STUDIES ON THE ROOT AND STEM ROT DISEASE OF CASTOR CAUSED BY Rhizoctonia bataticola (Taub.) Butl.

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PART FULFILMENT OF THE REQUIREMENTS FOR THE
AWARD OF THE DEGREE OF
MASTER OF SCIENCE IN AGRICULTURE

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1974.

C E R T I F I C A T E

This is to certify that this Thesis entitled "STUDIES ON THE ROOT AND STEM ROT DISEASE OF CASTOR CAUSED BY *Rhizoctonia bataticola*" submitted to the Andhra Pradesh Agricultural University for the award of the Degree of Master of Science in Agriculture in the Major subject Plant Pathology is a bonafide Research work done by Sri Hamed Ali Khan Sarwar under my guidance and supervision and that the thesis has not formed in whole or in part, the basis of the award of any Degree, Diploma or other similar Degree or distinction.

The assistance and help received during the work of investigation have been fully acknowledged.

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ACKNOWLEDGEMENTS

I express my deep sense of gratitude and indebtedness to Dr. D. Gopala Raju, Major Adviser Associate Professor and Head, Department of Plant Pathology for suggesting the problem, guidance, constant encouragement keen interest, constructive criticism and help in the conduct of the present investigation.

I express my grateful thanks and deep appreciation to Mr. D.V. Subba Rao, Oilseeds Pathologist, Agricultural Research Institute, Rajendranagar for the valuable suggestions in the preliminary work.

My sincere thanks are also due to Dr. K.Ramachandra Reddy Minor Adviser Assistant Professor Department of Plant Pathology, College of Agriculture for his constant encouragement and valuable suggestions throughout the period of investigation and in the preparation of the Thesis.

I express my sincere thanks to Mr. Satyanarayana, Instructor, Department of Plant Pathology for his keen interest and help rendered.

I am also grateful to Dr. Aftab Ahmed, Associate Professor and Head, Department of Entomology, College of Agriculture for his advise and help.

My thanks are due to Sri Ankineedu Breeding Assistant Regional Research Station for providing the seed material during the course of research.

I am also grateful to Sri Prabhakar Reddy, Research Assistant, Plant Pathology for providing the necessary Fungicides and Antibiotics.

Finally, I express my whole-hearted thanks to the other staff members of Department of Plant Pathology, my colleagues and friends for the kind and active assistance rendered in the preparation of this Thesis.

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C O N T E N T S

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INTRODUCTION

The castor plant (Ricinus communis L.) is one of the most important oil yielding crops of our country. It is believed to have its origin in India and North Africa (Watt, 1892). Hindus have known castor oil from very remote periods. This has been mentioned in Suruta Ayurveda, one of the oldest works of Ayurveda. Castor is grown in many parts of the world, such as Brazil, U.S.A. Egypt, Sudan and many Asian countries. The annual cultivated area under castor in India is about 4,16,000 hectares and production is 1,44 lakh tonnes of seed. Andhra Pradesh which accounts for the largest area cultivated under this crop in our country is estimated to have 3,17,000 hectares and producing 56,000 tonnes of seed. Thus the state ranks first in India not only in the area the crop occupies but also in yield production. In Andhra Pradesh the crop is mainly grown in the districts of Prakasam, Hyderabad, Nalgonda, Mahboobnagar, which all put together accounts for 85.2 percent of the total area under castor as per the Agricultural Statistics (1970-71).

Castor oil which is an important source of raw material of several industries and of economic importance in domestic medicinal and veterinary uses and has also got a great export value in earning foreign exchange. Such an important crop is subjected to a number of diseases causing severe losses. Some 150 organisms are known to be pathogenic on the plant (Rogers, 1954). Leaf spots, blights, rust, powdery mildew, stem and

root rot are some of the important diseases of the crop. With the introduction of high yielding and fertilizer responsive varieties some of the hitherto minor diseases have assumed serious proportions so much so their occurence have now become a limiting factor in stepping up yields. Recently a severe out-break of root and stem-rot of castor caused by Rhizoctonia bataticola was observed on one month old seedling of a popular variety "Aruna" during kharif 1973, in experimental plots of Agricultural Research Institute, Rajendranagar, Hyderabad, Andhra Pradesh.

Diseased symptoms observed were leaf yellowing, puckering and stunting of the plant with withered leaves remain attached and hang-down even after the death of the plant. In the final stage the plants turned pale, almost gleaming ash coloured with abundant black pin head like pycnidia and with small sized sclerotia. The roots of affected plants turned black and shredded.

The affected castor plants were examined and on isolation consistently yielded R. bataticola.

Although this particular disease appears to be relatively new to this tract, it was provicously described in our country. (Uppal, 1934, Prasad 1944, Thirumalachar 1953, Kulkarni et al., 1966). Sufficient work has been carried out on other crops affected with Rhizoctonia diseases ranging from cereals, pulses, fruits, vegetables to forest trees. But not enough of data has been accrued on Rhizoctonia affected castor crop.

The object of the present investigation are presented here under.

Isolation of the organism associated with disease and its pathogenicity on castor, varietal reaction, isolation and screening of antagonists, physiology of the pathogen, and efficacy of various fungicides and antibiotics against *R. batatticola in Vitro*.

The results obtained in the course of the investigation are presented in this thesis.

REVIEW OF LITERATURE

Root and stem rot of castor caused by Rhizoctonia bataticola (Taub.) Butl., is known in India since 1932. It was first reported from Poona by Uppal (1934). Later it was recorded by Prasad (1944) from Sind, Thirumalachar (1953) from Bihar and Kulkarni et al., (1966) from Mahabaleshwar. Lele and Pathak (1965) reported that a severe drought, high temperature and delayed monsoon rains preceeded the disease outbreak.

The disease was recorded for the first time in U S A by Cook (1958). Several varieties of castor was found to be susceptible to the ravages of the pathogen. Charles Hedges (1963) noted the appearance of the disease on one month old seedlings at the post-emergence stage. The classical studies of Plentje et al., at the Waite Agricultural Research Institute, Adelaide, have brought out several aspects of the disease (Saksena 1971).

Symptoms:

Thirumalachar (1953) observed that the charcoal rot of castor caused by R. bataticola was characterised by the grey discolouration followed by blackening of the stem which bears numerous sclerotia of the fungus. The pycnidial stage was also found on the dead stem.

In the case of Stalk rot of Strawberries caused by R. bataticola Lele and Pathak (1965) noted puckering and stunting of the plant.

Gangopadhyay and Iswar (1972) described the symptoms in the case of charcoal rot disease of Datura stramonium caused by R. bataticola, gradual withering of the leaves starting from the lower ones was noticed with the advance of the disease. The withered leaves remain attached even after the death of the plant. Black pin head like pycnidia and sclerotia were found all over dead stem. The pycnidia were observed to be produced in abundant externally on the bark and were macroscopic. The pith was full of sclerotia. Dead stem turned light, almost gleaming ash colour and could be broken easily and the bark was shredded.

Hansford (1928) observed that the root disease of coffee caused by R. bataticola (Macrophomina phaseoli), first destroys the minute feeding rootlets near the coil level and gradually works back along the larger roots towards the collar region of the tree. The tissue of the inner bark are then attacked, and only at a relatively latter stage of the disease the sclerotia of the fungus were found in woody tissues. The infected plant shows generally an unhealthy appearance with considerable dying back of the laterals and gradual shredding of the foliage and in some cases die very suddenly, with the leaves and berries still hanging on the branches.

Shukla et al., (1970) reported in case of root rot of Lentil caused by R. bataticola, that the infected plants showed a gradual change of colour from dull green to reddish brown, which latter changed to yellow. With the advancement of the

disease the whole plant dried up and wilted. Diseased plants when pulled up easily detached at the soil level. Roots were poorly developed, finer root-lets were either not fully developed, or destroyed due to rotting.

Taxonomy and Nomenclature of the pathogen:

The genus Rhizoctonia was established by De Candolle (1815,Flore Fr. 6: 110) as Rhizoctonia crocorum (Pers) D C. to accomodate Sclerotium crocorum Persoon (1801 Syn.Meth. Fung. P. 119).Fries (1822 Syst. Mycol. 2: 265). recognised the genus Rhizoctonia D C ex Fries. (Rhizoctonia crocorum) (Pers) D C ex Fries, as Macrophomina phaseoli (Maubl).

Taubenhaus (1913, Phytopathology 3: 164). Butler (1925 Briton Jones, Bull. Plantat. Agric. Dept. 29: 65), transferred the species bataticola to the genus Rhizoctonia and made a new combination as Rhizoctonia bataticola (Taub.). Butler.

A large number of species has been described in this genus by several authors. The genus is now characterised by the absence of any kind of fruiting body and the presence of sclerotia, varying in shape and size, cartilaginous to fleshy and uniform texture within.

Ashby (1927) showed that the fungus R. bataticola produces a pycnidial stage corresponding to Macrophomina philippinensis Petrak, the type species of the genus Macrophomina. The pycnidial stage of R. bataticola was described by Maublanc

(1905, Bull. Soc. Myc. France 21: 90) as Macrophoma phaseoli (Maubl.) was an earlier name: hence the new combination was Macrophomina phaseoli (Maubl.), Ashby.

Ashby (1927) and Young (1949) have reviewed the nomenclature of the charcoal rot inciting fungus and pointed out the need for avoiding frequent changes in the names of well known pathogens.

Thirumalachar (1953) studied the pycnidial stage of charcoal rot inciting fungus and discussed its nomenclature and made the new combination Botryodiplodia phaseoli (Maubl.), Thirum. without examining the type material of Macrophoma phaseoli Maubl-lance.

Morphology of the pathogen:

Vasudeva (1936) observed that Rhizoctonia bataticola produced white colony with plenty of aerial mycelium which turns dark gray with age and has numerous tiny black sclerotial bodies.

Godiarih and Caraici (1946) reported that in culture the fungus rapidly formed round colonies at first hyaline and finally becoming intense carbonaceous black with little, if any aerial mycelium. Microsclerotia developed in profusion measuring 90-120 μ in diameter.

Reichert and Hellinger (1947) studied the morphology and parasitism of Sclerotium bataticola and noted that the

culture from different hosts fall into two main types and designated as sub-species *typica* and *oryzae*. In the type *typica* the hyphae are at first white, gradually turning smoky grey. Following the formations of sclerotia, the mycelium breaks down. The tiny black sclerotia are uniformly distributed on the media. In the type resembling *oryzae* the hyphae are at first white and gradually turns smoky grey showing concentric zonations of growth. The sclerotia are few and irregularly grouped. The hyphae are not evanescent as in the former type but remain persistent. This second type has been isolated from potato, maize kernels, and castor. (Thirumalachar 1953).

Thirumalachar (1953) reported that the fungus grow very rapidly at the room temperature producing abundant funnecous hyphae.

Lele and Pathak (1965) described *R. bataticola* causing Stalk rot of Straw berries. The mycelium was profuse and white in colour, broad, septate and branched. Within three days of sub-culturing small black, horny fleshy sclerotia variable in form were produced in abundance in the culture. The sclerotia were formed among and connected by the mycelial threads. No sporulation was seen even after twenty weeks.

Raut and Bhonbe (1965) working with the leaf spot of Sorghum caused by *R. bataticola* recorded that the fungus produced abundant whitish aerial mycelium on P D A in the

beginning, but turned greenish or light brown after 3-4 days at 27-30°C. Mycelium showed, a characteristic branching parallel to the parent hyphae with a constriction at the point of union. Cells of older branches turned barrel shaped. Mature hyphae repeatedly divided giving rise to mass of irregular structures which formed sclerotial bodies. Sclerotia were smooth, black, round to oval and sometimes irregular, sclerotia measured 112-278 μ with an average of 191 μ. The pycnidia were not produced by the fungus either in culture or on the host.

Physiology of the pathogen:

Studies on the physiology of the fungus did not appear to have received much attention. A brief review of work done on Rhizoctonia sp., R. bataticola is presented below.

Vasudeva (1937) reported that R. bataticola on cotton was able to attack a considerable range of host plants and are not highly selective in the metabolism so much so that all carbohydrates, nitrogen sources tested yielded satisfactory growth.

Vasudeva (1936) studied the physiology of the cotton root rot fungus (R. bataticola) and reported that the pathogen had a very wide range of tolerance to acidity and alkalinity, ranging between p^H 2.4 and p^H 9.2 with fairly good growth between p^H 3.2 and p^H 8.5 and optimum growth near the neutral point.

Microbial antagonism:

Specific studies on the microbial antagonism of the castor root and stem rot pathogen have not been made so far. However, work on microbial antagonism to R. bataticola on other hosts have been reported by several workers.

Weindling (1932) reported that the culture of virulent fast growing strains of Rhizoctonia as well as other pathogens were parasitised by Trichoderma lignorum. In 1934 he showed that T. lignorum to be capable of parasitising R. bataticola and Armellaria mellea.

Hanseler and Allen (1934) studied the toxic action of Trichoderma on Rhizoctonia and other soil fungi and observed that Trichoderma sp. when added to soil which was heavily infested with Rhizoctonia reduced the "damping-off" of cucumber and peas. The culture filtrates of Trichoderma were found lethal to Rhizoctonia and Pythium.

Thomas (1939) reported that R. bataticola was completely inhibited in the presence of T. lignorum and its growth was retarted by filtrates from liquid culture of the latter.

Ghaffar (1938) found that T. viridae inhibits the growth of Macrophomina phaseoli with its hyphae coiling around those of the latter.

Vasudeva and Sikka (1940) while making studies on the effect of certain fungi on the growth of root rot fungi reported that the presence of T. lignorum and Aspergillus niger in the inoculum of R. bataticola interfere with its growth. The hyphae of T. lignorum and A. niger, showed a dissolving effect on the hyphae of R. bataticola. The activity of R. bataticola and R. solani was reduced when the fungi were grown mixed with T. lignorum and A. niger. It was demonstrated that T. lignorum had a markedly depressing effect on R. bataticola and there appeared a line of demarkation at the junction of the hyphae of the two fungi when grown opposite to each other in the same plate.

Brooks and Tisdale (1948) carried out a series of green house tests with inoculated soil, to see the effect of vegetative matter and soil fumigants and post emergence damping off caused by Rhizoctonia sp. He found that there was some indication of antagonism by Trichoderma towards Rhizoctonia.

Chemical control of the pathogen:

Regarding the control of root and stem rot of castor caused by R. bataticola Tropova (1928) suggested disinfection of seeds with a high concentration of formalin than usually used for the seed disinfection, considerably reduced the incidence and stimulated the germinability of the seed and subsequent growth of the seedlings. Except for this, there are no specific reports on the chemical control of root and

stem rot of castor although there are several reports on chemical control of R. bataticola on different hosts.

Thruston (1921) obtained a good control of Rhizoctonia sp., by corrosive sublimate treatment with mercuric chloride solution of 1: 1000 which was found to kill all sclerotia in five minutes.

Chanzit (1923) controlled black scurf of potato caused by R. solani by disinfecting seed tubers with formalin.

Ashby (1927) reported the control R. bataticola by an application of wood ashes and also by potash or ash in water in acid soils.

Sundararaman (1929) during his studies on control of groundnut wilted plants by R. bataticola, used lime as soil treatment at the rate of 1,000 lbs. per acre and recorded the reduction in the number of wilted plants.

Burkett (1938) found that two percent ceresan used at the rate of 8 g per 1 g of nutrient medium inhibited the growth of Rhizoctonia in pure culture. He also reported that Cuprocide, Copper, K.B. and Zinc - oxide gave satisfactory control on the growth of the origanism when used at higher rates.

Heiberg and Ramsay (1946) worked on the control of the pathogens of fruit and vegetable grown in pure culture on P D A . In the presence of vapour from diphenyl crystals at dosages ranging from 1 to 20 mg per plate were found to be

fungistatic to Rhizoctonia. Based on this he believed that the use of diphenyl impregnated wrap would control the decay of fruits and vegetables.

Vasudeva (1945) made a number of abortive attempts by soil fumigation, cultural treatments and the application of fertilizer to control root rot disease of cotton caused by R. bataticola. The use of paradichlorobenzene reduced the incidence of the rot, but delayed germination and caused smaller and stunted growth. While removal of diseased debris and additional farm yard manure, flooding and fine tillage treatments gave appreciable results. Calcium chloride (2, 231 lbs/acre), potassium chloride (210 lbs) and both together, among several chemical treatments tried, were the only ones to bring about a reduction of incidence on treated as compared with untreated plots.

Lyale and Williams (1947) studied the localised chemical application to the soil and their effect upon the root rot of Beans and peas caused by Rhizoctonia. He reported that 25 percent disodium ethylene bisdithiocarbamate (Dithane D_{14}) when applied at the rate of 1 gallon per acre or 2 lbs. of dry powder (Dithane D_{10}) per acre in the row of seedling was found to be quite effective.

Houll et al.,(1947) reported that the Zinc salts of 2,4-5 trichlorophenol have been very effective as a fungicide for seed treatment. The most effective concentration appears

to be fifty percent of the above salt with an inert diluent and usual dosage recommendations of 2 to 4 kg for larger seeds like cotton.

Sadasivan (1950) studied the action of malachite green on Pythium aphanidermatum and other soil fungi achieved fungistatic and fungicidal action of malachite green at a strength of 6 ppm.

Gruenhagen et al.,(1951) used chlorinated phenols for Pea and groundnut seeds, as a protectant against Rhizoctonia which gave varying degree of control.

Simkover and Schenfelt (1951) worked on the effect of benzene hexachloride and chlordane on certain soil organisms and found that crude benzene hexachloride sprinkled over agar plants of Rhizoctonia caused marked inhibition of mycelial growth.

Gauman et al., (1953) studied the fungicidal properties of some carbonic and thiocarbonic acid derivatives of hydrazine found that the growths of Rhizoctonia sp., cultured at room temperature on P D A containing diffusible amounts of carbonic or thiocarbonic acid derivatives of hydrazine are effectively inhibited at concentrations over 500 ppm.

Henry (1953) found that several fumigants applied to the nursery prior to seedlings, gave varying degree of control. The application of 24 gallons per acre of a 20 percent by

volume solution of ethylene dibromide, 2 to 3 weeks prior to spring seedlings control the disease on pine. It was equally effective when applied to soil, in good tilth and to wet soil.

Henry (1953) and Foster (1961) found that black root rot of pine caused by R. batatícola could be controlled easily by ethylene dibromide (EDB) and Methyle bromide.

Dharamvir, et al., (1967) while testing the relative effectivity of 14 fungicides for the control of R. batatícola found parzate liquid (Disodium ethylene bisdithiocarbamate and Vapon (Sodium methyl dithiocarbamate), effectively controlling the fungus.

Jhooty and Grover (1971) who studied the root rot of cucurbits and its control in India reported seed treatment with Vitavax and Brassicol gave best control.

MATERIAL & METHODS

The general laboratory techniques employed in the present study were the same as followed by Rawlins (1933), for the preparation of media, maintenance of cultures etc., In addition, some of the procedures given by Fred and Waksman (1928), Riker and Riker (1936), Lilly and Barnett (1951), Zentmyer (1955) were also adopted with some modifications wherever necessary.

Glassware - Cleaning and Sterilisation:

The glassware used were of "Corning" make. After thorough washing with washing soda and water, all glassware were placed in strong chromic acid solution for twenty-four hours, washed thoroughly with tap water and dried before use.

Glassware such as Petri plates and Pipettes were sterilized in hot-air-oven at 160°C to 180°C for atleast an hour. Petri plates and Pipettes were wrapped in paper before use, to prevent contamination.

Media - Preparation and Sterilisation:

The different media used were:

1. Potato Dextrose Agar (P D A)

Potatoes (Peeled and sliced)	..	200.0 g.
Dextrose	..	20.0 g.
Agar Agar	..	20.0 g.
Distilled water	..	1000 ml.

2. Oat-meal Agar Medium (O M A)

Oat-meal	..	100.0 g.
Agar Agar	..	20.0 g.
Distilled water	..	1000 ml.

3. Soil Oats Medium

Soil	..	90.0 g.
Oats	..	10.0 g.
Distilled water	..	30-35 ml.

4. Czapeks liquid Medium:

Sodium Nitrate	..	2.0 g.
Dipotassium monohydrogen phosphate.	..	1.0 g.
Potassium chloride	..	0.5 g.
Magnesium sulphate	..	0.5 g.
Ferrous sulphate.	..	0.01 g.
Sucrose	..	30.0 g.
Distilled water	..	1000 ml.

5. Czapeks Agar Medium.

The same ingredients of Czapeks liquid medium in addition 20.0 g. of Agar Agar was used.

6. Martins Agar Medium

Glucose	..	10.0 g.
Peptone	..	5.0 g.
Potassium dihydrogen phosphate	..	1.0 g.
Magnesium sulphate	..	0.50 g.
Rose Bengal	..	33.0 g.

Streptomycin sulphate	..	300 ml.
Agar Agar	..	15.0 g.
Distilled water	..	1000 ml.

7. Richards liquid Medium:

This medium was used for all physiological experiments.

Potassium Nitrate	..	10.0 g.
Potassium dihydrogen phosphate	..	5.0 g.
Magnesium sulphate	..	2.5 g.
Ferric chloride	..	Traces.
Sucrose	..	50.0 g.
Distilled water	..	1000 ml.

The media used were sterilized by autoclaving at 15 lbs pressure per square inch for 15 minutes.

p^H of the medium:

The initial p^H of the medium was adjusted to 6.0 before autoclaving for all the experiments with IN. NaoH and IN. Acetic acid, using B.D.H. indicator papers. The p^H measurements for the p^H experiments were made with a photovolt Model 110 electronic p^H meter.

Soil - Sterilization and growing the plants in pots:

Black clay loamy soil mixed with red soil and enriched with Farm Yard Manure, was passed through 1/16" mesh sieve and filled in earthen pots of 12" diameter for pathogenicity tests

and 6" diameter for varietal reaction tests. The pots were sterilized in an autoclave at 20 lbs. pressure per square inch for 2 hours.

Five seeds were surface sterilized by immersing in 1: 1000 mercuric chloride solution for a minute and washed in several changes of sterile water and sown in each pot.

Isolation of the pathogen:

Root and stem rot affected plants were pulled along-with roots showing black sclerotia and pycnidia. Freshly collected samples were used for the isolation of the pathogen. A bit of diseased portion from the affected stem and roots having small black sclerotia along with healthy tissue was taken and washed well with sterile water to get ride of foreign matter as much as possible and surface sterilized with 1:1000 mercuric chloride solution for a minute and then washed in several changes of sterile water. These bits were then plated out on P D A and incubated at room temperature for 48 hours. A portion of the mycelium developed from the plated bits was transferred to O M A plates. The fungus was maintained in pure culture on O M A medium throughout the period of investigation.

Pathogenecity studies:

Pathogenicity tests were conducted by adopting the following two methods.

Inoculum - Preparation of inoculum and method of inoculation:

1. Soil-Oats inoculum method:

Soil-oats medium was autoclaved at 20 lbs. pressure for two hours in 250 ml conical flasks. Prior to autoclaving 30 ml of water was added. The fungus (Rhizoctonia bataticola) was grown on the sterilized medium for a period of three weeks, after which it was mixed with sterilized soil filled in earthen pots, in the proportion of 20 parts of inoculum and 80 parts of sterilized soil (i.e.) 800 g of inoculum was thoroughly incorporated in 4 kg of sterilized soil. Sufficient water was added to the pots to provide optimum moisture. Pots were incubated for a period of 48 hours, 25 seeds of 'Aruna' variety of castor were surface sterilized in 1: 1000 mercuric chloride solution and 5 seeds per pot were sown in 5 pots. Observations were made on pre-emergence rotting of seed and also on the post-emergence root and stem rot incidence.

2. Stem tape inoculation method:

The stem pieces of castor were cut into small bits of about 1" length and immersed in 2% sucrose solution. These were then transferred to conical flasks and were autoclaved at 15 lbs. pressure per square inch for one and half hour. The sterilized pieces were inoculated with stock culture of the fungus (R. bataticola) maintained on Czapeks agar slants. The mycelial growth of the pathogen was very fast and within a week it covered all the stem pieces and formed small dark black sclerotia.

The method of inoculation developed at the All India Co-ordinated Rice Improvement Project which is known as "Stem tape inoculation technique" (Anonymous, 1970 and 1971) was followed here.

The plants at the Collar region just above the soil were injured with the needle or scalpal. Individual stem pieces alongwith mycelium and sclerotia were placed at the injured part on the collar region and finally wrapped with a celluphane tape. This keeps the inoculum in contact with the stem. Then a coating of absorbent cotton was wrapped over the inoculated portion so as to keep it moistured. Seedlings of 1 week to 8 weeks old were inoculated by the above method.

Varietal reaction:

The inoculum for varietal reaction experiment was prepared by soil oats inoculum method. 200 g of inoculum was thoroughly incorporated in 800 g of sterilized soil and placed in pots. Then the pots were incubated for 48 hours in the glass house. Care was taken to water the pots in order to maintain optimum moisture levels. After the incubation period, 25 surface sterilized seeds from each of the following 9 varieties, were sown at the rate of 5 seeds per pot.

1. Aruna,	4. R-63,	7. RC- 1377
2. 413 A,	5. HC-8,	8. V-2-9,
3. B-157,	6. 6-219-22,	9. S-248-2.

Isolation of Micro-organisms for Antagonistic studies:

In order to isolate fungi from soil for antagonistic studies against R.bataticola, isolations were made from the rhizosphere of castor plant found to be resistant to R.bataticola in varietal reaction studies.

Six weeks old seedlings of castor were brought alongwith the roots and shaken briskly to remove the soil particles adhering to the roots. 10 g of soil was taken in 100 ml of sterile water into 250 ml conical flask and serial dilutions were made using standard serial dilution technique (Wakeman 1952). On the basis of 1: 10 (ie) each higher dilution made by taking 10 ml of lower dilution and transferred to 90 ml of sterile water till a dilution of 10^{-4} was obtained. One ml of the prepared dilution was added to the Petri plates and 15-20 ml of melted Martins Agar Medium was poured over only after it was cooled and just before setting. The plates were rotated to mix up soil suspension and the medium and then incubated at room temperature for 5 days.

The colonies developed in the Petri plates on Martins Agar Medium were transferred to P D A slants. Isolations were made thrice and all the pure cultures were maintained by periodical sub-culturing and sorted out on the basis of differences in colony colour, growth habits and latter identified. The fungi which appeared frequently were selected and used for antagonistic studies against R. bataticola.

Screening of Micro-organisms for Antagonistic activity:

Eight fungi were selected for studying their antagonistic activity against R. bataticola. These fungi were tested in 4 replications keeping one as control. A bit of mycelium from each of the 8 selected isolates was inoculated in the centre of a Petri plates containing P D A. A bit of the fungus (R. bataticola) was inoculated on either side of the centrally inoculated fungus and incubated at room temperature.

The radius of the fungus growth developed from the inoculated point and the diameter of the inhibition zones if any on either side were measured 5 days after incubation.

Effect of culture filtrate of the Mycoflora isolated on the growth rate of the test fungus:

The isolated mycoflora were grown on Czapeks liquid medium and incubated at room temperature, for 15 days until the cultures were fully grown for obtaining the culture filtrates. The filtrates were mixed with freshly prepared P D A in the ratio of 1: 1.5 (Medium: filtrates) and were sterilized in the autoclave and plated out in the Petri plates. The test organism was inoculated in the centre of the Petri plates and incubated at room temperature for two weeks. Observation was made if there was any inhibition in the growth of the test fungus.

Physiology of the pathogen:

Inoculation and incubation of liquid media:

Discs of 2 mm diameter were cut from the peripheral zone of 6-7 days old R.bataticola culture. These discs were then inoculated in 250 ml conical flasks containing liquid medium. The flasks were then incubated in the laboratory at room temperature (26-32°C).

Determination of mycelial dry weight:

Mats were harvested in dried and tarred filter papers by filtering through a Buckner funnel under vaccum, thoroughly washed with distilled water, rolled and air dried. Latter they were dried at 70°C in an oven for 48 hours, cooled in a dessicator and weighed. The weight of the mycelium was calculated by deducting the weight of the filter paper from the total weight. The figures, given in the tables represents the mean of three replicates. All weights are corrected to a milligram.

Physiology of the pathogen:

Experiment - I. Determination of optimum incubation period for the growth of Rhizoctonia bataticola.

The experiment was conducted to determine the incubation period required for maximum growth of R. bataticola, in the Richards liquid medium.

The cultures were harvested from fourth day onwards and with an interval of two days upto 22 days.

In all the subsequent experiments, the cultures were incubated for 14 days.

Experiment - II. Influence of carbon sources on the growth of Rhizoctonia bataticola.

This experiment was conducted with a view to determine the best source of carbon for the growth of R. bataticola, under study. The carbon sources employed are given below:

1) Monosaccharides:
   a) Pentoses .. Xylose, Arabinose
   b) Hexoses .. Glucose, Fructose.
2) Disaccharides: .. Sucrose, Maltose.
3) Polysaccharides: .. Starch, Dextrin.
4) Sugar alcohols: .. Sorbitol, Mannitol.
5) Organic acids: .. Citric acid, Tartaric acid.

The control received no carbon source. The sucrose in the Richards medium was replaced with the above 11 carbon sources on the basis of their carbon content to give equal amount of the element in each case.

Experiment - III. Influence of different Nitrogen sources on the growth of Rhizoctonia bataticola.

This experiment was conducted to study the best source of Nitrogen for the growth of the fungus. The following nitrogen sources were employed by replacing potassium nitrate in the Richards medium on equal nitrogen basis. The following

are the different sources of Nitrogen employed in this experiment.

1. Organic Nitrogen compounds:

a) Amino acids	..	Glutamic acid.
b) Amids	..	Asparagine.
c) Other organic compounds	..	Urea.

2. Inorganic Nitrogen compounds:

a) Nitrates	..	Potassium nitrate, Sodium nitrate.
b) Nitrites	..	Potassium nitrite, Sodium nitrite.
c) Ammonium salts.	..	Ammonium chloride, Ammonium oxalate, Ammonium nitrate, Ammonium sulphate.

Experiment - IV. Growth of Rhizoctonia bataticola at various levels of p^H.

The p^H of the medium has a significant role to play in the growth of fungi. In general, fungi have a preference for slight acidity (Lilly and Barnett, 1951 a). Individual fungi, however, vary in their p^H requirements. Hence, this study was conducted with an object of finding out the optimum p^H requirement for the growth of the fungus under study.

The following p^H levels were employed in this experiment: 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. All the p^H levels were adjusted, either with IN acetic acid or IN Sodium hydroxide solutions, with a p^H meter before autoclaving. The medium was unbuffered.

Efficacy of various Fungicides and antibiotics against Rhizoctonia bataticola in Vitro:

The fungicides and antibiotics were assayed for their efficacy against the pathogen by adopting the soil Vial technique developed by Zentmyer (1955).

Fine sandy loam soil, was air dried passed through a sieve of 20 mesh per square inch and autoclaved for 45 minutes at 15 lbs.pressure per square inch. One inch soil was placed in a sterilized glass vial (3" in length and 1" in diameter). The fungus was grown on P D A in Petri plates for two weeks and the culture discs of 10 mm in diameter were cut with the help of a 10 mm cork borer, and placed on the soil in the glass vial and then covered with one inch of the same sterilized soil. Five ml. of the fungicidal dilution was added to the surface of the vial with a pipette, and the vial was plugged with cotton. In the case of control the same quantity of sterilised distilled water was added to the soil. The vials were incubated at 25°C for 24 hours. Three replications were provided for each of the treatments.

After the incubation period three vials in each treatment were emptied in a strainer and the soil was gently removed by washing with sterile water and the disc of the mycelium were placed on Potato dextrose Agar in the petri plates in order to determine the viability of the fungus by the presence or absence of growth indicating non-toxic or toxic effect of the concentrations.

The following are the details of fungicides representing different groups and the antibiotic employed in the study.

S.No.	Name of the fungicide	Recommended concentration in ppm.	Active ingradient
1.	Aureofungin	20 ppm-50 ppm	Antibiotic produced by Streptoverticillium cinnamoneium Var. terricolum Thirum a new aromatic group of heptaenes.
2.	Benlate	250 ppm.	Methyl 1-(butyl carbamoyal)-2 benzimidazole carbamate.
3.	Brassicol (PCNB)	5000 ppm.	Penta chloronitrobenzene.
4.	Blitox	3500 ppm.	Copper oxychloride.
5.	Captan	1500 to 2000 ppm.	N(Trichloromethyl thio)4 Cyclohexene-1,2 dicarboximide.
6.	Formaldehyde	1000 ppm.	Formalin.
7.	Topsin M-70	1000 ppm.	1,2 Bis(3-Methoxycarbonyl-2-thioureido) benzene (Thiophanate Methyl).
8.	Thiram	1500 ppm.	Tetra methyl thiurum disulphide.
9.	Zincomadine	1000 ppm.	Chelation complex of 1-hydroxypyridine-2-thione and Zinc.

RESULTS

The disease was observed during kharif 1973 in the experimental plots of "Aruna" variety of castor in the Agricultural Research Institute, Rajendranagar. The disease was noticed subsequently in other varieties of castor grown in the fields of Research Institute as well as in the fields of Agricultural College Farm, Rajendranagar.

Symptoms:

Sporadic incidence of the disease was observed in the field, when the crop was 30 days old. The disease could be easily identified from a distance by watching some of the dead seedlings found among the healthy green plants. Leaf yellowing, puckering and stunting of the plant with withered leaves remained attached to the plant was noticed. Black pin head like pycnidia and sclerotia were found on the dead bark and the stem turned black coloured. Roots were poorly developed and shredded.

Numerous pycnidia and sclerotial bodies were found in the shredded dead tissue. They were examined microscopically and described below.

Pycnidia are dark, olive to black, erumpent, globose to sub-globose, osticlate and measuring 105 μ - 200 μ in diameter. Numerous single-celled, hyaline, oblong or some what ellipsoidal conidia are present within the pycnidia and size varying from 16 μ - 30 μ x 5 μ - 10 μ .

Sclerotia are smooth, black, round to oval and sometimes irregular and consisted of polygonal pseudoparenchymatous

cells and measuring 75 μ - 150 μ in diameter.

Morphology of the pathogen:

The pathogen could be easily isolated by plating the diseased root and stem bits containing the sclerotia on P D A after sterilizing in 1: 1000 mercuric chloride solution. The fungus rapidly formed spherical hyaline colonies on P D A as well as on O M A at first and finally becoming intense carbonaceous black, showing concentric zonations covering the whole Petri plate within three days (Plate-1). A few sclerotia developed on the margins of the colonies after 4 days and latter a number of sclerotia developed gregariously on the edges of the colony. No pycnidia are formed even in the old cultures.

Mycelium consisting of fumacious, broad, septate and branched hyphae, branches frequently running parallel to each other, hyphae constricted at the septa, with cylindrical cells in the growing hyphae and latter the cells becoming barrel shaped in the old hyphae. Mature hyphae are repeatedly divided giving rise to irregular structures which formed sclerotial bodies. Sclerotia are smooth, black, round to oval and sometimes irregular measuring 86 μ in diameter (Plate-2). The pathogen was identified as Rhizoctonia bataticola.

Pathogenicity test:

The pathogenicity of the isolate was tested on its own host with a view to study its incubation period and the development of disease. Two methods were adopted for pathogenicity

Plate-1

Plate - 1

Petri plates showing three days old culture growth of *Rhizoctonia batatícola*.

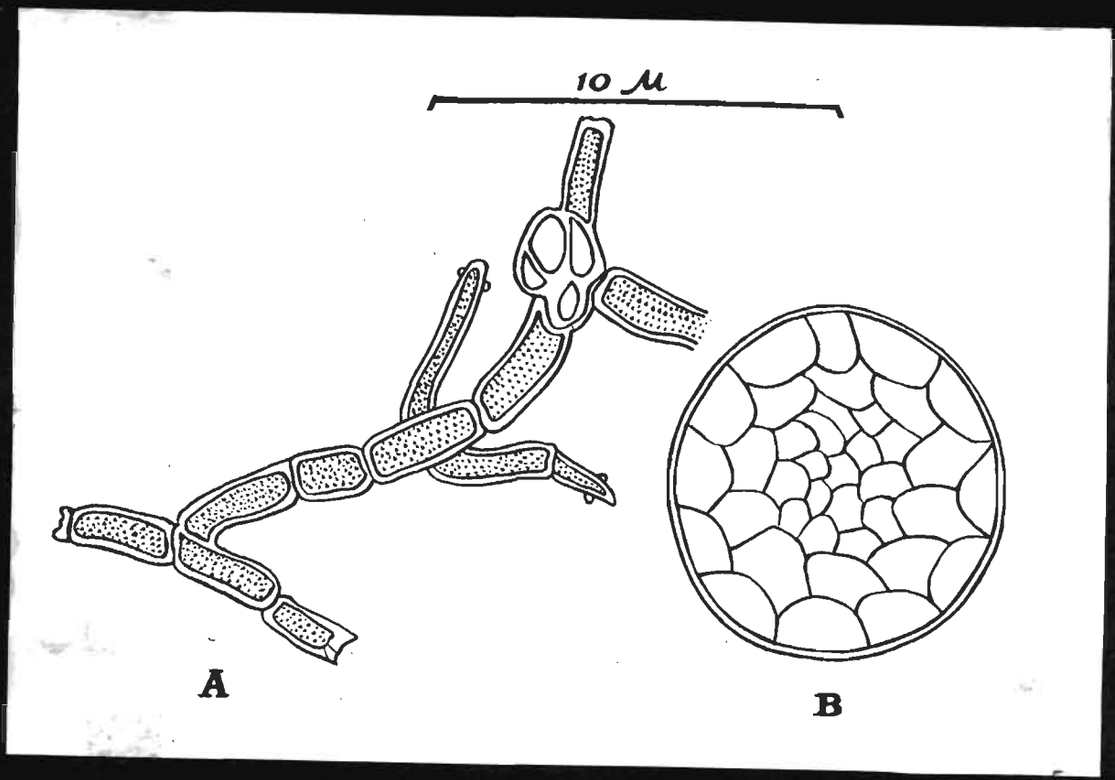


Plate-2

Plate - 2

Photograph showing

A = Hyphal structure of *Rhizoctonia bataticola*

B = Sclerotial body of *R. bataticola*

tests viz., soil-oats inoculum and stem tape inoculation method maintaining suitable control. The plants inoculated by the first method developed typical symptoms on 5 weeks old castor seedlings.

Leaf yellowing, puckering and stunting of the plants were observed (Plate-3). With the advance of the disease, gradual withering and drooping of leaves starting from the lower ones were noticed. The withered and drooped leaves remained attached to the plants even after the death of the plant (Plate-4). Shredding of the bark was observed on all the affected plants. Black pin head like pycnidia and small size sclerotia were fou d on the dead bark of the plant. The dead stem turned light, almost gleaming ash colour.

When such plants were pulled and examined it was seen that there was poor development of roots, finer rootlets were either not fully developed or destroyed due to rotting near the soil level and gradually spread to the larger roots towards the collar region, of the plant. Water soaked leisions were formed at the collar region. The leisions were light brown in the beginning and latter turned dark brown to black and at an advanced stage the plants withered and profusion of small size sclerotia and pin head like pycnidia were produced all over the dead stem.

The pycnidia developed extrnally on the bark. They are macroscopic, dark, olive to black, erumpent, globose

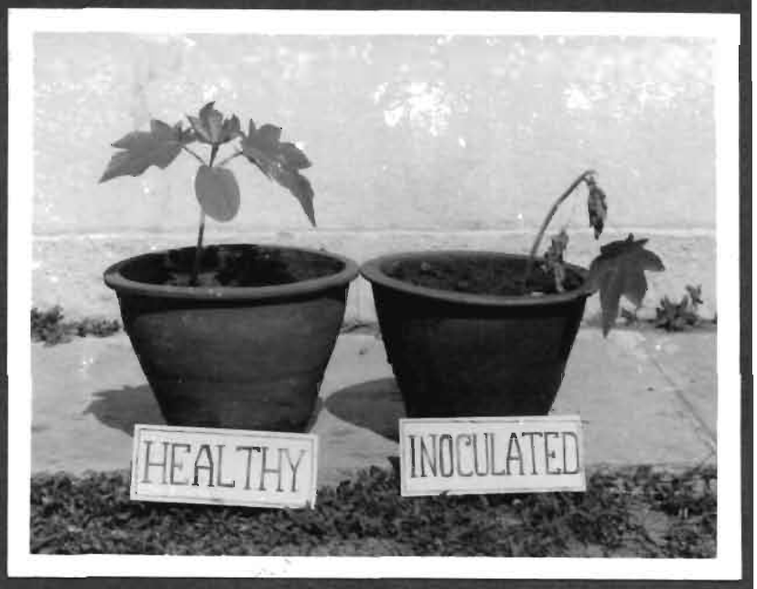


Plate-3

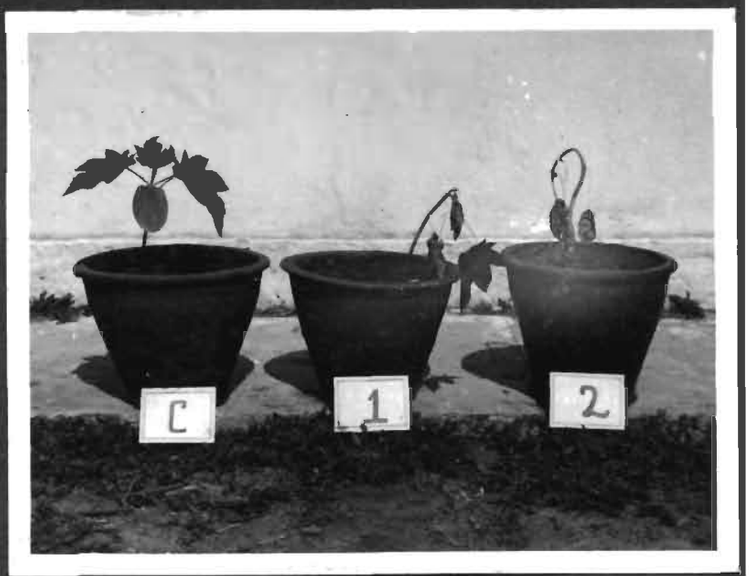


Plate-4

Plate - 3

Photograph showing Healthy and Inoculated plants of "Aruna" variety of Castor.

Plate - 4

Photograph showing

C = Control

1. Initial stage of infection (four weeks after germination)

2. Final stage of infection (five weeks after germination).

to sub-globose, ostiolate and measuring 155 μ in diameter.

Conidia are 1-celled, hyaline, emerging out through the ostiole when the pycnidia are pressed oval to ellipsoid and measuring 28.88 μ x 7.15 μ (Plate-5).

Based on the morphology of the fungus and the disease symptoms produced by it the fungus was identified as Rhizoctonia bataticola.

Reisolation of the pathogen was done from the roots and stems, of the diseased plants which consistantly yielded R. bataticola which was similar to the one used for the inoculations.

Varietal reaction:

With a view to find out the varietal reaction of castor to R. bataticola, nine varieties were screened under glass-house. Inoculations were made by soil-oats inoculum method. The inoculum was thoroughly mixed with sterilized soil in the ratio of 800 grams of soil and 200 grams of inoculum. 27 pots of 6" diameter were filled with the mixed inoculum and soil. Incidence of the disease in different varieties were recorded after 5 weeks. Twenty five seedlings were raised for each variety at the rate of 5 seedlings per pot. Two pots containing 10 seedlings for each variety was maintained as control.

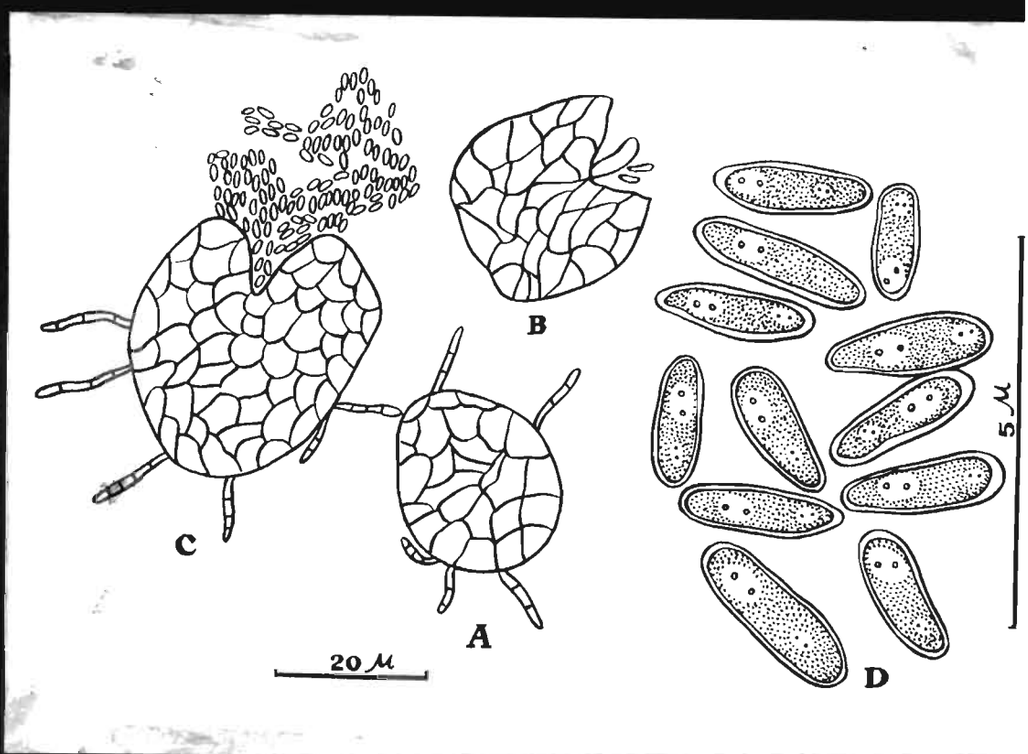


Plate-5

Plate - 5

Photograph showing the Pycnidia and Conidia of Macrophomina phaseoli.

A = Unruptured pycnidium
B = Half ruptured pycnidium
C = Ruptured pycnidium with emerging conidia.
D = Conidia.

The results of varietal reaction are presented in Table-1 (Plate 6 & 7). Out of the nine varieties tested 2 varieties viz., B-157 and 6-219-22 appeared to be resistant and the remaining seven varieties were found to be susceptible to the disease.

Isolation and screening of antagonists:

The fungal flora from the rhizosphere of 6 weeks old castor seedlings were isolated by Serial dilution technique Waksman (1952). The following genera of fungi were isolated.

1. Myrothecium sp.
2. Trichoderma sp.
3. Aspergillus flavus
4. Aspergillus niger
5. Fusarium sp.
6. Rhizopus sp.
7. Sclerotium sp.
8. Cladosporium sp.

The taxanomic position of the different isolates of fungi are presented in Table-2. All the eight fungi which were found to be of common occurrence were tested against R. bataticola for their antagonistic behaviour. Among them only four fungi viz., Myrothecium sp., Trichoderma sp., A Aspergillus flavus, A. niger, were found to be antagonistic.

Measurement of the Antagonistic potential:

Each of the eight fungi isolated was tested separately against the test fungus R. bataticola. Each Fungus was inoculated in the centre of plates containing P D A and inoculating the test organism on either side. The growth of the

Table - 1. Varietal Resistance of Castor to *Rhizoctonia bataticola*.

S.No.	Variety	Number of inoculated	Number infected	Percentage of infection.
1.	Aruna	15	15	100
2.	413-A	15	15	100
3.	B-157	15	Nil	0
4.	[illegible]-63	15	15	100
5.	HC-8	15	15	100
6.	6-219-22	15	Nil	0
7.	RC-1377	15	15	100
8.	V-2-9	15	15	100
9.	S-248-2	15	15	100

Plate-6

Plate-7

Plate 6 & 7.

Photograph showing varietal reaction.

C = Control
S = Suceptible.
R = Resistent.

Variety 3 and 6 are Resistent.

Table-2. Taxanomic position of fungi isolated from the Rhizosphere of Castor.

S.No.	Genus	Family	Order	Class
1.	*Myrothecium* sp.	Tuberculariaceae	Moniliales	Deuteromycetes
2.	*Trichoderma* sp.	Moniliaceae	Moniliales	Deuteromycetes
3.	*Aspergillus flavus*	Moniliaceae	Moniliales	Deuteromycetes
4.	*Aspergillus niger*	Moniliaceae	Moniliales	Deuteromycetes
5.	*Fusarium* sp.	Tuberculariaceae	Moniliales	Deuteromycetes
6.	*Rhizopus* sp.	Mucoraceae	Mucorales	Zygomycetes
7.	*Sclerotium* sp.	————	Mycelia sterilia	Deuteromycetes.
8.	*Cladosporium* sp.	Dematiaceae	Moniliales	Deuteromycetes.

organism was found to be restricted at a distance alroung and formed appreciable zones of inhibitions. A gap of 24 hours was given for slow growing fungi viz., Myrothecium sp., Aspergillus flavus, Fusarium sp., Sclerotium sp., A. niger, and Cladosperium sp., For Trichoderma sp., and Rhizopus sp., no gap was given as they are fast growing as seen in preliminary tests.

Clear zones of inhibition between the test fungus and the antagonist were formed within four days after incubation (Plate- 8 & 9). Due to lysis of mycelium along the edges of the zone, Trichoderma sp., produced yellow to brown coloured pigmentation and showed pronounced effect on the growth of R. bataticola, followed by Myrothecium sp., A. flavus and A. niger. The data regarding the total inhibition zone of the three replicates are given in Table-3.

Growth of Rhizoctonia bataticola on solid medium incorporated with culture filtrates of the fungi isolated from the rhizosphere of castor seedlings:

The growth rate of fungi can conveniently be studied and their linear growth measurements easily be recorded on solid medium than on liquid medium. To study the effect of antagonistic principle on the growth rate of the test fungus, this experiment was conducted on solid medium incorporated with the culture filtrates of the isolated fungi. The data pertaining to the mean diameter of the colonies of the three replicates are given in Table-4.

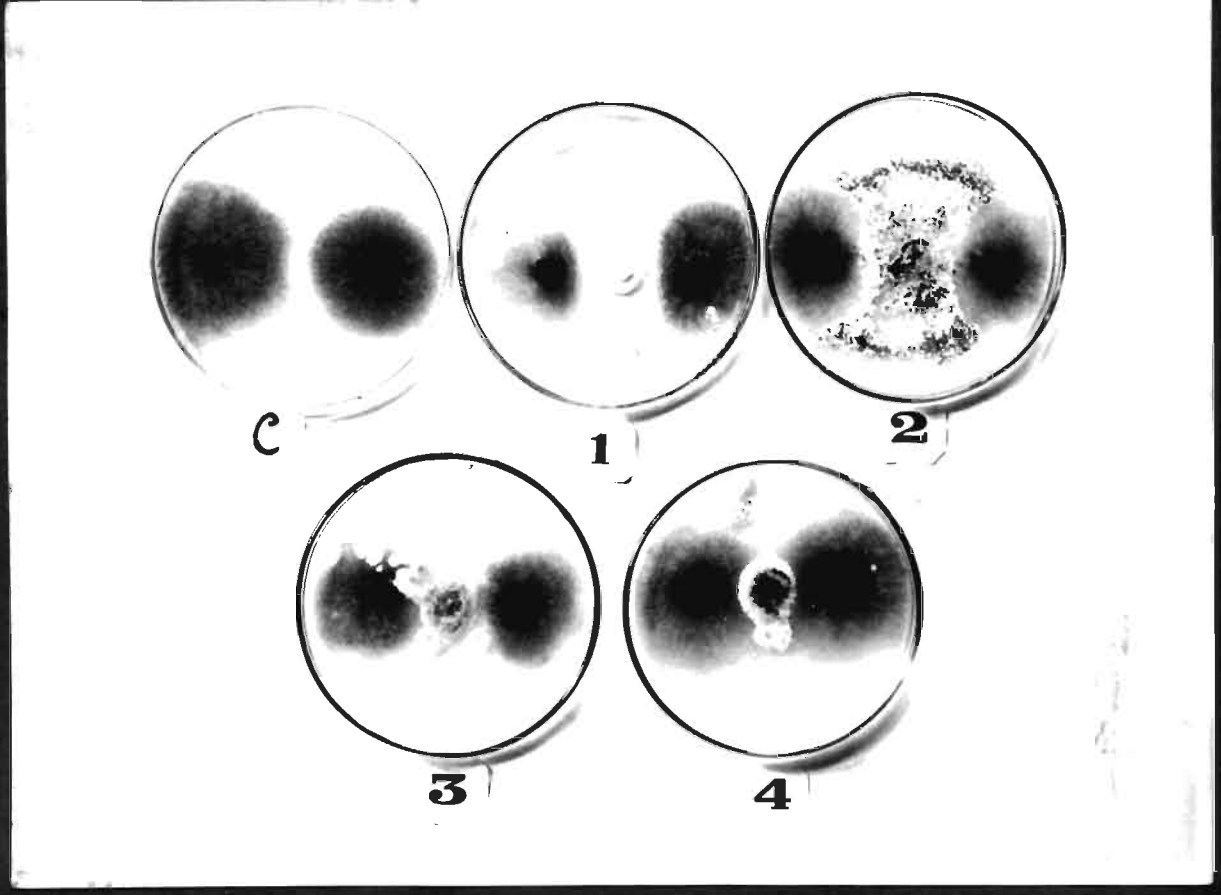


Plate-8

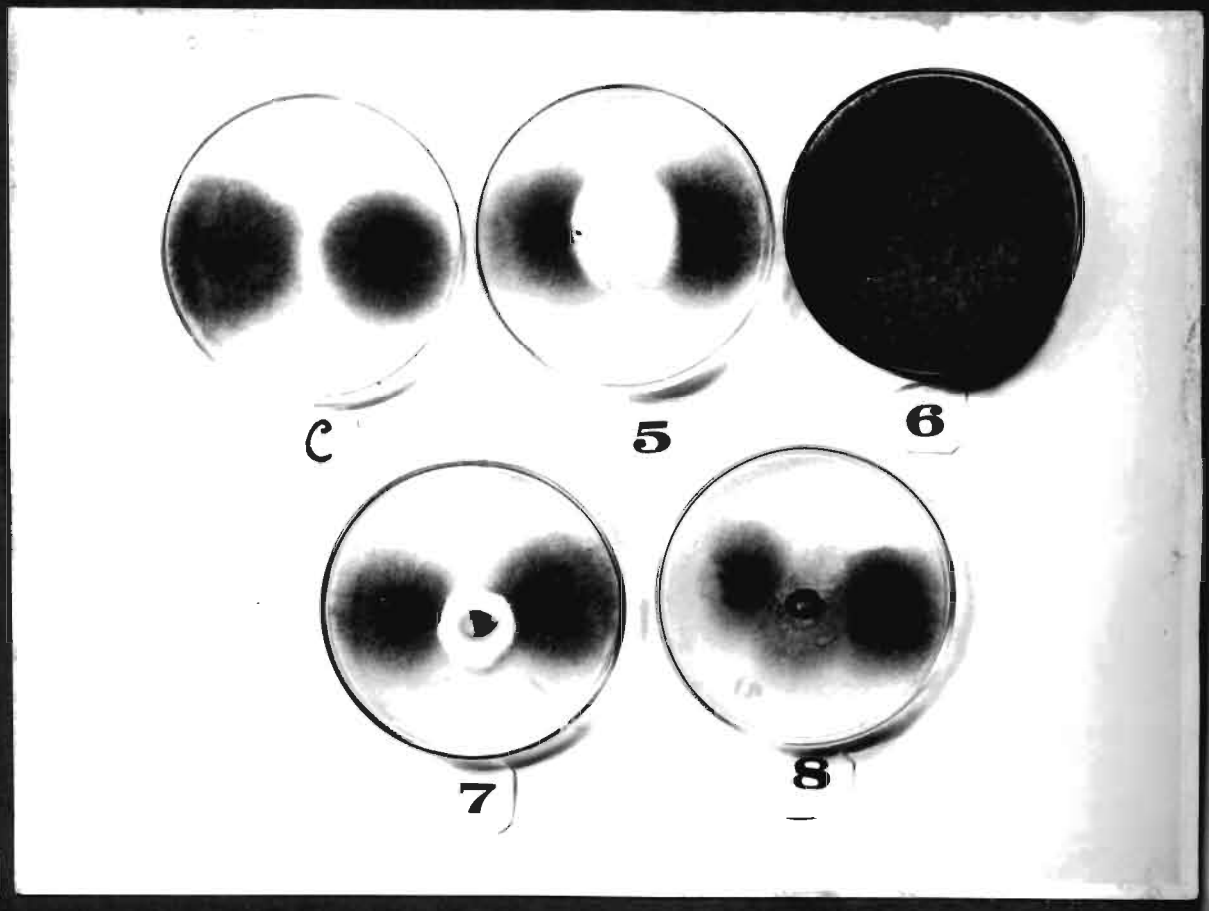


Plate-9

Plate - 8

Photograph showing 1 to 4 fungi antagonistic to Rhizoctonia bataticola as indicated by the presence of inhibition zone.

C. ... ... Control
1. ... ... Myrothecium sp.
2. ... ... Trichoderma sp.
3. ... ... Aspergillus flavus.
4. ... ... Aspergillus niger.

Plate - 9

Photograph showing non-antagonistic fungi as indicated by absence of inhibition zone.

C. ... ... Control.
5. ... ... Fusarium sp.
6. ... ... Rhizopus sp.
7. ... ... Sclerotium sp.
8. ... ... Cladosporium sp.

Table-3. Rate of inhibition of *Rhizoctonia bataticola* by the fungi frequent isolated from the rhizosphere of castor seedlings.

S.No.	Antagonistic organisms.	Radius of antagonists (in mm)	Clear zone (in mm)	Total inhibition (in mm)
1.	*Myrothecium* sp.	20	4	24
2.	*Trichoderma* sp.	33	3	36
3.	*Aspergillus flavus*	20	2	22
4.	*Aspergillus niger*	20	1	21
5.	*Fusarium* sp.	-	-	-
6.	*Rhizopus* sp.	-	-	-
7.	*Sclerotium* sp.	-	-	-
8.	*Cladosporium* sp.	-	-	-

Table-4. Growth of Rhizoctonia bataticola on solid medium incorporated with culture filtrates of the fungi isolated from the rhizosphere of castor seedlings.

S.No.	Fungi tested	Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	Control	25	70	90	90	90	90	90	90	90	90	90	90	90	90
1	Myrothecium sp.	10	30	60	70	80	80	80	80	80	80	80	80	80	80
2	Trichoderma sp.	10	27	45	56	65	75	75	75	75	75	75	75	75	75
3	Aspergillus flavus	12	40	45	63	72	85	85	85	85	85	85	85	85	85
4	Aspergillus niger	11	38	63	70	80	80	80	80	80	80	80	80	80	80
5	Fusarium sp.	14	49	80	85	90	90	90	90	90	90	90	90	90	90
6	Rhizopus sp.	10	35	60	90	90	90	90	90	90	90	90	90	90	90
7	Sclerotium sp.	12	42	70	90	90	90	90	90	90	90	90	90	90	90
8	Cladosporium sp.	12	45	72	90	90	90	90	90	90	90	90	90	90	90

Remarks: The data presented in the Table-4 shows there was rapid growth of the test fungus (Rhizoctonia bataticola) in the case of cultural filtrates of Fusarium sp., Rhizopus sp., Sclerotium sp., Cladosporium sp. within four days comparable to control reaching the maximum of 90 mm. The culture filtrate of Trichoderma allowed the test fungus to grow only upto 56 mm within 4 days and upto 75 mm on the 14th day followed by Aspergillus flavus, Aspergillus niger and Myrothecium sp. which allowed the test fungus to grow 63, 70 and 70 mm respectively. Evenafter two weeks the test fungus was restricted by these three fungi allowing to grow to a diameter of 80-85 mm.

It was generally noticed that the growth rate of the testo organism on staled medium was slowed down considerably when compared to control.

The growth of R. bataticola on solid P D A medium was ordinarily 90 mm on 3rd day itself as shown by the control. However, on medium staled with fungal filterates, the growth was severely reduced. The culture filterate of Trichoderma sp. isolate had a pronounced effect on the growth of R. bataticola followed by Myrothecium sp., Aspergillus flavus and A. niger. The remaining isolates had no effect on the growth of R. bataticola.

PHYSIOLOGY OF THE PATHOGEN:

Determination of optimum incubation period for the growth of Rhizoctonia bataticola.

Visible growth of the fungus occured within 24 hours after inoculation. The mycelial mat weight on the fourth day was 137 mg and latter there was a gradual increase in the weight of the mycelial mat upto the fourteenth day, where a maximum growth of 507 mg , then a gradual fall in mycelial mat weight was obtained.

It is thus evident that maximum growth was obtained on the fourteenth day. In all the subsequent physiological experiments, the cultures were incubated for fourteen days. The results obtained are presented in Table-5 and Text fig.I.

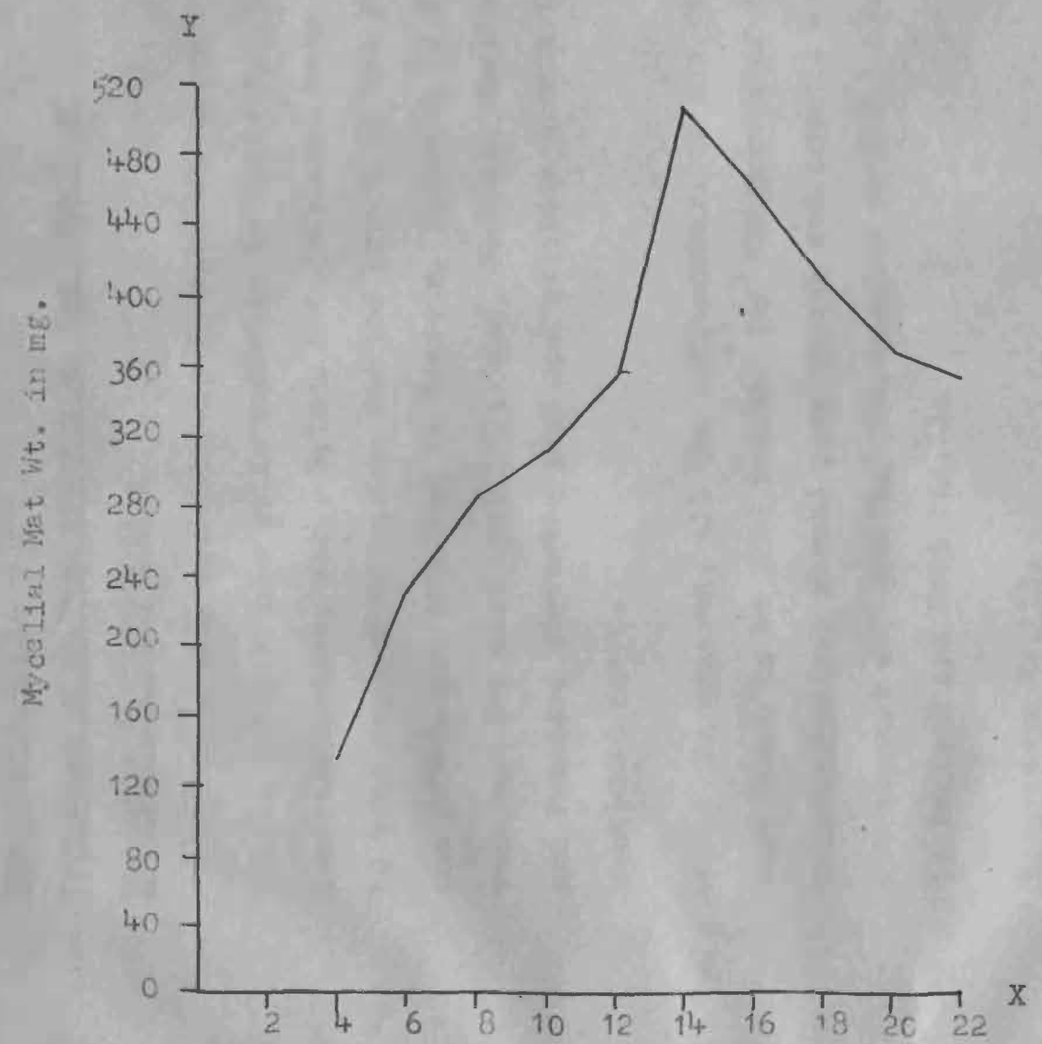
Table-5. Growth of Rhizoctonia bataticola at different incubation periods.

S.No.	Incubation period	*Mycelial mat weight in mg.
1.	4th day	137
2.	6th day	232
3.	8th day	286
4.	10th day	312
5.	12th day	354
6.	14th day	507
7.	16th day	463
8.	18th day	408
9.	20th day	369
10.	22nd day	353

*Mean of 3 replications.

Text fig.I

Growth of Rhizoctonia bataticola at different incubation periods.



Experiment - II.

Influence of carbon sources on the growth of Rhizoctonia bataticola.

No growth was obtained in both the organic acids viz., Citric and Tartaric acids and also in control. The two Monosaccharides, viz., Xylose and Arabinose were found to be poor sources of carbon for the growth of the fungus. Maximum growth was obtained in Dextrin, followed by Starch. Sucrose was the next best. Maltose, Glucose, Sorbitol, Mannitol and Fructose supported good and moderate growth in the order mentioned above.

In general, all the polysaccharides and disaccharides were found to be good sources for the growth of the fungus. Monosaccharides showed poor growth and organic acids could not support any growth. The results obtained are furnished in Table-6 and Text Fig.II.

Experiment No.III.

Influence of different nitrogen sources on the growth of Rhizoctonia bataticola:

Good growth of the fungus was obtained with Asparagine, followed by Ammonium salts (viz., Ammonium nitrate and Ammonium oxalate). Further it is significant to note that inorganic salts viz., Ammonium chloride and Ammonium sulphate were poorly utilised by the fungus growing in an unbuffered

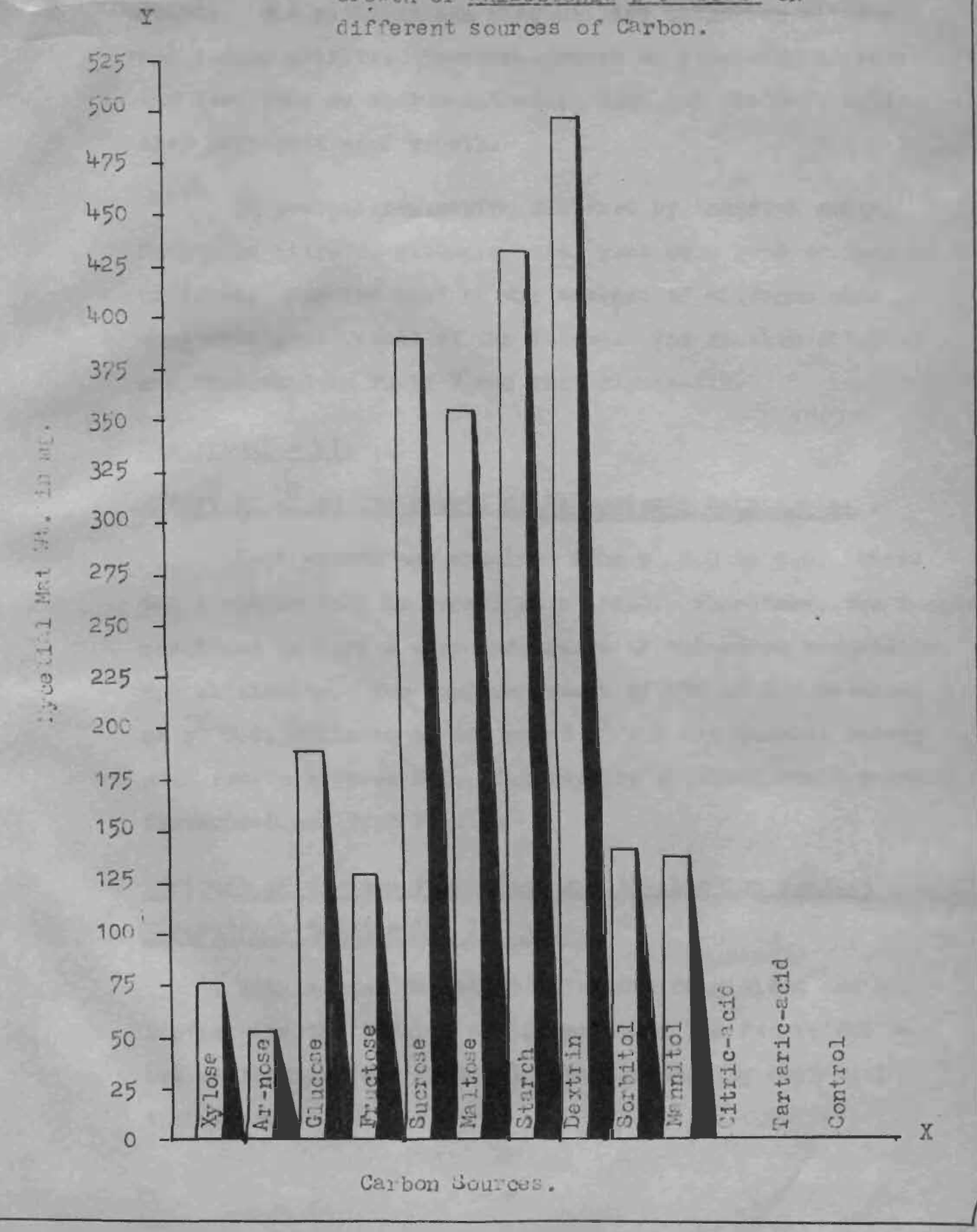
Table-6. Growth of *Rhizoctonia bataticola* on different sources of carbon.

S.No.	Carbon source	Mycelial mat weight in mg.*
1.	Xylose	76
2.	Arabinose	53
3.	Glucose	188
4.	Fructose	129
5.	Sucrose	390
6.	Maltose	355
7.	Starch	432
8.	Dextrin	498
9.	Sorbitol	142
10.	Mannitol	137
11.	Citric Acid.	-
12.	Tartaric Acid	-
13.	Control	-

*Mean of 3 replications.

Text fig.II

Growth of Rhizoctonia bataticola on different sources of Carbon.



medium. The pathogen can also utilise potassium nitrite and sodium nitrite. However, growth on potassium nitrate was less than on sodium nitrate. Urea and Glutamic acid also supported good growth.

In general Asparagine followed by Ammonium salts, Potassium nitrate, glutamic acid, urea were good sources of nitrogen. However rest of the sources of nitrogen also supported good growth of the fungus. The results obtained are furnished in Table 7 and Text Figure-III.

Experiment - IV.

Effect of p^H on the growth of Rhizoctonia bataticola.

Good growth was obtained from p^H 3.0 to 9.0. There was a sudden fall in growth at p^H 10.0. Therefore, the fungus was found to have a very wide range of tolerance to acidity and alkalinity. The maximum growth of 572 mg was obtained at p^H 6.0, followed by 448 mg at p^H 7.0 and showing fairly good growth between 5-8. The results obtained are furnished in Table-8 and Text Fig.IV.

Efficacy of various Fungicides and Antibiotics against Rhizoctonia bataticola, in Vitro.

With a view to select effective fungicides and antibiotics for the control of the pathogen the fungicides and one antibiotic (See Table-9) were screened by soil vial technique.

Table-7. Growth of Rhizoctonia bataticola on different Nitrogen sources.

S.No.	Source of Nitrogen	*Mycelial mat weight in mg.
1.	Potassium Nitrate	251
2.	Sodium Nitrate	196
3.	Potassium Nitrite	156
4.	Sodium Nitrite	138
5.	Ammonium chloride	143
6.	Ammonium Oxalate	272
7.	Ammonium sulphate	210
8.	Ammonium Nitrate	281
9.	Urea	207
10.	Glutamic Acid	239
11.	Asparagine	316
12.	Control	26

*Mean of 3 replications.

Text fig.III

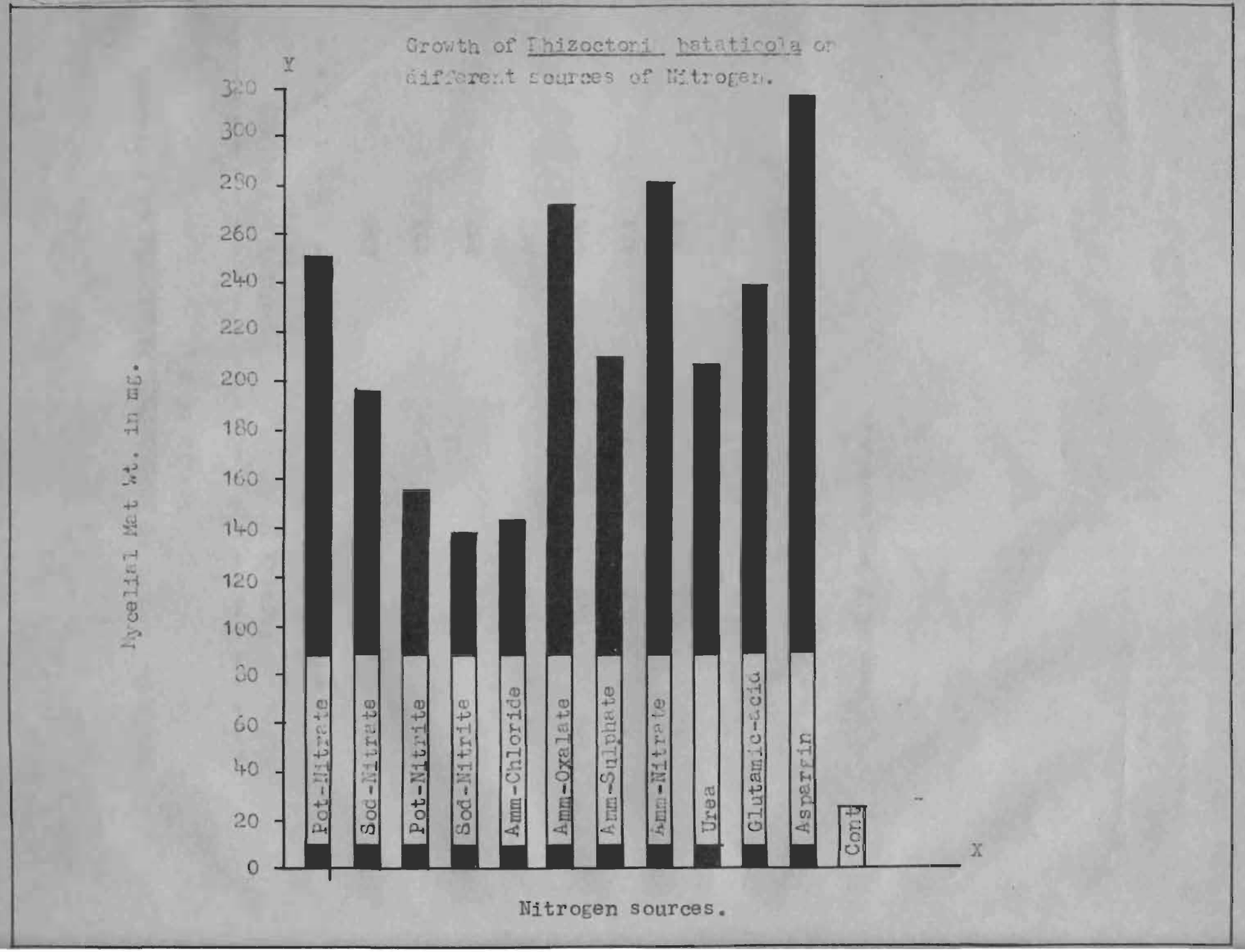
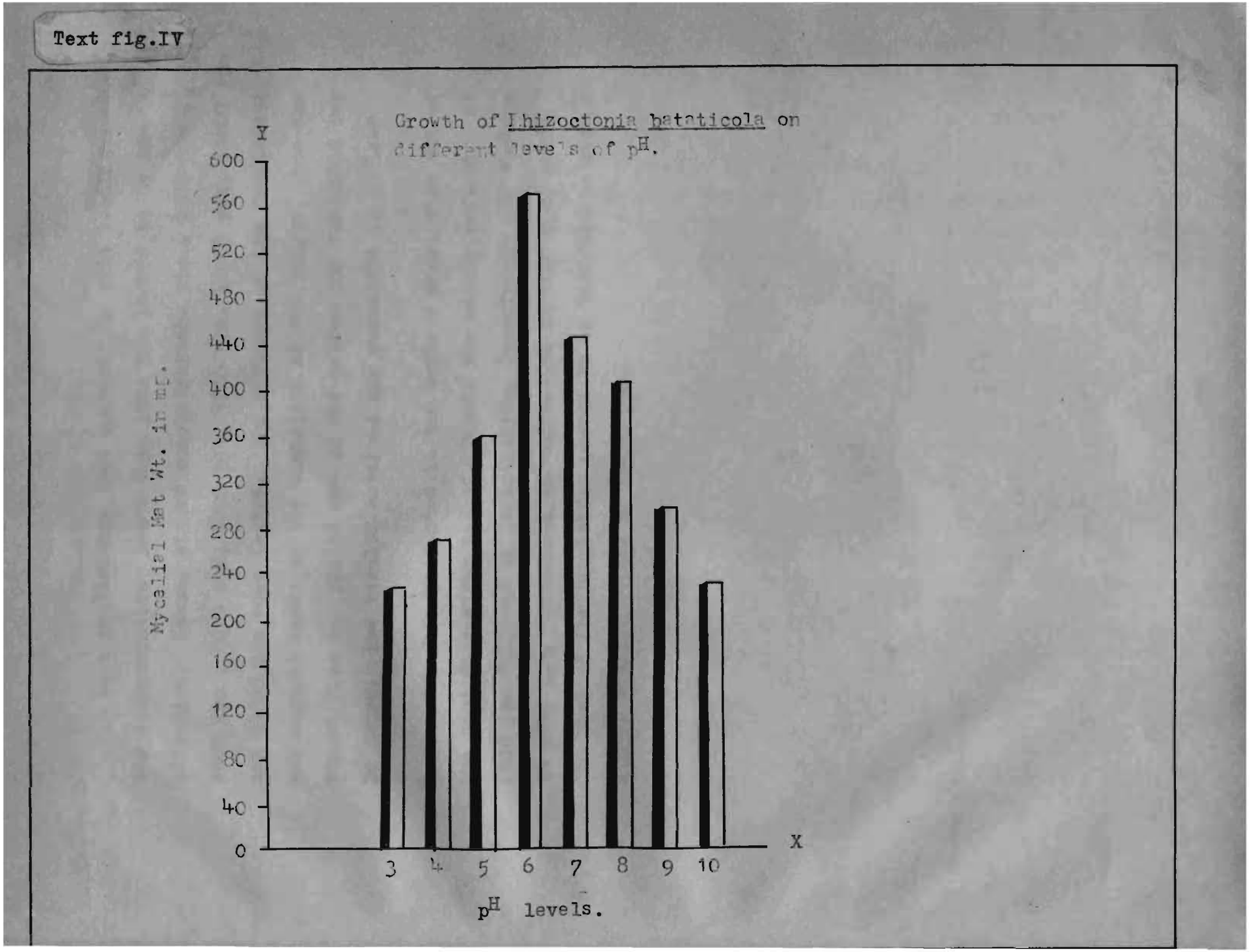

Growth of Rhizoctoni bataticola on different sources of Nitrogen.
Y
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
Mycelial Mat Wt. in mg.
Pot-Nitrate
Sod-Nitrate
Pot-Nitrite
Sod-Nitrite
Amm-Chloride
Amm-Oxalate
Amm-Sulphate
Amm-Nitrate
Urea
Glutamic-acid
Asparin
Cont
X
Nitrogen sources.

Table-8. Growth of Rhizoctonia bataticola at different levels of p^H.

S.No.	Initial p^H	*Mycelial mat weight in mg.
1.	3.0	230
2.	4.0	272
3.	5.0	363
4.	6.0	572
5.	7.0	448
6.	8.0	411
7.	9.0	300
8.	10.0	232

*Mean of 3 replications.

Text fig.IV


Growth of Rhizoctonia bataticola on different levels of pH.
Y
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
Mycelial Mat Wt. in mg.
3
4
5
6
7
8
9
10
X
pH levels.

In a preliminary test emphasizing this technique the concentration lower than 1000 ppm proved to be ineffective. Hence, higher concentrations viz., 1000 ppm , 2000 ppm , 3000 ppm, 5000 ppm, 7000 ppm, 10000 ppm, 15000 ppm and 20000 ppm were tested. The efficacy of the fungicides was assayed based on the viability of the fungus, when the fungal disc was plated out on the medium and incubated for 24 hours. The results obtained are presented in Table-9.

The data presented in the Table-9 shows that out of the nine fungicides tested, Topsin was highly effective at 1000 ppm followed by Formaldehyde, Aureofungin and Benlate at 3000 ppm. Brassical was effective at 7000 ppm, while the rest of the fungicides proved to be ineffective even at higher concentrations of 20000 ppm.

Table-9. Efficacy of various Fungicides at different concentrations (in ppm) as assayed by soil vial technique against *Rhizoctonia bataticola*.

S.No.	Fungicide tested	1,000	2,000	3,000	4,000	5,000	7,000	10,000	15,000	20,000
1.	Aurecfungin	+	+	-	-	-	-	-	-	-
2.	Benlate	+	+	-	-	-	-	-	-	-
3.	Brassicol (PCNB)	+	+	+	+	+	-	-	-	-
4.	Blitox	+	+	+	+	+	+	+	+	+
5.	Captan	+	+	+	+	+	+	+	+	+
6.	Formaldehyde	+	+	-	-	-	-	-	-	-
7.	Topsin	-	-	-	-	-	-	-	-	-
8.	Thiram	+	+	+	+	+	+	+	+	+
9.	Zinconidine	+	+	+	+	+	+	+	+	+

+ = Growth

- = No growth.

DISCUSSION

The root and stem rot disease of castor inciting pathogen was critically examined, isolated in pure culture and its pathogenicity was studied on castor. Based on the morphology and the disease symptoms produced by the pathogen on the host the organisms was identified Rhizoctonia bataticola (Taub.) Butl. with Macrophomina phaseoli (Maubl.) Ashby as its pycnidial state.

R. bataticola causing charcoal rot of castor was first reported from Poona by Uppal (1934) and latter it was recorded from Sind by Prasad (1944), from Bihar by Thirumalachar (1953) and from Mahabaleshwar by Kulkarni et al.,(1966). According to Lele and Pathak (1965) a severe drought, high temperature and delayed monsoon rains preceded the outbreak of the disease. In the present study severe incidence of the disease was noticed on "Aruna" and other castor varieties grown in "Kharif" 1973 in the fields of Agricultural Research Institute and Agricultural College Farm, Rajendranagar which was preceded by severe drought and high summer temperature experienced at Hyderabad. These observations regarding disease incidence confirmed the observations made by Lele and Pathak (1965).

Taxanomy and Nomenclature of the pathogen:

The pathogen produced sclerotial as well as pycnidial bodies in the diseased host tissues. The sclerotial state was described by Taubenhorst (1913, Phytopathology 3: 164),

as Sclerotium bataticola. Latter Butler (1925, Briton Jones Bull. Minniat. Agric. Expt. 42: 65), transferred the species to the genus Rhizoctonia D C. (1815, Flore. Fr. 6: 110). The genus Rhizoctonia was recognised by Frion as Rhizoctonia D C ex Frion (1822, Syst. myc. 3: 265). The pycnidial stage of R.bataticola was described by Maublanc (1905, Bull. Soc. Myc. France 21: 90) as Macrophoma phaseoli Maublanc. Ashby (1927, Trans. Br. mycol. Soc. 12: 141-147) transferred the species to genus Macrophomina established by Petrak and made a new combination as Macrophomina phaseoli (Maubl.) Ashby.

Thirumalachar (1953) discussed the nomenclature of the pycnidial stage of the charcoal rot inciting fungus and stated that it is in a state of confusion. Although Ashby (1927) and Young (1949, Texas. Agr. Expt. Sta. Bull. 712: 18-33) have reviewed the nomenclature of this fungus earlier and pointed out that there is no need for frequent changes in the name of this well known pathogen, Thirumalachar (1953) transferred the species Macrophomina phaseoli to the genus Botryodiplodia and made a new combination as Botryodiplodia phaseoli (Maubl.) Thirum. He proposed this new combination based on his observations of the hyaline amerosporae contained in the pycnidia becoming one septate, coloured spores, after incubation in moist chamber for 12-24 hours, characteristic of the conidia of Botryodiplodia. Moreover, Thirumalachar, without examining the type material of Macrophoma phaseoli Maublanc transferred the species to Botryodiplodia and hence his proposal for new combination does not appear to be sound.

Morphology of the pathogen:

The isolated organism Rhizoctonia bataticola (Taub.) Butl., rapidly formed luxuriant and fast growing colonies at first hyaline and finally intense carbonaceous black mycelium showing concentric zonations covering the whole petri plate on the third day itself. The sclerotia developed were few and irregularly grouped after four days in the medium and on the inner edges of the petri plates with the ageing of the culture. The hyphae are persistant, funaceous, broad, septate and branched parallel to the parent hyphae with a constriction at the point of union . Cells of older branches turned barrel shaped. Mature hyphae repeatedly divided giving rise to masses of irregular structures which formed sclerotial bodies and does not produce any pycnidia in the culture. Due to above mentioned morphological characters the pathogen can be included in the sub-species macrorica as reported similarly by Reichert and Hollinger (1947).

Thus the results obtained confirmed the observations made earlier by Lele and Pathak (1965) Godiarih and Carnici (1946), Luthra and Vasudeva (1938) and Thirumulachar (1953), Raut and Bhombe (1965).

Pathogenicity studies:

Studies regarding the pathogenicity test show that castor plants were highly susceptible to this disease at 20% inoculum potential by soil-oats inoculum method, at post emergence stage after 5 weeks of germination, of castor seedling.

and 100 percent mortality was recorded. It is significant to note that along with sclerotia, black pin head like pycnidia were produced externally on the bark. Pycnidia were macroscopic erumpent globose to sub-globose.

The results obtained are in accordance with the work done previously by Thirumulachar (1953) and Charles Hodges (1963).

However the method of stem tops inoculation adopted was a failure in this case.

Schmithinner and Schonfelt (1962) reported that in soil-oats inoculum potential method, the principle involved was multiplying the inoculum in flasks and incorporating the same in pots containing sterilized soil and incubating for about 48 hours before sowing. Contact between seedling roots and inoculum can be insured without injury to the roots, and the roots of emerging plants grow through the inoculum and become vulnerable to infection. Bateman (1963) reported that probably the polygolacturonase secreted by the pathogen in association with host roots become more active and exert a maserating effect on host tissue, thereby gaining entrance into the host resulting in further spread of the pathogen.

Fulton and Hansen (1960) reported that root reserves are largely consumed during the long winter dormancy, so that the plants are less able to resist the attack by the pathogen and the disease they incite develop very slowly under conditions favourable to host development.

Numerous papers published regarding the pathogenicity of R. bataticola on different hosts indicate that the pathogenicity of an organism is correlated with its ability to produce in Vitro., pectic enzymes and utilise pectic substances. (Barker and Walker, 1962, Waggoner and Dimond, 1955; Leal and Villanueva, 1962; Singh and Hussain 1964).

The results obtained in the present studies with some modifications with respect to pathogenicity test by soil-oats inoculum method revealed that it was the best method compared to the stem tape inoculation method. Similar results were obtained by Schimithinner and Hilty (1962) while studying the post emergence seedling root rot of forage legumes with Rhizoctonia sp., and Aphanomyces sp., and Bateman (1963) while studying the macerating enzyme of R. Solani.

Varietal Reaction:

There appears to be no information regarding the varietal resistance to root and stem rot of castor caused by R. bataticola in the literature. In the present investigation nine varieties of castor were tested for the varietal resistance and out of them only two varieties were found to be somewhat resistant viz., B-157 and 6-219-22. However, intensive studies on screening for disease resistance are required before concluding their resistance to the pathogen.

Timonin (1940) noted higher number of bacteria, and to less extent fungi, in the case of susceptible variety than

in the case of resistant varieties. These observations were made by him both in green house and field tests with flax susceptible to Wilt (Fusarium lini) and with tobacco susceptible to black root rot (Thielaviopsis basicola). He attributed this variation in the host susceptibility to inherent differences in physiological functioning of the plant roots. Some workers attributed the difference between the varieties to the excretion of certain toxic substances by the resistant varieties. Timonin (1940 a) and (1940 b) studied the root excretion of flax plant in relation to the resistance and susceptibility to Fusarial wilt and he found the presence of hydrocyanic acid in greater amounts in the resistant plants than in the susceptible. Harper (1950, 1950 a) working on Panama disease of banana, presented evidence to show that differences in microbial numbers between resistant and susceptible varieties were due to the presence of fungistatic organisms in greater numbers in the rhizosphere of resistant plants.

Hence, the resistance of the two castor varieties viz., B-157 and 6-219-22, could be attributed to the physiological functioning of the plant roots, differences in the rhizosphere microflora of the variety and also their root exudates liberated in the rhizosphere.

Isolation and screening of antagonists:

Soil dilution technique gave greater facility to sporulating fungi than to non sporulating ones. Nevertheless,

the sporulating fungi have a decided advantage in getting quickly established in the soil over non sporulating ones and in this way, would profusely influence the establishment of Rhizoctonia population in the soil. The presence of various common genera and species occurring in the soil affected with Rhizoctonia sp., have been reported by many workers, Abraham et al.,(1966) in their studies on soil and kernel of groundnut fields.

Thus the results obtained in the present study for the isolation and screening of antagonists are in confirmation with the results obtained by the above workers (Loc.cit.)

Out of eight isolated mycoflora four were found to be antagonistic to R. bataticola in the present studies, which were identified as Myrotheciun sp., Trichoderma sp., Aspergillus flavus, A.niger, similarly Brooks and Fiadale (1948) Hanseler and Allen (1934), Weindling (1932) and Vasudeva and Sikka (1940), reported that Trichoderma sp., and Aspergillus sp., are antagonistic to R. bataticola.

The width of the clear zone had long been considered to be an index of the antagonistic potential of the inhibitory organisms and also a measure of antibiosis between the test fungus and the antagonists. Thus it has been noticed that wider the zone the organism produces, the stronger will be its antagonistic activity. It has been found that with the test fungus R. bataticola the fungi produced distinct inhibitory zones in order mentioned (Vide Table-3).

Studies on the growth of R. bataticola on staled solid medium with hot sterilized culture filtrate of antagonists gave results that toxins are destroyed by heating and showed a little inhibitory effect and restrict the growth of the test organism. From the results obtained (Vide Table-4) it is evident that Trichoderma sp., showed maximum inhibitory effect followed by Myrothecium sp., A. niger, and A.flavus. The results showed that the inhibitory effect is not so much by mixing the cultural filtrates after autoclaving the filtrates and incorporating in the medium and growing the test fungus on the medium.

Similar results were obtained earlier by Haneeler and Allen (1934) while making studies on toxic action of Trichoderma sp., on Rhizoctonia sp., and other soil fungi.

The results obtained in the present studies on the microbial antagonism to R. bataticola, are interesting in that they indicate a possibility of utilising the various antagonists isolated, in the biological control of the fungus in the field.

Physiology of the pathogen:

The suitability of different sources of carbon on the growth of R. bataticola was studied. All the monosaccharides, Disaccharides and Polysaccharides were found to be good sources of carbon for the growth of the fungus. No growth was obtained with both the organic acids viz., Citric and Tartaric acids.

From the results it is clear that among polysaccharides Dextrin followed by starch are the best sources of carbon. The other sources of carbon such as Sucrose followed by Maltose, Glucose, Sorbitol , Mannitol and Fructose supported good growth.

In general all the carbon sources were utilised by the fungus. The results are in accordance with the work of Vasudeva (1937).

Nitrogen is an essential element used by the fungi for functional as well as structural purposes (Lilly and Barnett, 1951 a). Fungi generally grow and sporulate better on organic nitrogen than on inorganic (Cochrane, 1958). But from the results obtained (Vide Table-7), it is evident that inorganic nitrogen source of nitrogen were equally utilised by the fungus, except ammonium chloride and ammonium sulphate. Asparagine an organic nitrogen source and ammonium oxlate and ammonium nitrate, the inorganic nitrogen source, have supported best growth. Glutamic acid and urea are also good sources of carbon for the growth of P. bataticola.

In general all the sources of nitrogen tested are utilised well by the fungus. The results obtained are in accordance with the work of Vasudeva (1937). As the fungus utilised all the sources of nitrogen it can be grouped under Group 'A' of Robbins classification (1937).

Regarding the p^H requirement of the fungus (vide results Table-8) good growth was obtained in the p^H range of 3-9 there was a sudden fall in growth at p^H 10.0 with fairly good growth between p^H. 3-8 and maximum growth at p^H 6.0. Similar results were obtained by Vasudeva (1936).

In the experiment conducted to determine the toxic levels of various fungicides and antibiotic in Vitro. (vide results Table-9). It was found that Topsin was highly effective among the different fungicides used. It was found to inhibit the growth of the mycelium at 1000 ppm . Aureofungin, Formaldehyde and Benlate were next to Topsin and inhibited the growth of mycelium at 3000 ppm. Brassical (PCNB) was found to be effective only at 7000 ppm concentration. The rest were not effective even at high concentration than the recommended dosages.

Simkover and Schenfelt (1951) while making studies on effect of benzene hexachloride and chloradene on certain soil organism obtained marked inhibition of mycelial growth by sprinkling crude benzene hexachloride on agar slants. The results obtained (vide Table-9) showed that Topsin, a benzene compou d was highly effective to R. bataticola. The results obtained in the present studies are in confirmation with the results obtained by (Loc.cit).

It is quite possible in Vitro., toxicity of fungicides differ from the ability to control the disease in the complex environment in the soil or may also differ due to old samples or more inert material mixed by local distributors in India.

SUMMARY & CONCLUSIONS

The causal organism Rhizoctonia bataticola (Taub.) Butl. was isolated in pure culture. It produced round hyaline colonies which latter turned intense carbonaceous and black colour, producing sclerotia which were few and irregularly grouped. The hyphae are persistant and did not produce any pycnidia.

The pathogenicity of R. bataticola on castor was established by using soil-oats inoculum. The plants were highly susceptible to the disease in post emergence stage on five weeks old seedlings, and suffered cent percent mortality. Sclerotia and pycnidia were produced on the dead shredded stem.

Out of the nine varieties tested for varietal reaction, two varieties viz., B-157 and 6-219-22 were found to be resistant to the root and stem rot caused by R. bataticola.

With regard to the isolation and screening of antagonistic studies eight fungi were isolated, viz., Myrothecium sp, Trichoderma sp, Aspergillus flavus, A. niger, Fusarium sp, Rhizopus sp, Sclerotium sp and Cladosporium sp. Out of these first four fungi were found to be antagonistic to R.bataticola and produce clear zones of inhibition. Wider zones were produced by Trichoderma sp.

From the results (vide Table-4) it was evident that the culture filtrates of four fungi viz., Myrothecium sp, Trichoderma sp, A. flavus, and A. niger, were highly

antagonistic as the growth of R. bataticola was very much retarded. Myrothecium sp, was highly antagonistic as the growth of R. bataticola was very much retarded, followed by the latter three antagonistic fungi.

The optimum incubation period for the best growth of R. bataticola in Richards liquid medium was found to be fourteen days.

Of the various carbon sources tested for the utilisation by R. bataticola, Dextrin followed by Starch was the best source. The other sources of carbon such as Sucrose, Maltose, Glucose, Sorbital Mannitol, Fructose also supported good growth. The fungus could not utilise any of the organic acids viz., Citric and Tartaric acids, tested as sources of carbon.

The suitability of various nitrogen sources for the growth of R. bataticola was tested and it was found that Asparagine an organic source of nitrogen and ammonium oxalate and ammonium nitrate which are the inorganic nitrogen sources have supported best growth. The other nitrogen sources viz., Glutamic acid, Urea were also well utilised.

Maximum growth of R. bataticola was obtained at p^H 6.0 followed by p^H 7.0 and sudden fall in growth at p^H 10.0 in the Richards liquid medium.

Among nine fungicides tested in Vitro., to find out their efficacy on the growth of the pathogen, Topsin was

found to be the most effective fungicidal followed by Formaldehyde, Benlate Aureofungin and Bronicol (PCNB). The rest of the fungicides were ineffective even at the higher concentrations of the recommended dosages.

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