STUDIES ON A TOMATO FRUIT ROT CAUSED BY CLADOSPORIUM SP.

A

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MASTER OF SCIENCE IN AGRICULTURE

(MYCOLOGY AND PLANT PATHOLOGY)

BY

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THESIS ADVISOR — DR. A. NARAIN

DEDICATED TO MY MOTHER

C E R T I F I C A T E

This is to certify that the thesis entitled " STUDIES ON A TOMATO FRUIT ROT CAUSED BY CLADOSPORIUM SP." submitted in partial fulfilment of the requirements for the award of the degree of MASTER OF SCIENCE (AGRICULTURE) of the Orissa University of Agriculture and Technology, Bhubaneswar, is a faithful record of bonafide research work carried out by Sri Gagan Bihari Rout under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

The help and information as has been availed during the course of this investigation has been duly acknowledged by him.

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The 3rd September, 1979.

C O N T E N T S

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CHAPTER-I
INTRODUCTION

INTRODUCTION

Tomato is one of the most popular and nutritious vegetable crop grown through out the world. It is generally grown in almost all the home gardens and in a large percentage of market gardens and by Truck growers. As a processing vegetable crop it assumes first rank among all the other vegetables.

Few products attain to themselves such a great variety of uses in general as does tomato. It is one of the most common salad vegetables and is also used to prepare soups, pickles, catchups, sauces, and other miscellaneous products. From the nutritional point of view, tomato fruits are well known as a source of enriched vitamins, ascorbic acid and minerals.

The native home of tomato is Central America and South America where it was well known and highly prized prior to the discovery of America. Thus its use is very ancient. According to Sturtevant (1919) and Mc Cue (1952), the earliest mention of its use was by Mathhiolus in Italy in 1544. It was known in Germany, France, and other European countries prior to 1600. The first reference to the use of tomato for culinary purposes was introduced in the United States by Jefferson in the year 1781.

Tomato is generally considered to be an important vegetable crop of tropical climate which requires a long season to produce profitable yields. Usually it is considered that better tomato crop can be raised when the soil pH is relatively lower than it being higher. The yields may be increased when the pH ranges between 5 - 5.5. Experimental evidence has even revealed that yields may be increased to an extent of 4.2 tons of fruits when liming with 3 tons dolomite lime stone per acre was incorporated in to Fulton loam soil which had the congenial pH of 4.8 (Sayre,1947).

Like many other vegetable crops tomato plants are also parasitised by a number of phytopathogenic micro-organisms belonging to each category e.g. fungi, bacteria and viruses. Under ideal conditions of nutritional supply in the soil and optimal environmental factors most tomato varieties grow profusely with extensive vegetative growth and fruit development, as well. Such factors are also suitable for the multiplication and parasitism of a variety of pathogens which may come in contact to the roots, foliage, shoots or developing or developed fruits. The primary source of inoculum may be soil or the seed. Typical and some most common examples of widely occurring tomato diseases are, early blight, late blight, wilts (fungal or bacterial), damping off, leaf spots, leaf curl, mosaic and fruit rots etc.

Such diseases particularly in tropical countries have been considered to be great limiting factors to minimize the tomato production. Tomato being a delicate vegetable which is easily perishable after harvest when kept in storage or in the process of transit. Several storage fungi e.g., moulds are not uncommon to cause significant deterioration to tomato fruits after being picked from the plants.

During the course of a survey of vegetable diseases in a vegetable garden around the campus of Orissa University of Agriculture & Technology, Bhubaneswar a fruit rot was detected during the month of February in the current year. Since the symptoms expressed only on the green fruits were rather unusual than other common tomato diseases prevalent in this region it drew the attention so as to pursue systematic investigations. Hence the thesis problem herewith dealt was chosen to conduct a series of investigations relating to etiology, pathogenicity and host range of the pathogen. Furthermore, the causal organism which is a fungus was identified, its morphological characters were studied, fungus after being isolated in pure culture was grown on selected nutrient media so as to determine the best nutritional source for its growth and sporulation. Effects of few carbon and nitrogen, sources were determined on the degree

of growth and sporulation of fungus which was found to be associated with the tomato fruit rot. The influence of temperature and pH on growth, sporulation and conidial germination of the fungus was also determined in view to learn their optimum levels.

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CHAPTER-II
REVIEW OF LITERATURE

REVIEW AND LITERATURE

Tomato plants and fruits in a particular area are known to be parasitized by a large number of fungal and other diseases. Due to these, heavy losses occur generally in the crops which are grown particularly in tropical countries. Even the earliest literature concerned with an account of tomato diseases and their control in the journals of plant pathology has made reference to some important tomato diseases. Since the problem dealt in this thesis relates to a dry rot disease, only a selected review from the available literature is included concerning the disease incited by Cladosporium species. Almost all the previous workers in the past have found an association of C.fulvum with tomato, moreover, different common names have been given to the disease in various countries. During the course when literature was reviewed nowhere an account of C.tenuissimum, the fungus dealt in this thesis could be found. Hence this review consists of an account of other species of Cladosporium, parasitic on tomato.

DISTRIBUTION

Webber (1922) described that the principal disease of tomato is the rust of tomato. This disease is caused by Cladosporium fulvum and it was observed in Denmark.

Bewley (1922) gave a short description of chief tomato diseases in his paper "Tomato diseases" in England. Out of these diseases mildew of tomato caused by Cladosporium fulvum was one.

Hansford recorded the leaf mould disease of tomato caused by Cladosporium fulvum in 1923.

Adam (1935) recorded the occurrence of tomato leaf mould, a disease of glass house grown tomatoes caused by Cladosporium fulvum in south Australia. Besides, he also gave a popular account of disease and its control.

Kochman (1935) gave a brief and popular account of morphology and biology of Cladosporium fulvum Cooke which was first time recorded in Poland in 1930. Since then it has been observed some times even in epidemic form at several localities of that country both in glass houses and in field, thus causing serious damage to the crop.

Wit (1977) found by utilizing a light and scanning electron microscopic study that infection of tomato plants was caused by virulent and avirulent races of Cladosporium fulvum in Netherland.

Mac' (1977) in Kentucky (USA) studied the reaction of 23 cultivars of cucumber against a scab causing pathogen, Cladosporium cucumerinum. Presence of spore agglutinins was also investigated.

RACES AND PHYSIOLOGICAL SPECIALIZATION:

Cirulli (1968) reported the physiological specialization of Cladosporium fulvum in Italy. He described the racial composition of 43 isolates from green house grown tomatoes from different regions. Monofactorial races (0,1,3) were found in 28 isolates and mixture of two monofactorial races in 15 isolates.

Boukema (1978) identified a new physiological race of Cladosporium fulvum in tomato and made considerations in the manupulations of resistance genes. Race D of Cladosporium fulvum (Fulvia fulva), originating in Belgium was found to break resistance of gene cf_5. Degree of resistance was studied in response to several genes.

Leski (1979) identified races of C.fulvum,parasitic on tomato which were grown in Poland. Reactions of tomato varieties to several races viz. 1.4 and some from Netherlands was tested.

MORPHOLOGY:

Spangler (1924) carried out cultural and biological studies of the tomato leaf mould fungus, Cladosporium fulvum. In culture the fungus formed numerous small, almost spherical spores as well as the typical bicellular ones. Both kinds germinated rapidly and gave rise to similar mycelia which in both cases bear branching conidiophores on which spherical

continious conidia were formed, inaddition to the elongated ones, with one or less often, 2 septa. The conidia were in branching chains, connected by short isthmi and sometimes as many as six chains of the round and small oval type developed from a single, large, elliptical conidium. The conidia germinated rapidly after being kept dry during 4th January to 1st May. The minimum temperature during this period being 20°C. It is possible that the larger bicellular spores were really fragments of modified conidiophores.

ENVIRONMENTAL FACTORS FOR DISEASE DEVELOPMENT:

Taylor (1924) found the suitable conditions for disease development. He reported that tomato mildew (Cladosporium fulvum) was essentially a glass house disease favoured by insufficient ventilation, high temperature and a staturated atmosphere.

Poser (1924) reported that at Pillnitz (Saxony) tomatoes growing in a green house were attacked by Cladosporium fulvum chiefly during the abnormally hot weather in early July.

Brown (1926) determined that the disease was more prevelent during hot weather and abundant rain, especially when it causes cracking of fruits.

Winspear, Pstlethwaite and Cotton (1970) determined that high humidity favoured the development of Cladosporium fulvum. Environments both physically and biologically different were obtained with humidistats at 90 and 75% relative humidity in glass house maintaining two temperature regimes. Less infection occurred on tomatoes grown at a constant temperature of 20°C.

SYMPTOMS:

According to Brown (1926) a stem end and center rot has caused heavy losses in consignments of greenhouse tomatoes grown in Texas and Nebraska during recent years. The rusts usually started when the fruits were usually green, but it was usually noticiable until they began to turn colour. When it showed small brown spots or a thin browning round the stem end, or the fruit may simply become a sickly colour. When cut open the placentae were found partly or entirely decayed, and the diseased portions became hard brown or in ripen fruit an almost black mass which could be separated intact from the surrounding tissues. The fungus Cladosporium sp. was inoculated to green house tomatoes and it showed the stem end and centre rots. This give positive results only after repeated trials. Green tomatoes were favourable than ripen tomatoes owing to the lower acidity thus favouring the development of the disease.

MODE OF INFECTION:

Wit, (1977) reported that t when tomato cultivars carrying the cf_2 gene susceptible to races 1,2,3 and immune to race 4 of(Cladosporium fulvum) and the cf_4 gene (Immune to races 1,2,3 and susceptible to race 4), were inoculated with these races, no differences were observed in growth between compatible and incompatible combinations during germination, subsequent formation of runner hyphae and stomatal penetration. Runner hyphae did not show directional growth towards stomata. Penetration usually occurred on the 3rd and 4th day of inoculation. In compatible combinations the fungus grew intercellularly,often in close contact with spongy mesophyll cells. Among incompatible combinations fungal growth was arrested 1-2 days after penetration and confined to stomata and surrounding cells.

Salmon and Ware (1936) reported that the primary infection of Cladosporium fulvum was apparent in the cotyledons of recently potted tomatoes at Wye and the evidence indicated that the soil was the primary source of infection.

Spurr (1977) reported that C.cladosporoides (Fresen) de Vries as a second pathogen to Alternaria in frequency as an endophyte on leaf tissues of grape, prwit, sweet gum, sycamore,tomato, tobacco,squash soybeans and peanuts in north carolina.Parasitism on tobacco was studied. Parasitism was

favoured at 21°C and high humidity. Germination was 50% in water of 100% in 0.1% Tween 80 in 24 hours.

Both stomatal and direct penetration of tobacco leaf tissues were observed. Mycelium grew onleaf surface as well as in mesophyll tissues of leaves. Conidia were produced on leaves 3.7 days after inoculation. The fungus was considered a common and early established enophytic parasite of tobacco.

SPORE PRODUCTION

Harvey (1970) studied the spore production in Cladosporium. With the tests conducted on the cultures of 6 Cladosporium spp. many more spores were released in wet (mid laden) than in dry air. Spore numbers were intermediate in humid air.

BIOCHEMICAL RESISTANCE

Dow, Callow (1979) determined a possible role for α-tomatine in the varietal specific resistance of tomato to Cladosporium fulvum. By an assay of hyphal extension the glycoalkaloid was shown to be toxic to Cladosporium fulvum (Fulvia fulva). The degree of toxicity depended on pH and nutrient status of the assay medium. Tomatine exerted both fungistatic and fungicidal effects and caused an irreversible leakage of electrolytes from hyphae.

CONTROL MEASURES

A number of workers have attempted to control the disease of tomato, caused by Cladosporium fulvum by using fungicides.

Ebben (1969), applied Benomyl as a soil drench which reduced the disease incidence, however, some degree of phytotoxicity was also caused by this compound. Similarly, importance of Benomyl and one of its derivatives, B.C.M. to control the disease by soil drench application was mentioned by Locke and Green (1973).

Kankam (1973), evaluated two Maneb fungicides viz. Dithane M-45 and Dithane Z-78 which appreciably controlled the disease on tomato.

Jones (1974), reported that Maneb, Zinc and copper formulations and cupric hydroxide alone controlled the disease, however, the latter chemical caused some phytotoxicity.

Cirnuli and Montemurro (1979) determined that few systemic fungicides viz, Thiophorate methyl, Benomyl and Triforine completely checked the fungal sporulation in Cladosporium fulvum when the chemicals were applied as soil drench or as leaf spray. Fungitoxic activity was reported by translocation from leaf to leaf by Thiophorate methyl

(40-70g/100ml) which controlled the disease completely. however, Benomyl at 60g/100ml controlled the disease. Similarly, Triforine (70-100g/100ml) gave a partial control and Triadimefom gave no control by such translocation.

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CHAPTER-III
MATERIALS AND METHODS

MATERIALS AND METHODS

The causal organism of the dry rot disease of tomato under investigation was isolated from an extensively infected raw tomato fruit , collected from a kitchen garden of Bhubaneswar at the University campus. The diseased fruits were collected from vigorously growing tomato plants. They were kept overnight under moist chamber. The microscopic examination of the diseased portion of tomato fruit revealed the presence of characteristic mycelium and conidia of a fungus, which was identified as a species of Cladosporium . It was not possible to identify exactly the species of fungus, hence a pure culture slant on PDA was sent for its identification to C.M.I. , England.

ISOLATION OF THE PATHOGEN:

Small samples of diseased tomato fruit tissue were removed by gently cutting out with a clean blade and washed in distilled water. Small pieces of diseased tissue, preferably from the margin of the indiscriminately developing fresh diseased area including both apparently healthy and diseased tissues were dipped in 1:1000 mercuric chloride solution for about 2 minutes. This was done to surface sterilize the tissue. After washing thoroughly with sterile

distilled water, the small bits of diseased tissue were aseptically transferred to culture tubes containing sterilized Potato Dextrose Agar. The culture tubes were incubated at room temperature (25± 1°C). After 5 days of inoculation characteristic growth and development of the fungus Cladosporium sp. was observed under microscope. The culture was further purified by single spore isolation.

SINGLE SPORE ISOLATION

A spore suspension was made under aseptic condition in sterile water. The presence of spores in the suspension was ensured by examining a drop of the suspension under the microscope. One loopful of the suspension was transferred to a culture tube containing 15 ml of sterilized water agar medium and then the culture tube was shaken several times to get a uniform spore suspension. One loopful of suspension from this tube was transferred to the second tube of melted water agar and like wise several dilution plates were prepared by pouring the melted medium from tubes to sterilized petridishes. All the petridishes were incubated at room temperature (25± 1°C). After 12 hours of incubation a petridish with its lid off was examined under microscope and few germinated spores were marked. From the petridish few

germinating and isolated spores were located which were marked with a glass marking pencil. A germinating spore with medium was transferred in to a Potato Dextrose Agar slant with the help of an inoculating needle. These culture tubes were incubated at the room temperature. After 4 days the growth was visible. The characteristic mycelium and spores were examined under microscope. Thus a single spore pure culture was obtained.

MORPHOLOGY:

In order to study the morphological characteristics of the fungus, a small portion of fungal mass growing vigorously in culture was mounted on the surface of a slide and examined under microscope.

After properly adjusting under microscope the measurements of the size of conida were taken with the help of an ocular micrometer. The value of each ocular division was calibrated with a stage micrometer which was 3.33 μ. under high power. With the help of camera lucida spores were drawn.

PATHOGENICITY:

The pathogenicity of causal organism, namely, Cladosporium sp. was proved both in vitro and in vivo.

For this purpose a 14 day old pure culture was used. For testing in vitro the fresh and healthy green and ripe tomato fruits were first thoroughly washed in water and then dipped in 1:1000 mercuric chloride solution for 2 minutes. Finally, they were washed in sterile water several times so as to remove the traces of surface disinfectant.

The fruits were slightly injured by making minute pin pricks and placed inside a sterile petriplate. On the surface of injured fruits a drop of heavy spore suspension of fungus was placed. The spore suspension was made from a PDA culture slant suspended in sterile water. In view to provide high moisture for initiating the infection process a wet cotton swab was placed adjacent to the inoculated tomato fruit inside the petriplate. It was covered with a clean beljar and incubated at room temperature. For comparison, a fresh raw and ripe tomato fruit was similarly placed which was left uninoculated.

In order to establish the pathogenicity of Cladosporium sp. on the fruits attached to the living tomato plants under natural field conditions, another experiment was conducted. Same conidial suspension of Cladosporium sp. was used. After sterilizing with 1:1000 mercuric chloride and washing several times with sterile water, the previously injured fruits on plants were similarly inoculated with the

same inoculum as described above. The artificially inoculated green fruits were covered with perforated polythene bags inside which wet cotton was placed,in order to provide moisture. In the similar manner few tomato leaves of experimental plants were also inoculated. After a week the inoculated tomato fruits were examined for infection. Typical disease symptoms were observed on the artificially inoculated tomato fruits as in nature. After the development of disease in vitro and in vivo, it was compared with the naturally infected fruits. The pathogen was reisolated in pure culture on PDA. Its morphological and cultural characteristics were compared to the previously maintained culture which was obtained from the naturally infected tomato fruits.

Although under the natural conditions none of the tomato plants showed the occurrence of disease on foliage as on the fruits; it was considered desirable to determine if the fungus is capable of inciting the disease on foliage if artificially parasitized. For leaf inoculation also the same procedure was followed as described above. After 5 - 7 days, the leaf samples were also examined microscopically, for the presence of fungus.

SPORE GERMINATION:

The conidial germination of fungus Cladosporium sp. was examined in water by using cavity slide. A conidial

suspension in sterile water was prepared from a 10 day old sporulating culture grown on PDA. A sterilized cavity slide was placed inside sterile petriplate, fitted with a moist filter paper so as to provide humid condition. A 0.1 ml of conidial suspension was transferred to the depression of cavity slide. It was incubated at 25± 1°C. Observations on conidial germination were regularly made at the intervals of 4, 8 and 12 hours under high power of the microscope. The number of germinated and ungerminated conidia were detected under several microscopic fields and the average number estimated.

STUDIES ON SOME FACTORS INFLUENCING SPORE GERMINATION

(a) Effect of pH. on germination:-

To determine the effect of Hydrogen-ion concentration on conidial germination, the conidia were germinated at different pH, ranging from 2 to 8. The pH of distilled water was adjusted by a Beckman pH meter using N/10 HCL and NaOH buffers. The number of germinated conidia were counted microscopically after incubating at 25± 1°C for 4 and 8 hour intervals.

(b) Effect of Carbohydrates on germination:-

In view to find the effect of certain carbohydrates on spore germination, one percent solutions of different sources of carbon such as, glucose, dextrose, sucrose, maltose,

mannitol, fructose, lactose and starch were prepared by dissolving one gram of each solute in 100 ml of sterile distilled water. Conidial suspensions were made with the above solutions and a 0.1 ml portion of spore suspension from each test sample was filled in the depression of cavity slides. These were incubated at 25± 1°C inside a B.O.D. Incubator. The percentage of conidial germination was estimated at 4,8 and 12 hour intervals.

(c) Effect of Temperature on germination:-

With an objective of determining the optimum temperature required for conidial germination an experiment was carried out. The conidial suspension of Cladosporium sp. prepared as described earlier from an actively growing PDA slant was also used for this investigation. Effect of 5 temperatures viz, 10°C , 20°C, 25°C, 30°C and 35°C was determined. A drop of conidial suspension was placed on cavity slide which was placed within petriplate moist chamber. At the desired temperatures they were incubated within B.O.D. Incubators and the degree of germination was determined at 4,8 and 12 hour intervals.

GROWTH OF FUNGUS ON NUTRIENT MEDIA

To study the nutritional requirement for the growth of fungus, some common solid and liquid nutrient media were used. The method described by the Riker and Riker (1936) was

followed with slight modifications, whereever necessary. The composition and procedure for the preparation of different media are as follows:

(a) Potato Dextrose Agar:-

Peeled and sliced potato	.. 200 gms.
Dextrose	.. 20 gms.
Agar Agar	.. 20 gms.
Distilled water	.. 1000 ml.

(b) Czapek's Agar Medium:-

Sodium Nitrate	.. 2 gms.
Potassium Phosphate (dibasic)	.. 1 gm.
Potassium chloride	.. 0.5 gms.
Ferrous sulphate	.. 0.01 gm.
Magnesium sulphate	.. 0.5 gms.
Sucrose	.. 30 gms.
Agar Agar	.. 20 gms.
Distilled water	.. 1000 ml.

(c) Tomato Fruit Extract:-

Peeled tomato (Ripe)	.. 100 gms.
Dextrose	.. 20 gms.
Agar Agar	.. 20 gms.
Distilled water	.. 1000 ml.

(d) Tomato Leaf Extract Agar Medium:-

Tomato leaves (green)	..	100 gms.
Dextrose	..	20 gms.
Agar Agar	..	20 gms.
Distilled water	..	1000 ml.

(e) Richard's Agar Medium:-

Potassium Nitrate	..	10 gms.
Potassium Phosphate (Monobasic)	..	5 gms.
Magnesium sulphate	..	2.5 gms.
Ferric Chloride	..	0.02 gms.
Sucrose	..	50 gms.
Agar Agar	..	20 gms.
Distilled water	..	1000 ml.

(f) Nutrient Agar Medium:-

Yeast Extract	..	5 gms.
Peptone	..	5 gms.
Asparagine	..	1 gm.
Sucrose	..	20 gms.
Agar Agar	..	20 gms.
Distilled water	..	1000 ml.

All the ingredients of each medium in desired quantities were mixed and boiled. In case of solid medium

agar agar was melted in 500 ml water and rest of ingredients were mixed in another 500 ml of distilled water. Both were mixed and the final volume was made up to one litre.

In case of liquid media except agar agar all the other ingredients were mixed together in one litre of distilled water to make the desired medium.

STERILIZATION OF SOLID AND LIQUID MEDIA:

For solid media 15 ml portion was poured into culture tubes while they were in melted conditions. The pouring of the liquid medium to the culture tubes was done by funnels keeping it over a funnel stand. The culture tubes containing the media were adequately plugged with dry non-absorbant cotton and sterilized at 15 lbs pressure for 30 minutes in an autoclave. Media in tubes were stored in a Refrigerator for its consequent use. When required, the medium in culture tubes was melted in a water bath and poured in petriplates which were previously sterilized at 160°C for 2 hours.

In case of liquid media 50 ml of the medium was poured in to the 100/250 ml Erlenmeyer conical flasks, they were plugged with dry non-absorbant cotton and sterilized as described above.

INOCULATION AND INCUBATION OF CULTURES ON SOLID AND LIQUID MEDIA

The petridishes were poured with the melted nutrient . agar medium aseptically in side a UV light sterilized culture room and the medium was allowed to solidify. With the

help of a sterilized inoculating needle, one loopful inoculum from vigorously growing culture of the fungus on PDA slant was transferred to the surface of agar medium, which was poured previously within the petriplates. Sometimes the inoculum was placed at 3-4 places on the surface of medium within the petriplates. This was done to facilitate the excessive growth and sporulation of fungus within a short time.

For determination of growth on liquid media an equal quantity of inoculum was picked up by means of a sterilized 0.5 mm diameter cork borer from one week old cultures of the fungus previously grown in a petridish. A single such agar disc was transferred into each of the conical flasks containing the requisite nutrient media.

Both the solid and liquid media were kept inside a low temperature B.O.D. Incubator, preferably at 25± 1°C for a specified period of time. For each treatment at least 3 replications were generally used.

DETERMINATION OF GROWTH IN LIQUID MEDIA

Growth of fungus was estimated by growing it on selected nutrient media for a limited period of time and dry weights were evaluated. A 50 ml portion of the medium was poured in 250 ml conical flasks and sterilized in usual manner. The nutrient media used for this investigation were, tomato leaf extract, tomato fruit extract, Richard's ,Czapek's ,

potato dextrose and nutrient. After a period of 14 days of incubation the mycelial mat of the fungus was harvested by filtering through Whatman filter paper (No.1, 11 cm dia). The initial weight of the filter paper was taken by means of a Single pan Analytical Balance before filtration. The mycelial mat with filter paper were dried in a hot air oven at 60°C for about 48 hours and weighed by means of a single pan Analytical balance. The weight of filter paper was substracted from the total weight of filter paper plus mycelial mat so as to get the dry weight of the fungus.

DETERMINATION OF SPORULATION IN LIQUID MEDIA

For determining the degree of sporulation on liquid media used in different experiments, this study was carried out. After 2 weeks of growth, the growing cultures on the liquid media were shaken thoroughly for 30 minutes by means of a shaker to get a homogenous suspension of spores. A 0.5 ml of the homogenized suspension was taken and further diluted in 5 ml of sterile distilled water from each treatment. From this sample a0.1 ml portion was taken on a slide and the average number of spores present in three microscopic fields was counted. The number of spores present was counted and estimated by using the following scale.

No. of spores / Microscopic field		Grade
Nil	...	-
1-10	...	+
11-20	...	++
21-30	...	+++
31-40	...	++++
41-50	...	+++++
51-60 and above	...	++++++

BASAL MEDIUM

For Nutritional studies a synthetic medium was always used. Among the different synthetic media tried for the growth and sporulation of fungus, Richard's medium was found to be the best for the growth of a Cladosporium sp. So in all the cases Richard's medium was taken as a basal medium.

EFFECT OF HYDROGEN ION CONCENTRATION ON GROWTH AND SPORULATION

To study the minimum, optimum and maximum pH requirement of the fungus, Richard's medium was adjusted to different pH levels ranging from 3.0 to 6.0 with the help of a Beckman's pH meter. The Buffers used were N/10 Hcl or NaOH. Fifty ml of the medium was poured in each 100 ml Erlenmeyer flasks. The inoculation and incubation of the organism was done by the above described manner. The dry

mycelial weight was taken after 14 days of incubation. Each treatment was replicated thrice. Observations on the degree of sporulation were taken in the manner described above.

INFLUENCE OF TEMPERATURE ON GROWTH AND SPORULATION.

To study the optimum temperature required for growth and sporulation of fungus, this experiment was conducted. Fifty ml of the Richard's medium was taken in each 100 ml conical flask. Inoculation with fungus was done in the above described manner. For each temperature it was replicated thrice. The inoculated flasks were incubated at different temperatures such as 10°C, 20°C, 25°C, 30°C, which were adequately adjusted in the B.O.D. Incubator. After 14 days of incubation the weights of dry mycelial mat harvested from each treatment were taken. Before harvesting the fungus, spores were counted from each sample. Accordingly the optimum temperature for growth and sporulation was determined.

EFFECT OF DIFFERENT CARBON SOURCES ON GROWTH AND SPORULATION

In view to determine the requirement of carbon source for growth and spore production by the fungus a study was undertaken to grow the fungus on a basal medium (Richard's medium) provided with seven different sugar

sources individually. These were, starch , Mannitol, Sucrose, Lactose, Fructose, Glucose, and Dextrose. Richard's basal medium was used. Sucrose, one of the ingredients of medium was substituted by the different carbon sources as given above, on the basis of equivalent amount of carbon present in each compound.

The quantities of carbon compounds for 100 ml of the medium were determined on the basis of their molecular weights; so as to contain an equivalent of carbon present in 5 grams of sucrose in the following manner.

The molecular weight of sucrose ($C_{12} H_{22} O_{11}$) is 144 + 22 + 176 = 342. So 342 g of sucrose contain 144 g of carbon. Hence 5 gm. of sucrose contain $\frac{144 \times 5}{342}$ = 2.105 g of carbon. Accordingly the quantities of different carbon sources were added to 100 ml of basal medium so as to contain 2.105 g of carbon. Following carbon compounds were taken for the purpose:-

Name of the carbon compounds	Molecular formula	Molecular weight	Weight of carbon compounds in g/100ml of medium
<u>Polysaccharides</u>			
Starch	$C_6H_{10}O_6$	184	5.3794
<u>Hexhydricalcohol</u>			
Mannitol	$C_6H_{14}O_6$	182	5.3209

Disaccharides			
Sucrose	$C_{12}H_{22}O_{11}$	342	5.0000
Lactose	$C_{12}H_{22}O_{11}$	342	5.0000
Hexose sugar			
Fructose	$C_6H_{12}O_6$	180	5.2625
Glucose	$C_6H_{12}O_6$	180	5.2625
Dextrose	$C_6H_{12}O_6$	180	5.2625

After incorporating the desired quantity of sugar to the Richard's basal medium, and sterilization in each flask, same quantity of inoculum was transferred with the help of a sterile cork borer. Source of inoculum was a profusely growing culture of Cladosporium sp. grown in petriplate on PDA. A uniform size agar disc was cut out with the aid of sterilized cork borer, one such disc was placed on the surface of medium within each flask. For each treatment 3 replications were used. The culturers were allowed to grow at 25°C for 10 days. The growth was estimated by previously described dry weight procedure. A record was also made on the degree of spore production with respect to each treatment.

EFFECT OF DIFFERENT NITROGENOUS COMPOUNDS ON GROWTH AND SPORULATION

With a view to determine the best nitrogenous source for growth and sporulation of the fungus, Richard's medium was

again used as basal medium. Its nitrogen source, potassium nitrate was substituted by other selected nitrogenous compounds, such as Urea, Ammonium Chloride, Potassium Nitrate, Ammonium Oxalate, Sodium Nitrate, Ammonium Nitrate and Asparagine. One hundred ml of the basal medium was taken and the amount of different nitrogenous compounds added in 100 ml of the medium was calculated in the following manner.

The molecular weight of KNO_3 is 39+14+48=101. Hence 101 g of KNo_3 contains 14 g of Nitrogen. So 1 g of KNo_3 contains 14/100 = 0.1366 g of nitrogen. Accordingly the quantities of different nitrogenous compounds were added in each 100 ml of basal medium so as to contain 0.1366 g of nitrogen. The following nitrogenous compounds wre taken for the purpose-

Name of the Nitrogenous Compounds	Molecular formula	Molecular weight	Weight of the N.compounds in g/100 ml of the medium
1.Urea	$Co(NH_2)2$	60.00	0.2927
2.Ammonium Chloride	NH_4Cl	53.50	0.5220
3.Potassium Nitrate	KNo_3	101.00	1.0000
4.Ammonium oxalate	$(CooNH_4)2H_2O$	142.12	0.7000
5.Sodium nitrate	N_aNo_3	85.00	0.8293
6.Ammonium nitrate	NH_4No_3	80.00	0.3928
7.Asparagine	$NH_2.Co.CH_2.CH(NH_2)$ $CooH.H_2O$	150.13	0.7350

Each treatment with different nitrogen sources was replicated thrice. Procedures for inoculation, and incubation, were exactly same as described above. Then observations were taken after 10 days and accordingly the best nitrogenous source was determined.

HOST RANGE STUDY

The pathogen, Cladosporium sp. used in the present investigation as mentioned earlier was isolated from the diseased tomato fruits. Hence to determine if it could parasitize other species of plants, this investigation was carried out. The healthy fruits of brinjal, chillies, potato, carrot and beans were used.These were washed in distilled water to remove surface borne dirts. The surface of the fruits was sterilized by 1:1000 mercuric chloride solution for 2 minutes and washed in sterile water for several times. These were minutely injured by making pin-prick injuries. The inoculation was done by placing drops of conditial suspension of Cladosporium sp., which was made in sterile water.Inoculated samples were kept inside the sterilized petridishes. Inorder to supply sufficient moisture water soaked cotton was kept inside the petridishes. These were kept inside the beljars with slight openings for proper ventilation. After one week the observations were taken to record the disease intensity on each host.

EFFECT OF CLADOSPORIUM SP. ON GERMINATION OF DIFFERENT CROP SEEDS

This experiment was conducted to find the effect of fungus on different host seeds. To test this seeds of tomato, brinjal, chilli and cucumber were taken and washed in water. They were kept inside the petridishes which were properly wrapped inside, with moist filter paper to provide moist chamber. A heavy spore suspension was made and the seeds of different crops were dipped inside the spore suspensions for 1 minute and then kept in the petridishes as above mentioned. These inoculated seeds were incubated for one week of 25± 1°C inside the B.O.D. Incubator after which the observations were taken.

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CHAPTER-IV
EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

SYMPTOMS

On naturally growing tomato plants the symptoms of disease were noticed only on the green fruits. At the lower end of fruit, opposite to the stalk a depressed, somewhat sunken and brown coloured lesion was formed. It increased in size gradually and attained a spherical shape. As the disease progressed larger fruit area was covered with the disease lesion which became darker in color, turning greyish black. The surface of diseased fruit became diseased, shrivelled , velvety and with erumpent areas in the middle. Apparently in the regions where profuse sporulation took place the texture of diseased lesion was somewhat velvety and dense. The diseased fruit remained small in size without reaching maturity. With age the parasitized tissue dried up and it was much depressed in comparison to the very small fruit surface which remained green. Within a course of 5-7 days the entire fruit dried up, discolored to black both externally and internally, thus resulting in a dry rot. No other part of tomato i.e. leaves, stem or root exhibited the disease symptoms. However , if one plant showed green fruit infection, others

also were parasitized to a variable degree, giving typical disease symptoms.

PATHOGENICITY

As mentioned earlier, the pathogenic potential of the causal organism was determined both in vivo and vitro. Fruits on experimentally grown tomato plants in pots were suitably inoculated with the fungus, similarly leaves were also inoculated. It was observed that at the infection sites the typical disease symptoms were developed on green fruits which exactly resembled to those observed on naturally infected tomato fruits. The uninoculated fruits in control however, remained completely disease free throughout the experimental period (Fig - 1). The inoculated tomato fruits showed small, round and brown colored lesions after 4-5 days of inoculation. These enlarged in size turning dark greyish brown in color. The diseased surface somewhat shrinked and was depressed. At the end of experimental period the infected tomato fruits dried up without showing an increase in their size.

Unlike the disease-free condition of leaves in nature, the artificially inoculated leaves on the experimental plants however, showed disease symptoms. During the intial stages the leaf tip expressed yellowish-brown discolouration, it extended downwards along the margins. Discrete and small size

Fig.1. Pathogenicity prof of the fungus Cladosporium tenuissimum

1. 15 days after inculation.
2. 7 days after inculation.
3. Control.

lesions were also seen in the intervenial areas of leaves. The neighbouring regions discoloured to yellow in color. The untreated leaves in control remained complitely disease free.

The ripe and green detached tomato fruits which were tested in vitro for confirming the pathogenicity also showed the diagnostic disease symptoms as described above.

From the artificially parasitized fruit as well as leaf tissues abundant typical conidia and branched, septate mycelium of Cladosporium sp. were observed when examined under the microscope. These exactly resembled the characters to the naturally parasitizing fungus. Hence the pathogenic potential of caused organism was thus established; keeping in view the Koch's postulates.

MORPHOLOGY

The microscopic studies of the fungus from affected tissues as well as from pure culture revealed the presence of typical mycelium and conidia. The mycelium was hyaline, branched and septate. Conidia were oval or round, hyaline, mostly one-celled but in very rare cases they were two-celled. (Fig-2)The conidiophores had an appendage at the tip to bear the conidia. The conidia were easily detachable from the conidiophores.

CAMERA LUCIDA DRAWINGS OF CONDIA AND MYCELIA OF CLADOSPORIUM TENUISSIMUM

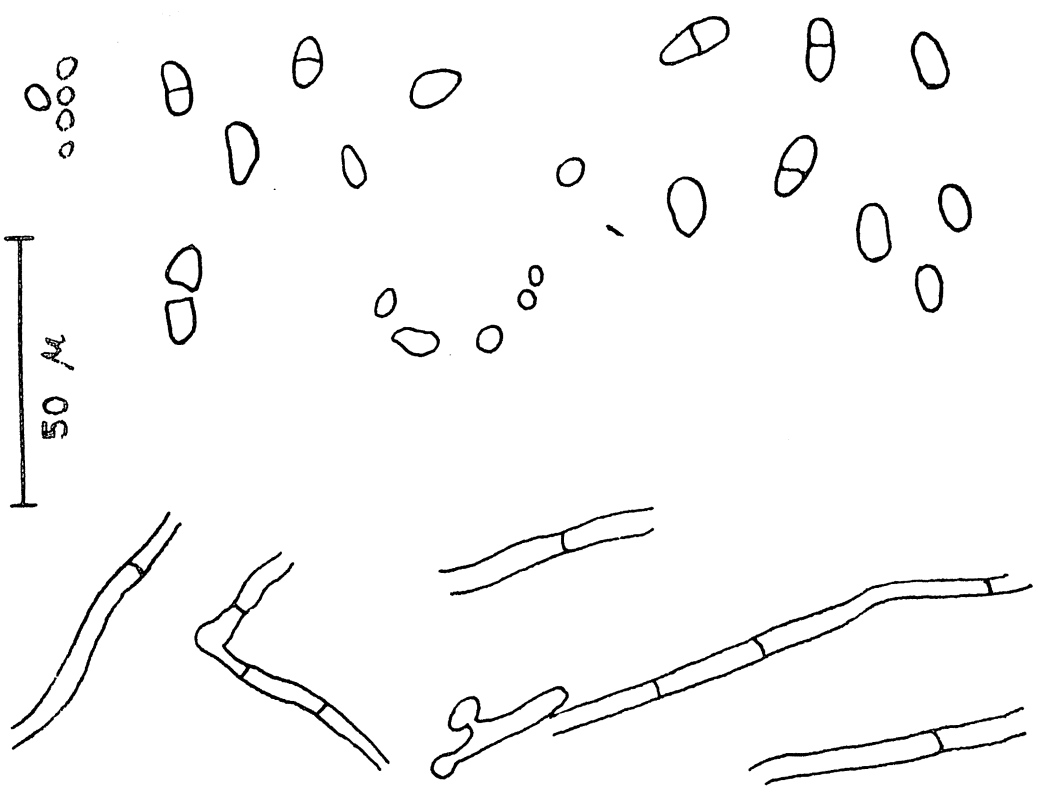

Fig-2.

The conidia which developed in culture measured 4.16-8.32x3.33-6.66 microns in size and their average size being 6.66x3.66 microns. The average length of conidia measured to 6.66 microns and the average breadth was 3.66 microns.

The identification report on the species of fungus received from Dr.Paul M.Krik, Mycologist C.M.I. England revealed that the fungus is Cladosporium tenuissimum Cooke. Hence the causal organism from now onwards will be referred with this name.

GERMINATION OF SPORES

The conidia of Cladosporium tenuissimum started to germinate after an initial lapse of 4 hours in distilled water. This was evidenced by the development of a very short hyaline germ tube. The small germ tubes emerged out generally from the end cells. The percentage of conidial germination was counted after 4, 8 and 12 hour intervals in the foregoing experiments.

(a) Effect of pH on germination:-

The experiment relating to the effect of different pH on conidial germination of Cladosporium tenuissimum

indicated that it varied at different pH levels when estimated at 4 and 6 hours intervals (Table-1). The data shows that highest degree of conidial germination took place at pH -5 (65.5%) which was closely followed by pH-4 (63.7%). At pH-8 the poorest degree of germination was recorded (17%) and at pH-7 it was 32%. Comparatively, the two lower pH, i.e, 2,3 supported a higher degree of conidial germination than the two higher pH of 7 and 8. Apparently, it appears that for intrating the germination of conidia, in Cladosporium terrissimum a lower degree of pH range is more suitable relatively in comparision to the higher pH. The optimum pH, however, for this fungal activity is 4-5 (Fig-3). The influence of pH on conidial germination may reflect upon the infection by pathogen while involved in pathogeneeis on tomato fruits at different stages of their maturity.

TABLE - 1 Percentage of conidial germination of Cladosporium tenuissimum at different levels of Hydrogen-ion-concentration.

pH	% Conidial germination	
	4 hours	8 hours
2	38.5	42.0
3	39.7	45.5
4	51.0	63.7
5	53.0	65.5
6	39.0	58.3
7	25.0	32.0
8	15.0	17.0

The above % conidial germination was counted out of 45 spores per microscopic field of 15 days old culture.

PERCENTAGE GERMINATION OF CLADOSPORIUM IN DIFFERENT HYDROGEN-ION CONCENTRATION.

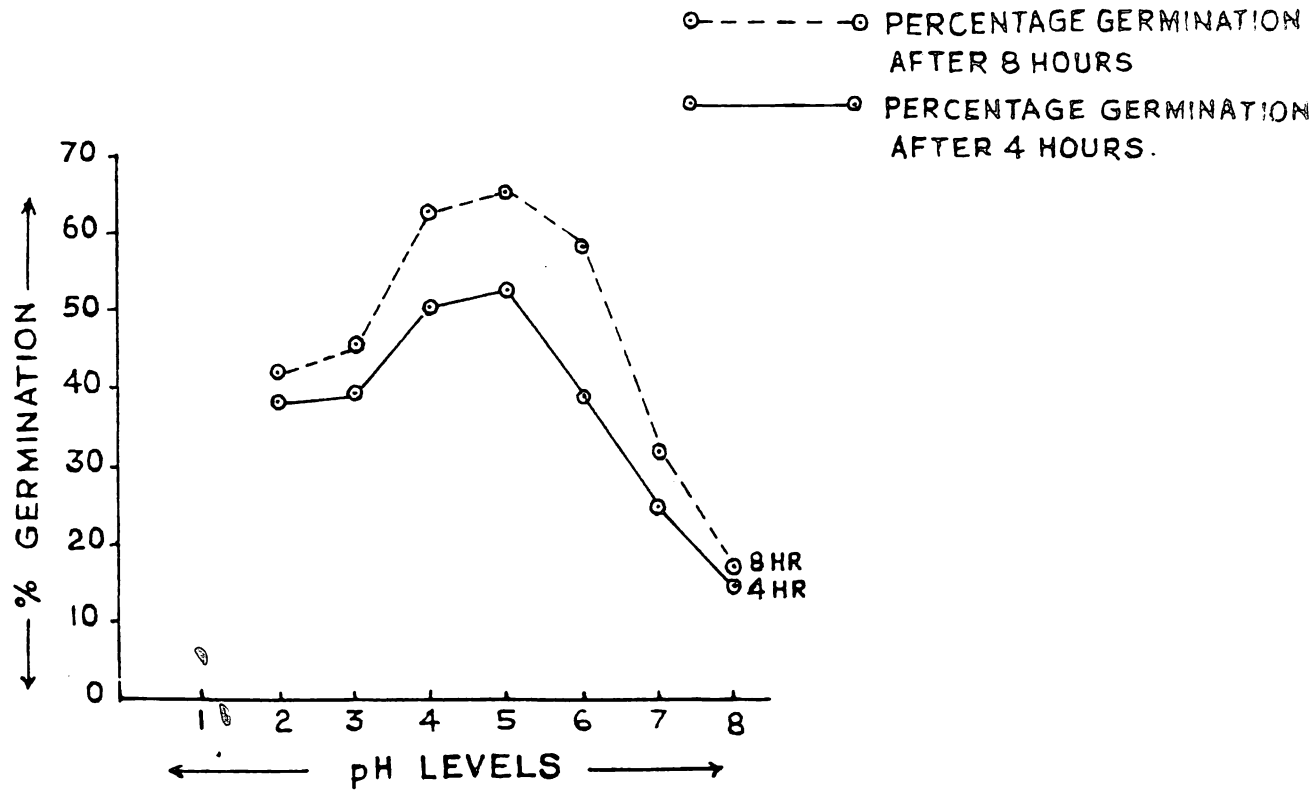

Fig-3.

(b). Effect of different sources of carbon on conidial germination.

The influence of 8 carbon sources (1%) was determined on the degree of conidial germination of Cladosporium tenuissimum after being incubated at 25(± 1°C) for 4 , 8 and 12 hours duriation. The experimental results (Table-2) revealed that the highest degree (92%) of conidial germination was supported by sucrose at the end of 12 hours, this was also highest from the beginning when the first observation was taken after 4 hours. Maltose, glucose and dextrose after 12 hours supported the conidial germination to the same degree (84-85%) which was slightly better than that observed in respect to sucrose. Similarly no striking difference could be found in response to mannitol and fructose, the former was little superior to fructose. Lactose and starch somewhat reduced the degree of germination when compared to that in control at each time interval (Fig-4).

It is interesting to note from the Table-2 that of each treatment the degree of increase in conidial germination from 4 to 8 hours interval was much higher than the total increase note in the latter experimental period of 8-12 hours.

Even the fairly high degree of germination in plain distilled water(control)without the availability of any sugar source suggests that Cladosporium tenuissimum does not necessarily require a carbon rich nutrient solution to intiate the proce of conidial germination.

TABLE-2 Percentage of conidial germination of *Cladosporium tenuissimum* in different solutions of Carbohydrates.

Carbohydrates	Conidial germination (%) Intervals		
	4 hours	8 hours	12 hours
Glucose	38	72	85
Dextrose	39	71.5	84
Sucrose	64	85	92
Maltose	58	78	85
Mannitol	42	73	78
Fructose	36	68	75
Lactose	26	54	63
Starch	24	58	69
Distilled water (Control)	42	73	79

The above % conidial germination was counted out of 50 spores per microscopic field of 14 days old culture.

PERCENTAGE GERMINATION OF CLADOSPORIUM TENUISSIMUM IN DIFFERENT SOLUTIONS OF CARBOHYDRATES.

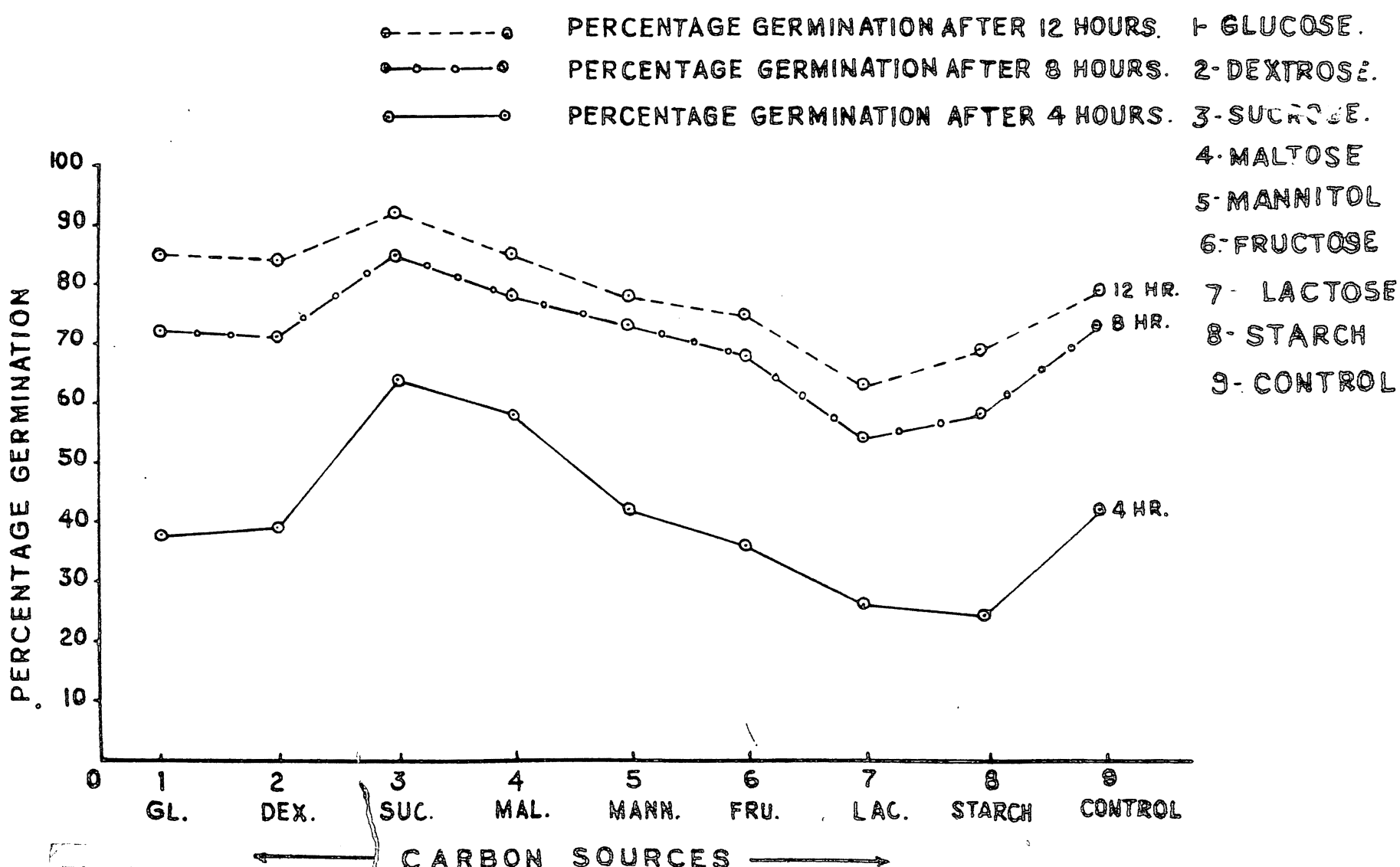

Fig-4

c) Effect of temperature on the degree of conidial germination

Besides the nutrient factors the temperature is another important factor which generally affects the spore germination in most fungi which is of great importance towards the infection process.

The effect of 5 different temperatures, viz, 10°C 20°C, 25°C, 30°C and 35°C was determined on conidial germination of Cladosporium tenuissimum by the previously described cavity slide procedure. The observations were recorded at 4,8 and 12 hours interval. The data (Table-3) shows that at the lowest temperature tried, i.e. 10°C there was no conidial germination throughout the experimental period. At 25°C, however, the highest population (67%) of conidia germinated after 12 hours; this was such even at 4 and 8 hours, (48% and 55%, respectively). At 20°C, 30°C and 35°C the degree of conidial germination was 35%, 52% and 25%, respectively after the end of 12 hours. Relatively, among all the 5 treatments, the poorest response to conidial germination was observed at 35°C. Apparently, 25°C-30°C which supported a higher percentage of conidial germination appear to be the optimum temperature for this fungal activity in vitro, (Fig-5).

TABLE-3 Effect of 5 different temperatures on degree of conidial germination of Cladosporium tenuissimum at 3 time intervals.

Temperature (C°)	% Conidial germination Time intervals (Hrs.)		
	4	8	12
10	0	0	0
20	25	30	35
25	48	55	67
30	30	42	52
35	17	23	25

The above % conidial germination was counted out of 50 spores per microscopic field of 14 days old culture.

PERCENTAGE GERMINATION OF CLADOSPRIUM TENUISSIMUM AT FIVE DIFFERENT TEMPERATURES.

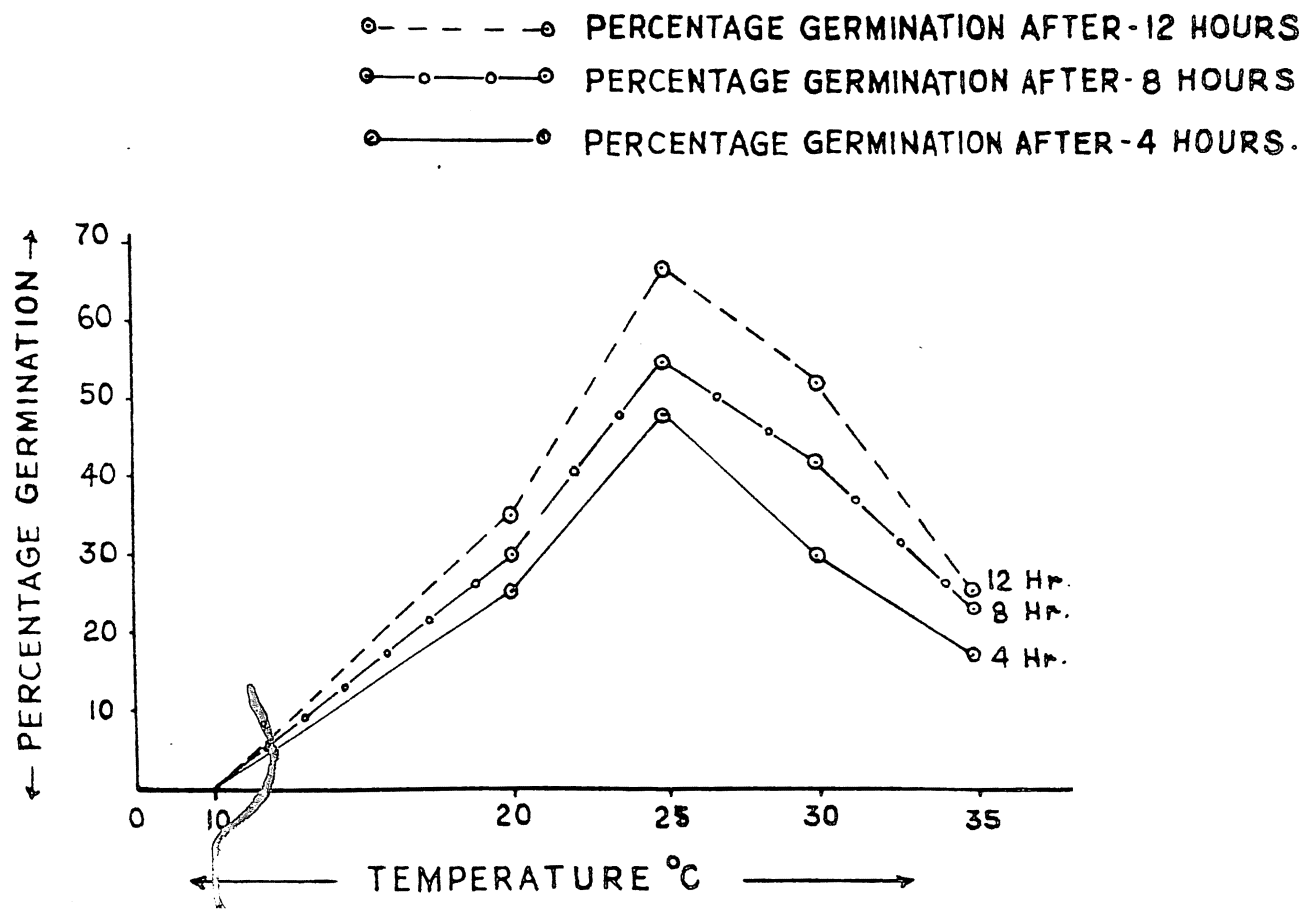

Fig-5.

GROWTH OF FUNGUS ON NUTRIENT MEDIA

a) Dry weight growth and sporulation on liquid nutrient media-

In view to determine the best growth-supporting nutrient medium for Cladosporium tenuissimum this investigation was carried out. The fungus in pure culture was allowed to grow for 14 days on 6 nutrient media(liquid) which were tomato leaf extract, tomato fruit extract, Richard's medium, Czapek's medium, potato dextrose medium and nutrient medium. After end of experimental period simultaneously with growth estimation the degree of sporulation was also determined in response to each nutrient medium.

The experimental results (Table-4) indicate that the most profuse degree of growth (1080 mg) of the fungus was supported by Richard's nutrient medium; even it allowed the highest number of conidia development.Potato dextrose was next suitable medium which provided a fairly high amount (407) of dry weight growth however, it was much lesser than Richard's medium. Among the 2 natural extract media, i.e., tomato leaf extract and and tomato fruit extract, the latter was much superior than the former; actually the tomato leaf extract provided the least degree of growth (43 mg) of

TABLE-4 Average* dry weight (mg) of fungal mat and degree of sporulation by *Cladosporium tenuissimum* on 6 different liquid media.

Media	Dry weights (mg)	Degree of sporulation
Tomato leaf extract	43	+
Tomato fruit extract	336	++
Richard's medium	1080	++++
Czapek's medium	53	++
Potato dextrose medium	407	+++
Nutrient medium	-	-

* Each weight was estimated from 3 replication for each treatment and cultures were grown for 14 days.

GROWTH AND SPORULATION OF CLADOSPORIUM TENUISSIMUM ON DIFFERENT LIQUID MEDIA

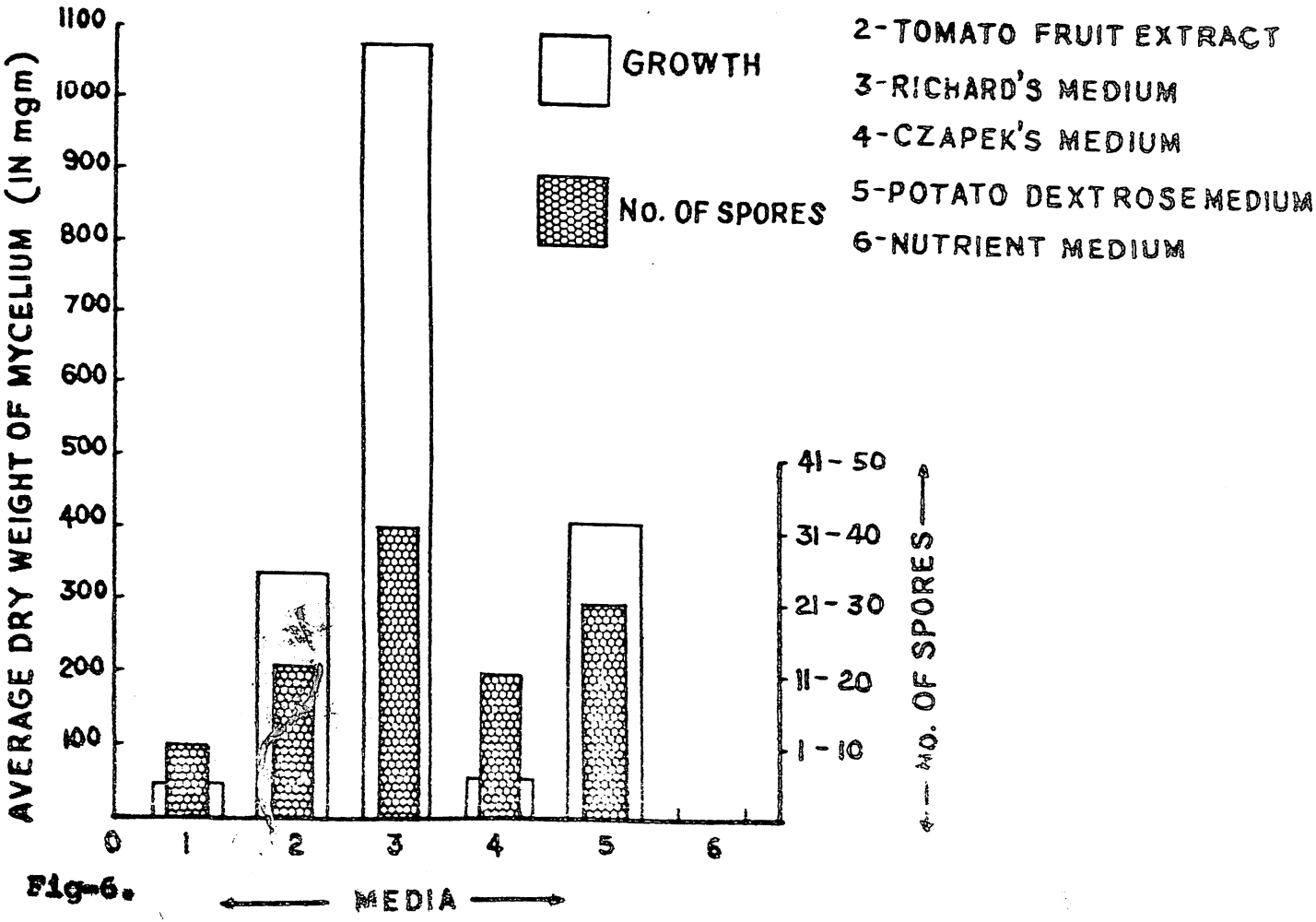

Fig-6.

Cladosporium tenuissimum which was almost the same as on Czapek's medium. The nutrient liquid medium did not support any growth of the fungus till the d end of experimental period. On the basis of experimental evidence it is evident that the ingredients of Richard's synthetic medium were most suitable for fungal metabolism so as to support its maximum growth. On the contrary, the nutrient medium was poorest since it could not even allow the growth in traces. (Fig-6)

In regard to the degree of sporulation observed on various nutrient media the largest number of conidia were developed on Richard's medium which was followed by potato dextrose agar, Czapek's and tomato fruit extract media. The tomato leaf extract medium developed the conidia only in traces.

(b) Effect of Hydrogen ion-concentration on growth and sporulation -

Another important factor i.e. pH is also critical in many fungi for their pathogenicis as well as, for growth and sporulation in vitro. Hence to obtain such information on Cladosporium tenuissimum the effect of 6 different pH viz. 3,3.5, 4,4.5, 5 and 6 was determined. Richard's medium suitably adjusted with each pH was used for this investigation and the cultures were grown at 25(± 1°C).

The data (Table-5) shows that the highest degree of growth (1162 mg) and sporulation could take place when the pH of medium was 4.5. At 3 and 3.5 pH the growth and sporulation degree was same and these were next to the previous pH level in their superiority. Fairly high degree of growth (1058 mg) was also recorded when the medium had its pH 4. Relatively, least degree of growth (710.7 mg) was recorded at the highest pH. 6 among all the pH levels used in this experiment.

The degree of sporulation was same at 3,3.5 and 4 pH . It was relatively least at 6 pH and more or less same at 4.5 and 5. Like growth the sporulation also was heaviest at 4.5 pH. It appears that fungus prefers lower range of pH for its growth and sporulation (Fig-7).

TABLE-5 Effect of 6 different pH on growth [a] and sporulation of Cladosporium tenuissimum after 14 days

pH range	Average dry weights(mg)	Degree of sporulation
3.0	1080	+++
3.5	1081	+++
4.0	1058	+++
4.5	1162	+++++
5.0	829.6	++++
6.0	710.7	++

a. Each reading is based upon an average of 4 replications for each treatment.

GROWTH AND SPORULATION OF CLADOSPORIUM TENUISSIMUM IN DIFFERENT H-IO N-CONCENTRATION.

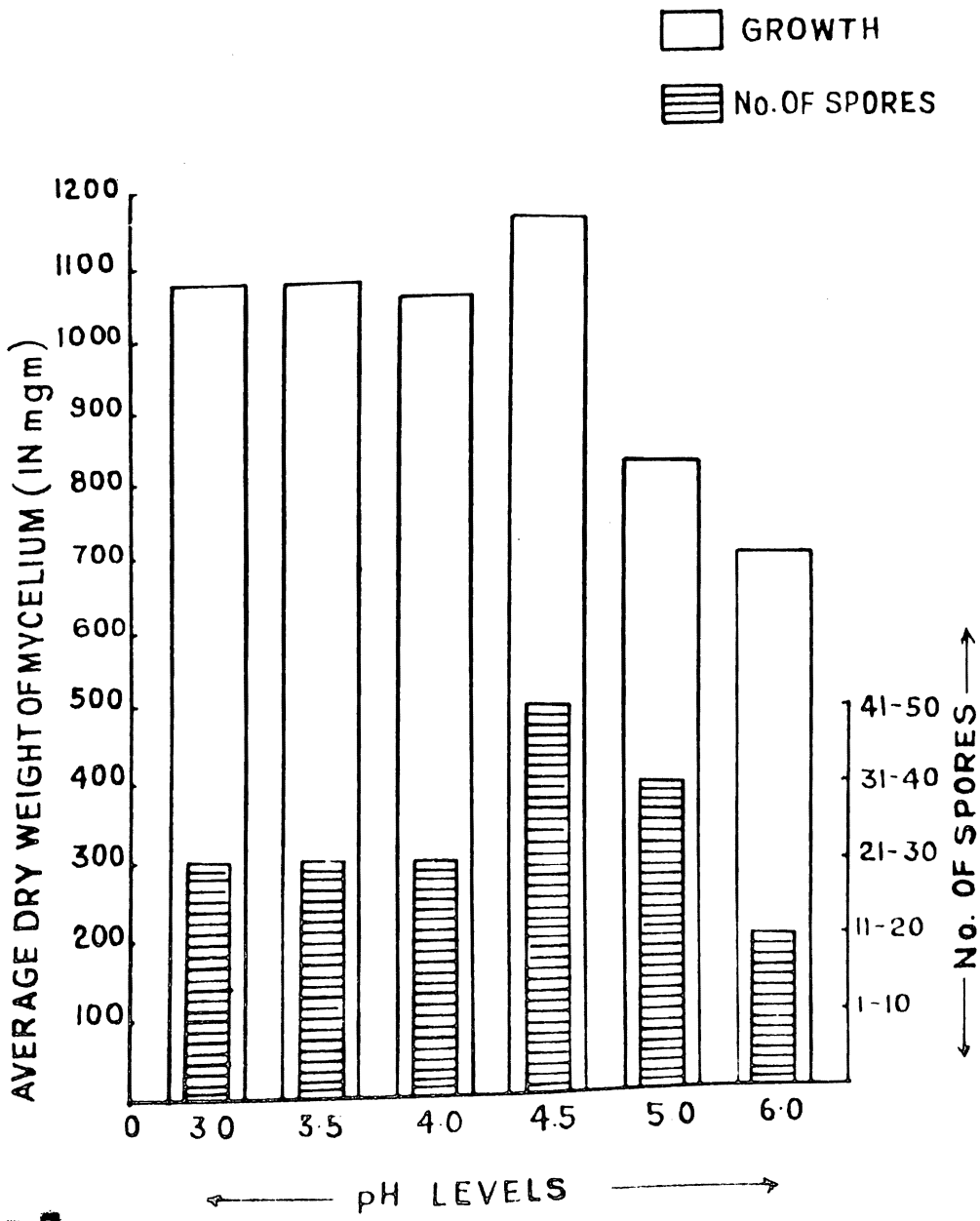

Fig-7.

(c) Influence of temperature on growth and sporulation of Cladosporium tenuissimum.

For estimating the most suitable temperature to support the growth and sporulation of Cladosporium tenuissimum this experiment was conducted. This was carried out by incubating the growing fungal cultures on Richard's medium at 5 different temperatures viz; 10°, 20°, 25°, 30° and 35°C. The experimental results (Table-6) show that at 25°C most profuse growth (1357 mg) and heavy sporulation was observed whereas, it was lowest (232 mg) at 10°C. The growth at 30°C was relatively superior than at 25° and 35°C. However, the degree of sporulation was more or less same. On the basis of experimental results it is apparent that the optimum temperature for growth and sporulation of fungus is 25°-30°C. (Fig-8).

TABLE-6 Average dry weight[a] of mycelial mat of *Cladosporium tenuissimum* and sporulation at 6 different temperature.

Temperature (°C)	Average dry mycelial weight (mg)	Degree of sporulation
10	232	+
20	579	+++
25	1357	++++
30	917	++++
35	399	+++

a. Each reading is an average estimated from 3 replications for all treatments when cultures were grown for 14 days.

GROWTH AND SPORULATION OF CLADOSPORIUM TENUISSIMUM AT DIFFERENT TEMPERATURES.

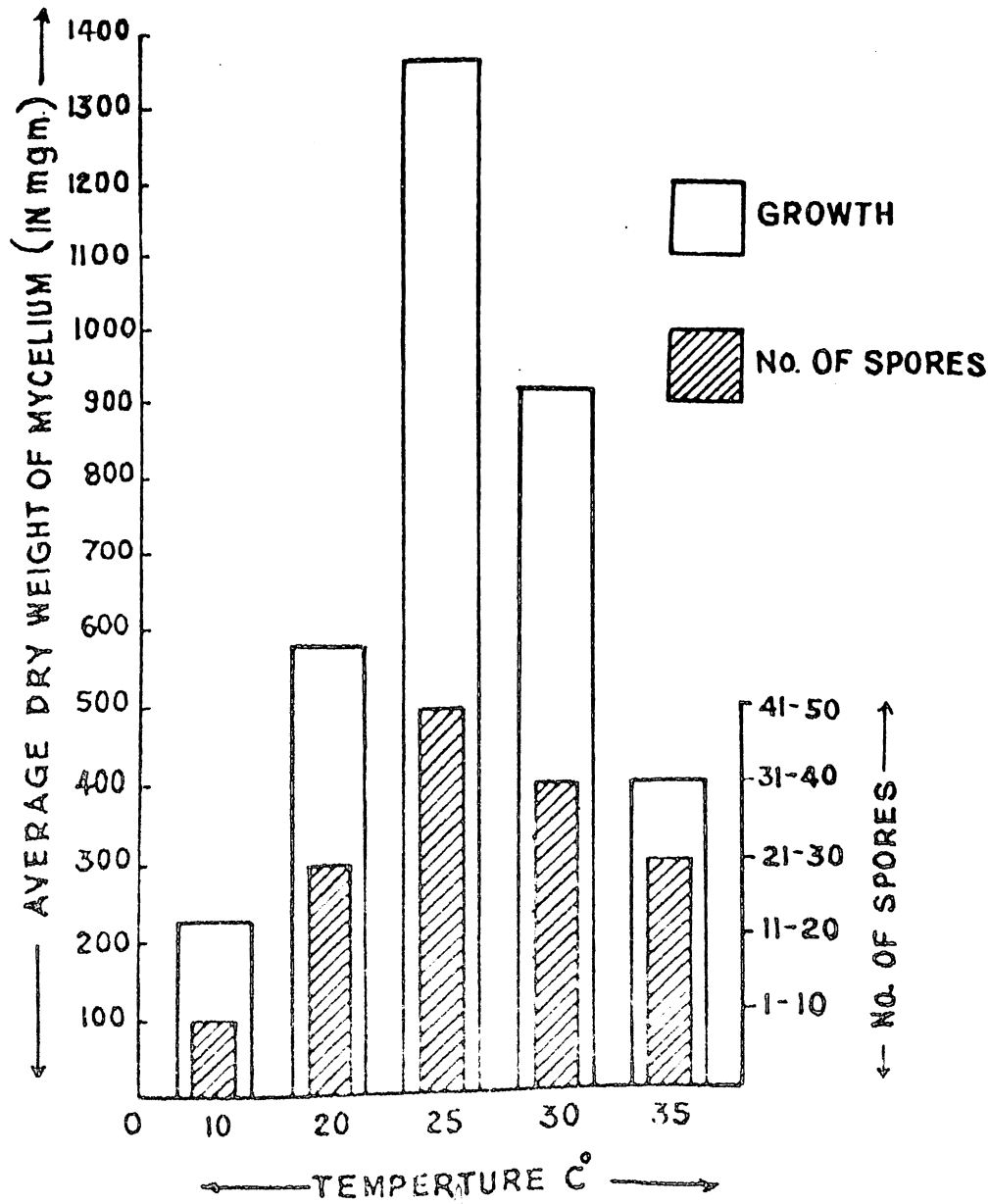

Fig-8.

(d) Effect of different sources of carbon on growth and sporulation of the fungus Cladosporium tenuissimum.

Among the basic and important nutrient sources required by fungi for their development the role of carbon sources is of importance. Hence, in view to determine the best source of carbohydrate required for the growth and sporulation of Cladosporium tenuissimum this experiment was conducted. To Richard's basal medium seven different sugars namely, starch, mannitol, sucrose, fructose, lactose, glucose and dextrose were individually added in estimated quantities by substituting the original carbon source of medium.

Experimental results (Table-7,Fig-9) show that after 10 days of growth the fungus exhibited highest degree of growth (880 mg) on sucrose-supplemented medium which was followed by Manitol, glucose, lactose, fructose, dextrose and starch. In control, where no sugar was added the fungus failed to grow even in traces and there was no sporulation, as well. The highest degree of growth on the medium enriched with sucrose and least with starch suggests that the fungus is capable of utilizing most efficiently, the former for its vital metabolic activities whereas this was contrary in regard to latter.

While considering the degree of sporulation the data (Table-7) shows that again sucrose was most superior; glucose

TABLE-7 Effect of 7 carbon sources on dry weight growth[a] and sporulation of *Cladosporium tenuissimum* after 10 days.

Carbon sources	Average dry weight (mg)	Degree of sporulation
Control	-	-
Starch	412.5	+
Mannitol	705.0	+++
Sucrose	880.0	+++++
Fructose	477.5	-
Lactose	585.0	++++
Glucose	615.0	++++
Dextrose	455.0	++

a Each reading is based upon an average estimated from 3 replications for all the treatments.

GROWTH AND SPORULATION OF CLADOSPORIUM TENUISSIMUM IN DIFFERENT CARBON SOURCES.

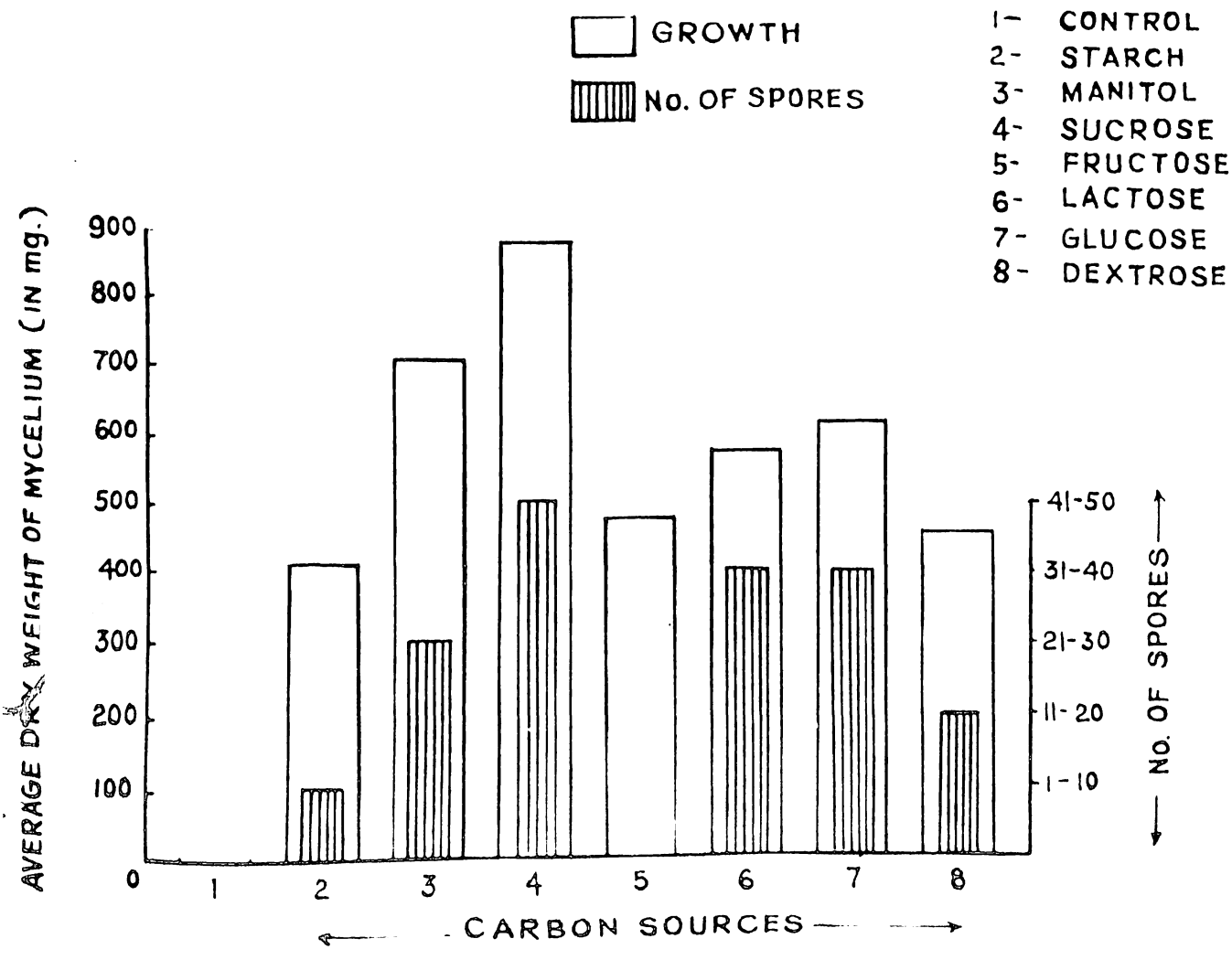

Fig-9.

and lactose were next best and these supported for same degree of sporulation. Mannitol and dextrose could provide relatively lower degree of sporulation. Starch and fructose were not found suitable for spore development of Cladosporium tenuissimum.

(e) Effect of different nitrogen sources on growth and sporulation of Cladosporium tenuissimum.

The capacity of the fungus to utilize nitrogen from different sources was investigated using different nitrogenous compounds mentioned below. They were incubated at 25± 1°C for about 10 days by incorporating different nitrogenous sources in the basal Richard's medium. The observations were taken after 10 days of incubation.

The experimental data (Table-8,Fig-10) reveals that highest degree of growth (885 mg) by fungus was exhibited in ammonium oxalate-supplemented medium whereas it was least (330 mg) in response to ammonium chloride. Potassium nitrate and sodium nitrate (675 mg and 670 mg, respectively) supported almost the same degree of growth which was relatively higher than on the medium enriched with asparagine,urea and ammonium nitrate. Almost the same degree of growth in response to both nitrate forms (KNO_3 and $NaNO_3$) suggests that they were utilized in a

TABLE-8 Effect of 7 nitrogen sources on dry weight growth [a] and sporulation of Cladosporium tenuissimum.

Sources of nitrogen	Dry weight (mg)	Degree of sporulation
Control	-	-
Asparagine	655	+
NH_4Cl	330	-
KNo_3	675	++++
Urea	640	+
Ammonium oxalate	885	+
$NaNo_3$	670	+
NH_4No_3	565	-

a Each reading is based upon an average estimated from 3 replications for each treatment when cultures were grown for 10 days.

GROWTH AND SPORULATION OF CLADOSPORIUM TENUISSIMUM IN DIFFERENT N. SOURCES.

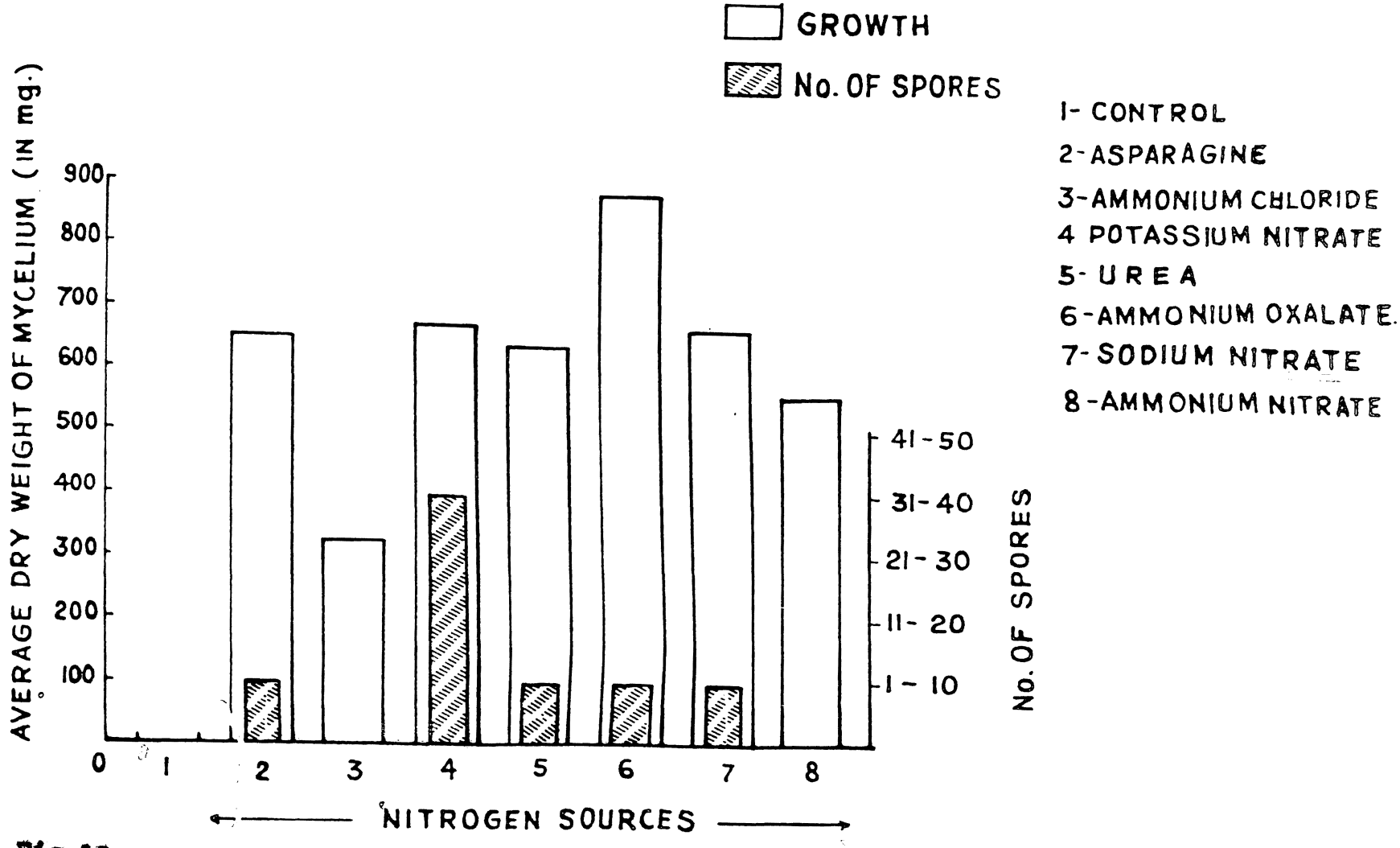

Fig-10.

similar proportion and possibly their metabolism was also same by the fungus. The only organic form of nitrogen used, Asparagine supported a higher degree of growth when it was compared to ammonium chloride and ammonnium nitrate. The absence of any nitrogen source in control neither provided growth nor sporulation.

In regard to the extent of sporulation the results indicate that the only sources of nitrogen which supported this fungal activity was potassium nitrate. Other sources namely, asparagine, urea, ammonium oxalate, and sodium nitrate only allowed the conidia to develop in traces. Ammonium chloride and ammonium nitrate did not support sporulation to any degree in Cladosporium tenuissimum. Such experimental evidence suggests that this fungus is specific in its nitrogen requirement towards sporulation.

HOST RANGE STUDY

The pathogen, Cladosporium tenuissimum used in the present investigation was isolated from the diseased tomato fruits. Hence to determine if it could parasitize the fruits of other species of plants, this investigation was carried out. The healthy fruits of Brinjal, Chillies,potato, carrot and beans were used. After seven days of inoculation to the above hosts oveservationr were taken. From the results it

was seen that there was no development of fungus on any of the hosts. However, after about 10 days saprophytic deterioration of the inoculated vegetables was apparent.

EFFECT OF CLADOSPORIUM TENUISSIMUM ON SEED GERMINATION OF DIFFERENT CROPS

In view to investigate if Cladosporium tenuissimum could colonise the seeds of different crops this study was conducted. The seeds of tomato, brinjal, chilli and cucumber were dipped in heavy conidial suspension of the fungus and kept inside the moist petridishes under beljars. After 7 days of inoculation they were examined. The following results were obtained in each case-

a. Chilli- Out of 20 seeds that were inoculated, 6 seeds germinated and rest remained as such. Out of the remaining 14 seeds which were not germinated, 6 seeds showed colonization by the fungus around the seeds but without any sporulation.

b. Cucumber-Out of 20 seeds which were inoculated only 13 seeds germinated. Among all the ungerminated 7 seeds, 4 showed fungal coloniaation without any sporulation.

c. Brinjal- Out of 20 seeds, 3 seeds germinated and the remaining 17 seeds showed vegetative colonisation and sporulation by the fungus.

d. Tomato- Out of 20 seeds 17 seeds germinated only 3 seeds were infected. Among the 3 infected seeds only 2 showed the colonization of fungus and also sporulation.

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CHAPTER-V
DISCUSSION

D I S C U S S I O N

Tomato disease dealt with this thesis on the basis of its typical symptoms, particularly under natural conditions on fully established tomato plants was of moderate intensity. On the basis of disease affects localised particularly on green fruits thus making them somewhat reduced in size, discoloured and drying the large infected area, suggest to catagorise it as a dry fruit rot. Such infection on tomato under Orissa conditions apparently has not been detected earlier nor, reported in any literature as to the knowledge of investigator of this problem as well as others associated with the plant disease problems in Orissa state.

Presence of abundant small size conidia as well as, septate mycelium in the disease tissues confirmed that the disease was of fungal origin. To confirm the pathogenic potential when fungus from pure culture was inoculated to the healthy experimental plants, the typical disease symptoms as in nature were reproduced on green fruits. However, all the untreated fruits remained healthy in control. Unlike the healthy condition of foliage as observed in nature, when the leaves were artificially inoculated with fungal spores, the disease could be induced. Such observations indicate that under

high infection pressure under experimental conditions even tomato leaves may be succeptible to the fungus which was observed to a cause dry fruit rot under natural conditions.

On the basis of morphological characteristics the conidia when were compared to the original description of the fungi infecting tomato it was tentatively identified to the genus Cladosporium. The number of investigators have reported from the other parts of the world that C.fulvum is a common parasite on tomato. However, it could not be decided if Cladosporium which was isolated in the present investigation was really C.fulvum. Most of the reports, by workers(Spangler, 1924) have described the presence of mostly 2-celled conidia and only few single-celled conidia in C.fulvum. This appears to be a significant difference between the species dealt in present thesis and C.fulvum on tomato described by others. Through the courtesy of Paul M.Kirk, Mycologist C.M.I.,England, the species has been identified as C.tenuissimum Cooke. According to his comment this fungus is only a common saprophyte in tropics but this view seems to be contradictory keeping in view the experimental evidence that indicates that the isolate C.tenuissimum used in the present investigation was virulent and associated in pathogenisis under the natural and experimental conditions. The capacity of fungus to parasatized by expressing typical disease symptoms

even on detached green, semiripe and ripe fruits suggest that it is capable of causing significant damage to fruits under storage.

The rapid germination of conidia within 4 hours in distilled water suggest that the conidia do not require a resting period, and they germinated easily without any nutrition. However, the degree of conidial germination could be enhanced by providing enriched nutrient solutions, as well as, the desirable pH to the substratum. The results had shown that highest degree of conidial germination was supported by sucrose which was closely followed by glucose, maltose and dextrose. Most of the sugars either promoted the conidial germination of fungus and others influenced to the same degree as in untreated control. However, lactose and starch were found to reduce the conidial germination. Apparently, the data suggest that fungus prefers some source of carbon as a typical factor for spore germination. Spangler (1924) reported a rapid conidial germination in C.fulvum at 20°C under dry conditions.

Among the pH range which generally influenced the degree of conidial germination to larger extent, those which were lower, enhanced the degree of germination to more than those which were higher (pH 7 and 8). The optimum pH range

falls between 4 and 5 since at this level highest degree of conidial germination was observed after 8 hours duration.

On the basis of such experimental evidence it may be atributed that highest degree of spore metabolism of spore food reserves was activated at pH 4 to 5, whereas it was at the lowest order when pH of the water during spore germination was maintained at 8.

As regards the effects of temperature on conidial germination is concerned the data suggest that highest degree of germination was supported at 25°C. This may be referred as the optimal temperature for conidial germination of C. terminalimum. The least degree of germination was about 35°C. Such information may imply that initially for conidial germination the lowest temperature around 25°C is essential to initiate the germ-tube growth even for parasitism. Since this is a temperature which prevails on an average during the month of February when the disease originally was detected for the first time in nature under Bhubaneswar conditions.

Among six nutrient media which were used to grow the fungus it was determined that the balanced nutritional ingredients of Richard's medium supported the highest degree of fungal growth and its sporulation during 14 days period. It is rather peculiar to note that another synthetic medium

that is, Czapek's was considerably poor in supporting the growth of the fungus. Comparatively, the nutritional sources of tomato fruit extract medium were found more growth stimulatory when compared to tomato leaf extract medium. Better growth response on tomato fruit extract medium may be due to its qualitatively and quantitatively richer nutrient factors. No growth of the fungus could be observed in the nutrient media. Furthermore the least growth of fungus on tomato leaf extract medium may be explained due to some growth inhibitory substance , which may be present in tomato leaves, and these could explain the disease free nature of tomato leaves to C.tenuissimum under natural conditions.

Like spore germination even the growth and sporulation of C.tenuissimum was best supported with the lower pH of 4.5. similarly, the highest pH 6 used in this study supported the growth relatively to a least degree. Thus even for the growth the best metabolism of the fungus for its nutritional requirement, cell make up and sporulation seems to be supported by an acidic medium.

Among all the 5 temperatures tried to determine the effect of growth and sporulation, 25^{o}C was found to be most stimulatory, thus it may be referred as optimum temperature

for growth as well. The lowest range of temperature was found to be 10°C as it supported almost 1/6th of the growth incomparision to that at 25°C. The highest temperature for growth was found to be 35°C. Such information in regard to temperature may be useful for studying the role of physical factor, temperature and disease development in nature.

Most fungi require some degree of carbon source to support growth and spore production. Thus the study pertaining to the influence of 7 carbon sources for growth and sporulation revealed useful information. Similar to spore germination even the best growth and sporulation were most highly supported by sucrose. Dextrose and starch on the other hand, were found to be least supporting for growth and sporulation. Mannital was next best to sucrose. It is possible that sucrose was most efficiently metabolised carbon source as a consequence of enzymatic degradation by the fungus. It is interesting to note that where no sugar was added in control there was no growth of the fungus throughout the experimental period. Thus it seems fungus is dependent for its growth and sporulation on some sugars as carbon source.

As regard to the nitrogen nutrition the most growth stimulatory substance was recorded as ammonium oxalate.

Although potassium nitrate was less efficient in producing the growth of fungus but the degree of sporulation in this nitrogen source was the highest. The least growth supporting nitrogen source on the basis of experimental evidence was ammonium chloride. Excepting potassium nitrate, no other nitrogen source was found to support the sporulation of C. temisseimum. In the absence of nitrogen nutrition no spore production as well as growth could be observed in control This suggests that a source of nitrogen is critically essential for cellular buildup, spore development and other biochemical activities of the fungus.

The artificial inoculation of some non-host vegetable crops for example brinjal, chilli, potato, carrot and beans revealed that none of the could be parasitised by C. temisseimum. This suggests the specific pathogenic potential of fungus only on tomato.

However, the artificially parasitized seeds of some non-host plants for example, chilli, cucumber and brinjal with C. temisseimum were found to be colonized by the fungus which inhibited the germination. Perhaps the nutrients available on such seeds did not allow the fungus to maintain its specific nature, at least under artificial experimental

conditions. However, this aspect needs further investigations by using other isolates of the pathogen and making extensive surveys to detect the possible parasitism of C.terminalimum on other crop plants under natural conditions in various agroclimatic regions of the country.

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CHAPTER-VI
SUMMARY

S U M M A R Y

During the year 1979 in the month of February, a dry rot of tomato was observed parasitizing green tomato fruits in a vegetable garden at Bhubaneswar. Under natural conditions the disease caused considerable damage to the fruits. The symptoms showed the presence of brownish color discoloration at the distal-end of fruits, which was small in the initial stages but gradually the disease lesion increase in size and attained a uniformly round, and depressed area. As a consequence of disease the affected tissue became shrivelled, sunken and erumpent at places. The colour changes almost to black. Such infected fruits with such symptoms did not increase in size nor reach they maturity.

The microscopic examination of disease tissue revealed the presence of fungal spores and mycelium, which resembled to the description of Cladosporium. On the basis of morphological characteristics of the fungus, have been identified as C.tenuissimum.

Its pathogenicity was established on healthy experimental tomato plants by following Koch's Postulates. Under artificial conditions in addition to fruits the tomato leaves could also be parasitized.

Under natural conditions as well as in culture on PDA the fungus sporulated heavily. Mycelium was hyaline, branched and septate. Conidia mostly single celled and also hyaline, were of oval or round in shape, rarely they were two celled. The conidia are easily separated from conidiophores having an apendage at their tip. Under high power the average size of conidia was found to be 6.66x3.66 microns. The conidia germinated in distilled water within 4 hours. Most suitable pH, carbon source and temperature determined for highest conidial germination, where determined as 4-5, sucrose, and 25°C respectively.

Among the 6 nutrient media used for the growth and sporulation of C.tenuissimum, Richard's medium was found to be the best. Among the various factors influencing the degree of growth and sporulation the most suitable are, pH 4.5 , best temperature 25°C, best carbon source sucrose and best nitrogen source for growth was Ammonimum oxalate, however, the highest degree of sporulation was supported by KNo_3.

When the seeds of chilli, cucumber, brinjal, and tomato were artifically inoculated with the conidial suspension of fungus it was observed that all seeds were colonized with fungal growth and the percentage of germination was also affected to a variable degree.

When the other non-host vegetables like chilli, brinjal, potato, carrot and beans were artificially inoculated by C.tenuissimum none could exihibit any signs of disease injury, thus revealing that the fungus is apparently host specific.

C.tenuissimum on tomato unlike C.fulvum seems to be a new record of disease in India.

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