase (PG) at different pH levels

S. No	Time interval (in minutes)	Per cent loss in viscosity at different pH level		
		pH 4	pH 7	pH 9.2
1.	0	0	0	0
2.	60	40.12	20.99	15.65
3.	120	50.37	21.06	17.01
4.	180	55.23	25.62	20.67
5.	240	65.78	33.93	35.15
6.	300	67.43	50.06	38.23
7.	360	69.09	57.56	45.55
8.	420	70.43	59.40	50.15
9.	480	70.56	66.53	53.45
10.	540	72.08	75.87	55.04
11.	600	73.73	77.48	56.00
12.	660	77.62	79.88	60.32
13.	720	79.65	80.58	66.15
14.	780	79.65	90.67	75.73
15.	840		90.67	77.56
16.	900		90.67	80.01
17.	960			80.01

4.5.3 Effect of culture filtrate of *Myrothecium roridum* on macerating of potato and carrot pith.

Effect of culture filtrate of *M. roridum* on macerating of potato and carrot pith presented in Table10 indicates that the culture filtrate of *M. roridum* at 100%

concentration was macerated in all vegetable pith of potato and carrot at 20 hrs. and 24 hrs respectively. While 46 hrs and 40 hrs were taken by 50 % concentration in comparision to control 121 hrs and 124 hrs respectively.

The similar work done by Kapat *et al*. (1998) and Binjhare (2002) both reported that liquid culture filtrate *B. cinerea* produced both conductive and inductive forms of hydrolic enzymes and also suggested that enzymes may have important role in penetrating the cutin layer and extensive cell death in potato tissue.

Table 10: Effect of culture filtrate of *Myrothecium roridum* on macerating of potato and carrot.

S.No	Concentration of culture filtrate (%)	Rotting of vegetable pith in hours	
		Carrot	Potato
1	100	24	20
2	50	46	40
3	Control	124	•

4.5.4 Influence of culture filtrate of *Myrothecium roridum* on attached leaf of soyabean

Data on influence of culture filtrate of *Myrothecium roridum* on attach leaf of soyabean presented in Table 11 indicates that slight necrosis and browning of leaf appeared after 6 days and these Brown colour changed to darker color after 10 days and maximum appearance of the necrosis and death of the plant (100 % leaf area cover) recorded after 15 days.

Mukarami (2005) found that the culture filtrate of *Myrothecium roridum* consist of toxin Myrotoxin B of Trichothecenes that causes necrosis on leaf of Mulberry.

Table 11: Influence of culture filtrate of *Myrothecium roridum* on attached plant leaf of soyabean.

S. No.	Disease range	Disease development after inoculation (in days)
1.	Initiation of necrosis and browning of the leaf	6
2.	Brown colour changes to darker color	10
3.	Maximum appearance of necrosis and death of the plant	15

- **Influence of culture filtrate of *Myrothecium roridum* on detached leaf of soyabean :**

The result presented in Table 12 indicates that the culture filtrate of *M. roridum* influenced on leaf surface of soyabean by pin-prick method, leaf scratch method & plain leaf method in comparison to control.

Uniform brown colour appeared on the surface of the leaf in 3 days by leaf scratch method while pin- prick and plain leaf method were taken 5 days and 7 days respectively. Brown surface darkened and chlorotic area get enlarged in 9, 11 and 15 days by leaf scratch, pin-prick and plain leaf respectively while no symptom was produced in control leaf. 100 percent leaf area turned blackish brown in leaf scratch method at 11 days while 80 % and 55 % blackish brown were recorded at 15 and 20 days in pin-prick and plain leaf method respectively.

Kuti *et al.*(1989) inoculated the culture filtrate of the *M. roridum* by detached method on the leaves of muskmelon in which susceptibility was determined by necrotic

and chlorotic symptoms. Mukarami *et al*. (1998) observed necrotic and browing symptoms on mulberry plants leaves by culture filtrate of *Myrothecium roridum* by detached methods. Mmbaga (2010) used culture filtrate of *M. roridum* on detached leaves of Garden Hydrangea where necrotic lesions started 4-5 days after inoculation of 5 mm mycelia plugs.

Table 12: Influence of culture filtrate of *Myrothecium roridum* on detached leaf of soybean.

S. No.	Inoculation method	Symptoms developed after inoculation in days		
		Appearrance of uniform brown colour of leaf surface	**Enlargement of chlorotic and necrotic area**	**Appearance of blackish brown of leaf surface**
1.	Pin- prick method	5	11	After 15 day s 80% of leaf area appeared blackish brown
2.	Leaf scratch method	3	9	After 11 days 100% of leaf area appeared blackish brown
3.	Plain leaf method	7	15	After 20 days 55% of leaf area appeared blackish brown
4.	Control	No symptoms appeared.	-	

- **Influence of culture filtrates of *Myrothecium roridum* on germination of soyabean seeds tested by standard blotter method :**

The data on the influence of culture filtrate of *Myrothecium roridum* on seed germination of soybean are given in Table 13.

The data revealed that seed germination and mortality varied with the treatment. Total mortality of the seeds were recorded in seed + blotter treated (100%) followed by blotter treated (90%). The total mortality in seed treated alone was 85%. Minimum mortality (15%) and maximum germination (87%) were recorded in broth treated control.

Reduction, delayed in germination and death of seeds due to the application of spore suspension of *M. roridum* recorded by Duke (1980) on cultivated varieties of cotton seeds. The similar work was done by Siddaramaiah *et al.* (1979) who reported the culture filtrate of *A. carthami* Chowdhary inhibited seed germination up to 50 percent, with toxic effect to all 25 varieties.

Table13: Influence of culture filtrate of *Myrothecium roridum* on germination of soyabean seed (tested by standard blotter method)

S. No.	Treatment	Germination (%)	Percent mortality of seeds		
			Before germination	After germination	Total mortality
1.	Seed treated	30	70	15	85
2.	Blotter treated	40	60	30	90
3.	Seed + blotter treated	25	75	25	100
4.	Control	87	13	2	15

4.6 Management of test pathogen:

4.6.1 Efficacy of different medicinal plant leaf extracts on inhibition of radial growth of *Myrothecium roridum* :

Hot water extracts of different plant species were evaluated to observe the inhibitory activity of *M. roridum* under *in-vitro* condition.

The data was recorded and presented in Table 14 and Fig6. It is clear from the data that all extract of medicinal plants were significantly superior in reducing the radial growth over control. The per cent inhibition in growth of *M. roridum* was ranged from

(70.76% to 95.32%) at 5 DAI. The maximum inhibition in growth at 5 DAI was recorded in the extract of neem (95.32%) followed by onion (93.45%), karanj (92.28%), lemon (91.81) and garlic (90.64%) and statistically at par with each other. Least inhibition by tulsi (70.76%) and pudina (80.50%).

The per cent growth inhibition of 15 DAI by different plant extract ranged between (70.00 to 90.77%). The maximum radial growth inhibition was recorded in plant extract of neem (91.11%) followed by onion (90.77%), lemon (89.00%) and garlic (87.77%) and least by pudina (74.07%), karanj (70.00%) and tulsi (68.52%). The maximum average inhibition in growth was recorded in the extract of neem (93.21%) followed by onion (92.11%) , lemon (90.40%) and garlic (89.20%). Minimum inhibition was recorded by tulsi (69.64%).

An average % growth inhibition indicates that maximum inhibition of growth of *M. roridum* recorded in neem, onion, lemon and garlic and least in tulsi.

Olufolaji (1999) tested antifungal property of medicinal plant extract under *in-vitro* and *in-vivo* condition and found *Azadiracta indica* (neem) to be effective in inhibition of mycelial growth (13.85%) & sporulation (82.50%) of various fungus.

Leaf extract of onion is supported best by Manas *et al.* (2005) against growth of *M. roridum* which is followed by leaf extract of neem.

Table 14 Efficacy of different medicinal plant leaf extracts on inhibition of radial growth of *Myrothecium roridum.*

S. No.	Medicinal Plant	Radial growth (mm)				Average of inhibition (%)
		5 DAI		15 DAI		
		Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	
1.	Pudina	8.30	80.58 (63.54)	23.33	74.07 (59.37)	77.32
2.	Tulsi	12.50	70.76 (56.94)	28.33	68.52 (55.76)	69.64
3.	Onion	2.80	93.45 (74.82)	8.30	90.77 (72.29)	92.11
4.	Lemon	3.50	91.81	9.30	89.00	90.40

			(72.81)		(70.63)	
5.	Garlic	4.00	90.64 (71.94)	11.00	87.77 (69.30)	89.20
6.	Neem	2.00	95.32 (77.07)	8.00	91.11 (72.57)	93.21
7.	Karanj	3.33	92.28 (73.66)	27.00	70 (55.54)	81.14
8.	Control	42.76		90.00		
	SEm ± CD (5%)		0.16 0.50		0.09 0.28	

Arc sine Transformation in parenthesis
Average of three replications

- **Evaluation of fungicides at different concentration on inhibition of radial growth & sporulation of *M. roridum* by Poisoned food technique :**

Seven fungicides at three concentrations (250, 500 and 1000 ppm) were tested under *in-vitro* condition against *M. roridum* and observation on inhibition of radial growth and sporulation were recorded and presented in Table 15,16,17 and 18 and Fig 7,8 and 9. Data indicates that all fungicides were significantly superior in reducing the mycelial growth in comparison to control. Percent growth inhibition of *M. roridum* recorded ranged between 100% to 20.32% in 250 ppm at 10DAI while it was 100% to 43.44% in 15 DAI. The complete inhibition in growth was recorded in the Benomyl, Saaf and Vitavex power (100%) at 10 DAI as well as 15 DAI. On the basis of Average inhibition Dhanustin, Dhanucop and Dithan M-45 were inhibited the mycelial growth by 47.25%, 47.06% and 37.27% respectively. Minimum inhibition was recorded by Curzate M-8 (31.88%).

In 500 ppm concentration all fungicides were significantly superior in reducing the mycelial growth over control at 10 DAI and 15 DAI (Table 16 and Plate 18). Percent inhibition in growth of *M. roridum* recorded in ranged 100% to 26.88% in 10 DAI and 100% to 27.77% in 15 DAI. Complete average growth inhibition was found in Benomyl,

Saaf, Vitavex power and Dhanustin (100%). Dithan M-45 inhibited by 58.10 % and Dhanucop inhibited by 53.34%. Minimum average of mycelial growth inhibition recorded in Curzate M-8 (27.33%).

In 1000 ppm concentration percent inhibition in growth of *M. roridum* recorded in ranged 100% to 35.69% in 10 DAI and 100% to 49.38% in 15 DAI. Complete inhibition in average growth was found in Benomyl, Saaf, Vitavex power, Dhanustin and Dhanucop(100%). Minimum average of mycelial growth inhibition recorded in Curzate M-8 (41.72%).

Data presented in Table 18 indicates that all the fungicides were significantly superior in reducing the sporulation of M. roridum in 250, 500 and 1000 ppm. Complete inhibition of sporulation was recorded in Benomyl, Saaf and Vitavex power at all the concentration tested. While Dhanustin also completely inhibited the sporulation at 500 ppm and 1000ppm in comparison to control (335.50 and 400.33 spores). Cent percent inhibition of sporulation of sporulation was recorded by Dhanucop in 1000ppm. Maximum sporulation was recorded in Curzate M-8 at all concentration (78.66, 84.00 and 86.67 spores).

Maximum sporulation was recorded in Curzate (78.66) at 250 ppm and Curzate (84.00) at 500 ppm. Minimum sporulation was recorded in Benofit, Vitavex, Saaf (0.00)at 250 ppm and Dhanucop, Benofit, Vitavex, Saaf (0.00) 500 ppm and Dhanucop , Benomyl, Vitavex, Dhanustin, Saaf(0.00) at 1000ppm.

Carter (1980) confirmed that a mixture of Benomyl and Zinc ion- maneb complex significantely controlled *Myrothecium roridum* on cantaloupe leaves and stems. Banik *et al*. (2004) reported that carbendazim could effectively control *Myrothecium*

roridum causing leaf spot of black gram. Murthy *et al.* (2004) confirmed that disease caused by *Myrothecium roridum* on teak saplings could be effectively managed by bavistin (carbendazim), captan, captafol, *Trichoderma harzianum*, *Pseudomonas fluorescens*, Urea, Diammonium phosphate and NPK. Bavistin, *P. fluorescens* and NPK fertilizer gave the lowest mean disease incidence percentage of *Myrothecium roridum*. Tomar and Shastry (2006) also reported that Carbendazim is the most effective in controlling seed born diseases caused by *M. roridum* in cotton.

Table 15 Effect of different Fungicides on radial growth of *Myrothecium roridum* (250 ppm)

S. No	Fungicide	Radial growth (mm)				Average of inhibition (%)
		10 DAI		15 DAI		
		Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	
1	Dhanucop	30.00	29.69 (32.58)	32.00	64.44 (51.86)	47.06
2	Benomyl	0.00	100.00 (85.45)	0.00	100.00 (85.45)	100.00
3	Saaf	0.00	100.00 (85.45)	0.00	100.00 (85.45)	100.00
4	Vitavex power	0.00	100.00 (85.45)	0.00	100.00 (85.45)	100.00
5	Dhanustin	27.00	36.72 (37.29)	29.00	57.78 (49.17)	47.25
6	Dithan M-45	30.30	28.99 (32.34)	49.00	45.55 (41.27)	37.27
7	Curzate M-8	34.00	20.32 (30.22)	50.90	43.44 (40.84)	31.88
8	Control	42.67		90.00		
	SEm± CD(5%)		0.06 0.18		1.77 0.58	

Arc sine Transformation in parenthesis

Average of three replications

Table 16: Effect of different fungicides on radial growth of *Myrothecium roridum* (500 ppm).

S. No	Fungicide	Radial growth (mm)				Average of Inhibition (%)
		10 DAI(mm)		15 DAI(mm)		
		Growth(mm)	Inhibition %	Growth(mm)	Inhibition %	
1	Dhanucop	25.05	44.68 (41.92)	25.05	62.16 (52.02)	53.34
2	Benomyl	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
3	Saaf	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
4	Vitavex power	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
5	Dhanustin	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
6	Dithan M-45	25.00	45.10 (42.15)	26.00	71.11 (57.46)	58.10
7	Curzate M-8	33.30	26.88 (31.18)	65.00	27.77 (31.62)	27.33
8	Control	45.54		90.00		
	SEm± CD(5%)		0.01 0.05		0.06 0.18	

Arc sine Transformation in parenthesis

Average of three replications

Table 17: Effect of different fungicides on radial growth of *Myrothecium roridum* (1000ppm)

S. No	Fungicide	Radial growth (mm)				Average of Inhibition (%)
		10DAI		15 DAI		
		Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	
1	Dhanucop	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
2	Benomyl	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
3	Saaf	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
4	Vitavex power	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
5	Dhanustin	0.00	100.00 (85.94)	0.00	100.00 (85.94)	100.00
6	Dithan M-45	20.01	56.02 (48.45)	21.00	66.66 (54.73)	61.34
7	Curzate M-8	30.00	34.06 (35.69)	45.55	49.38 (44.74)	41.72
8	Control	45.50		90.00		
	SEm± CD(5%)		0.01 0.06		0.01 0.04	

Arc sine Transformation in parenthesis

Average of three replications

Table 18: Effect of fungicides on sporulation of *Myrothecium roridum*

S. No.	Fungicides	No of spores per microscopic field in different concentrations of fungicides		
		250 ppm	500 ppm	1000 ppm
1.	Dhanucop	20.00	14.10	0.00
2.	Benomyl	0.00	0.00	0.00
3.	Saaf	0.00	0.00	0.00
4.	Vitavex power	0.00	0.00	0.00
5.	Dhanustin	54.56	0.00	0.00
6.	Dithan M-45	75.78	23.33	20.00
7.	Curzate M-8	78.66	84.00	86.67
8.	Control	355.50	400.33	399.95
	SEm ±	2.44	0.09	0.12
	CD (5%)	7.34	0.27	0.37

Average of three replications

4.6.3. Screening of different isolates of *Trichoderma spp.* against *Myrothecium roridum*

Seventeen isolates of *Trichoderma* spp. were used to test antagonistic performance in dual culture with a test pathogen *Myrothecium roridum*. All the seventeen

isolates of *T.*spp (T93, T73, T66, T31, T29, T27, N, T132, T120, T114, T101, T110, T7, T158a, T16, T15 and T14) were used for the study. The data on the inhibition of growth of *M. roridum* by various *Trichoderma* spp. was presented in Table 19. Among various Trichoderma spp, highest inhibition of the growth of test pathogen by T31 (37.5%) followed by T7 (32.5%), T15 (32.5%) and T27 (27.5%). T101 and T158a could not inhibit the test fungus.

Trichoderma spp. isolates was found most effective species to inhibit the mycelial growth of other fungus *Sclerotium rolfsii.* This coincides with the findings of Wells *et al.* (1972), Upadhyay and Mukhopadhay (1986), Patel and Anahosur (2001) who also tested the efficacy of bio-agents under *in vitro* condition.

Murthy *et al.* (2004) determined that *Myrothecium roridum* causing disease in teak saplings could be effectively controlled by bioagents like Trichoderma *harzianum* and Pseudomonas *fluorescens* that gave the lowest mean disease incidence percentage of *Myrothecium roridum.*

Table 19 Screening of different species of *Trichoderma* spp. for mycoparasitic efficacacy against *Myrothecium roridum* of soyabean

S. No.	*Trichoderma* spp	Radial growth (mm)		% inhibition over control
		Trichoderma spp	*Myrothecium roridum*	
1.	T93	55	34	15.00 (22.94)
2.	T73	57	33	16.25 (23.70)
3.	T66	55	37	7.50 (15.72)
4.	T31	68	25	37.50 (37.68)
5.	T29	50	35	12.50 (20.76)
6.	T27	67	29	27.50 (31.52)
7.	N	50	32	20.00 (26.73)
8.	T132	55	30	25.00 (30.07)
9.	T120	53	33	17.50 (24.61)
10.	T114	56	30	25.00 (30.07)
11.	T101b	49	40	0.00 (4.05)
12.	T110	47	35	12.50 (20.76)
13.	T7	59	27	32.50 (34.75)
14.	T158a	46	40	0.00 (4.05)
15.	T16	50	30	25.00 (30.05)
16.	T15	55	27	32.50 (34.75)
17.	T14	35	35	12.50 (20.76)
18.	Control		40	
	SEm±			0.10

	CD (5%)			0.29

Arc sine Transformation in parenthesis
Average of three replications

- **Evaluation of host resiatant of different soyabean varieties against *Myrothecium roridum* :**

The data presented in Table 20 indicates that out of 56 varieties, 50 varieties showed highly resistant reaction while remaining 6 varieties were found moderately resistant. None of the varieties found in absolutely resistant, moderately susceptible, susceptible and highly susceptible categoties.

Maximum PDI was observed in KS0 245 (18%) followed by JS-93-05 and Shivalic (15%), minimum PDI was recorded in PK-472 (4.66%) under highly resistant grouped.

Maximum PDI in highly resistant group was recorded in NSO-81 (11%) and minimum was recorded in PK-472 (4.66%).

From the result, it is clear that maximum varieties exhibited highly resistant reaction for the disease of Myrothecium leaf spot of soyabean.

Present finding are in accordance with Singh and Srivastava (1994) who reported only two of the 26 cultivars as resistant for three consecutive years (1984-86), three were moderately resistant and the rest were moderately to highly susceptible against Myrothecium leaf spot disease.

Srivastava *et al*. (1995) recorded JS 81-303, JS 81-1619 and JS 81-335 as highly resistant varieties against *Myrothecium* leaf spot of soyabean.

Table.20: Evaluation of host resistant in different soyabean varieties against *Myrothecium roridum*.

S. No.	PDI	Disease reaction	No of variety	Variety
1.	0	AR	0	
2.	0.01- 11.11	HR	50	JS-72-44, JS-75-46, 71-05, R2-72-280, PK-262, PK-472, MACS-58, Punjabi-1, Bragg, KHSB-2, MRC0-7, VLS-58, JS-335, RKS-63, NSO-81, PS1477, JS(SH)2003-8, DS-15-2, MAUS-449,SL-778, P51480, JS 20-34, VLS 76, AMS-MB-5-18, MACS-1311, DSB-20, CSB08-08, KS103, MAUS 453, NRC-86, AMS-MB-5-19, MACS 1336, RKS 61, JS20-29, NRC-85, TS10, DSb 18, SL871, DS27-11,NRC-87, Himso 1680, AMS 243, MACS 1201, BAU 540, KDS 344, KBS-8, NRC 88, JS-335, Bragg, NRC-3
3.	12.22-33.33	MR	6	JS 20-30, VLS77, KS0 245, PS1476, Shivilic, JS-93-05,
4.	34.44-55.55	MS	0	-
5.	56.66-77.77	S	0	-
6.	78.88-100.00	HS	0	-

Location severity index (LSI) =1.13

4.8 Effect of date of sowing on disease severity of *Myrothecium roridum* on soyabean under field conditions :

The results presented in Table 21 and Fig. indicates that Myrothecium leaf spot intensity was found to differ with respect to sowing dates and varieties. Average severity recorded at ranged from 7.45 to 15.19 % in crops sown at different dates. Significant reduction in disease severity (7.45%) was recorded in 31st July sowing (D_1) followed by D_2 8th August sowing (8.50%). Maximum severity (15.19%) was recorded in late sown i.e. 24-8-10 (D_4).

In context to variety, minimum disease severity (8.31%) was recorded in JS-9752 with significant difference followed by JS-335 (10.56%) and JS-9305 (15.22%). Interaction between date of sowing and variety indicates that D_1V_2 effectively reduced the disease severity (5.0%) of Myrothecium leaf spot followed by D_2V_2 (5.67%). In interaction of D×V maximum disease severity (20.33%) was recorded in D_4V_3.

The present findings are in accordance with the Chauhan and Suryanarayan (1970) who reported that disease severity of Myrothecium leaf spot disease increased and yields decreased with deley in sowing date of cotton. Significant differences in disease severity was recorded by Singh (1999) between sowing date and varieties against leaf spot of soyabean caused by *M. roridum*. They reported that the early sown soyabean crop (25 June) exhibited less severe disease than that sown on July 8 and 23.

Table 21 Effect of date of sowing on disease severity of Myrothecium leaf spot of soyabean

Date of sowing	PDI			
	JS-335(V_1)	JS-9752(V_2)	JS-9305(V_3)	Average
D1 31.07.10	7.33	5.00	10.00	7.45
D2 08.08.10	7.34	5.67	12.55	8.52

D3 16.08.10	12.55	10.00	18.00	14.30
D4 24.08.10	15.00	12.56	20.33	15.19
Mean PDI	10.56	8.31	15.22	11.36
	SEm±	**CD**		
1. Date (D)	0.09	0.25		
2. Variety(V)	0.07	0.22		
3.Interaction(D×V)	0.15	0.43		

4.9 Disease development in variety in relation to environmental factors: Variety JS-335 was selected for studying quantative relationship between mean temperature, relative humidity and cumulative rainfall as three independent variables and the percent disease indicence of Myrothecium leaf spot of soyabean. Dates presented in Table 22 indicates that with an increase in maximum temperature, rainfall and maximum relative humidity, there is an increase in disease incidence of Myrothecium leaf spot of soyabean. Favourable temperature for this disease is mean air temperature between 25.10 to 31.50 ^{0}C, RH 77-94% and cumulative rainfall 78.20-129 mm.

Chauhan and Suryanarayan (1970) concluded that the disease incidence of Myrothecium leaf spot of soybean highly affected by climatic conditions. Singh *et al.* (2003) determined the effects of environmental factors, namely atmospheric temperature, relative humidity, and rain on Myrothecium leaf spot (*M. roridum*) of pigeon pea cultivar T-21 (susceptible). They found that the disease started to appear in traces in the second fortnight of July which gradually increased. The maximum disease intensity was observed in the first fortnight of September (45.6%) when the average atmospheric temperature, relative humidity, and rain were 27 degrees C, 84.7%, and 11.4 mm. Rain

coincided with the rapid increase in disease intensity. The present findings were also supported by Tomar and Shashtri (2006) who studied Myrothecium leaf spot in cotton.

Table 22: Disease development in relation to environmental factors

Observation (Date)	PDI	Temperature ^{0}C		Rainfall (mm)	Relative humidity	
		max	min		max	min
31st July	2.00	31.2	25.10	78.20	92	82
8th Aug	4.55	30.10	24.90	41.20	92	70
16th Aug	7.93	31.00	25.30	129.60	94	77
24th Oct	10.65	31.50	23.2	00.00	87	54
	Correlation coefficient	0.40	-0.69 *	0.64 *	0.90 *	-0.7 *

CHAPTER V

SUMMARY, CONCLUSION AND SUGGESTION FOR FUTURE WORK

The finding of the investigation on "**Studies on Myrothecium Leaf Spot (*Myrothecium roridum*) Tode ex. Fries. of Soyabean"** are summarized below:

The symptoms of Myrothecium Leaf Spot of soyabean were observed under natural field condition on leaves, stem and pods. Isolation was done from these plant parts and showed presence of *Myrothecium roridum* which was identified on the basis of its morphological characters. The pathogenicity of the fungus was tested on both leaf and seed. On leaf it was tested on field condition by attached method and laboratory condition by attached and detached methods and Koch's postulates were confirmed. Pathogenicity test was done on seed by *Myrothecium roridum* where 100% total mortality was observed in both surface sterilized+ inoculated & without surface + inoculated.

The most suitable $_{P}$H for mycelial growth and sporulation of *Myrothecium roridum* was found to be $_{P}$H 6 followed by $_{P}$H 7.

The mycelial growth of *Myrothecium roridum* was higher in Potato sucrose agar media followed by Malt extract and Potato dextrose agar media. Maximum sporulation was found in Malt extract followed by Potato sucrose agar media.

The best mycelial growth was found at 25^0C followed by 20^0C and maximum sporulation was noticed at 30^0C.

Maximum degradation (88.57 %) of CMC was found at $_{P}$H 7 by culture filtrate of *Myrothecium roridum*. It was late enzymatic activity up to 360 minutes in the reaction mixture of culture filtrate for Carboxy methyl cellulase.

Maximum degradation (90.67%) of PG was found at $_{P}$H 7 by culture filtrate of *Myrothecium roridum*. It was late enzymatic activity up to 780 minutes in the reaction mixture of culture filtrate for Polygalacturonase.

The toxic metabolites of *Myrothecium roridum,* maximum total mortality was found in seed + blotter treated (100%) followed by blotter treated (98%).

The maceration of potato and carrot pith 1mm thickness varied with varying concentration of culture filtrate. 100 % pure culture filtrate was taken less time as compared to 50% concentration for macerating of vegetable pith (potato 40 hrs and carrot 46 hrs). Influence of culture filtrate was studied on leaf by attached and detached methods as well as on seed of soyabean. Toxic effect of culture filtrate was very high as initiation of necrotic symptoms appeared after 6 days in attached method and uniform browning appeared in 3 days on detached method. In detached method, leaf scratch method was most suitable for disease development. Influence of culture filtrate was studied on soyabean seeds by slandered blotter method where there was total mortality of 85 % in treated seed, 90 % in blotter treated and 100 % in seed blotter treated. Germination percentage in control was 87%.

Seventeen isolates of *Trichoderma* spp. was taken for study among which T31 (*Trichoderma* isolates belonging to Section longibrachiatum) was found to be most effective against *Myrothecium roridum* followed by T7 belonging to same section that overlapped by the test pathogen.

All the seven treatments of medicinal plant leaf extract showed their inhibitive influence of growth of *Myrothecium roridum.* The maximum inhibition of radial growth was in

neem (93.21%) followed by onion (92.11%) and lemon (90.40%) and minimum in extract of tulsi (69.64 %).

All the seven fungicide treatments reduced the radial growth of *Myrothecium roridum* where maximum inhibition was found in Benomyl, Saaf and Vitavex power at 250ppm , Benomyl, Saaf, Vitavex power and Dhanustin at 500ppm and Benomyl, Saaf, Vitavex power, Dhanustin and Dhanucop at 1000ppm. Minimum inhibition was recorded in Curzate M-8 at all concentration. Maximum sporulation was found in Curzate M-8 at all concentrations.

56 different varieties of soyabean were screened under natural field condition for resistance of Myrothecium Leaf Spot of Soyabean. 50 varieties exhibited highly resistant and while remaining 6 varieties were found moderately resistant. None of the varieties showed moderately susceptible, susceptible and highly susceptible reaction.

Study was conducted on effect of date of sowing on disease severity of *Myrothecium roridum* on soyabean under field condition where maximum disease severity was recorded in late sown crop i.e 24/8/10. Maximum disease severity was recorded in variety JS-9035 (15.22) followed by JS-335(10.56) and lowest in JS-9752.

Percent disease index increased with increase in maximum temperature, rainfall and humidity. So PDI is positively correlated with maximum temperature , rainfall and maximum relative humidity.

Conclusion:

In the light of finding of present investigation, some conclusion could be drawn like, the fungus *M. roridum* grows well in synthetic and non-synthetic media particularly

on PSA, Malt extract and PDA media. The well suited temperature, $_{P}$H for mycelial growth of *Myrothecium roridum* are 20-30^{0}C and $_{PH}$ 6 respectively.

Medicinal plant leaf extracts like neem, onion and lemon plays an effective role in reducing mycelial growth of the fungus. Benomyl, Saaf and Vitavex power are very effective in all three (250 ppm, 500 ppm and 1000 ppm) concentrations in reducing mycelial growth of the fungus.

Trichoderma isolate T31 and T7 were comparatively effective in reducing mycelial growth of the fungus.

Disease can be managed effectively by use of resistant material. Out of 56 varieties, 50 varieties exhibited highly resistant reaction and remaining 6 varieties exhibited moderately resistant reaction. These varieties can be utilized in resistant breeding programme also. The efficacy of some plant extract, combination of fungicides and other indigenous methods and technological knowledge etc. have significantly effective in minimizing the Myrothecium leaf spot of soyabean.

Suggestions for future work:

- Survey to record the prevalence and severity of *Myrothecium roridum* in different parts of Chhattisgarh state.
- To find out the source of resistance against *Myrothecium roridum* of soyabean at inoculated condition.
- To find out the lower concentration of fungicides and non conventional chemicals.

- Evaluating integrated management practices specially bio agent or botanicals to manage disease and minimize the disease.

"Studies on Myrothecium Leaf Spot of (*Myrothecium roridum*) Tode ex. Fries. of Soyabean"

by

Diganggana Talukdar

ABSTRACT

The present investigation entitled "Studies on Myrothecium Leaf Spot (*Myrothecium roridum*) Tode ex. Fries. of Soyabean" was carried out at the Department of Plant Pathology, IGKV, Raipur (C.G.). Myrothecium Leaf Spot is very destructive disease of soyabean caused by *Myrothecium roridum* .

Pathogen was derived from the infected leaf and culture was maintained in PDA media and pathogenicity of fungus was conducted in leaf by attached and detached method as well as in seed and Koch's postulates confirmed. Typical symptom developed first on leaf then spread to stems and pod. Out of seven media, maximum radial growth was recorded in PSA followed by Malt extract and PDA and sporulation was highest in malt extract. The most suitable $_{P}H$ for mycelial growth of *Myrothecium roridum* was at $_{P}H$ 6 and $25^{0}C$ temperature. Influence of culture filtrate of *Myrothecium roridum* on enzymatic activity of CMC and PG was given maximum % loss of viscosity in culture filtrate in CMC and PG at 7 $_{P}H$. The seed germination was minimum (42%) and post seed mortality was maximum (100%) in without surface sterilized and inoculated treatment. Culture filtrate play important role in development of disease on leaf and seed. When use of culture filtrate in attached leaf of soyabean necrosis and browning appeared after 6 days. Leaf scratch method was most suitable for disease development in detached method as symptoms of uniform browning appeared after 3 days of inoculation. By standard blotter method there was total mortality of 85 % in treated seed, 90 % in blotter treated and 100 % in seed and blotter treated. Germination percentage in control was 87%. The 100 % pure concentration of culture filtrate was most effective for macerating of vegetable pith.

The maximum inhibition of radial growth of the fungus was in neem (93.21%) followed by onion (92.11%) and lemon (90.40%) and minimum in extract of tulsi (69.64

%). In fungicides treatment Benomyl, Saaf and Vitavex power proved to be most effective in inhibiting the mycelial growth of the fungus. *Trichoderma* isolates T31 followed by T7 was most effective against Myrothecium *roridum*.Out of 56 varieties of soybean screened, 50 varieties showed highly resistant, 6 varieties showed moderately resistant.Maximum disease severity was recorded in late sown crop i.e. 24/8/10. Maximum disease severity was recorded in variety JS-9035 (15.22) followed by JS-335(10.56) and lowest in JS-9752.Percent disease index increases with increased in maximum temperature, rainfall and humidity. So PDI is positively correlated with maximum temperature, rainfall and maximum relative humidity.

Department of Plant Pathology
IGKV, Raipur C.G.

(Dr R. K. Dantre)
Major Advisor

REFERENCES

Agrawal, S.C. and Kotasthane, S.R. 1971, Resistance in some of soybean varieties against *Sclerotium rolfsii*. Sacc. *Indian Phytopathology*, **24**: 401-403.

Anderson, K.I. and Hallett, S.G. 2004. Herbicidal spectrum and activity of *Myrothecium verrucaria*. *Weed-Science*, **52**(4): 623-627.

Annonymous. 2009. Annual Report of Kharif crop, Department of Plant pathology IGKV, Raipur.

Anonymous, 2010-11. All India Coordinated Research Project on soybean. Director's Report and summary table of experiment. Directorate of soybean Research ICAR, Indore (M.P.) pp: 9-14.

Baker, K.F. and Cook, R.J. 1974. Biological control of plant pathogens. W.H. Freeman and co. San Francisco, pp 433.

Banik, S.; Chatterjee, S.C. and Krishnamurthy, K.V.M. 2004 Effects of plant age mycoflora in Vigna mungo. *Annals of Plant Protection Sciences*, **12**(1) : 227-229.

Bean, G.A.; Jarvis, B. and Aboul, N. 1992. A biological assay for the detection of *Myrothecium* pp. produced macrocyclic trichothecenes. *Mycopath*., **116**:175-180.

Bhatnagar,P.S. and Tiwari,S.P. 1996. Soyabean In: Genetics , Cytogenetics and Breeding of crop plants. Oxford and IBH Publishing Co Pvt.Ltd, New Delhi and Calcutta, pp: 195.

Binjhare, L. P. 2002. Studies on grey rot (*Botrytis ricini godfrey*) of castor (*Ricinus communis L.*) M.sc. Thesis Department of Plant Pathology, I.G.A.U. Raipur, (C.G.).

Byrne, J. and Raymond A. C. 2007 . A pocket guide for IPM Scouting in herbaceous perennials. MSU Extension (publication E-2981).

Carter, W.W. 1980. Incidence and control of *Myrothecium roridum* on cantaloup in relation to time of fungicide application. *Plant Disease,* 64:872-874.

Chase, A.R. 1983. Influence of host plant and isolate source on *Myrothecium* leaf spot of foliage plants. *Plant Disease,* **67**: 668-671.

Chase, A. R., and Poole, R. T. 1984. Development of Myrothecium leaf spot of *Dieffenbachia maculata* perfection at various temperatures. *Plant Dis*. **68**:488-490.

Chase, A. R. 1992. Myrothecium Leaf Spot. Compendium of Ornamental Foliage, APS press,Minnesota. *Plant Disease*, pp: 35-37.

Chauhan, M.S. and Suryanayan, D. 1970. Effect of temperature, pH and light on growth and sporulation of *Myrothecium roridum*, the causal organism of leaf spot disease of Cotton in Haryana State. *Indian Phytopathology*, **23** (4): 660- 663.

Dey, S. (2010). Area & production estimates of soyabean in India Kharif (monsoon). www.myriis.com.

Datar, V.V. 1994. Investigation of purple blotch of onion in India. *Acta Horticulture*., **358**: 259- 263.

Di Menna, M.E.; Mortimer, P.H.; Smith, B.L. and Tulloch, M. 1973. The incidence of the genus *Myrothecium* in New Zealand Pastures and its relation to animal disease. *J Gen Appl Microbiol*., **79**:81-87.

Duke, G.N. 1980. Effect of *Myrothecium roridum* on the germination of cotton seeds. *Indian Phytopathololy*, **33**(4). 591-593.

Duvel, A.M.Q.; Henz, G.P.; Lima, P.M.L.; Medeiros, A.R.; Miranda, B.E.C.; Pfenning, L.H. and Reis, A. 2010. New hosts of *Myrothecium* spp. in Brazil and a preliminary in vitro assay of fungicides. *Brazilian Journal of Microbiology*, **41**(1): 246-252.

Escalona, F.; Maciel, N. and Renaud, J. 1992. A spotting of the inflorescences of heliconias. *Fitopatologia Venezolana*, **5**(2): 30-32.

Horinouchi,H.; Taguchi,Y.; Hagiwara,H. and Izutsu,S. 1999 . Occurrence of Myrothecium rot and leaf spot of Kalanchoe. *Proceedings of the Kansai Pl.Protection Society*, **41**: 33-3.

Jarvis, B. B.; Comezoglu, F.; Lee, Y.W.; Anderson, J. L.; Gilardi, R. D. and George, C. F. 1985, Novel Macrocyclic Trichothecenes from *Myrothecium roridum*. *Des Sociétés Chimiques Belges*, **95**: 681–697.

Kapat, A.; Zimand , G. and Elad, Y. 1998. Biosynthesis of pathogenicity hydrolytic enzymes by *B. cinerea* during infection of bean leaves *in- vitro. Mycological Res*., **102**(8): 1017-1024.

Kassim, E.A. 1983. Effect of *Myrothecium* sp. cellulase on different types of cellulose and cellulosic materials. *Folia Microbiol (Praha)., **28**(1):36-40.*

Kumar, P.M.P.; Pal, S.C. and Qadri, S.M.H. 2003. Screening of mulberry germplasm for resistance to leaf spot caused by Myrothecium roridum. Journal of Mycopathological Research, **41**(1): 105-106.

Khisal, A. A.; Rabenstein, J.; Woodard, J.; Dwight, D.B.; Jennifer, D.; Bergthold, J.L.;Kuo L.L. and Braude, I.A. 2002. 14‘-Hydroxymytoxin B and 16-

Hydroxyroridin E, two new cytotoxic trichothecenes from *Myrothecium roridum* . *J. Nat. Prod*., **65** (5): 742–744.

Kuti, J.O., and Bean, G. A. 1989. Possible involvement of a pathogen- produced trichothecene metabolite in Myrothecium leaf spot of muskmelon. *Physiol. Mol. Plant Pathol*., **34**:41-54.

Kuti, O. J.; Jarvis, B.B.; Molchtari, R. and Bean, G.A. 1990. Allelochemical regulation of reproduction and seed germination of two Brazilian *Baccharis* species by phytotoxic trichothecenes. *J. Chem. Ecol*.,**16**: 3441-3453.

Lakshminarayana, C. S. and Joshi, L.K. 1978. Myrothecium disease of soybean in India. *Plant-Disease*, **62**(3): 231-234.

Lakshmanan, P.1990. Effect of certain plant extract's against *Corynespora cassiicola*. *Indian J. Mycol. Pl. Pathol.,* **20**(3):267-269.

Lee, H.B.; Kim, J.; Hong, K. and Kim, C. 2008. Evaluation of a Fungal Strain, *Myrothecium roridum* F0252, as a Bioherbicide Agent. *Plant Pathol. J*. 24(4): 453-460.

Lucas, G.B. and Sherwood, R.T. 1966. Role of pectic enzymes of *Alternaria tenuis* in brown spot disease of tobacco. *Tobacco Science,* (**10**):410-416.

Manas ,D.M.; Chattopadhyay, S.; Pratheesh K. and Saratchandra, B. 2005. *In vitro* screening of some plant extracts against fungal pathogens of mulberry (*Morus* spp.). *Archives Of Phytopathology And Plant Protection*, **38**(3) :157 – 164.

Mangandi, J.A,; Seijo, T.E and Peres, N.A. 2007. First Report of *Myrothecium roridum* causing Myrothecium Leaf Spot on *Salvia* spp. in the United States. *The American Phytopathological Society* . **91** (6): 772.

Margaret, E. Di. Menna; Mortimer, P.H.; Smith, B.H. and Margaret. T. 1973. The Incidence of the Genus Myrothecium in New Zealand Pastures and Its Relation to Animal Disease. *Journal of General Microbiology*, **79**:81-87.

Mmbaga, M.T. 2010. First report of *Myrothecium roridum* causing leaf spot on Garden Hydrangea in the United States. School of Agriculture and Consumer Science, Otis Floyd Nursery Crops Research Center, Tennessee State University, McMinnville, **94**(10):1266.1

Mohan, S.; Lakhman, P and Jeyarajan, R.1988. New leaf spot and fruit rot of papaya caused by *Myrothecium roridum*. Tode.Ex. Fries. *Current Science,* **57**(24): 1345.

Moreira, F.G.; Simone, R.; Costa, M.A.F.; Marques de Souza, C.G. and Peralta, R.M. 2005. Production of hydrolytic enzymes by the plant pathogenic fungus *Myrothecium verrucaria* in submerged cultures. *Braz. J. Microbiol.*, **36**: 7-11.

Movahedi, S. and Heale, J.B. 1990. The roles of aspartic proterinase and endo- pectin lyase enzymes, in the primary stages of infection and pathogenesis of various host tissues by different isolases of *B.cinerea*. *Physiological and Molecular Plant Pathology*, **36**(4):303-324.

Murakami, R. Shirata, A. and Inoue, H. 1998. A selective medium containing the toxins of *Myrothecium roridum* for isolation from soil. *J. Seric. Sci. Jpn.*, **67**(5):381-387.

Murakami,R.; Shirata,A.; Inoue,H. 2000. Survival and fluctuation in density of *Myrothecium roridum* in mulberry (*Morus alba*) field **soil *J.*** *Gen.Pl. Path. Jpn.*, **66**(4) : 299- 302.

Murakami, Y. T. and. Shindo, K. 2001. Roridin L, M and verrucarin M, new macrocyclic tricothecene group antitumor antibiotics, from *Myrothecium verrucaria*. *American Phytopathol*., **54**:980–983.

Murakami, R. and Shirata, A. 2005. Myrotoxin B detection from mulberry leaves nfected with *Myrothecium roridum*, cause Myrothecium leaf spot of mulberry, and possible roles in pathogenicity. *Japan. J.Phytopath*., **71**: 91- 100.

Murakami, R.; Shirata, A. and Inoue, H. 1998. A selective medium containing the toxins of *Myrothecium roridum* for isolation from soil. *J. Seric. Sci. Jpn*., **67**(5): 381-387.

Murthy, N.; Lokesh, S. and Rai,V.R. 2004. Pathogenicity of *Myrothecium roridum* in teak saplings and its management. *Advances in Plant Sciences*, **17**(2): 541- 548.

Nguyen, T. H.; Mathur, S.B. and Neergaard, P. 1973. Seed-borne species of Myrothecium and their pathogenic potential. *British Mycological Society*, **61**(2): 347-354.

Norman, D.J.; Henny, R. J.; Yuen , J.M.F. and Mellich,T.A. 2003. Screening for resistance to Myrothecium leaf spot among *Syngonium* species and cultivars. *Hort Science*, **38**(1):75-76.

Okigbo, R.N. and Ikediugwu, F.E.O. 2001. Biological control of tuber surface mycoflora of yam (*Dioscorea rotundata*). *Tropical Science,* **41**(2):85-89.

Okunowo, W.O.; Gbenle, G.O.; Osuntoki, A.A.; Adekunle,A.A.; 2008. Survey,evaluation and molecular characterization of Nigerian native fungus for potential biocontrol of water hyacinth. *Phytopathology*, **98**: 115.

Okunowo, W.O.; Gbenle, G.O.; Osuntoki, A.A.; Adekunle, A.A. and Ojokuku, S.A. 2010. Production of cellulolytic enzymes by a phytopathogenic *Myrothecium roridum* and some avirulent fungal isolates from water hyacinth. *Afr. J. Biotechnol*., **9**(7): 1074-1078.

Olufolaji, D.B. 1999. Control of wet rot disease of *Amaranthus sp*. caused by *Choanephara cucurbitarum* with extraxt of *Azadirachta indica* (neem). *Journal of Sustainable Agriculture and the Environment*, **1**(2):183-190.

Patel, S.T. and Anahosur, K.H. 2001. Potential antagonism of *Trichoderma harzianum* against *Fusarium* sp.; *Macrophomina phaseolina* and *Sclerotium rolfsii.J. Mycol. Pl. Pathol*. **31** (3):365.

Ponnappa, K.M. 1970. On the pathogenicity of *Myrothecium roridum-Eichhornia crassipes* isolate. *Hyacinth Control J.*, **8**: 18-20.

Preston, N.C. 2009. The parasitism of *Myrothecium roridum* tode**.** *Transactions of the British Mycological Society* 20(4): 242-251.

Purohit, R. and Purohit, D. K. 2002 . Mycoflora of minor millets under different storage structures. *Journal-of-Eco-Physiology*, **5**(3/4): 137-138

Reddy, B.R. (1998). Cellulolytic activity of fungal cultures. *Journal of Scientific and Industrial Research*, 57(10/11): 671-620.

Seebold, K.W: Langston, D.B and Kemerait, R.C. 2005. First report on leaf and stem canker caused by *M. roridum* on water melon. United States Deptt of Plant Pathology, University of Georgia,*Plant Disease*, **89**(3): 342.

Sengupta,G.K.; Sharma,D.D.; Gargi and Gunasekhar,V. 1989. A new leaf spot disease of Mulberry caused by *Myrothecium roridum* Tode. Ex. Fries. *Current Science,* **58**(7): 398.

Shrivastava, S.K. and Khan, S.U .1994. Impact of host age at infection time on the severity of Myrothecium leaf spot disease of soybean. *Indian Phytopatho.*, **47**(2): 190-191.

Shrivastava , S.K.; Singh, P.P. and Khan , A.U. 1995. Effect of plant populations and varieties on *Myrothecium* leaf spot (*Myrothecium roridum*) of soybean (*Glycine max*). *Indian Phytopathology*, **48** (3): 360-362.

Siddaramaiah, A.L.; Bhat, P.P.; Desai S.A. and Basavarajaiah. 1979. Effect of culture of *Alternaria carthami* chowdhary on seed germination, root and shoot elongation of safflower. *Curr. Res*., (**8**):170-171.

Siddaramaiah, A.L. and Hegde, R. K. 1984. Occurrence of *Alternaria alternat* (Kissler) on safflower and sesamum from India. *Journal of oilseed Research,* **1**:83.

Siddiqui, Y. 2008. *Trichoderma* fortified compost extract for the control of Choanephora wet rot in okra production crop production. **27**(3-5): 385-390.

Singha, A.K and Narain, U .1993. Chemical control of leaf spot of soybean. *Ann. Pl. Prot.Sci*.,**1**(1): 48-50.

Singh, B.K.; Singh, J. and Narain,U. 2003. Role of environmental factors for development of Myrothecium leaf spot of pigeonpea. *Annals of Plant Protection Sciences*. **11**(2): 401-402.

Singh, B.K.; Singh,J. and Narain,U .2004. Role of diseased plant debris and seed in the perpetuation of Myrothecium leaf spot of pigeon pea. *Ann. Pl.Prot.Sci.*, (India), **12**(1) : 96-98.

Singh, S.M. and Shrivastava , S.K. 1994. Screening of soybean varieties against leaf spot disease caused by *Myrothecium roridum*. *Indian Journal of Mycology and Plant Pathology*, **24**(3): 222.

Singh, S. N .1999. Influence of sowing date and plant population of soybean on the disease intensity due to *Myrothecium roridum* and *Phyllosticta sojicola*. *Ann. Pl.Prot.Sci*., **7**(1): 55-58.

Sultan, N and Ghaffar, A (2009). Pathogenesis and control of *Myrothecium* spp., The cause of leaf spot on Bitter Gourd (*Momordica charantia* Linn). *Pak. J. Bot*., **41**(1): 429-433.

Thakur, K.D.; Khune, N.H. and Sabley, J.E. 1995. Inhibition of some cotton Pathogen's by plant extracts. *PKV Res. J*., **19**(1):39-41.

Tomar, D.D and Shashtri, P.P. 2006. Efficacy of fungicides against recovery of *Myrothecium roridum* in cotton seed. *Internat. J. agric. Sci*. , **2**.(2): 408-410.

Tsay, J. G.; Yeuh, C. S. and Tung, B. K. 1996. Occurrence of Myrothecium leaf spot on *Vriesea poelmannii* in Taiwan. *Plant Pathol*., **5**:163-167.

Umana , E.J. and Ikotum, T. 2000. Effect of pH on enzyme productivity by three pathotypes of *Choanephora cucurbitarum*. *Global Journal of Pure and Applied Sciences*., **6** (3):413-418.

Upadhyay, J.P. and Mukhopadhyay, A.N. 1986.Biologocal control of *Sclerotium rolfsii* by *Trichoderma Harzianum* in sugarbeet. *Tro. Pest Management*. **32** (3): 215-220.

Wells H.D., Durham, B.K. and Jaworski, C.A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol of *Sclerotium rolfsii*. *Phytopath*. **62**: 442-447.

Worapong, J.;Sun, J. And Newcombe,G. 2009. First report of *Myrothecium roridum* from a gymnosperm. *North American Fungi*, **4**(6):1-6.

Yam, K. J. and Park, E.W. 1990. Soybean leaf disease caused by *Myrothecium roridum* Tode. Ex. Fries. *Korean Journal of Plant Pathology*, **6** (3): 313-317.

Zhao, Y.J.; Li, B.J.; Shi, Y.X. and Xie, X.W.(2010). First Report of Myrothecium Leaf Spot of Common Bean in China caused by *Myrothecium roridum. Plant disease*, 94 (1):127.

Zhang, F and Smith, D.L. 1995. Soyabean (*Glycine max*(L) Merr). Physiology and Symbiotic dinitrogen fixation. In Crop yield, Physiology and Processess.

AVI Publishing Company, pp: 375-395.

Fig 4 : Influence of culture filtrate of *M. roridum* on the activity of CMC at different $_{P}$H level

Fig 5: Influence of culture filtrate of *M. roridum* on the activity of PG at different $_{P}$H level

Fig 3 : Effect of different temperature on radial growth and sporulation of

M. roridum

Fig 1 : Effect of different pH on radial growth and sporulation of

M. roridum.

Fig 2 : Effect of different media on radial growth and sporulation of *M. roridum*

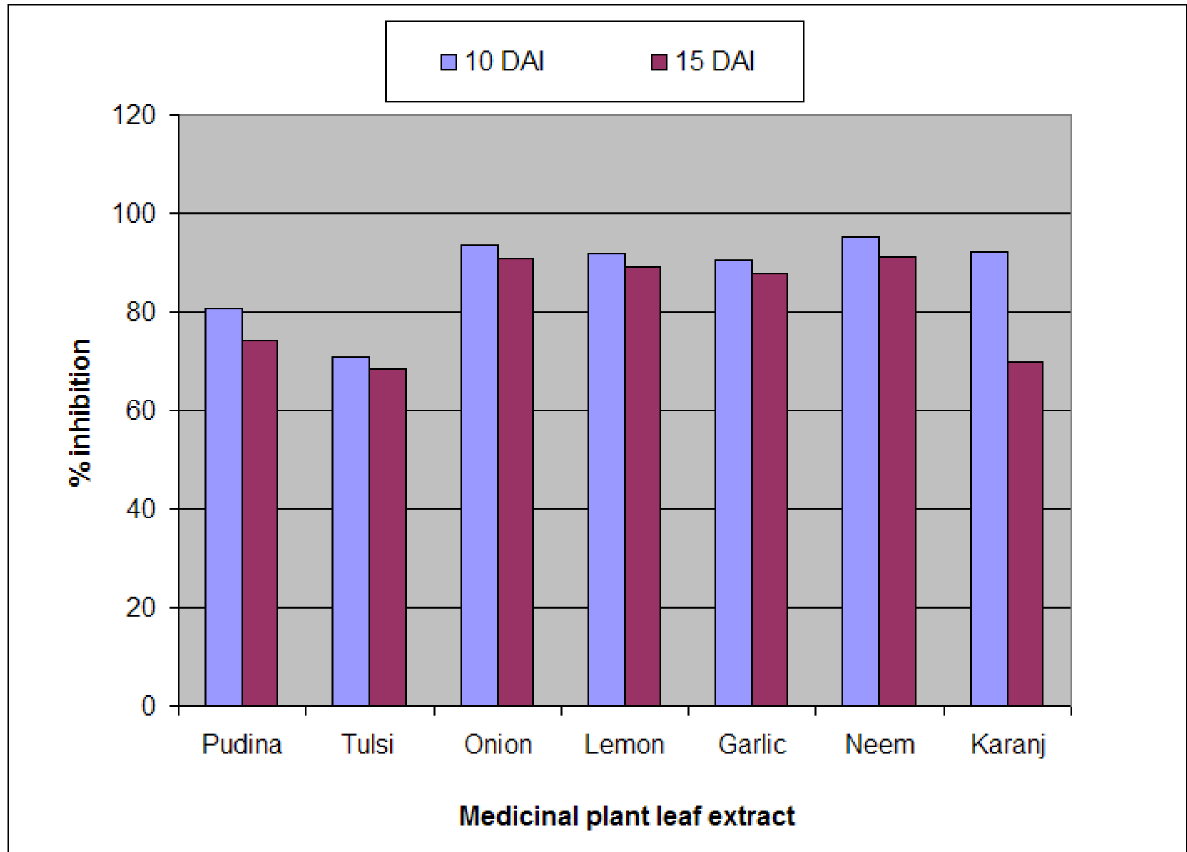


Fig 6 : Effect of different medicinal plant extracts on radial growth of

M. roridum

Fig 9 : Effect of different fungicides on radial growth of *M.roridum* (1000ppm)

Fig 7 : Effect of different fungicides on radial growth of *M. roridum* (250 ppm)

Fig 8 : Effect of different fungicides on radial growth of *M. roridum* (500 ppm)

***Trichoderma* spp .**

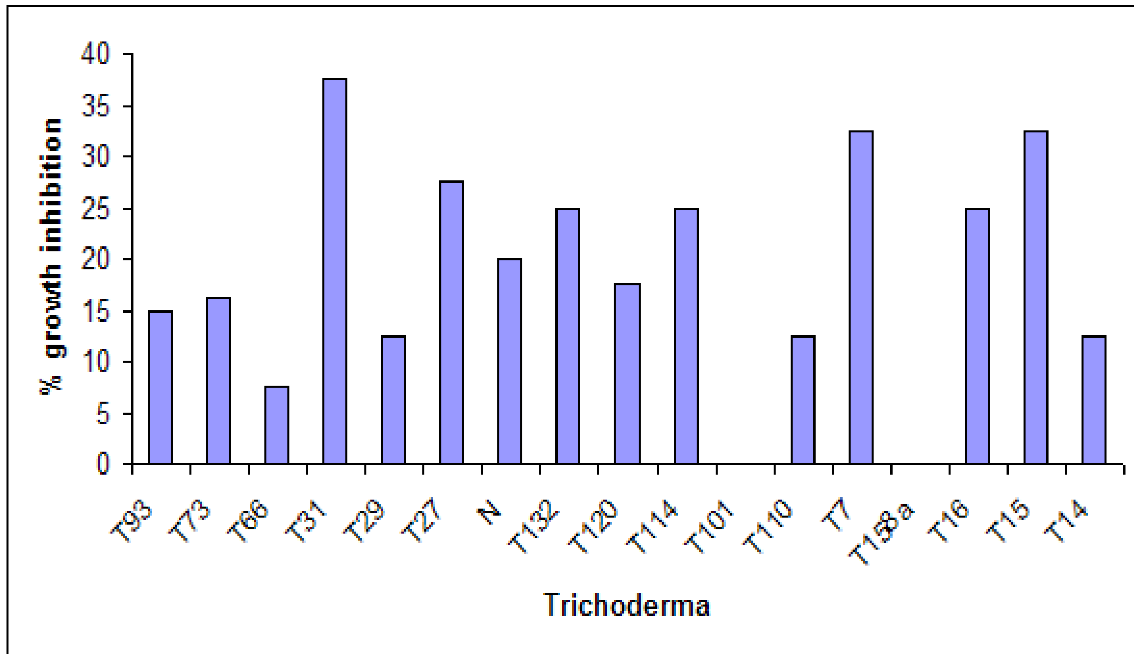


Fig 10 : Effect of different *Trichoderma* spp . on radial growth of *M. roridum*

C

B

A

A – Healthy plant of soyabean

B – Initiation of necrosis and browning

C – Dead plant

Plate 11: Influence of culture filtrate on attached plant leaf of soyabean

Close view of infected leaf by pinprick method

Close view of infected leaf by scratch method

Close view of infected leaves by normal method (without pinprick or scratch method)

A A

A

B

1.Normal, 2. Pinprick, 3. Scratch. **Before treatment**

Normal, pinprick, scratch **After treatment**

D D

C Close view of infected leaves by normal method (without pinprick or scratch method)

F D

Plate : 5 Testing of Pathogenicity on soyabean leaf by detached method

Plate 1: Symptoms of Myrothecium leaf spot of soyabean

Plate 1: Symptoms of Myrothecium leaf spot of

soyabean

A **-** Innitial symptom of leaf spot of soybean

B - Merging of spots to form irregular shape and drying of leaf

C - Formation of sporodochia on the spots

D - Formation of shot holes at the later stage

E - Symptom found in stem

F - Symptom found in pods

PLATE 2:

A – 20 days old culture

B – One month old culture with a concentric ring pattern of white mycelia and dark green sporodochia.

C – Hyaline and non septate mycelium of *M. roridum*

D – Microscopic morphology of conidia

PLATE: 5

A – Before treatment

- Normal
- Pinprick
- Scratch

B – After treatment

- Normal
- Pinprick
- Scratch

C- Close view of infected leaf by pinprick method

D - Close view of infected leaf by scratch method

E - Close view of infected leaves by normal method (without pinprick or scratch method)

F – Close view of control leaf

PLATE 13

A – View of treated seeds with culture filtrate in plastic box

B – Germinated and ungerminated seeds of soybean

a.Germinated seeds

b.Culture filtrate affected seeds

c.Ungerminated and unaffected seeds

PLATE 14

A – View of soyabean seeds on blotter treated with culture filtrate

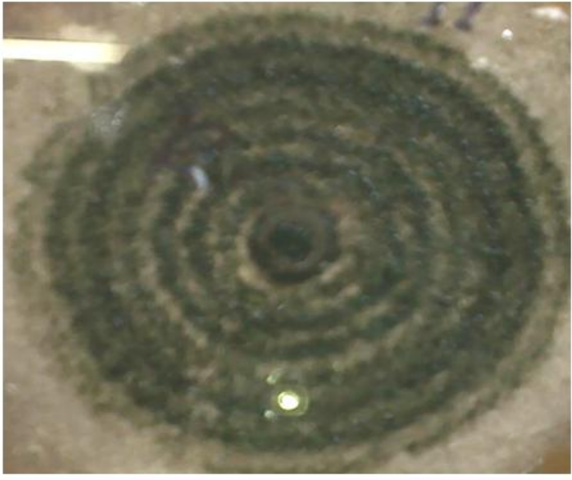
B - Germinated and ungerminated seeds of soyabean

- Ungerminated and unaffected seeds
- Germinated seeds
- Culture filtrate affected seeds

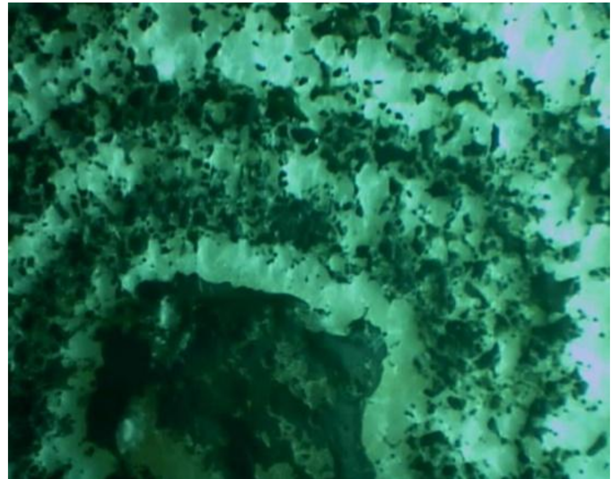
PLATE 15

- View of seed and blotter treated with culture filtrate
- Germinated and ungerminated seeds of soyabean
- Germinated seeds
- Ungerminated seeds

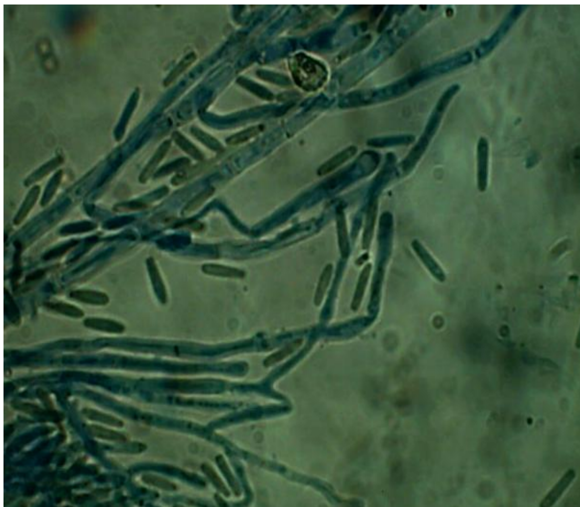
C - Control



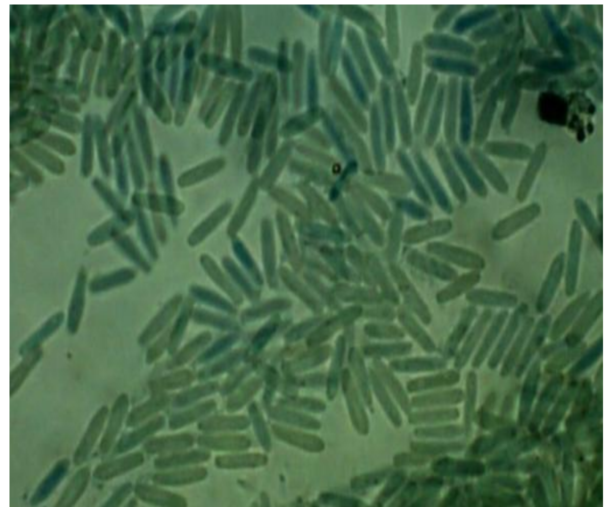
A. 20 days old culture



B. 1 month old culture



C. Mycelium



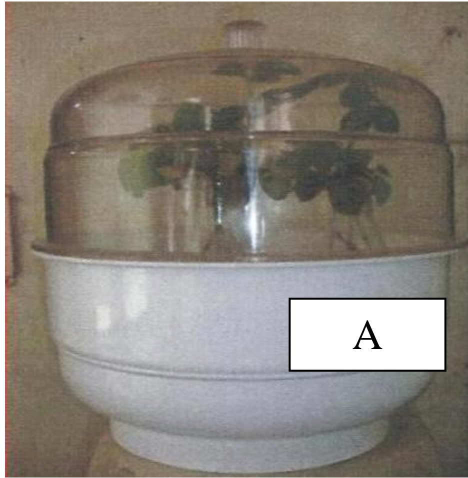
D. Conidia

Plate 2 : Culture of *M. roridum*

A- Inoculated plant covered with perforated polythene bag

A- Inoculated leaves showing lesions after 8 days

Plate 3: Testing of pathogenicity of *M. roridum* under field conditions



Plants kept in desiccators **Initial symptoms**

Developed symptoms

Plate 4: Testing of pathogenicity of *M. roridum* under laboratory condition by attached method

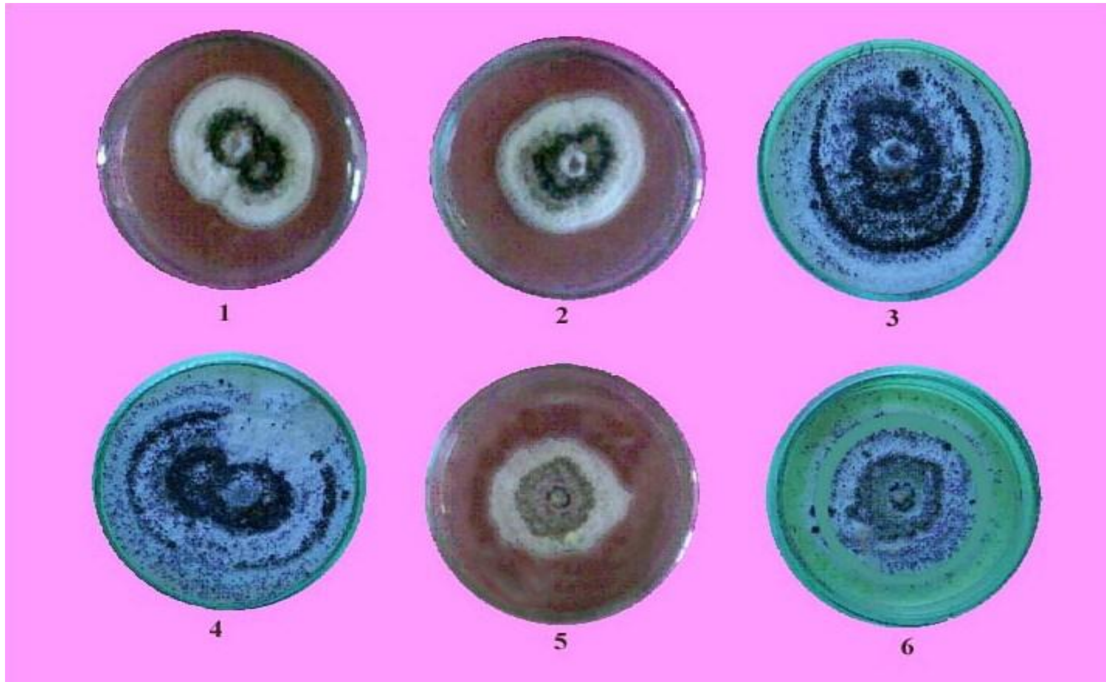


Plate 7 : Effect of different pH on radial growth of *M. roridum*

1. 4 pH	2. 5 pH	3. 6 pH
4. 7 pH	5. 8 pH	6. 9 pH

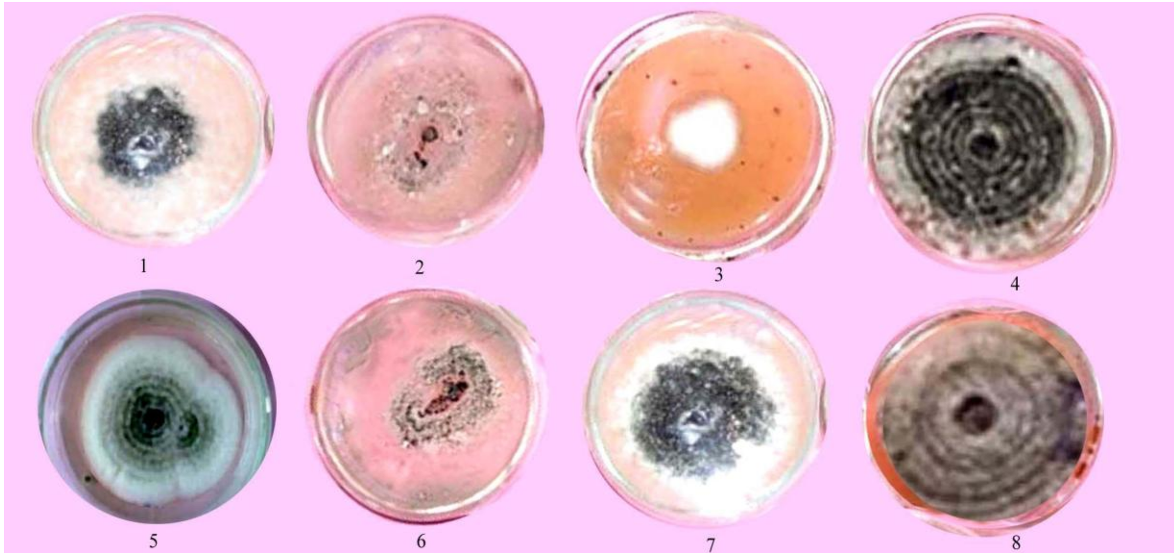


Plate 8: Effect of different media on mycelial growth of *M. roridum*

1.	**Czapex dox agar**	**5. Malt extract**
2.	**Dextrose nitrate agar**	**6. Asthana & Hawker's**
3.	**Kerr's**	**7. Richards**
4.	**Potato sucrose agar**	**8. Potato dextrose agar**

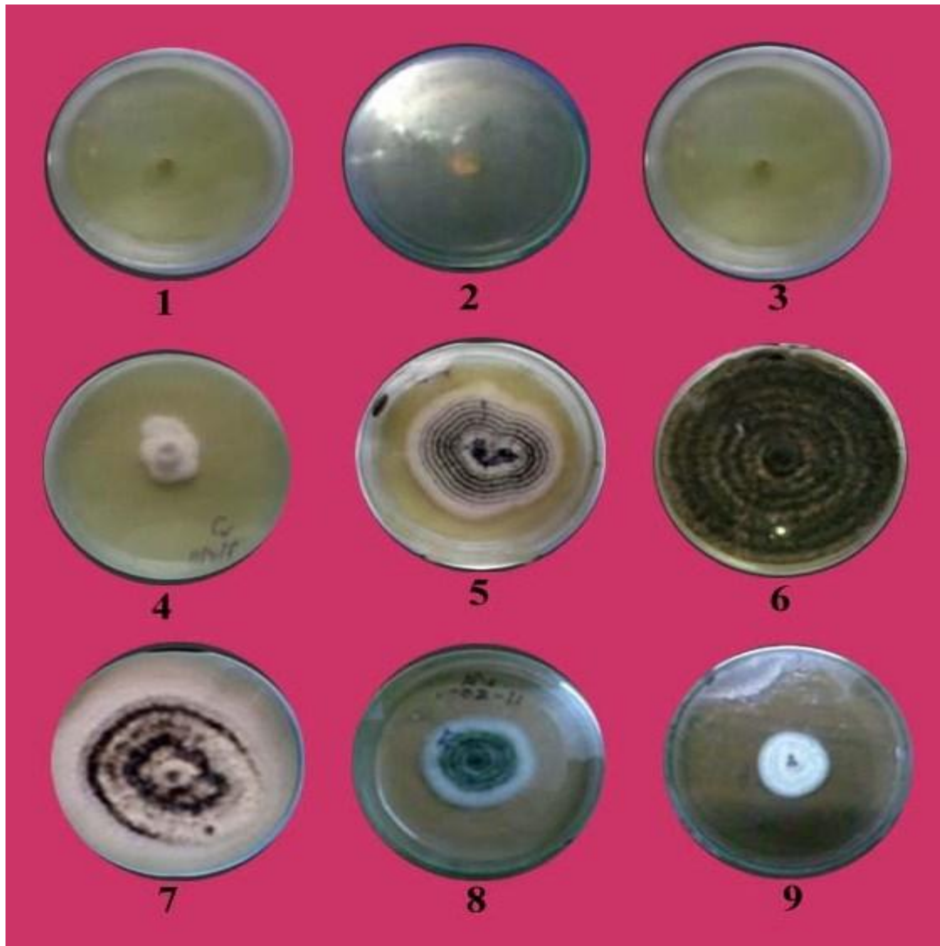


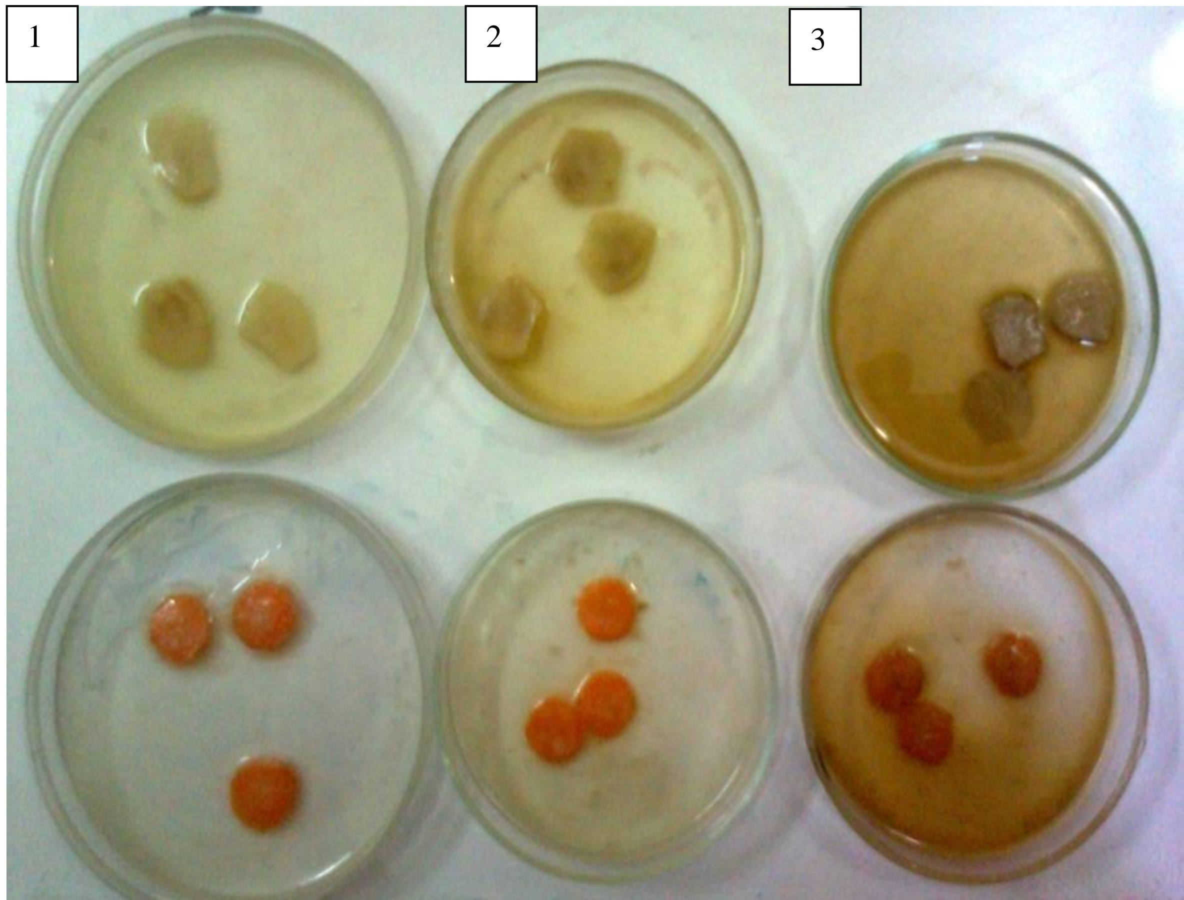
Plate 9: Effect of different temperatures on radial growth of *M. roridum*

1. 0^0C	**2. 5^0C**	**3. 10^0C**
4. 15^0C	**5. 20^0C**	**6. 25^0C**
7. 30^0C	**8. 35^0C**	**9. 40^0**

.

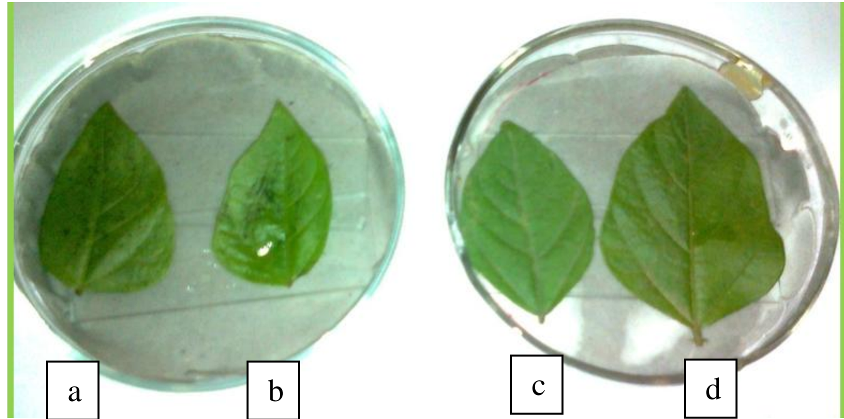
4 5 6

Plate 10 : Effect of culture filtrate of *Myrothecium roridum* on macerating of potato

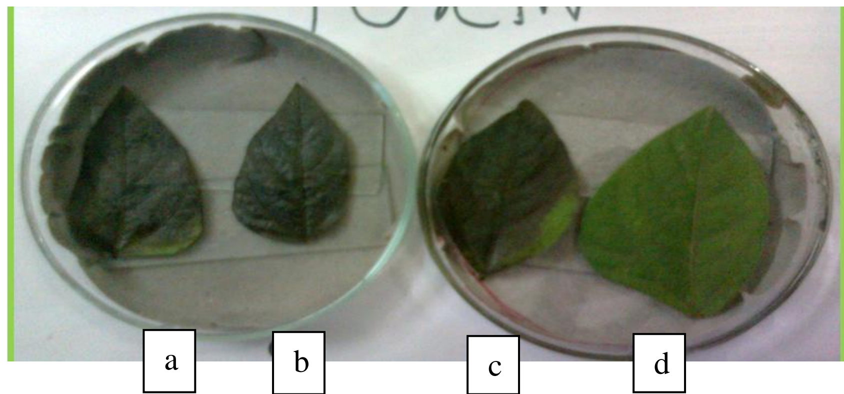


and carrot pith

1. **Potato in 100% culture filtrate concentration**
2. **Potato 50% culture filtrate concentration**
3. **control**
4. **Carrot in 100% culture filtrate concentration**
5. **Carrot in 50% culture filtrate concentration**
6. **control**



A. Before treating with toxins



B. After treating with toxins

Plate 12. Effect of culture filtrate on detached leaves of soyabean

A) Before treating with toxins

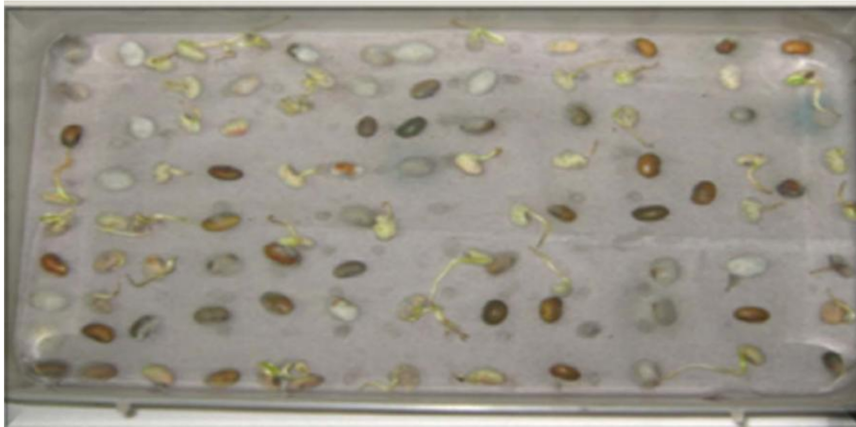
B) After treating with toxins

a) Pinprick

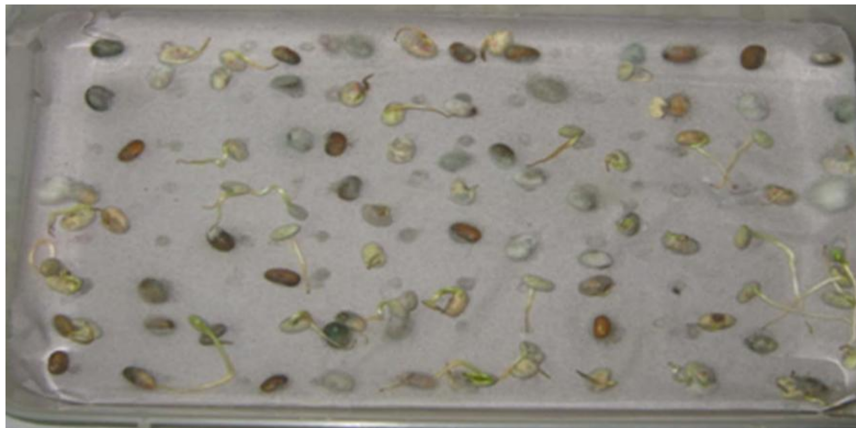
b) Scratch

c) Normal

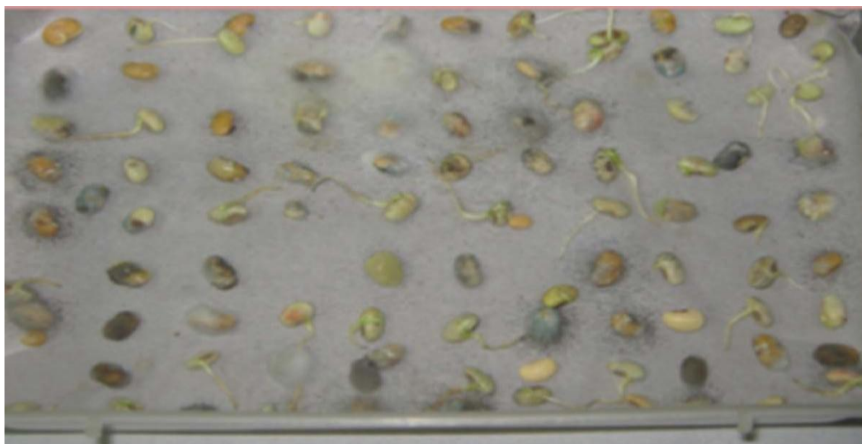
d) Control



(A) SURFACE STERILIZED+INOCULATED SEED



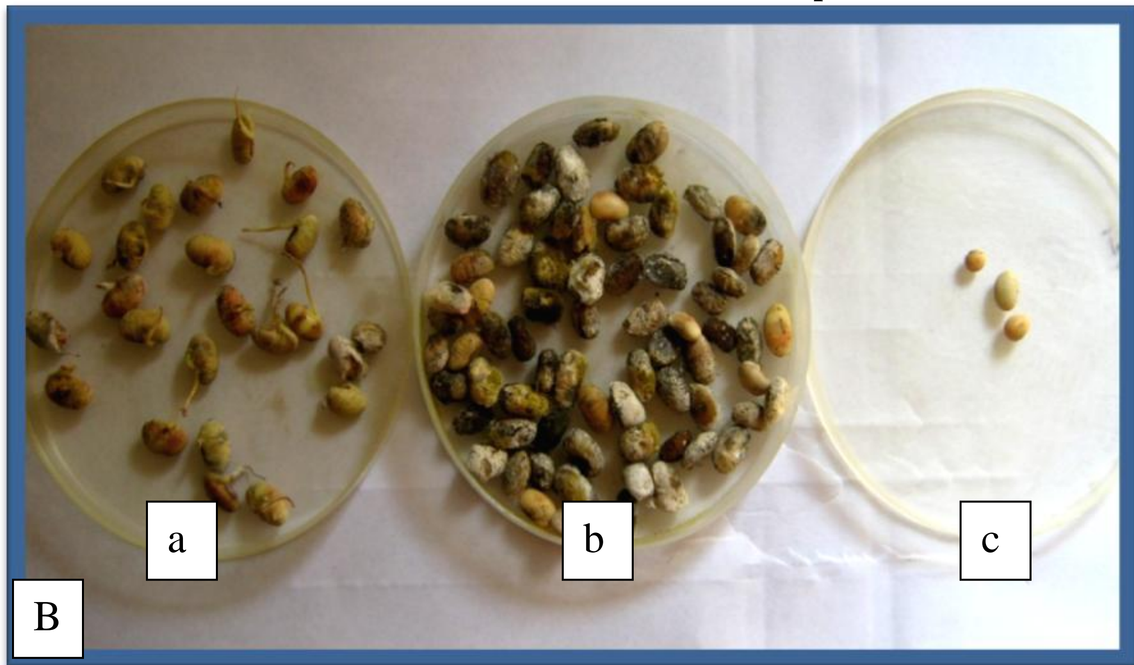
(B) WITHOUT SURFACE STERILIZED + INOCULATED SEED



(C) WITHOUT SURFACE STERILIZED + UNINOCULATED CONTROL

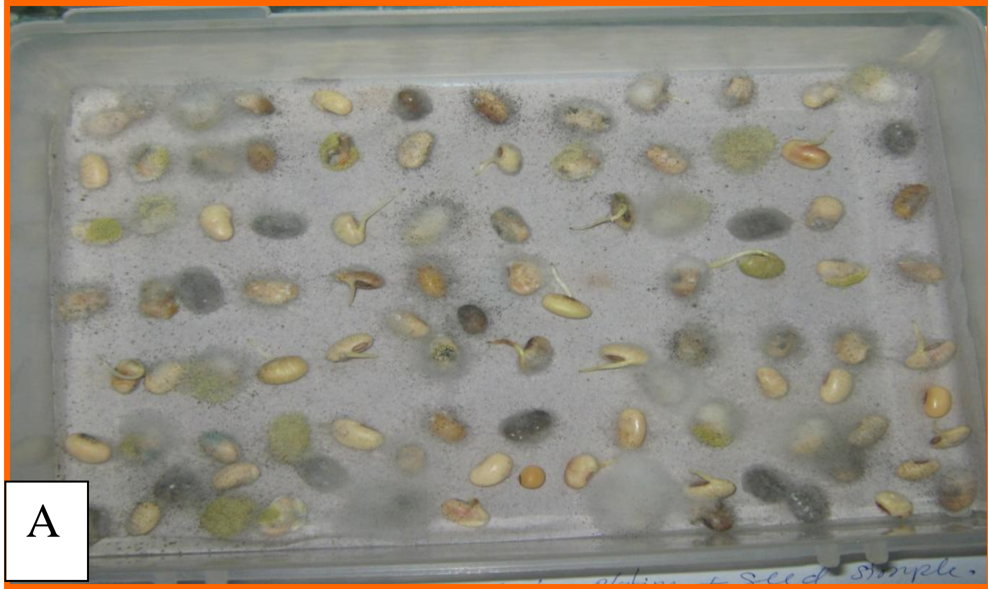
Plate 6 : Testing of pathogenicity on soyabean seed

A – View of treated seeds with culture filtrate in plastic box



B- View of germinated and ungerminated seeds

Plate 13 : Influence of culture filtrate of *M. roridum* on germination of soyabean seeds (seed treated)



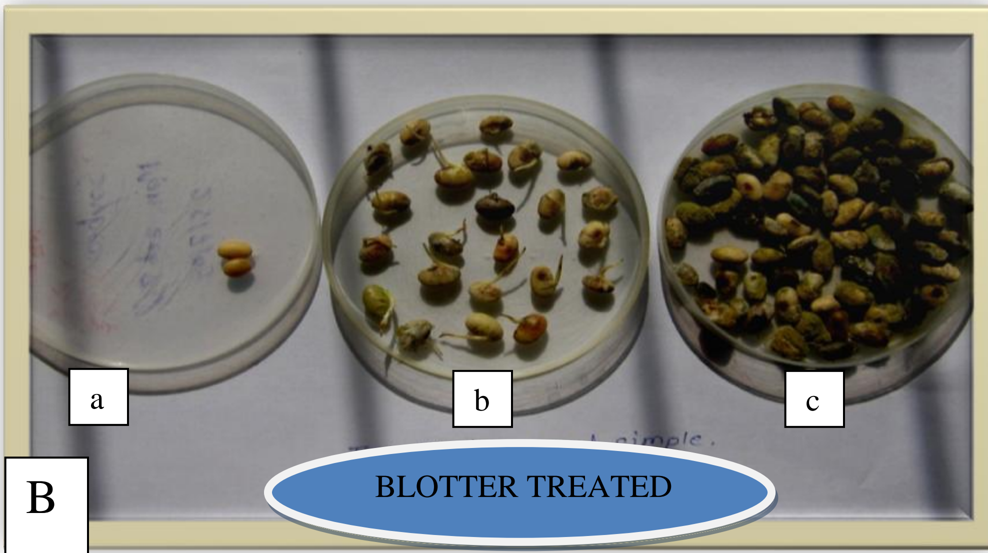
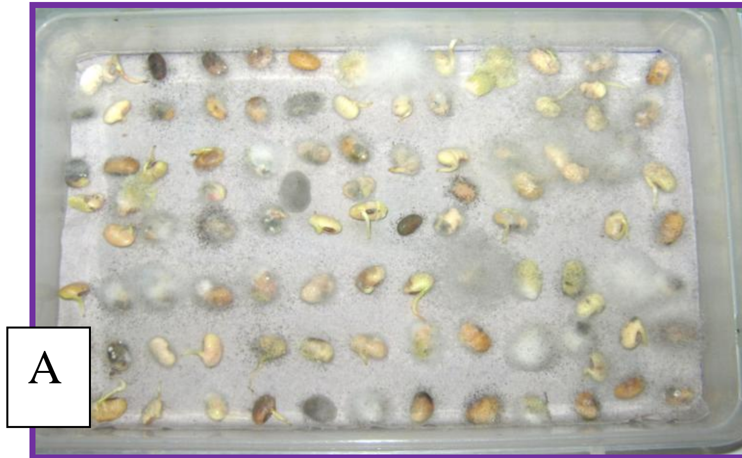
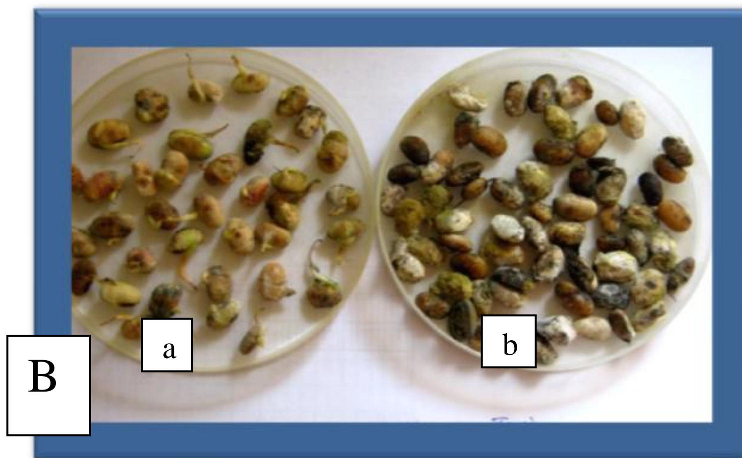


Plate 14 : Influence of culture filtrate of *M. roridum* on germination of soyabean seeds (blotter treated)



View of seed and blotter treated with culture filtrate



Germinated and ungerminated seeds

CONTROL

Plate 15 : Influence of culture filtrate of *M. roridum* on germination of soyabean seeds (seed+blotter treated)

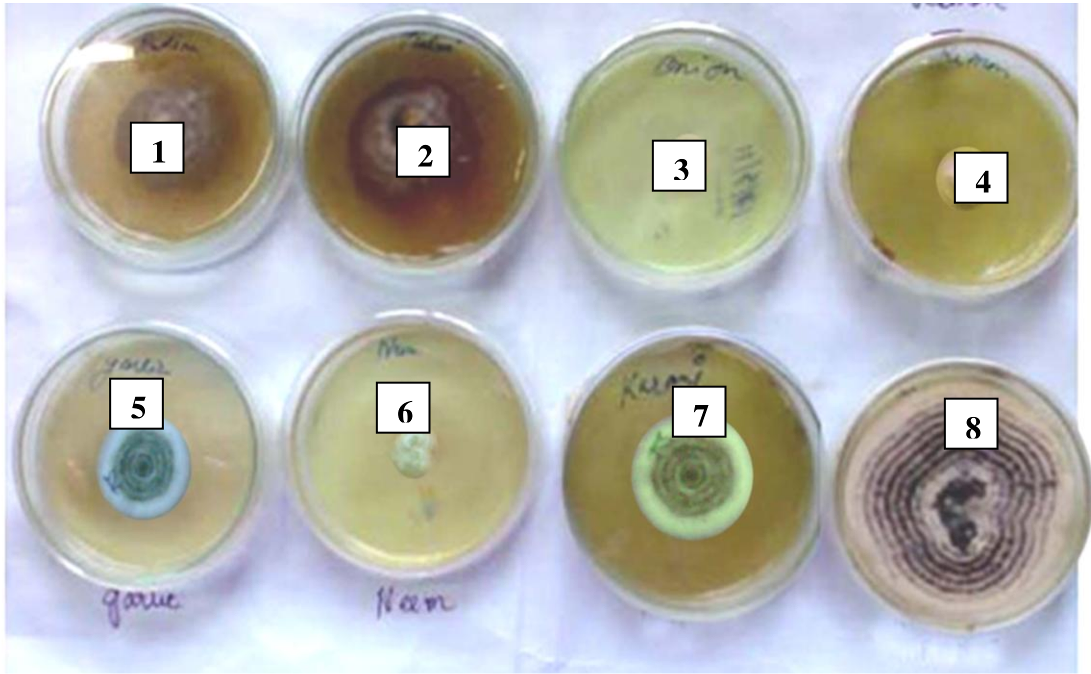


Plate 16: Efficacy of different culture medicinal plants extracts on growth of *Myrothecium roridum*

1. Pudina	2. Tulsi	3. Onion	4. Lemon
5. Garlic	6. Neem	7. Karanj	8. Control

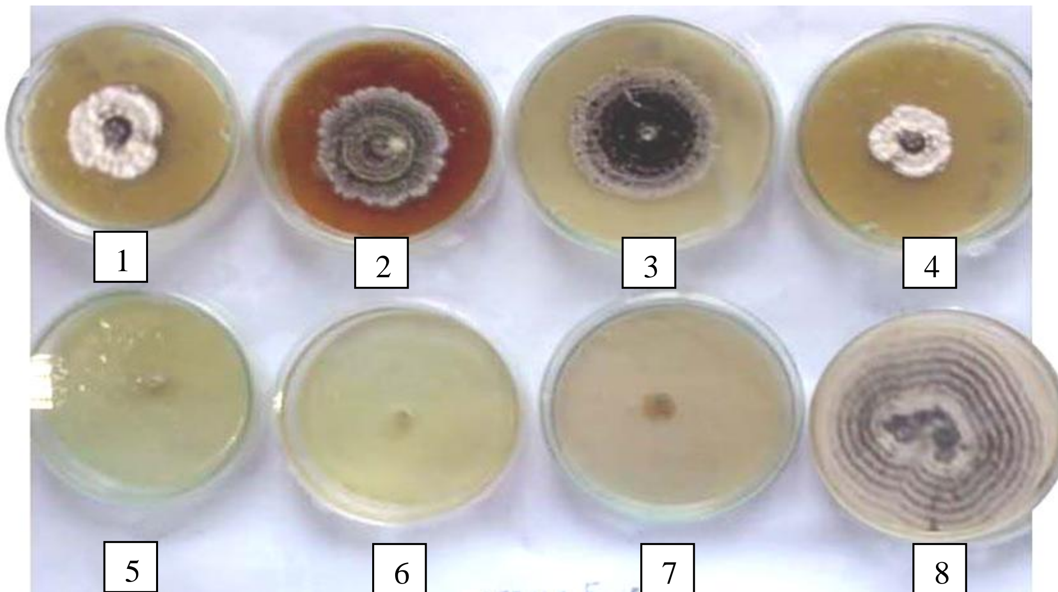


Plate 17: Efficacy of different fungicides on inhibition of radial growth of *M. roridum* (250 ppm)

1. Dhanustin	2. Curzate M-8	3. Dithan M-45	4. Dhanucop
5. Benomyl	6. Saaf	7. Vitavex power	8. Control

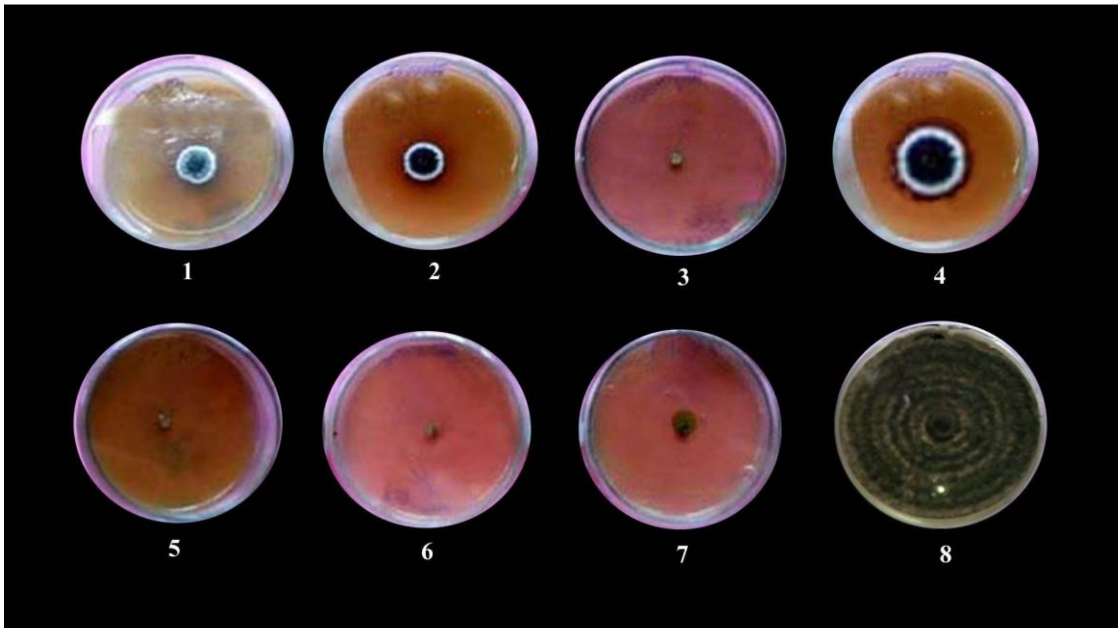


Plate 18: Efficacy of different fungicides on inhibition of radial growth of M. *roridum* (500 ppm)

1. Dhanucop	2. Dithan M-45	3. Dhanustin	4. CurzateM-8
5. Saaf	6. Benomyl	7. Vitavex power	8. Control

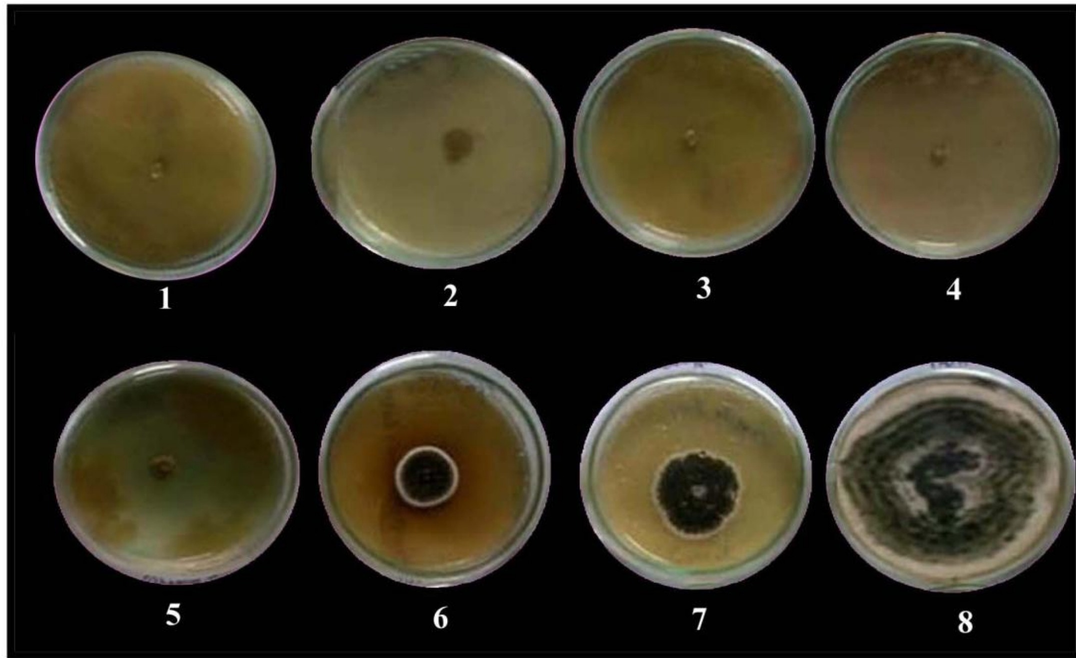


Plate 19: Efficacy of different fungicides on inhibition of radial growth of M. *roridum* (1000 ppm)

1. Dhanucop	2. Benomyl	3. Dhanustin	4. Vitavex power
5. Saaf	6. Dithan M-45	7. Curzate M-8	8. Control

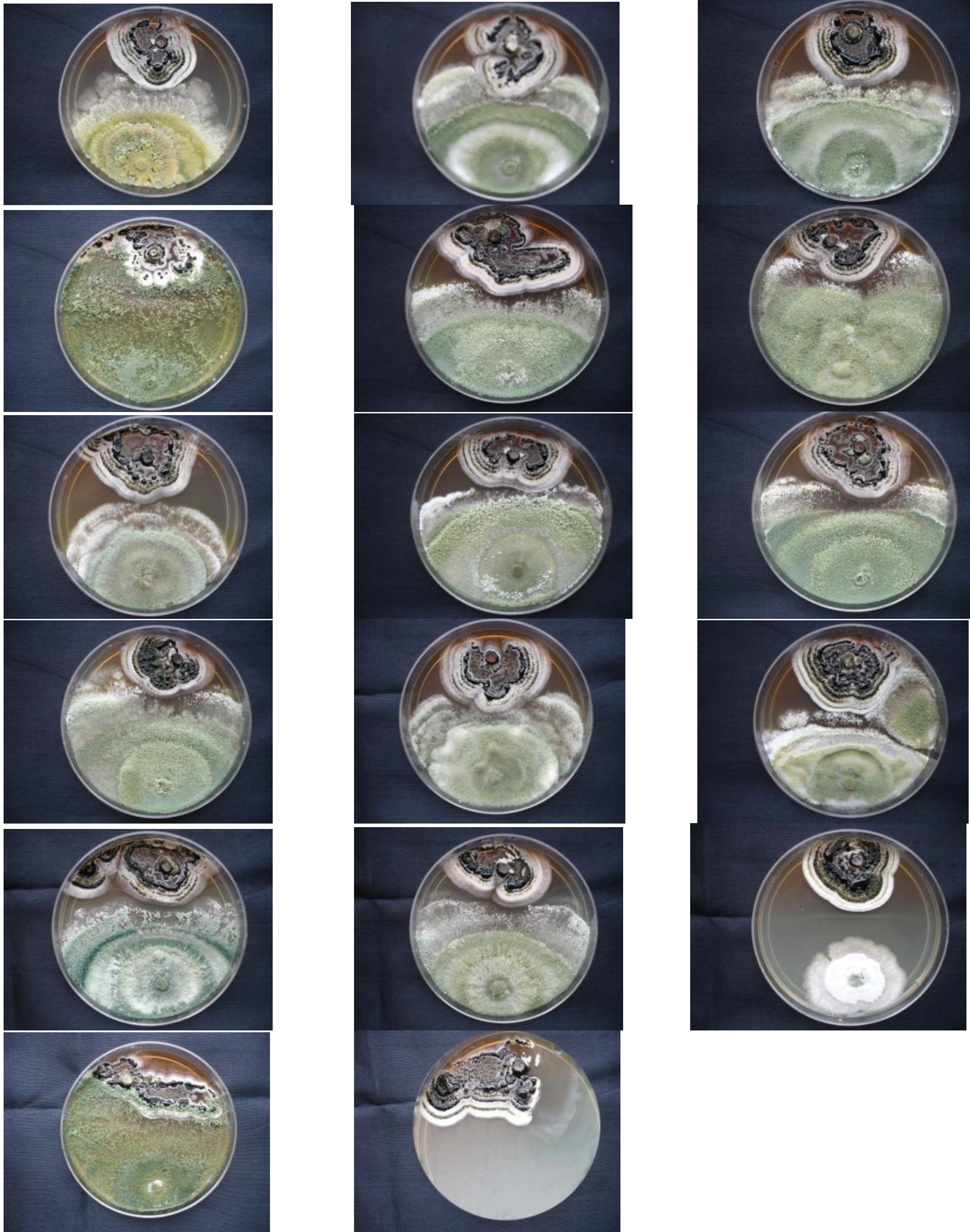


Plate : 20 Screening of different *Tricoderma* spp. against *Myrothecium roridum*

A

B

D

C

F

E

Plate 1: Symptoms of Myrothecium leaf spot of soyabean

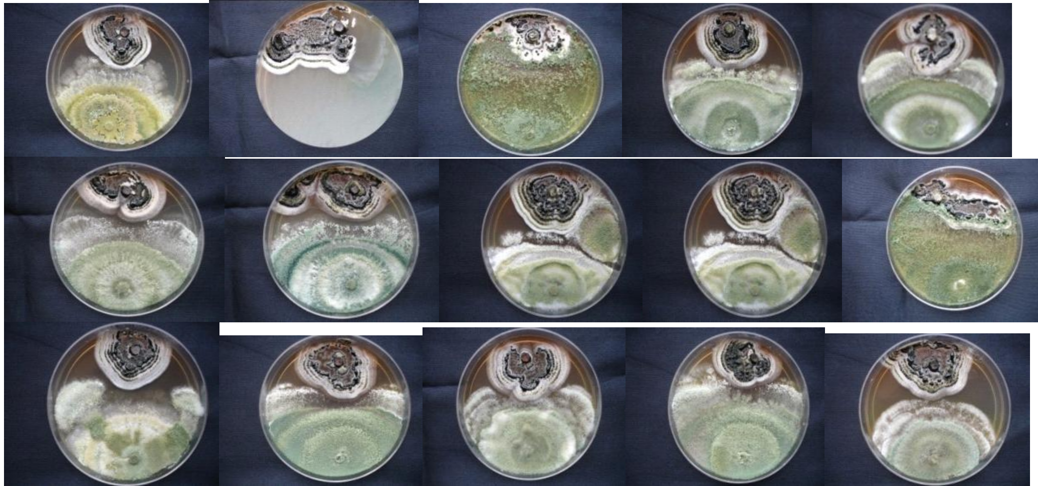
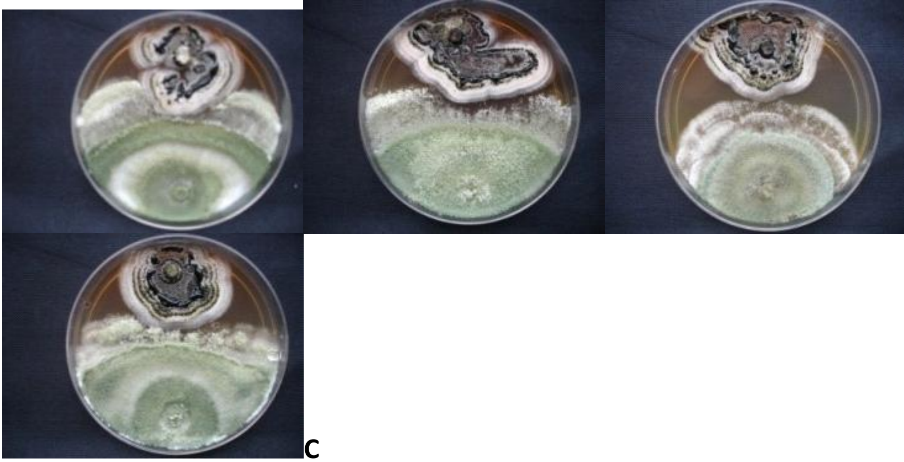


PLATE 20 : Screening of different *Trichoderma* spp. against *M. roridum*

• T93	• T73	3. T66	4.T31	5.T29	6.T27	7.N	8.T132	9.T120
10. T114	11. T101	12. T7	13. T110	14.T158a	15.T16	16.T15	17.T14	18.Control

PLATE 20 : Screening of different isolates of *Trichoderma* spp. against *M. roridum*

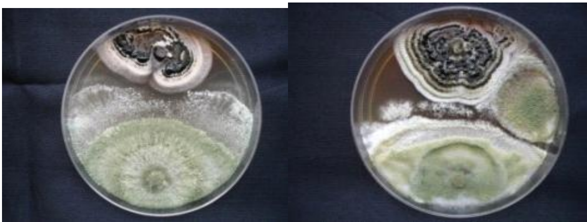
T93



C

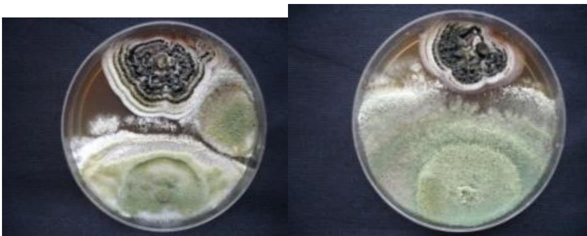
17

16



14

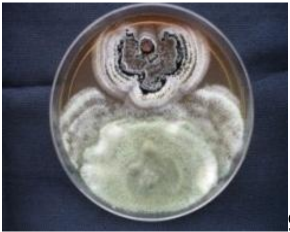
13



12

11

1000


9

8

4

7

6

5

3

2

15

PLATE 20 : Screening of different *Trichoderma* spp. against *M. roridum*

1. T93	2. T73	3. T66
4. T31	5. T29	6. T27
7. N	8.T132	9. T120
10. T114	11. T101	12. T7
13. T110	14.T158a	15. T16
16. T15	17. T14	18. Control