PART I

STUDIES ON LEAF BLIGHT OF CHRYSANTHEMUM (Chrysanthemum indicum, L.) CAUSED BY Alternaria alternata (Fries) Keissler

PART II

STUDIES ON LEAF-SPOT OF CHIKU (Achras sapota, L.)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH

[AGRICULTURAL UNIVERSITY]

RAHURI, DISTRICT :- AHMADNAGAR

(MAHARASHTRA STATE)

in partial fulfilment of the requirements for the degree of

Master of Science (Agriculture)

in

PLANT PATHOLOGY

By

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Post-Graduate School, Rahuri

NOVEMBER, 1979

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November, 1979

Approved by the Advisory Committee :

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C E R T I F I C A T E

This is to certify that the thesis entitled PART-I " STUDIES ON LEAF BLIGHT OF CHRYSANTHEMUM (Chrysanthemum indicum,L.) CAUSED BY Alternaria alternata (Fries) Keissler", PART-II "STUDIES ON LEAF-SPOT OF CHIKU (Achras sapota, L.)", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth (Agricultural University), Rahuri, District Ahmadnagar (Maharashtra State) in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of bonafide research work carried out by Shri Dnyandeo Gangaram Kalane under the guidance and supervision of Dr. B.C. Patil and that no part of the thesis has been submitted anywhere for any other degree or publication.

(G.K. Zende)

Rahuri,

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Maharashtra State.

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Rahuri,
Dated : 14, November, 1979.

(Dr. B.C. Patil)
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A C K N O L E D G E M E N T S

I consider myself to be the most fortunate for the opportunity I got to work under the guidance of Dr. B.C. Patil, Project Officer (Agricultural Technology), Mahatma Phule Krishi Vidyapeeth, Rahuri with buoyant heart, I express my sincere gratitude to him for suggesting the problem and for his valuable guidance and criticism during the research work and preparation of manuscript.

It is incumbent upon me to express my profound sense of gratitude to Dr. B.B. More, Head, Department of Plant Pathology and Agricultural Microbiology; Prof. M.B. Joi, Vegetable Pathologist, All India Co-ordinated Vegetable Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, for their keen interest and useful suggestions in the systematic accomplishment of this course of investigation.

I acknowledge with thanks the help of Prof. Y.K. Hande.

I will be failing in my duties, if I do not mention thanks to Dr. P.A. Shinde, Professor of Plant Pathology, College of Agriculture, Pune-5 and to all staff members of the department.

Last but not the least, the author is grateful to Sarvashri R.V. Awachat, G.T. Shivale and his colleagues for their healthy co-operation.

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Chapter I

INTRODUCTION

Chapter I

I N T R O D U C T I O N

Chrysanthemum (*Chrysanthemum indicum* L., *Chrysanthemum morifolium*, L.) is an important ornamental crop grown for its highly decorative flowers. It is considered as native of China and Japan. According to Searle and Machin (1968) Chrysanthemum were first cultivated in China at least 3000 years ago. It was introduced from Japan in Britain and in North America respectively in the seventeenth and eighteenth century. Bose and Mukherjee (1972) mentioned that, chrysanthemum is called the 'Glory of the East' as it has truly all the delicacy of the East. The flower is also called the 'Queen of the Autumn' as it blooms in November and December when usually there is no such colour in the garden. So far there is no other perennial plant that yields such a brilliant range of colour tones, fantastic flower shape and height variations. There is probably no flower that enjoys a greater world wide popularity than the chrysanthemum. It is found all over the Europe. It has been grown with great success in Australia and South Africa and is much admired in America. It is the national flower of Japan.

According to Pal (1963) Chrysanthemum is one of the few flowers grown on a commercial scale in India. The small flowered, scented variety is in great demand, particularly in Peninsular India, for the decoration of the hair of ladies, for making garlands and for purpose of worship. The large flowered types are grown for garden purpose and for exhibition. Vishnu Swarup (1963) pointed out that in our country, it has

assumed economic importance as cut flower for garlands, veni etc. like marigolds and roses. This is evidenced by the organisation of displays of chrysanthemum varieties and flowers by the Agri-Horticultural society in collaboration with the Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi.

In India, the cultivation of chrysanthemum has spread over throughout the country with considerable concentrated area in the States of Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra (Singh and Srivastava, 1968; Desai, 1978). In Maharashtra, the chrysanthemums are grown over an area of about 150 hectares (Phadnis, 1970). The cultivation is restricted mostly to Ahmadnagar district. Phadnis opined that the major cultivation is in drought-prone area and is climatically ideal for the cultivation of this flower. Acharya and Patil (1972) studied the economics of cultivation and market for the chrysanthemum. In their opinion, this crop is of high commercial value with a net profit of Rs. 4626 per acre. The flower is sold primarily in Pune and Bombay markets. The flowers from Ahmadnagar district are despatched even to distant markets of Nagpur, Dharwar, Indore, Baroda and Delhi. The varieties grown in Ahmadnagar district are Zipri, Pandhari Revadi, Pivali Revadi and Rajn. Thus there is potential for increased area under this crop not only in Ahmadnagar district but also in adjoining districts of Pune and Nasik.

A number of diseases viz. leaf spots, leaf blight,

flower or blossom blight, downy mildew, powdery mildew, rust, collar rot, root rot, wilts, ray speck, crown gall tumours, virus stunt, mosaic, aspergy virus etc. have been recorded to cause losses in yields. Many of these are observed in India and Maharashtra.

During the year 1978, cultivators from Parner Taluka, and Vilad area in Ahmadnagar district complained of the appearance of a disease on chrysanthemum crop. It appeared on leaves and flowers and caused heavy losses resulting in the reduction of yield and also reducing the market value of the flowers. The disease was also noted in the horticultural garden at the Central Campus of the University. The microscopic examination of the affected parts revealed the presence of Alternaria spp. Rao (1963-65) recorded Alternaria chrysanthemi T. Schmidt and Alternaria tenuis Auct on Chrysanthemum indicum. Srinath and Sarwar (1965) reported Alternaria tenuissima (Fr.) Wiltshire on Chrysanthemum cinerariifolium. These two workers made the records but did not investigate into the disease problem. Since the disease was reported for the first time from the area where it is grown commercially, the investigations were undertaken to study the symptomology, the causal organism, its mode of perpetuation and to evolve suitable control measures.

Chapter II

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Literature review on leaf blight of chrysanthemum is briefly summarised pertaining to occurrence and fungi associated with, fungicidal control, and other disease records of chrysanthemum.

Occurrence and fungi associated with :

A. Outside India :

The first report of Alternaria spp. on chrysanthemum was probably from Denmark (Anonymous, 1946). Neergaard (1948) reported Alternaria sp. on Chrysanthemum carinatum. Schmidt (1958) reported Alternaria chrysanthemi on C. maximum in the Austrian Tyrol. According to Rodebaugh (1963) Alternaria tenuis caused blight of chrysanthemum. A high humidity in the green house favoured the development of Alternaria. Simmons (1965) gave the description and latin diagnosis to validate the name A. chrysanthemi Simmons and Crosier for the fungus on C. maximum. Tammen (1965) reported that dust like specks on the ray floret of chrysanthemum were found to be due to combined infection with Stemphylium and Alternaria species. He observed that most of the commercial varieties were proved susceptible to the disease. Epidemics of the disease could occur when day and night temperature ranged between 70° and 80°F and the flower remained moist for more than 12 hours. Sober (1966) recorded Alternaria

chrysanthemi on Chrysanthemum maximum in Florida. Waterworth and Povish (1968) reported Alternaria tenuis on C. yiezidi-hirtum. Cox (1969) observed a flower blight of chrysanthemum in South Florida and stated that the blight which caused heavy losses was connected with persistant cold weather following mild growing season. The symptoms were delayed in flowering, resultant senescent tissue and petal necrosis. Alternaria solani, A. tenuis (A. alternata) and Stemphylium species were associated with the disease. Engelhard (1970) observed Ascochyta blight, rust and three petal spot diseases on cultivars of commercial chrysanthemums. Differences were recorded in the reactions of 52 cultivars to inoculation with Mycosphaerella lingulata, natural infection by Puccinia chrysanthemi and flower petal necrosis. He classified petal necrosis into three types viz. tiny spots, large spots and necrotic petal tips. Alternaria sp. sporulated on the large spots and on the necrotic tips. Holcomb (1976) reported a new flower spot and blight of chrysanthemum in U.S.A. He classified the symptoms and identified the causal agent as Alternaria alternata.

B. In India :

Rao (1963) reported Alternaria chrysanthemi T. Schmidt inciting leaf and blossom blight of Chrysanthemum indicum. In his later report he (1965) recorded Alternaria tenuis Auct on leaves and blossoms of Chrysanthemum indicum. Srinath and

Sarwar (1965) reported Alternaria tenuissima (Fr.) Wiltshire on leaves of C. cinerariifolium.

Fungicidal control :

Hodebaugh (1963) reported that protective spray of Botran, captan or Phaltan gave good control of chrysanthemum blight and that the flower susceptibility was not related to nitrogen, phosphorus or potash nutrition. Tamsen (1965) mentioned that, the use of overhead watering should be avoided or where this is impracticable zineb dusts or sprays should be applied. Sober (1966) stated that application of copper compounds, maneb or zineb to the leaves of Chrysanthemum maximum when lesion first appeared and then at 7-14 day intervals provided adequate control. Increasing ventilation and reducing the amount of overhead watering also lessened the incidence. Cox (1969) reported that flower blight of chrysanthemum could be better controlled by zineb, maneb or daconil than captan.

In laboratory experiments, Singh and Milne (1974 a) evaluated 15 fungicides against five fungi causing chrysanthemum flower blight. The study included Alternaria alternata, Botrytis cinera, Itersonilia perplexans, Mycosphaerella linqulata and Stemphylium vesicarium. They stated that no one fungicide was outstanding at low concentration against all five fungi, but several performed well at higher concentrations. Captafol, chloroneb, mancozeb and thiram appeared most promising.

In their later report (1974 b) 17 fungicides were evaluated to determine their efficacy under field condition in controlling blight of chrysanthemum. They observed appreciable disease control with all fungicides tested, chlorothalonil being the most satisfactory individual chemical. Mancozeb and Captafol also gave reasonable control, but combination of two fungicides did not offer any major advantage.

Other diseases of chrysanthemum :

A. Outside India :

Walkden (1921) and Smith (1922) reported Bacterium tumefaciens, while Welles and Holden (1922) reported Bacillus solanacearum on chrysanthemum. Chifflot (1923) recorded Septoria chrysanthemi and Oidium sp. Jorstad (1924) enumerated the fungus diseases of various wild, garden and green house ornamental plants and shrubs observed in Norway. He reported Erysiphae cichoracearum on green house and outdoor chrysanthemums. Perti (1924) recorded Cladosporium. Martin (1925) reported wilt of chrysanthemum caused by Fusarium sp. and Verticillium alboatrum. Van Der Meer (1925) reported Verticillium wilt. Hemmi and Nakamura (1927) reported Septoria chrysanthemella and S. obesa. Wormald (1927) recorded leaf blotch of Shasta Daisy (C. maximum) caused by S. chrysanthemi. Ikata (1928) noted Diplodia spp. and Sclerotinia spp. Pape (1928) reported rust due to Puccinia

chrysanthemi. Chivers (1929) isolated Sclerotinia minor. Unamuno (1929) recorded Uredo pimardiae. Voglino (1929) recorded wilt due to Ramularia bellunensis. White (1929) isolated Fusarium culmorum. Muller (1930) gave the etiology, symptoms and control of three destructive fungus diseases affecting chrysanthemum in Germany viz. Mildew (Oidium chrysanthemella), rust (Puccinia chrysanthemi), leaf spot (Septoria chrysanthemella). Sydow (1930) reported Puccinia leucanthemi in two localities of northern Italy, but it was also reported in Palatinate (Germany) on C. leucanthemum. Curzi (1933) isolated Fusarium and F. javanicum var radicicola from C. cinerariaefolium. Duplessis (1933) isolated Epicoccum chrysanthemi from Chrysanthemum sp. from leaf characterised by circular brown-spots with a dark brown margin, sometimes incircled by a pale-yellow halo. Marchal (1933) reported Sclerotinia fuckeliana, Septoria chrysanthemi, Puccinia chrysanthemi, S. rostrupii and Fusarium dianthi on chrysanthemum. Van Der Goot (1935) reported Cercospora causing leaf and stem rot on C. coronarium crop in Priangan, Java and Sclerotium rolfsii on Pyrethrum (C. cinerariifolium). Sarejanni (1935) reported Macrophomina phaseoli on larged flower chrysanthemum on which chlorosis was produced. Tai (1936) reported Cercospora chrysanthemi on C. coronarium and C. circumscissea. Oyler (1937) reported Phytophthora cryptogea caused black neck disease of marguerite (C. frutescens)

in which the leaves of the whole or part of the plant wilted and the lower parts of the stems blackened, the roots showed a slight browning. Further, he reported leaf spot of marguerite (C. frutescens) caused by Ramularia bellunensis. Obee (1930), Arwidsson (1940), Greene (1949), Frezzi (1950) and Mayer and Viennot (1950) recorded respectively Sclerotium delphinii, Puccinia sp., Septoria macrospora and Cylindrosporium chrysanthemi, Phytophthora drechsleri and Entyloma scaliarum. Diehl (1952) gave brief description of symptoms and control of rust (Puccinia chrysanthemi), mildew (Oidium chrysanthemi), blotch (Septoria chrysanthemella gray mold (Botrytis cinera), wilt (Verticillium dahliae) and leaf gall (Corynebacterium fasciens) in Great Britain. Burkholder et al. (1953) made mention of three previously reported bacterial blight of chrysanthemum namely Pseudomonas solanacearum, P. syringae and Erwinia atroceptica/carotovora. Temmen (1959) reported ray speck of chrysanthemum caused by Stemphylium floridanum on C. morifolium. Zachos et al. (1960) reported Cephalosporium chrysanthemi on chrysanthemum caused by chlorosis of the leaves which eventually dry, pink longitudinal linear necrosis on the stem, latter turning reddish brown and a pink discolouration of the vessels. Mcfadden (1961) reported Pseudomonas cichorri on florists' chrysanthemum and stated that circular or elliptical, slightly sunken spots reach 1 cm diam. and coalesce into large necrotic areas. These spread

upward from the older leaves. Flower bud infection also occurred. The report of Wilcox (1961) was the first record in America of downy mildew on cultivated chrysanthemum caused by Perospora radii. Tyler (1962) noted Deuterophoma sp. Petersen and Spencer (1963) reported chrysanthemum flower rot caused by Fusarium tricinctum f poae. Hellmers (1964) made mention about the control of Japanese chrysanthemum rust caused by Puccinia horiana and stated that rust pustules were killed on plants kept at 35° for 20 hours and 40° for 12 to 20 hours. The recommended treatment is 37° to 40°C for 20 hours followed by application of maneb spray. Vermeulen and Van Kesteren (1964) reported that Pythium ultimum caused root and stem rot of chrysanthemum and stated that the control is limited to preventive measures. Prillwitz (1970) reported bacterial infection in chrysanthemum namely Erwinia chrysanthemi. He described the symptoms and gave details of recommended control measures. Schneider and Plate (1970) described symptoms of root and basal stem rot caused by Phoma chrysanthemicola. Kachaunova (1970) listed the most important diseases of chrysanthemum and their control. Groust (1971) made mention of problems posed at that time in France by fungal diseases of chrysanthemum. He discussed seven fungal organisms, viz. Ascochyta chrysanthemi, Botrytis cinera, Puccinia horiana, Pythium debryanum, Rhizoctonia solani, Sclerotinia minor and Verticillium spp. together with control measures. Hansen and

Dunez (1971) gave brief review of work on chrysanthemum mosaic, stunt, asperagy viruses including symptoms, transmission, meristem culture and indexing. Wolts and Engelhard (1973) reported Fusarium wilt of chrysanthemum and stated that use of nitrate nitrogen decreased the severity of wilt caused by Fusarium oxysporum f sp. chrysanthemi. Kohn (1974) reported Agrobacterium tumefaciens, as the causal agent of leaf and stem tumours on chrysanthemum. This was the first report in Berlin and Hamburg. Garibaldi and Gullino (1975) stated that Fusarium oxysporum f sp. tracheiphilum and F. O. f sp. chrysanthemi differing in their pathogenicity to certain cultivars were newly recorded. Control measures were indicated. Engelhard et al. (1976) reported stem rot as new disease on chrysanthemum incited by Fusarium solani in U.S.A. Menzies and Colhoun (1976) made mention about control of Phoma root rot of chrysanthemum by use of fertiliser. They stated that heavy fertilisation with nitrogen and phosphorus gave good control of root rot caused by Phoma chrysanthemicola.

B. In India :

Subba Rao (1944) reported wilting of Chrysanthemum cinerariaefolium associated with species of Fusarium from Nilgiris. Patel et al. (1949) reported Cercospora chrysanthemi, Puccinia chrysanthemi, Oidium chrysanthemi on leaves of chrysantherum sp. Vasudeva (1960) listed Fusarium

sp., Phytophthora cambivora and Rhizoctonia solani on Chrysanthemum cinerariafolium vis and Puccinia chrysanthemi, Septoria chrysanthemella on leaves of C. indicum and Cercospora chrysanthemi, Fusarium solani var. minus, Oidium chrysanthemi, Phyllostieta chrysanthemi and Rhizoctonia solani on Chrysanthemum sp. Rangaswami Gowda (1963) studied some bacterial diseases of ornamental and vegetable plants in Madras State (India) and reported Pseudomonas syringae for the first time on Chrysanthemum indicum. Wehmeyer (1963) recorded Leptosphaeria modesta and Pleospora richtophensis on Chrysanthemum richteria. Rao (1964) reported Phyllostieta chrysanthemi onl leaves of C. indicum and Patwardhan (1966) Agrosporium sp., Sarwar and Srinath (1966) reported new species of Stemphylium on C. cinerariaefolium. He reported Stemphylium nabarii M. Sarwar, causing large black brown, irregular spots.

Roy (1968), Govindu et al. (1970), Prasada et al. (1971), Thakur and Husain (1971), later Roy (1972, 73) recorded respectively Oidium chrysanthemi, Cercospora chrysanthemi, Leveillula taurica, Fusarium solani, Sclerotium rolfsii and Sclerotinia sclerotiorum. Patil and Rao (1973) reported a new leaf spot disease of

chrysanthemum from Maharashtra (India) caused by Septoria chrysanthemella. Natarajan and Srivastava (1975) studied the fungi associated with chrysanthemum leaf blight and its control. They isolated Septoria obesa, S. chrysanthemella, Phyllosticta chrysanthemi and Cladosporium sp.

Chapter III

SYMPTOMS OF THE DISEASE

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In nature the disease appeared as dark brown to black spots generally in the region of leaf apex and leaf-edges. The spots were circular and oval to irregular having greyish brown concentric rings at the centre. The size of the spots varied in diameter and ranged from 4 mm to 15 mm. In nature severely attacked leaves developed large spots which coalesced to form a large irregular necrotic patch, such necrotic patches gave appearance as blight. The leaves dry-off, curled and finally dropped down. Symptom also appeared on blossom. The colour of the affected floral parts turned dark brown to black. Thus the disease deteriated the quality of flower.

On artificial inoculation the symptoms appeared 10 days after inoculation by way of small dark brown to blakish spots circular, or oval to irregular in shape, and blighted in about 15 days. The spots were found scattered over leaf lamina. Generally infection started from apex and edges of the leaves. The blighted leaves dropped down prematurely after 25 to 30 days.

Chapter IV

MATERIALS AND METHODS

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Isolation :

Chrysanthemum leaves showing typical symptoms of the disease were collected from the Horticultural Farm at the, Central Campus of Mahatma Phule Krishi Vidyapeeth, Rahuri.

These leaves were then washed with tap water so as to remove dirt. After drying in the air, the affected parts were cut into small uniform pieces, disinfected with 1:1000 solution of mercuric chloride for 1 to 2 minutes and then washed in three changes of sterile water in order to remove traces of the disinfectant. These pieces were then planted in petriplates, poured with potato dextrose agar which was previously sterilised at 15 lbs pressure for 15 minutes. The plates were then incubated at room temperature (28-30°C). A typical grey to olive-grey fungus growth was observed on the third day, this growth was transferred to potato dextrose agar slants and cultures were maintained on it for further studies.

Pathogenicity :

Pathogenicity of the fungus was proved by inoculating one month old suckers of the chrysanthemum. Plants of chrysanthemum were raised in earthen pots, filled with sterilised soil. Plants were inoculated by spraying fifteen

days old culture with good conidial and mycelial growth of the fungus. Spore and mycelial suspension was prepared in sterilised water and sprayed on plant with the help of automiser. The plants sprayed with sterilised water served as control. These pots were kept under moist chamber for 24 hours before and after the inoculation separately. All the inoculated as well as control plants were transferred to the glass house benches for the disease development.

Reisolation :

Reisolations were made from the artificially inoculated leaves. Culture obtained from reisolation was transferred on potato dextrose agar slants for comparison with original culture.

Host Range :

Host range studies were undertaken in order to determine the ability of the fungus to infect other plants besides its own host. The chrysanthemum belongs to family compositae, five plants from this family and other economic plants were included in the study. The plants to be tested were raised in 9" height earthenware pots filled with sterilised soil. One month old plants so raised were inoculated by spraying spore and mycelial suspension of the fungus. These plants were then kept under moist chamber for 24 hours before and after inoculation and they were subsequently transferred to glass house for observing the disease symptoms. Adequate control plants for each host

were also provided, which were also sprayed with sterilise water. Periodical observations upto one month were recorded and are given in Table 1.

Varietal Resistance :

The object of this study was to find out the relative performance of the selected varieties of chrysanthemum against Alternaria sp. The available varieties of chrysanthemum were obtained from M.R.S. Hirayat Bag, Aurangabad. The suckers of these varieties were planted in sterilised soil filled in pots. When plants were about 4 months old, they were inoculated with the fungus as usual. The plants were kept 24 hours prior and after inoculation in the moist chamber. Observations on the number and size of the spots, were periodically recorded in order to find out the reaction of these varieties. The size and number of spots were considered in judging the varieties but most important considerations were stressed on size of the spots. The following score card was prepared.

Description of symptoms for judging the performance of varieties.

	Description	Size of spot	Performance of variety
I	No infection	-	Immune
II	Pin point spots, covering area of 10 % (approx.) of leaf.	A	Highly resistant

Contd.

	Description	Size of spot	Performance of variety
III	Slightly elongated to circular spots. Total number of spots covering 25 % leaf area.	B	Moderately resistant
IV	Bigger spots developing into irregular patches and total number of spots covering a leaf area of 40 %.	C	Moderately susceptible
V	Bigger type of spots with big patches covering a leaf area of 50 % or more	D	Highly susceptible

Performance of variety along with their resistance is presented in Table 2.

Cultural characters of the fungus :

The studies on cultural characters of the fungus were undertaken with the object of noting the growth behaviour and also to note its sporulation ability on different cultural media. For this purpose following media were tried.

1. Coon's agar
2. Czapeck's agar
3. Host leaf extract agar
4. Kirchoff's agar.
5. Leonian's agar.
6. M_2 agar
7. Nutrient agar

8. Oat meal agar
9. Potato dextrose agar
10. Richards' agar.

The above media were prepared according to the standard method, sterilised at 15 lbs pressure for 15 minutes. Duplicate plates were poured for each of the media and these plates were then inoculated with uniform bit of mycelium with the help of cork borer. Fresh culture of seven days growth was used for inoculation. The inoculated plates were then incubated at room temperature (28-30°C). Observations on colony diameter, sporulation and growth characters were recorded after 7 days. Rigway's (1912) "colour standard and colour Nomenclature" was followed for describing the colour of the colonies and substratum mycelium. The results are given in Table 3.

Spore Germination :

Studies on the germination of conidia were undertaken to note the mode, time required and effect of different substrata on germination of conidia of the fungus. For this purpose conidia secured from young culture of the fungus grown on potato dextrose agar was placed in a drop of each substratum on a cover glass, mixed thoroughly and the cover glass was placed upside down over the cavity slide. These were then placed in sterile petridishes with thin layer of moist cotton to maintain humidity. Such petridishes were incubated at room temperature (28-30°C). Observations on germination

percentage were recorded at an interval of two hours, and results are given in Table 4.

Morphology of the fungus :

Morphological characters of the pathogen grown on potato dextrose agar were studied. Slide culture technique was followed for preparation of slides. Mycelial width, septation and conidial measurements were taken after seven days of growth. Separate slides were also prepared for measurement of conidia from 20 days old culture. Five slides were prepared and randomly selected 20 conidia from each slide were measured.

Physiological characters :

Temperature growth relationship :

Studies on temperature growth relationship were undertaken to know the optimum, minimum and maximum temperature required for the growth of the fungus. For this purpose potato dextrose agar medium was used. The sterilised medium was poured (20 ml/plate) into duplicate plates for each temperature. Plates were inoculated with uniform bit of young growing culture of the fungus and incubated at 0°, 5°, 10°, 15°, 20°, 25°, 28-30° (room temperature), 35° and 40°C for 7 days. Observations on colony diameter and sporulation were recorded and results are given in Table 5.

Utilisation of carbon compounds :

This study was undertaken with the object to know the ability of fungus to utilise different carbon sources. A basal synthetic medium, Richards' agar without sugar, was prepared. Different carbon compounds were added to the basal medium on the molecular weight basis, taking sucrose as a standard in the Richards' medium. Media were sterilised and duplicate plates for each carbon compound were poured. The plates were then inoculated with an uniform bit of 10 days old culture and incubated at room temperature (28-30°C). Basal medium without sugar served as a control. Observations on colony diameter, growth characters and sporulation were recorded after 7 days and are given in Table 6. Following carbon compounds were used for the study.

I. Monosaccharides :

A. Pentose

1. Arabinose
2. D-xylose

B. Hexoses

1. Dextrose
2. Fructose
3. Mannitol

II. Disaccharides :

1. Lactose

2. Maltose

3. Sucrose

III. Trisaccharides :

1. Raffinose

IV. Polysaccharides :

1. Dextrin

2. Glycerol

3. Control

Utilization of nitrogenous compounds :

This experiment was carried out to find out the effect of different organic and inorganic nitrogenous compounds on growth and sporulation of the fungus. For this purpose Richards' medium without potassium nitrate was used as a basal medium, distributed in 100 ml aliquots in 250 ml Erlenmeyer flasks. The adequate quantities of organic and inorganic nitrogen compound calculated on molecular weight basis just to give nitrogen equivalent to potassium nitrate of the Richards' medium were added separately, to each flask. Richards' medium without potassium nitrate served as a control. These flasks were then sterilised and poured duplicate plates for each nitrogen compound, inoculated at centre with uniform size of fungus bit and incubated at room temperature (28-30°C), for 7 days. Observation on colony diameter, sporulation and growth characters etc. were recorded. The results are presented

in Table 7. Following nitrogenous compounds were used for the studies.

I. Inorganic nitrogenous compounds :

1. Ammonium nitrate
2. Ammonium tartarate
3. Ammonium sulphate
4. Ammonium oxalate
5. Calcium nitrate
6. Magnesium nitrate
7. Potassium nitrate
8. Sodium nitrate

II. Organic nitrogenous compounds :

9. Urea
10. Control

Enzyme production :

The enzymatic activity of the fungus as evidenced by the production of extracellular enzymes in the culture media, was studied by following the method described by Crabill and Reed (1915). For this purpose a basal medium of the following composition was prepared.

1. Magnesium sulphate	=	0.5 g
2. Potassium dihydrogen phosphate	=	1.09 g
3. Potassium chloride	=	0.5 g

4. Ferrous sulphate	=	Trace
5. Agar	=	30 g
6. Distilled water	=	1000 ml

The medium did not contain any carbon compound and hence, does not support any fungus growth.

The stock solution of above medium was distributed in 100 ml aliquots in 250 ml Erlenmeyer flasks. The following zymolates were added separately.

1. Potato starch	-	2 g
2. Egg albumin	-	1 g
3. Casein	-	1 g
4. Gelatin	-	1 g
5. Asparagin	-	1 g
6. Cellulose	-	2 g

The medium was sterilised, poured in duplicate plates for each zymolates, inoculated and incubated at room temperature (28-30°C) for 7 days. Uninoculated petriplates served as control in each case. The production of enzyme was judged either by colour reaction or by ability of the fungus to grow on a particular compound, which was added as a sole source of carbon in the medium. These results are presented in Table 8.

Effect of hydrogen ion concentration :

The growth of the fungus in culture medin as influenced by its hydrogen ion concentration was studied on Richards' liquid media.

The stock solution of Richards' liquid medium was prepared and distributed in 100 ml quantities in 250 ml Erlenmeyer flask. The medium was adjusted to different pH values colorimetrically by adding to it approximate quantities of N/10 sulphuric acid and/or N/10 sodium hydroxide solution. The test tubes filled in with the medium for each pH value were sterilised along with the flask at 15 lbs pressure for 15 minutes. After sterilisation the final pH of the medium in the flask was determined accurately by testing the sample solution from the tubes with Philip's pH meter. These flasks were then inoculated with the fungus bit and incubated at room temperature (28-30°C) for 21 days.

The mycelial mat was separated from the solution with the help of filter paper and dried at 40°C until a constant weight was obtained. The weight of the dried mycelial mat obtained at each pH level is given in Table 9.

Thermal death point :

Duplicate tubes of uniform size and shape were filled in with 10 ml of potato dextrose broth. These tubes were then sterilised and inoculated with an uniform bit of young growing culture. The inoculated tubes were kept in water bath at constant temperature of 35°, 40°, 45°, 50°, 55°, 60°, 65° and 70°C for ten minutes. An uniform and constant temperature of water was maintained by continuous stirring of the water in the water bath. A test tube with potato dextrose liquid medium

was used for keeping the thermometer for recording temperature. After an exposer of 10 minutes at a particular temperature, tubes were immediately placed in cold water and then incubated at room temperature (28-30°C). The observations on fungus growth were recorded after 7 days and are given in Table 10.

Efficacy of fungicides :

In vitro test :

Although evolving resistant varieties to different disease is an ideal method of disease control, the task of evolving resistant varieties by breeding involves technical skill and long term arduous investigation often leads to unsuccessful attempts due to several/reasons.

In absence of resistant varieties the next best alternative to resort to chemical method of control, which is being followed on an extensive scale in number of crops all over the world. Using over different fungicides and antibiotic with the object in view, in vitro test was undertaken to find out the efficacy of different fungicides and antibiotic for controlling the leaf blight of chrysanthemum. Following fungicides and antibiotic were used for trials.

Details of the fungicides are as follows :

1. Dithane M-45 (Mancozeb) :

a. Chemical name - Zincion Manganese ethylene-bisdithiocarbamate.

b. Active ingredient - 75 % wettable powder

c. Concentration used - 2,000 PPM.

d. Name of the firm - Indofill Chemicals Ltd., Bombay.

2. Vitavax :

a. Chemical name - 2, 3-Dihydro-6-carboxanilido 6-methyl 1-1, 4 Oxathilin

b. Active ingredient - 75 % wettable powder.

c. Concentration used - 1,000 PPM.

d. Name of the firm - Rallies India Ltd., Bombay.

3. Dithane Z-78 (Zineb) :

a. Chemical name - Zinc ethylene-bisdithiocarbamate.

b. Active ingredient - 75 % wettable powder.

c. Concentration used - 2,000 PPM.

d. Name of the firm - Indofill Chemicals Ltd., Bombay.

4. Cuman^{L} :

a. Chemical name - Ziram based organic fungicide.

b. Active ingredient - 30 % L.C.

c. Concentration used - 2,500 PPM.

d. Name of the firm - CIBA India Ltd., Bombay.

5. Difolatan :

a. Chemical name - N (1, 1, 2, 2 tetrachloroethyl) Thio-4-Cyclohexane 1, 2-dicarboxymide.

b. Active ingredient - 80 % wettable powder.

c. Concentration used - 1,000 PPM

d. Name of the firm - Rallies India ltd.,Bombay.

6. Aureofungin :

a. Chemical name - N-methyl P-amino acetophenone and mycosamine.

b. Active ingredient - 70 % wettable powder.

c. Concentration used - 50 PPM.

d. Name of the firm - Hindustan Antibiotics Ltd., Pimpri, Pune.

7. Fytolan :

a. Chemical name - Copper oxychloride

b. Active ingredient - 50 % wettable powder

c. Concentration used - 3,000 PPM

d. Name of the firm - Travancore Chemical and Manufacturing Co. Ltd., Alwaye.

8. Daconil 2787-W-75 :

a. Chemical name - Tetrachloro isophthalonitrile

b. Active ingredient - 75 % wettable powder

c. Concentration used - 2,500 PPM

d. Name of the firm - Diamond Shamrock India Ltd., Bombay 400 038.

9. Ziram :

a. Chemical name - Zinc dimethyl Dithiocarbamate

b. Active ingredient - 80 % wettable powder

c. Concentration used - 3,000 PPM

d. Name of the firm - Rallies India Ltd., Bombay.

10. Blitox-50 :

a. Chemical name - Copper oxychloride

b. Active ingredient - 50 % wettable powder.

c. Concentration used - 3,000 PPM

d. Name of the firm - Bandor India Ltd., Bombay.

11. Bavistin :

a. Chemical name - 2 (Methoxy-carbamoyl)-benzimidazole.

b. Active ingredient - 50 % wettable powder (It is formulated as a wettable powder containing 50 g a.i./kg).

c. Concentration used - 1,000 PPM

d. Name of the firm - BASF India Ltd., Bombay.

12. Kitasin :

a. Chemical name - O, O,Diisopropyl-S-benzyl thiophosphate.

b. Active ingredient - 30 % wettable powder.

c. Concentration used - 1,000 PPM

d. Name of the firm - Pesticides India Ltd., Udaipur.

13. Benlate :

a. Chemical name - Benomyl-(Methyl-1(butylcarbamoyl) 2-benzimidazole carbamate).

b. Active ingredient - 50 % wettable powder.

c. Concentration used - 500 PPM

d. Name of the firm - Agromore ltd., Bangalore.

Potato dextrose agar was prepared and distributed in 100 ml lots of 250 ml Erlenmeyer flasks and sterilised. After sufficient cooling i.e. 40°C, to each of the flask measured quantity of different fungicides and antibiotic of the recommended concentration was added and shaken thoroughly and then poured in duplicate plates for each chemical. The plates were inoculated with the young growing culture of the fungus and incubated for 7 days at room temperature (28-30°C). Plates with potato dextrose agar without fungicide served as control. Observations on colony diameter and sporulation were recorded after 7 days after inoculation and results are given in Table 11.

Chapter V

EXPERIMENTAL RESULTS

Chapter V

EXPERIMENTAL RESULTS

Isolation and pathogenicity of the fungus :

Isolation :

Isolations were made from diseased leaves of chrysanthemum depicting the typical symptoms. The culture obtained were maintained on potato dextrose agar slants for further studies.

Pathogenicity :

On artificially inoculated plants symptoms appeared 10 days after inoculation. The disease manifested as small circular to oval, irregular, brown to dark brown or black spots measuring 5 to 8 mm in diameter. Several such spots coalesced and formed big patches or necrotic areas, measuring 15-20 mm in diameter. Few leaves showed blightening after 15 days. Generally infection started from the apex and edges of the leaves. The symptoms developed due to artificial inoculation were similar to those observed under natural field conditions (Plate-I, II).

Reisolation :

Reisolations were made from artificially inoculated leaves which yielded the fungus identical in all respects with the original culture.

Host Range :

The results given in Table 1 (on page 32) indicated that Brinjal, Chilli, Cotton, Dahlia, Sunflower and Tomato were found

PLATE I

D H

Pathogenicity of the fungus

D = Diseased plants

H = Healthy plants

PLATE II

A B

Natural infection

A = Blossom

B = Leaves

Table 1 : Host range of the pathogen.

Sr.No.	Name of the host	Infection
1.	Bajra (Pennisetum typhoides, Stapf.)	-
2.	Bean (Phaseolus vulgaris, L.)	-
3.	Bhendi (Abelmoschus esculentus, L.)	-
4.	Brinjal (Solanum melongena, L.)	+
5.	Cabbage (Brassica oleracea var. Capitata, L.)	-
6.	Cauliflower (Brassica oleracea var.botrytis,L.)	-
7.	Chilli (Capsicum annum, L.)	+
8.	Cotton (Gossypium arborium, L.)	+
9.	Coriander (Coriandrum sativum, L.)	-
10.	Dahlia (Dahlia variabilis, DESF.)	+
11.	Groundnut (Arachis hypogaea, L.)	-
12.	Guar (Cyamopsis tetragonoloba, Tamb.)	-
13.	Jowar (Sorghum vulgare, Pers)	-
14.	Limabean (Phaseolus limensis, Macb.)	-
15.	Maize (Zea mays, L.)	-
16.	Methi (Trigonella foenum-graecum, L.)	-
17.	Mug (Phaseolus radiatus, L.)	-
18.	Peas (Pisum sativum, L.)	-
19.	Safflower (Carthamus tinctorius, L.)	-
20.	Sunflower (Helianthus annus. L.)	+
21.	Sunnhemp (Crotalaria juncea, L.)	-
22.	Tagetes (Tagetes erecta, L.)	-
23.	Tomato (Lycopersicum esculentum, Mill.)	+
24.	Tur (Cajanus cajan (L.) Millsp.)	-
25.	Urid (Phaseolus mungo, L.)	-
26.	Wheat (Triticum sativum, L.)	-
27.	Wal (Dolichos lablab, L.)	-
28.	Zinnia (Zinnia elegans, L.)	-

Note : + = Positive infection. - = Negative infection.

infected by the fungus, besides chrysanthemum. This showed that the culture under study was pathogenic on six host plants besides chrysanthemum, while it was non-pathogenic on the remaining 22 host plants.

Varietal Resistance :

The results in Table 2 revealed that variety Golden Darwar was found to be moderately susceptible while remaining all varieties viz. Barbara Phillips, Cormation Yellow, Garden State, H.V. West, Raja, Revadi, Snow queen, Surprise D'orsay and Zipri were found to be highly susceptible. No variety was immune or resistent.

Table 2 : Relative performance of chrysanthemum varieties against *Alternaria* sp.

Sr. No.	Name of the variety	Reaction	Size of the spot
1.	Barbara Phillips	Highly susceptible	D
2.	Cormation yellow	Highly susceptible	D
3.	Garden State	Highly susceptible	D
4.	Golden Darwar	Moderately susceptible	C
5.	H.V. West	Highly susceptible	D
6.	Raja	Highly susceptible	D
7.	Revadi	Highly susceptible	D
8.	Snow queen	Highly susceptible	D
9.	Surprise D'orsay	Highly susceptible	D
10.	Zipri	Highly susceptible	D

Note : A = Highly resistant
B = Moderately resistant
C = Moderately susceptible
D = Highly susceptible.

Cultural Characters :

The results presented in Table 3 (on page 35) indicated that the colony was circular having irregular margin and olive green in colour. The rate of the growth was slowest on Coon's agar, Czapeck's agar, Leonian's agar and maximum on Kirchoff's agar followed by Richards' agar. The fungus grew well on Kirchoff's agar, M_2 agar, oat meal agar, potato dextrose agar and nutrient agar. The development of aerial hyphae was on Kirchoff's agar. There was no aerial hyphae on other media. Concentric zonation were found on leonian's agar and Kirchoff's agar while ring formation at the periphery of the colony was noted on M_2 agar and host leaf extract agar. The sporulation was least on leonian's agar, Czapeck's agar and Coon's agar and maximum on Richards' agar, potato dextrose agar, Nutrient agar and host leaf extract agar (Plate III).

It is concluded that the fungus could have very wide range of selection in deriving nutrition. The growth and sporulation however was good on Kirchoff's agar, Richards' agar, potato dextrose agar, nutrient agar and host leaf extract agar followed by oat meal agar and M_2 agar. On remaining media growth was poor with moderate to scanty sporulation.

Spore Germination :

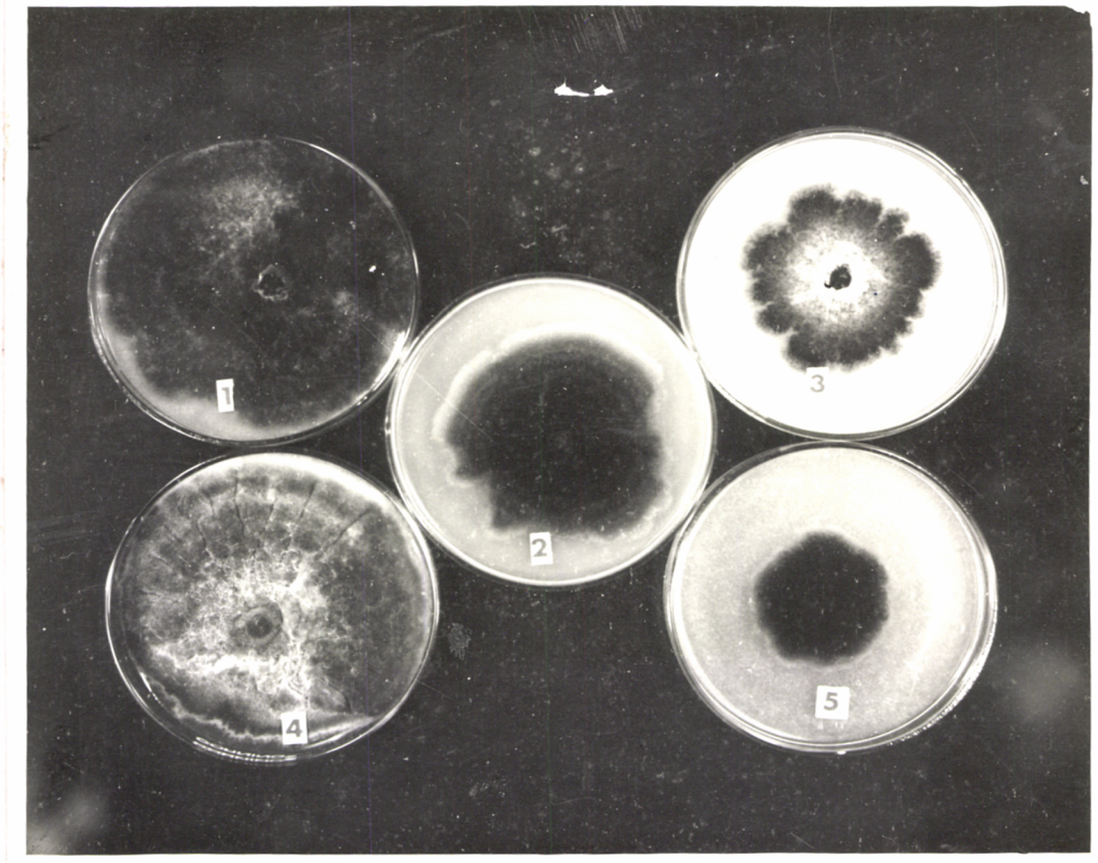
Results given in Table 4 (on page 37) revealed that the germination was noticed before two hours and almost completed at the end of eight hours. Germ tubes were emerging out from

PLATE III

Growth of the fungus on different culture media

1. Richards' agar
2. Leonian's agar
3. Czapeck's agar
4. Kirchoff's agar
5. Coon's agar
6. M_2 agar
7. Potato dextrose agar
8. Nutrient agar
9. Oat meal agar
10. Host leaf extract agar.

PLATE III



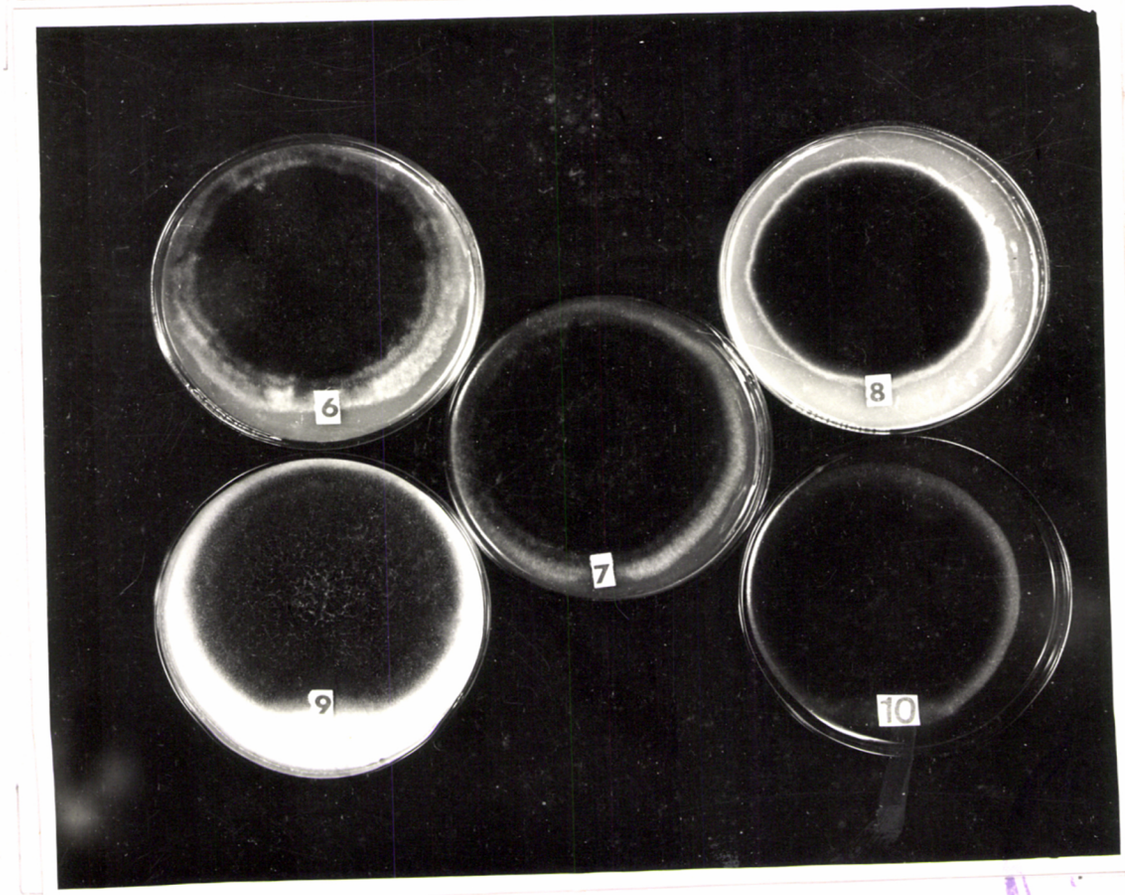


Table 3 : Cultural characters of the fungus on different media.

Sr. No.	Name of the media	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
1.	Coon's agar	41	++	Colony flat, circular with wavy margin, deep olive grey colour at the centre, mycelium poorly developed, no aerial hyphae.
2.	Czapeck's agar	56	++	Colony flat with irregular margin, dirty white in the centre, olive green colour around the centre, poor mycelial growth no aerial hyphae.
3.	Host leaf extract agar	74	++++	Colony flat, circular with entire margin, olive black in the centre and pale olive green at periphery with concentric ring at periphery. Moderate mycelial growth, no aerial hyphae.
4.	Kirchoff's agar	90	+++	Colony circular with entire margin, pale brown at the centre, olive greenish grey concentric zonation, substratum colour change to pale orange, mycelium moderate with aerial hyphae throughout the colony.
5.	Leonian's agar	62	+	Colony flat with irregular margin, colour pale olive green with concentric zonation, olive grey colou at periphery poor mycelial growth, no aerial hyphae.

Contd..

Table 3 (Contd.)

Sr. No.	Name of the media	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
6.	M_2 agar	81	+++	Colony flat circular with wavy margin, dark olive green and olive grey at periphery with concentric ring at periphery, mycelium poorly developed, no aerial hyphae.
7.	Nutrient agar	68	++++	Colony circular with entire margin, dark olive black at the centre and olive green at periphery. Moderate mycelium, no aerial hyphae.
8.	Oat meal agar	76	+++	Colony flat and circular, dark olive green with olive grey at periphery mycelial growth poor and no aerial hyphae.
9.	Potato dextrose agar	80	++++	Colony circular and raised with entire margin, concentric zonation having dark olive green colour with olive grey to whitish mycelium at periphery, good mycelium growth, no aerial hyphae.
10.	Richards' agar	85	++++	Colony circular with irregular margin, colour of the colony olive green with olive grey at periphery mycelium growth poor, compact, no aerial hyphae.

Note : - = Nil,
+ = Scanty.
++ = Moderate.
+++ = Good.
++++ = Abundant.

both the ends as well as from both the sides of conidia. Maximum seven germtubes were observed. Germtubes started branching after 6 hours from emergence. The host leaf extract was the best medium on which maximum germination was obtained, followed by tap water, 2 per cent sugar solution, 1 per cent sugar solution, potato dextrose broth, sterilise water and distilled water respectively (Plate IV).

Table 4 : Germination of conidia on different substrate.

Sr. No.	Name of the substratum	Germination % after 2 hours	4 hours	6 hours	8 hours
1.	Host leaf extract	45	74	92	100
2.	Potato dextrose broth	32	56	66	78
3.	Tap water	38	60	76	94
4.	Distilled water	35	52	60	70
5.	Sterilise water	40	48	65	72
6.	Sugar solution 1 %	25	59	70	80
7.	Sugar solution 2 %	42	56	68	88

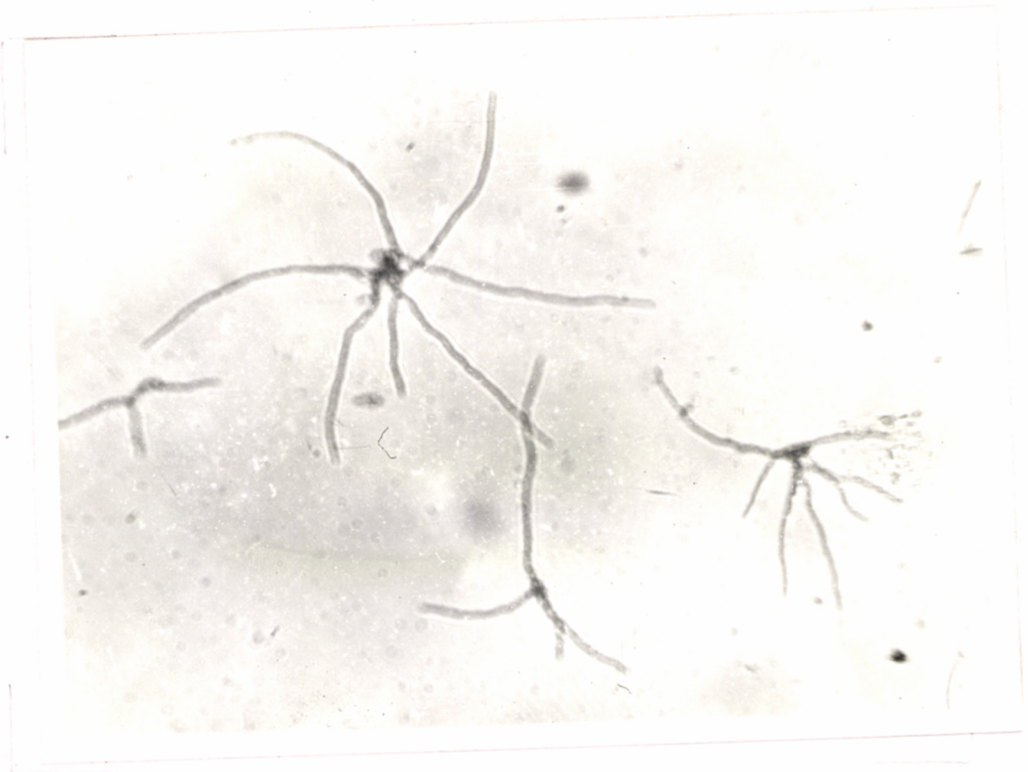
Morphology of the fungus :

The morphological characters of the fungus viz. mycelium, conidiophores and conidia were studied from the culture grown on potato dextrose agar and also from infected host.

I. Mycelium :

The fungus produce good mycelial growth in culture media.

PLATE IV



Photomicrograph showing the germination of conidia of the fungus under study

The young mycelium was poorly aerial, hyalins, septate, and irregularly branched, turning brown when old and measured 4.63 µ (3.12 - 6.24 µ) in width.

II. Conidiophores :

Conidiophores emerged from host tissue either singly or in groups of 2-5 with prominent apical scars. They were short, straight to slightly bent with 2-4 septa. They were slightly constricted at septa and swollen at base, light brown to dark brown in colour.

Length of the conidiophores from the host was 43.40 µ (15.60 - 81.90 µ) and from potato dextrose agar it was 35.76 µ (7.80 - 105.30 µ). Width of the conidiophores from the host was 5.27 µ (3.12 - 7.80 µ) and from potato dextrose agar it was 5.02 µ (3.12 µ - 6.24 µ) (Plate V).

III . Conidia :

There was variation in size and shape of conidia. The conidia obtained from host were relatively bigger in size and shape, beaks were also longer than those obtained from potato dextrose agar. Conidia were light olivaceous to dark brown, obclavate to muriform, with rudimentary or short beaks. They were in chains of 2-12 or more usually 5 to 8. They have 2 to 7 transverse septa, usually 3 to 5 and 0-3 longitudinal septa usually 1 to 2.

Length of conidia from the host was 41.34 µ (19.50 µ - 74.10 µ) and from potato dextrose agar it was 24.10 µ

(7.80 µ - 37.44 µ). Breadth of conidia from the host was 10.99 µ (4.68 µ - 19.50 µ) and from potato dextrose agar it was 8.88 µ (4.68 µ - 13.26 µ) (Plate V).

Physiological characters :

Temperature growth relationship :

The results given in Table 5 showed that the fungus did not grow at 0° and 5°C. The range of temperature in relation to growth was 10° to 40°C. The optimum growth and sporulation was at room temperature i.e. 28-30°C (Plate VI).

Table 5 : Temperature growth relationship.

Sr. No.	Temperature in °C	Mean colony diam. in cm after 7 days	Sporulation
1.	0	-	-
2.	5	-	-
3.	10	14	+
4.	15	18	+
5.	20	36	++
6.	25	60	+++
7.	28-30 (Room temp.)	76	++++
8.	35	34	++
9.	40	16	+

Note :

- = Nil.
+ = Scanty.
++ = Moderate.
+++ = Good.
++++ = Abundant.

PLATE V

Photomicrograph showing conidiophore with conidia in chain

Utilisation of carbon compounds :

The results given in Table 6 (on page 41) showed that fungus could utilise all carbon compounds under study. Excellent growth was obtained on dextrin, sucrose, maltose, lactose and arabinose. Good growth was obtained on fructose and glycerol. There was relatively moderate to scanty growth on D-xylose, raffinose, mannitol, dextrose and in control. Maximum sporulation was noted on sucrose, maltose and dextrin while good sporulation was observed on fructose, lactose, dextrose and raffinose, and on remaining carbon compounds moderate to scanty sporulation was observed.

It is concluded that fungus could utilise a wide range of carbon compounds, but sucrose, maltose and dextrin were found to be the best on which fungus could grow and sporulate abundantly.

Utilisation of Nitrogenous compounds :

Results in Table 7 (on page 43) showed that the fungus could utilise all inorganic and organic nitrogenous compounds. Excellent growth was observed on potassium nitrate and magnesium nitrate followed by sodium nitrate, ammonium tartarate. Good growth was observed on ammonium nitrate while scanty growth was noted on ammonium oxalate, urea, ammonium sulphate and on control.

Table 6 : Utilisation of carbon compounds.

Sr. No.	Source of carbon	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
I.	Monosaccharides :			
A.	Pentoses :			
1.	Arabinose	71	++	Colony flat, ring formation at the centre, irregular margin, pale grey mycelium growth at periphery and deep grey at centre. No aerial hyphae.
2.	D-xylose	56	++	Colony flat irregular margin, compact poor mycelium growth, deep olive grey colour at Centre and pale olive at periphery. Poor aerial hyphae at the centre.
B.	Hexoses :			
1.	Dextrose	55	+++	Colony flat, circular with entire margin, deep olive green to black colour, whitish mycelium ring at periphery, no aerial hyphae.
2.	Fructose	63	+++	Colony flat, concentric at periphery, entire margin, olive green colour, poor mycelial growth and no aerial hyphae.
3.	Mannitol	56	+	Colony flat with irregular margin, pale grey colour, poor mycelium growth, poor aerial mycelium at centre whitish mycelium at periphery.
II.	Disaccharides :			
1.	Lactose	70	+++	Colony flat with irregular margin, poor aerial mycelium at centre, pale olive colony, whitish mycelium at periphery.

Contd.

Table 6 (Contd.)

Sr. No.	Source of carbon	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
2.	Maltose	76	++++	Colony flat with irregular margin, poor mycelium at centre, colour of the colony deep olive grey, poor mycelium growth.
3.	Sucrose	82	++++	Colony flat irregular margin, colour deep olive green to black, olive grey at periphery, poor mycelium growth, no aerial hyphae.
III.	Trisaccharides :			
1.	Raffinose	50	+++	Colony flat with irregular margin, pale olive grey at centre and deep olive grey at periphery. Poor mycelium growth. Aerial hyphae absent.
IV.	Polysaccharides :			
1.	Dextrin	85	++++	Colony flat, compact with irregular margin, whitish mycelium at the centre and olive grey at the periphery, moderate mycelium growth, Aerial hyphae absent.
2.	Glycerol	61	+	Colony flat, circular and entire margin, poor and thin mycelium growth, whitish thread like growth at periphery with poor aerial hyphae.
3.	Control (Without any carbon compound)	31	+	Colony flat, compact, thin, scanty and hairy growth of mycelium with pale grey colour, irregular margin, no aerial hyphae.

Note : - = Nil.
+ = Scanty.
++ = Moderate.
+++ = Good.
++++ = Abundant.

Table 7 : Utilisation of nitrogenous compounds.

Sr. No.	Source of nitrogen	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
I.	Inorganic nitrogenous compounds			
1.	Ammonium nitrate	50	++	Colony slightly raised with irregular margin, olive grey at the centre and orange colour at periphery, moderate mycelium growth aerial hyphae absent.
2.	Ammonium tartarate	64	+++	Colony flat with irregular margin, olive grey colour with yellowish tinge, colony having zonation, substratum orange grey coloured, poor mycelium, no aerial hyphae.
3.	Ammonium sulphate	34	+	Colony flat irregular margin, mycelium compact and poor, pale olive in colour, no aerial hyphae.
4.	Ammonium oxalate	41	++	Surface uneven, colour colour olive with greyish at the centre, wavy margin, mycelium poorly developed, no aerial hyphae.
5.	Calcium nitrate	47	+++	Colony flat, irregular margin, colony having dark olive grey colour, with poor mycelium, no aerial hyphae.
6.	Magnesium nitrate	76	+	Colony flat, irregular margin, scanty hairy mycelium growth, pale grey colour, mycelium poorly developed, no aerial hyphae.

Contd.

Table 7 (Contd.)

Sr. No.	Source of nitrogen	Mean colony diam. in mm after 7 days	Sporulation	Growth characters
7.	Potassium nitrate	81	++++	Colony flat with irregular margin, dark olive grey colour, poor mycelial growth, dirty aerial hyphae at the centre.
8.	Sodium nitrate	68	++++	Colony flat, entire margin dark olive grey colour blackish at the centre with poor mycelial growth and no aerial hyphae.
II.	Organic nitrogenous compounds			
9.	Urea	46	+++	Colony flat, irregular margin, olive grey with yellowish tinge, green colour at periphery, mycelium poorly developed, no aerial hyphae.
10.	Control (without KNO_3)	36	+	Colony circular, entire margin, mycelium thin, scanty and pale grey in colour, no aerial hyphae.

Note :

- \- = Nil.
- \+ = Scanty.
- ++ = Moderate.
- +++ = Good.
- ++++ = Abundant.

Excellent sporulation was observed on potassium nitrate and sodium nitrate. Ammonium tartarate, calcium nitrate and urea developed good sporulation. While moderate to scanty sporulation was noted on ammonium nitrate, ammonium oxalate followed by magnesium nitrate and control.

It was observed that most of the nitrogenous compounds supported growth and sporulation.

Enzyme production :

a. Diastase :

Two grams of potato starch were added to 100 ml of the basal medium. The fungus made moderate growth and produced distinct 'halo' around the colony when treated with 1 per cent iodine solution. This indicate that fungus had the ability to hydrolise starch which was indicated by production of enzyme 'diastase'.

b. Trypsin :

One gram of egg-albumin was added to 100 ml of the basal medium, which was sterilised at 15 lbs pressure for 30 minutes, so as to all coagulation of proteins. Fungus grew well on this medium showing thereby its ability to produce enzyme 'trypsin'.

c. Erypsin :

One gram of casein was added to 100 ml of the basal medium to prepare casein agar. The fungus made excellent

growth and developed clear zone around the colony indicated the production of enzyme 'erypsin'.

d. Gelatinase :

One gram of gelatin was added to 100 ml of basal medium, moderate growth was observed, when plates were flooded with acified $HgCl_2$, clear 'halo' was produced around the colony, showing ability of the fungus to produce 'gelatinase' in small quantity.

e. Amidase :

One gram of asparagin was added to 100 ml basal medium. Fungus made very scanty growth indicating its ability to produce enzyme 'amidase' in very small quantity.

f. Cystase :

Two grams of cellulose was added to 100 ml basal medium. The fungus made scanty growth, showing its ability to produce enzyme 'cystase' in very small quantity.

The data presented in Table 8 (on page 47) showed that good growth of the fungus was in media containing casein and egg albumin indicating thereby the production of enzyme erypsin and trypsin respectively. In other media moderate to scanty growth was observed, which indicated that fungus could produced enzyme gelatinase, diastase amidase and cystase in small quantities.

Table 8 : Production of enzymes.

Sr. No.	Medium	Mean colony diam. in mm after 7 days	Enzyme produced	Sporulation
1.	Potato starch agar	43	Diastase	++
2.	Egg-albumin agar	54	Trypsin	+
3.	Casein agar	56	Erepsin	+++
4.	Gelatin agar	46	Gelatinase	++++
5.	Asparagin agar	36	Amidase	++
6.	Cellulose agar	31	Cystase	+

Note : - = Nil.
+ = Scanty.
++ = Moderate.
+++ = Good.
++++ = Abundant.

Effect of hydrogen ion concentration :

The results presented in Table 9 (on page 48) showed that the fungus could grow within a wide range of pH viz. 2.2 - 9.6 but the optimum pH for the growth was 4.8 to 7.1. The maximum growth was obtained at pH 6.4, thus indicating the fungus tendency to grow well in acidic media than the alkaline media.

Thermal death point :

There was no growth in tubes exposed to the temperature of 60°, 65° and 70°C, while in other test tubes growth was

observed. In order to find out exact T.D.P. same procedure was repeated at constant temperature of 56°, 57°, 58°, 59° and 60°C.

Table 9 : Effect of hydrogen ion concentration.

Sr. No.	pH of the medium		Dry weight of the mycelial mat in mgs after 21 days.
	Before sterilisation	After sterilisation	
1.	2	2.2	518
2.	3	2.6	686
3.	3.5	3.3	701
4.	4	3.6	829
5.	4.5	4.2	834
6.	5	4.8	1163
7.	5.5	5.3	1358
8.	6	5.7	1480
9.	6.5	6.0	1532
10.	7	6.4	1605
11.	7.5	7.1	1207
12.	8	7.7	946
13.	8.5	8.1	899
14.	9	8.8	771
15.	9.5	9.4	381
16.	10	9.6	352

Table 10 : Thermal death point.

Sr.No.	Temperature in °C	Fungus growth after 7 days
1.	35	+
2.	40	+
3.	45	+
4.	50	+
5.	55	+
6.	56	+
7.	57	+
8.	58	+
9.	59	+
10.	60	-
11.	65	-
12.	70	-

Note : - = Nil.
+ = Growth.

The results given in Table 10 showed that the thermal death point of the fungus was 60°C.

<u>In vitro test</u> :

It was observed from the data presented in Table 11 (on page 50) that Dithane M-45, Vitavax, Dithane Z-78 and Cuman[1] inhibited the growth and sporulation of the fungus. Difolatan was also found to be effective in checking the growth and

sporulation while aureofungin, fytolan, daconil, Ziram, Blitox-50, bavistin, kitazin and benlate did not prove effective in checking the growth of the fungus (Plate VII).

Table 11 : Effect of different fungicides and antibiotic on the growth and sporulation of the fungus *in vitro*.

Sr. No.	Name of the fungicides and antibiotic	Mean colony diam.in cm after 7 days	Sporulation
1.	Dithane M-45	-	-
2.	Vitavax	-	-
3.	Dithane Z-78	4	-
4.	Cuman[1]	8	-
5.	Difolatan	6	+
6.	Aureofungin	11	++
7.	Fytolan	17	++
8.	Daconil	21	++
9.	Ziram	34	+++
10.	Blitox-50	48	+++
11.	Bavistin	51	+++
12.	Kitazin	48	+++
13.	Benlate	46	+++
14.	Control	82	++++

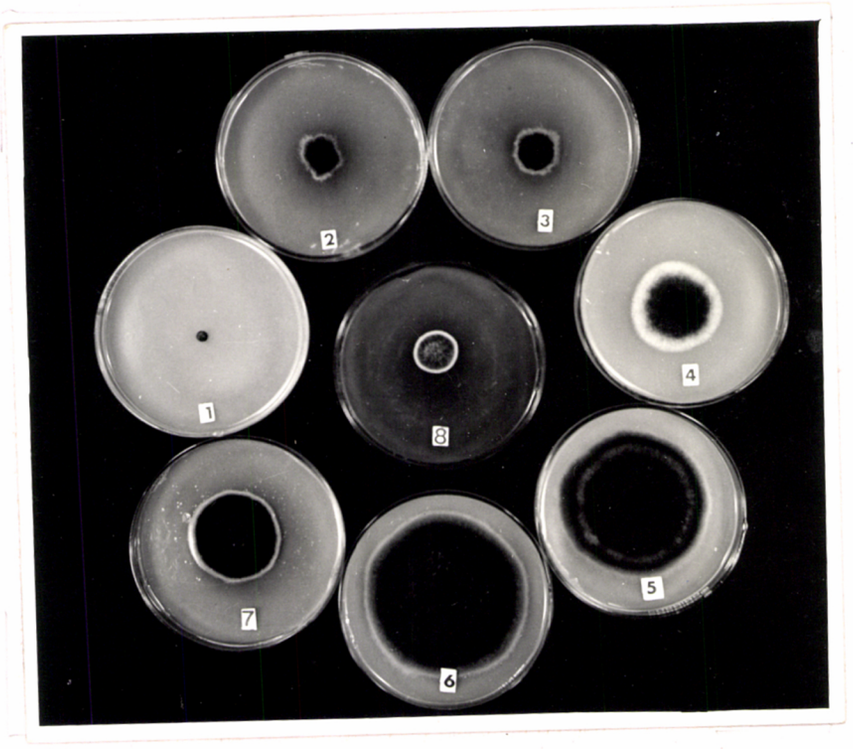
Note : - = Nil.
+ = Scanty.
++ = Moderate.
+++ = Good.
++++ = Abundant.

PLATE XII

Effect of different fungicides and an antibiotic on the fungus growth *in vitro*.

1. Dithane M-45
2. Vitavax
3. Dithane Z-78
4. Cuman[1]
5. Difolatan
6. Aureofungin
7. Fytolan
8. Daconil
9. Miram
10. Blitox-50
11. Benlate
12. Kitazin
13. Benlate
14. Control

PLATE VI



Effect of different temperatures on the fungus growth

1.	0°C	.. 5	25°C
2.	10°C	.. 6	28-30°C (Room temp.)
3.	15°C	.. 7	35°C
4.	20°C	.. 8	40°C

Chapter VI

DISCUSSION

Chapter VI

D I S C U S S I O N

The present investigations on the leaf blight disease of chrysanthemum were undertaken to study the symptoms, causal organism, its morphology and physiology and to find out suitable control measures.

The pathogen was easily isolated from the diseased leaves by usual method. Pathogenicity was proved by inoculating one month old healthy plant of chrysanthemum. The fungus was mainly responsible for inciting the leaf spot and blight. Disease appeared as dark brown to black spots generally in the region of leaf tips and leaf edges. The spots were circular and oval to irregular having greyish concentric rings at the centre. The size of the spots varied in diameter and ranged from 4 mm to 15-20 mm. Under severe conditions of infection spots coalesced to form a large irregular necrotic patch. Such necrotic patches gave appearance of blight. The leaves dry off, curled and dropped down prematurely. Symptoms also appeared on blossom and deteriated the quality of flower.

Schmidt (1958) stated that Alternaria chrysanthemi T. Schmidt. affected all green parts, causing round, initially pale grey spots. They enlarge rapidly to their final diameter of approximately 1 cm. When fully developed the spots were grey to brownish-black, often with a pale fleck at the centre and more or less distinct light and dark zonation where the

spots were sufficiently numerous, entire leaves withered. Rodebaugh (1963), Tennen (1965), Sober (1966), Cox (1969), Ingelhard (1970) and Holcomb (1976) described the disease as leaf-spots, leaf blight, flower blight, dusty specks on the ray floret, petal necrosis etc. In India Rao (1963) described the leaf spots as dark-brown to black circular to irregular, often coalescing to form large patches. These spots were due to A. chrysanthemi T. Schmidt. Later in 1965, he described similar leaf spots, oval to irregular or angular, dark-brown to black, as leaf blight, blossom blight and defoliation. These were due to A. tenuis Auct. Srinath and Sarwar (1965) noted leaf spots due to A. tenuissima (Fr.) Wiltshire as brown patches, 2-4 mm in diameter, mostly near the apical parts of the leaves. These patches enlarged irregularly turning blackish-brown in colour, eventually the necrosis covered the entire leaf area and proved fatal to the plant. The symptoms observed in the present study were similar to those reported by earlier workers. The symptoms noted were mostly on the leaf apex and leaf edges. Concentric zonations as described by Schmidt (1958) were observed only when spots were isolated and restricted in size. The pale fleck or white at the centre of the zonation was however absent on naturally affected diseased specimen as well as on the artificially inoculated plants.

The host range studies indicated that brinjal, chilli, cotton, dahlia, sunflower and tomato could be collateral hosts

for the pathogen under study as the fungus proved to be pathogenic on these hosts, and non-pathogenic on the remaining 22 hosts. Rao (1971) reported A. tenuis Aust. as pathogenic on 51 hosts. He however, did not carry cross inoculation test and that his identification was based on morphological characters. The present studies agreed with those of Rao in that the fungus under study was pathogenic on 6 host plants that were included in his list of host plants for A. tenuis Aust.

Several workers reported Alternaria spp. particularly, A. tenuis on a number of host plants. Thus Faulwetter (1918) reported it on cotton and wheat, Rosella (1930) on barley grains, Abramoff (1932) on soyabean, Groves and Skolko (1944) on alfalfa, Pucci (1947) on brinjal and chilli, Joshi (1952) on Cordia myxa. Agnihotri (1963) showed Alternaria tenuis isolated from Areca palm to be pathogenic on draucaena, egg plant, Solanum nigrum, Datura metal, and fruits of apple and pear. Roton (1965) showed tomatoes and potato to be the host plants of A. tenuis. Quebral and Shurtleff (1966) reported A. tenuis on bell paper, Joly (1967) on citrus fruits, Sulaiman and Jadhav (1967) on potato, Bhowmik (1969) on wheat. Patil (1970) studied the leaf blight of Safflower caused by A. tenuis, Dutta et al. (1971) studied the leaf spot of corn flower caused by A. tenuis and stated that the organism attacked a large number

of ornamental and other plants of family Compositae. Jadhav (1974) studied the leaf spot of sunflower caused by A.tenuis. Tandon and Shivkumar (1974) reported A.alternata causing brown rot of Carissa carandas and stated that fungus could also infect fruits of tomato, apple and beans. It however, failed to parasitise on amla, guava and brinjal.

Thus the pathogen under study, A. tenuis had a very wide host range and reported to be a mild pathogen at times developing into serious proportions. It would be worthwhile therefore, to isolate the fungus from the diseased specimen collected from different localities where chrysanthemum is grown and conduct detailed studies with respect to symptoms developed and the host range.

The cultivar golden Darwar was proved to be moderately susceptible, while all others viz. Barbara Phillips, Cormation yellow, Garden state, H.V. West, Raja, Revadi, Snow queen, Surprise D'orsay and Zipri were found to be highly susceptible.

Morphological characters viz. colour, septation, shape and size of the mycelium, conidiophores and conidia were studied. These characters indicated that the pathogen under study belonged to class Hyphomycetes and the genus Alternaria.

Nees (1817) described the genus Alternaria with A. tenuis as a type species. Although the characterisation of the genus was somewhat incomplete, the broad concept and definitions have been generally accepted by subsequent workers

in the field. Fries (1825) described the genus Macrosporium and subsequently (1832) gave more detailed account and description of this genus. He did not, at that time, recognise the genus Alternaria Nees but considered it synonymous with 'Torula'. Chevallier (1826) recognised the genus Alternaria and differentiated it from 'Torula' on the basis of the presence of beak in the former. Corda (1839) recognised the genus Alternaria and gave illustration and a description of A.tenuis Auct. Later Fries (1849) acknowledged the genus Alternaria in his work 'Summa vegetabilium Scandinaviae'. He thought the genus Polydesmus Mont to be a synonym of the genus Alternaria Nees and distinguished the genus Macrosporium FR. from the genus Alternaria Nees. Elliott (1917) reported detailed investigation on the generic characters and limitations of the genus Alternaria. He emphasised the conidial characters viz. form, shape and the presence of beak as a distinctive generic characters. He observed that the catenulation of conidia was determined and modified by environmental conditions. He divided Alternaria into six groups on the basis of morphological differentiations and dimentions of the spores. He recognised Alternaria and Macrosporium as two distinct genera. Wiltshire (1933, 38) and later, Groves and Skolko (1944 a & b) after an extensive study came to the conclusion that the generic name Macrosporium should be dropped and Stemphylium should be recognised instead for all muriform spores produced singly and the name Alternaria be resumed for beaked spores produced either

singly or in chains. He considered that the description given by Nees was sufficiently accurate for recognising the modern concept of the genus Alternaria. He suggested that since the name Alternaria is universally accepted and known term, it should be used to designate the beaked conidia irrespective of their formation in chains or singly on conidiophores although such procedure did not strictly comply with the provisions of the 'priority' accepted by the 'International Rules of Nomenclature'. Neergaard (1945) after a thorough study of many authentic specimens agreed to wiltshire's recommendation. He completely revised the taxonomy of Alternaria through a critical examination of over 70 species of this and several closely related genera, eliminating all but 20 species of the genus Alternaria. Some of the species classified under Macrosporium, Helminthosporium, Cladosporium, Sporodesmium, Polydesmus and Puccinia were rejected and made synonyms of the genus Alternaria.

The conidia of the fungus under study were obclavate, muriform having 2 to 7 transverse septa and 1 to 3 longitudinal septa. The conidia were borne mostly in chains to the extent of 5-12 conidia. Therefore the fungus under study was identified as belonging to the genus Alternaria.

In order to identify the Alternaria species under study, comparative data of the description of the Alternaria spp. viz.

A. chrysanthemi, A. tenuis, and A. tenuissima reported to be pathogenic on chrysanthemum was compiled. These descriptions along with those of the type species of A. tenuis and A. alternata are presented in Table 12. It was revealed from the Table 12 that the morphological characters of the pathogen under study were not in complete agreement with the description of A. chrysanthemi and A. tenuissima parasitic on chrysanthemum. The fungus under study differed from these two Alternaria species in respect of size, shape, septation, conidial measurements and other morphological characters.

According to Simmons and Crosier (1965) and C.M.I. descriptions of pathogenic fungi and bacteria No. 164, the conidia of A. chrysanthemi were often cylindrical with upto 9 transverse septa and sometimes one or more longitudinal septa. In this description there was no mention of the beak. Conidial length was 70 µ (25-130 µ). According to Rao (1963) conidia of A. chrysanthemi were in short chains of 2-5 obovate to oblong with medium septate beak. Conidial length was 27-130 µ. The shape of the conidia of the fungus under study was obclavate and not cylindrical or obovate. The portion of the conidium which was proximal to the cell which bore it was broader than the distal portion. The beak was non-septate and short on culture medium. The length of the conidia was 19-74 µ. Thus the conidial characters did not agree with those of A. chrysanthemi. Similarly the fungus under study was

differentiated from A. tenuissima on the basis of conidial chain. According to Srinath and Sarwar (1965) A. tenuissima was bravicatenulate or short-chained of 2-4.

The conidia of the fungus under study were light olivaceous to dark-brown, obclavate to muriform with rudimentary or short beak. There was variation in size and shape of conidia. The conidia obtained from host were relatively bigger in size, shape and beak length in comparison to those obtained from potato dextrose agar. Misaghd et al. (1978) noted the influence of environmental and culture media on spore morphology of Alternaria alternata and stated that the conidia formed in natural habitats usually were bigger, had long beaks and more uniform in size than those produced in vitro on common agar media. He also mentioned that morphology of conidia produced in vitro was influenced by composition of substrate. The fungus under study formed chains upto 12 or more usually 5-8, conidia with 2-7 transverse septa, usually 3-5 and 0-3 longitudinal septa, usually 1 to 2. Length of conidia from host was 41.34 µ (19.50 - 74.10 µ) and from potato dextrose agar 24.10 µ (7.80 - 37.44 µ). Breadth of conidia from host was 10.99 µ (4.68 - 19.50 µ) and from potato dextrose agar it was 8.88 µ (4.68 - 13.26 µ). Thus the morphological characters of the pathogen under study agreed to those reported for A. tenuis.

Simmons (1967) referred to the work of Wiltshire (1929, 30) and stated that Wiltshire was unable to locate

the type specimen of A. tenuis for examination. Simmons examined the typed specimen of A. tenuis which he referred as neotypus and gave the description of the organism. He presented the evidence based on International Rules of Nomenclature and stated that the valid name for A. tenuis is A. alternata. The morphological characters of the pathogen under study also agreed with those for A. alternata given by Simmons. The pathogen therefore was identified as Alternaria alternata (Fries) Keissler.

The fungus could grow on a variety of culture media, but the growth and sporulation was good on Kirchoff's agar, Richards' agar, potato dextrose agar, host leaf extract agar and nutrient agar. Ashour and El-Kadi (1959) studied the cultural characters of A. tenuis and reported that potato dextrose agar and Richards' medium was the best. Jadhav (1974) studied the leaf-spot of sunflower and stated that A. tenuis grew well on nutrient agar, potato dextrose agar and host leaf extract agar.

Fungus could germinate within 2 hours. Maximum germination was obtained on host leaf extract. The results obtained in the present study were in agreement to those reported by Chougule (1973) and Jadhav (1974). Chougule studied A. tenuis isolated from leaf-blight of soyabean and stated that percentage of germination was highest in host leaf decoction, while Jadhav found that the germination of

Table 12 : Comparative study of *Alternaria* spp. with fungus under study.

1		*Alternaria chrysanthemi* Simmons & Crosier
Habitat	:	*Chrysanthemum maximum* L.
Symptoms	:	On stem, leaves, flowers and seeds of *Chrysanthemum maximum*, on the leaves spots are round, at first pale grey and upto 1 cm diam., later grey or brownish black, often with whitish spot in the centre surrounded by pale and dark concentric rings.
Mycelium	:	Immersed, hyphae branched, septate, hyaline to rather pale olivaceous brown, 4-8 µ thick.
Conidiophore	:	The conidiophores arising singly or in fascicles of upto 6 terminally and laterally on the hyphae, simple (occassionally branched in culture), erect or ascending straight or flexuous, cylindrical, septate, pale or mid olivaceous or golden brown, smooth, upto 100 µ long, 6-11 µ thick, usually bearing 1-3 conidial scars.
Conidia	:	Solitary, acropleurogenous, arising through small pores in the conidiophore wall, straight or very slightly curved, often cylindrical, sometimes, obclavate with upto 9 (12 in culture) transverse septa and sometimes one or more longitudinal septa, often slightly constricted

at the septa, hyaline to pale olivaceous brown or golden brown, smooth, 25-130 (70) µ thick in the broadest part.

2		Alternaria chrysanthemi T. Schmidt
Habitat	:	Chrysanthemum indicum L.
Symptom	:	Incite leaf spot and blossom, leaf spots, dark-brown to black circular to irregular, often coalescing to form large patches, scattered, which turn dirty-brown to sooty brown, petals becoming brittle and fall away easily, imparting a blighted appearance to the crop. The disease is very common and destructive in cold season (November-January) damaging the flower and greatly reducing their market value.
Mycelium	:	--
Conidiophore	:	Conidiophores simple, dark-brown, bulged at the base rounded and scarred at apex, 1-5 septate, solitary or in group of 2-5, emerge through stomata or host tissue, measure 25.2-84 x 4.76 µ.
Conidia	:	Conidia in short chains of 2-5, obovate, to oblong with a medium septate beak, brownish with 1-5 longitudinal and 3-10 cross-septa, constricted at septa, scarred at base, measure 27.5-130.2 x 12.6 - 18.9 µ.

3		*Alternaria tenuis* Auct.
Habitat	:	*Chrysanthemum indicum* L.
Symptom	:	Infection spots oval to irregular or angular, dark-brown to black inciting leaf-blight, blossom blight and defoliation.
Mycelium	:	--
Conidiophore	:	Conidiophores short to long, septate, few geniculate with apical scars, solitary or in fascicles of 2-6, simple or branched, straight to slightly wavy, emerging through the host epidermis or stomata with a range of 46.2 - 147 x 4.2 - 8.5 µ.
Conidia	:	Conidia obclavate to typically muriform, dark-brown, thick-walled, sometimes verruccse, in long chains (10-25), cross, vertical as well as oblique septa common, constricted at cross-septa, prominently scarred at base and apex, majority non-beaked, few with short rudimentary dark-brown beaks, with a range of 23.0 - 90.0 x 8.5 - 23.0 µ.

4		Alternaria tenuissima (Fr.) Wiltshire
Habitat	:	Chrysanthemum cinerariaefolium
Symptom	:	Brown patches, 2-4 mm in diam. were observed near the apical parts of the leaves, these patches enlarged irregularly turning blackish-brown in colour eventually the necrosis covered the entire leaf area and proved fatal to the plant.
Mycelium	:	--
Conidiophore	:	Conidiophores dark-brown, septate, septa 8-10 µ apart, sub-erect to erect, unbranched, measuring 30-110 x 3-4 µ.
Conidia	:	Conidia dilute brown produced in short chains of 2-4 acrogenous, smooth, obclavate, 30-40 x 11-19 µ , with 3-9 transverse and 2-4 longitudinal septa, smooth-walled, beak 7-14 x 1-2 µ with an obtuse end.

5		Alternaria alternata (Fries) Keissler
Habitat	:	--
Symptom	:	--
Mycelium	:	--
Conidiophore	:	Conidiophores dilute yellow brown to medium golden brown in colour, simple, straight or curved, smooth, 1-3 septa, 20-46 x 4-6 µ,

apically uniperforate, sometimes with basal cell slightly swollen.

Conidia : Conidia ovoid, obclavate, obpyriform or rarely simply ellipsoidal in shape, usually with an easily visible basal pore; beakless when ellipsoidal, or with a short conical, narrowly tappered, or cylindrical beak 2-3 µ in diam., the apex of which may be narrow and rounded without a terminal pore or abruptly blunt with a well defined pore, beak length upto 25 µ, near equaling the length of conidium body but commonly representing one-fourth to one-third of the total conidium length, beak usually lighter in colour than the body conidium body (10-)18-47 x (5-) 7-18 µ) av. 30.9 x 12.6 $\frac{l}{b}$ = 1.7 - 3.4, av. 2.4; with (1-) 3-8 transverse septa, one or two longitudinal septa in each of the 1-6 of the transverse divisions, and commonly a strongly oblique septum in the basal division, distinctly but not deeply constricted at major transverse septa. Conidium wall smooth or very minutely roughened.

6		Fungus under study
Habitat	:	Chrysanthemum indicum L.
Symptom	:	Symptom appear as dark-brown to black spots

generally in region of leaf apex and leaf edges. The spots were circular and oval to irregular, greyish brown, concentric ring at the centre, size of the spots varied in diam. from 4 mm to 15-20 mm. Severely attacked leaves developed large spots which coalesced to form necrotic patch, gave the appearance of blight. The leaves dry-off, curled and dropped down prematurely. Symptoms also observed on blossom. The colour of the affected floral parts turned dark brown to black, detoriating the quality of flower.

Mycelium : The young mycelium was poorly aerial, hyaline, septate and irregularly branched turning brown when old and measured 4.63 µ (3.12 - 6.24 µ) in width.

Conidiophore : Conidiophores emerged from host tissue either singly or in groups of 2-5 with prominent apical scars. They were short, straight to slightly bent, and having 2-4 septa, slightly constricted at the septa and swollen at the base, light to dark brown in colour. Length of conidiophores from the host was 43.40 µ (15.60 - 81.90 µ) and from potato dextrose agar it was 35.76 µ (7.80 - 105.30 µ) width of the conidiophores from the host was 5.27 µ

(3.12 - 7.80 µ) and from potato dextrose agar it was 5.02 µ (3.12 - 6.24 µ).

Conidia : There was variation in size and shape of conidia. The conidia obtained from the host were relatively bigger in size and shape, beaks were also longer than those obtained from potato dextrose agar. Conidia were light olivaceous to dark-brown obclavate to muriform with rudimentary or short beaks. They were in chains of 2-12 or more usually 5-8. They have 2-7 transverse septa, usually 3-5, and 0-3 longitudinal septa usually 1-2. Length of conidia from host was 41.34 µ (19.50 - 74.10 µ) and from potato dextrose agar it was 24.10 µ (7.80 - 37.44 µ). Breadth from the host was 10.99 µ (4.68 - 19.50 µ) and from potato dextrose agar it was 8.88 µ (4.68 - 13.26 µ).

the conidia isolated from sunflower was best on host leaf extract and good germination in 4 per cent or 3 per cent sugar solution and in tap water.

The temperature growth relation studies revealed that the fungus could grow within the range of 10° to 40°C however maximum growth and sporulation was noted between 28-30°C (room temperature) and the growth slowed with increasing temperatures. The fungus did not grow at 0° and 5°C. Arya and Prasada (1952) studied A. tenuis on linseed and observed the temperature range of 10° to 35°C to be suitable with optimum at 26-30°C. There was no growth at 4° and 42°C temperatures.

The fungus could utilise all the carbon compounds under study but sucrose, maltose and dextrin were found to be best on which fungus could grow and sporulate abundantly. Uppal et al. (1938) studied Alternaria blight of cumin. His studies showed that the fungus grew profusely on maltose, dextrin, levulose, lactose and sucrose.

Fungus could utilise variety of nitrogenous compounds. Excellent growth was observed on potassium nitrate and magnesium nitrate followed by sodium nitrate and ammonium tartarate. The studies on utilisation of nitrogen compounds by A. tenuis isolated from cumin as reported by Uppal et al. (1938) indicated that the fungus made the best growth on modified Richards' agar and on medium containing potassium nitrate and ammonium tartarate. Singh and Tandon (1970)

studied the nitrogen requirement of certain isolates of A. tenuis Auct and showed that there were marked differences in their nitrogen requirements.

Fungus could produce enzymes like erypsin and trypsin in good quantity while it could produce enzymes gelatinase, diastase, amidase and cystase in small quantities. Chougule (1973) studied A. tenuis causing leaf blight of soyabean, and stated that the fungus could produce enzymes like diastase, trypsin, inulase and erypsin in good quantity, but it could produce the enzymes amidase, cystase and gelatinase in very small quantities.

The fungus could grow with wide range of pH viz. 2.2 - 9.6 but the optimum pH for the growth was 4.8 to 7.1. The maximum growth was obtained at pH 6.4. Arya and Prasada (1952) reported that the linseed Alternaria tenuis could grow with wide range of pH 3-8.5 with optimum 5-6.5.

The thermal death point of the fungus under study was 60°C. Jadhav (1974) studied the leaf-spot of sunflower caused by A. tenuis and stated that thermal death point lies between 59-60°C.

The relative efficiency of fungicides and antibiotic was tested in vitro. In this test Dithane M-45, Vitavax, Dithane Z-78 and Cuman^{L} yielded promising results. Difolatan was also found to be effective in checking the growth and sporulation while Aureofungin, Fytolan, Daconil, Ziram, Blitox-50, Bavistin, Kitazin and Benlate did not prove

effective in checking the growth and sporulation of the fungus. Rodebaugh (1963), Tammen (1965), Seber (1966), Cox (1969) and Singh and Milne (1974 a & b) reported the laboratory and field experiments on fungicidal control of Alternaria on chrysanthemum. Thus Rodebaugh (1963) found protective spray of botran, captan or phaltan as effective control. He observed that the flower susceptibility was not related to nitrogen, phosphorus or potash nutrition. Tammen (1965) recommended zineb dust or spray. Seber (1966), stated that the application of copper compounds, nameb or zineb provided adequate control. Cox (1969) reported that blight of chrysanthemum could be better controlled by zineb, nameb or daconil than captan. Singh and Milne (1974 a) noted that in laboratory experiments captafol, chloroneb, mancozeb and thiram at higher concentrations appeared to be the most promising. In their field experiments (1974 b) chlorothalonil was found to be most satisfactory. Mancozeb and captafol gave reasonable control. The results obtained in the present studies were in agreement with those of Tammen (1965), Seber (1966) and Cox (1969) in respect of Dithane Z-78 (zineb) and with Singh and Milne (1974 a) in respect of Dithane M-45 (Mancozeb). Since the varieties grown in Maharashtra State were very susceptible to the disease, the results obtained in the present studies would be very useful in recommending fungicides for control of the disease. It would be necessary, however, to conduct field trials with the promising fungicides.

Chapter VII

SUMMARY

Chapter VII

S U M M A R Y

Investigations on leaf blight disease of chrysanthemum were undertaken. The disease was noted for the first time in concentrated areas of Ahmednagar district where crop is grown commercially.

Dark brown to black spots generally in the region of leaf apex and leaf edges were developed. Spots were circular and oval to irregular, measured 4 to 8 mm in diameter. Several such spots coalesced and formed a big patch measuring 15 to 20 mm in length. These gave blight appearance to the leaves. Symptoms were also noticed on blossom.

Besides chrysanthemum, the fungus was pathogenic to brinjal, chilli, cotton, dahlia, sunflower and tomato.

The variety Golden Darwar was moderately susceptible while Barbara Phillips, Cornation yellow, Garden State, H.V. West, Raja, Revadi, Snow queen, Surprise D'orsay and Zipri were found to be highly susceptible.

The fungus produced good mycelial growth in culture media, mycelium was poorly aerial, hyaline, septate and irregularly branched turned brown when old and measured 4.63 µ (3.12 - 6.24) µ.

Conidiophores were short, straight to slightly bent, 2-4 septa, slightly constricted at septa, light brown to dark brown in colour. Length from the host 43.40 µ (15.60 - 81.90) µ and from potato dextrose agar 35.76 µ (7.80 -

105.30) µ. Width from host 5.27 µ (3.12 - 7.80) µ and from potato dextrose agar 5.02 µ (3.12 - 6.24) µ.

Conidia were light olivaceous to dark-brown, obclavate to muriform with rudimentary or short beak, forms chains upto 12 or more, 2-7 transverse septa (usually 3 to 5) and 0-3 longitudinal septa (usually 1-2). Length of conidia from host was 41.34 µ (19.50 - 74.10) µ and from culture it was 24.10 µ (7.80 - 37.44) µ. Breadth from host 10.99 µ (4.68 - 19.50) µ and from culture 8.88 µ (4.68 - 13.26) µ.

On the basis of these morphological characters, the pathogen was identified as Alternaria alternata (Fries)Keissler.

The fungus could grow well on Kircheff's agar, Richards' agar, potato dextrose agar, host leaf extract agar and nutrient agar.

The temperature requirement of the pathogen was 28-30°C (10-40°C). The pathogen utilise dextrin, sucrose and maltose. Potassium nitrate, sodium nitrate, magnesium nitrate, ammonium tartarate were found to be the best source of nitrogen. It utilised gelatin, casein, egg-albumin and potato starch indicating the production of enzyme gelatinase, erypsin, trypsin and diastase respectively. The favourable pH range was between 4.8 to 7.1 and the optimum was 6.4.

Dithane M-45, Vitavax, Dithane Z-78 and Cuman[L] inhibited the fungus growth and sporulation in vitro test.

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* Original not seen.

PART-II

Leaf spot of Chiku

(*Achras sapota*, L.)

I N T R O D U C T I O N

Chiku (Achras sapota, L.) also called Sapodilla or sapota is a native of South America. It is cultivated extensively in Mexico and has now spread to other tropical countries (Sandhu 1973; Cheema et al. 1954). In India the cultivation of this fruit crop on commercial scale is in Maharashtra, Gujrat, Andhra Pradesh, Tamil Nadu, Karnataka, Kerala, Bengal, Uttar Pradesh and Punjab (Randhawa and Kohli, 1966, and Madhava Rao et al. 1975). As quoted by Randhawa and Kohli (1966) the area under this crop in India was 833 hectares in 1954 while it was 1250 hectares during 1966. Since then the area is increased nearly six to eight times.

In Maharashtra, the crop was grown on an area of about 1100 hectares during 1974-75 in five districts viz. Thane, Ahmadnagar, Pune, Nanded and Aurangabad (Anonymous, 1977). In Thane it is on 700 hectares while on 100 hectares in each of the remaining four districts. Gholvad area in Thane district is well-known for chiku cultivation.

Cultivation of chiku is highly profitable as it does not need much spraying and pruning etc. and is long-lived fruit tree. Moreover, the chiku fruit fetches good price as it ripens in July and August, when mango and litchi fruits are scarce. The chiku fruit has good storage and transport qualities (Sanghavi and Brar, 1974; Sandhu, 1973).

Karat et al. (1971) listed five fungi viz. Fusarium sp. Macrophomina phaseoli, Rhizopus nigricans, Phyllosticta sapoticola, Phytophthora palmivora on chiku. During the year 1978 a new leaf-spot was observed on chiku plants on the cultivators' fields in Pune district, at the Regional Fruit Research Station, Ganeshkhind, Pune and at the Horticultural Farm at the Central Campus of this University. The symptoms observed were similar to those reported by Chinnappa (1968) in Dharwar. The investigations were therefore undertaken as the leaf-spot is a new record in Maharashtra.

Leaf spot of chiku (Achras sapota, L.)

SYMPTOM :

In nature, numerous leaf spots scattered all over the lamina were observed. These spots were circular. The colour of the spots was initially pinkish which gradually turned reddish brown. The centre of the spot was thin and light in comparison to the periphery which was dark brown to black.

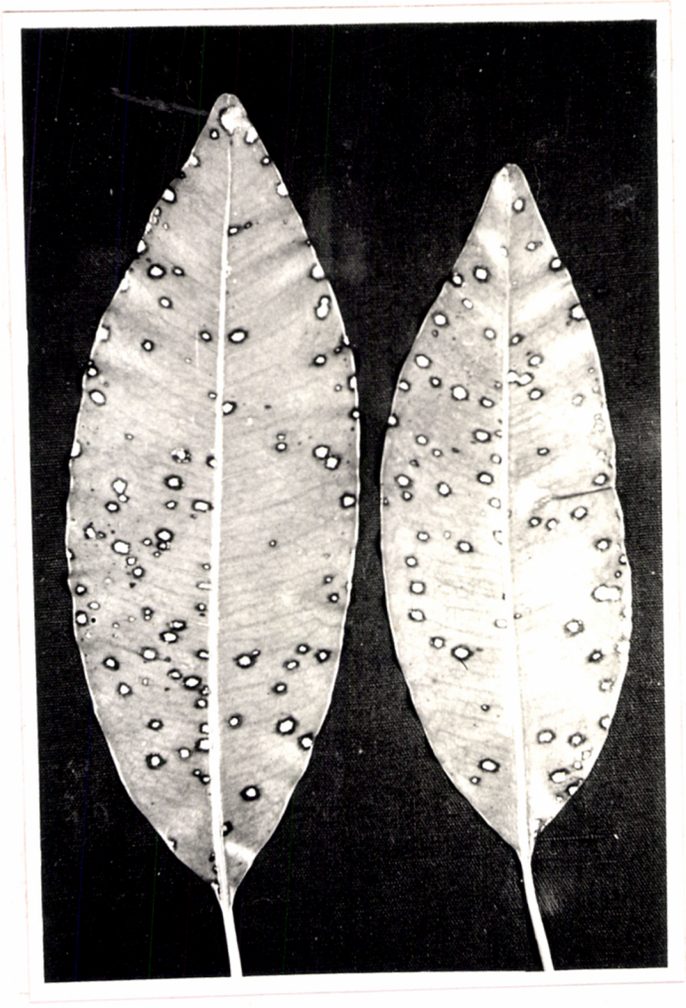
Microscopic examination :

The microscopic examination of the affected part did not reveal the appearence of spores or of pycnidia or any other fruiting body.

Isolation :

The sapota leaves having typical symptoms of the disease were collected from the horticulture garden of Mahatma Phule Krishi Vidyapeeth Farm, Baburi, and Regional Fruit Research Station, Ganeshkhind, Pune. The leaves were then washed with tap water so as to remove dirt and dried in air. The affected parts were then cut into small pieces, disinfected in 1:1000 $HgCl_2$ solution for 2-3 minutes and washed in three changes of sterilized water to remove the traces of poison. These small pieces were then put on sterilized filter paper to remove excess of water and then planted on petridishes poured previously with potato dextrose agar. The whole procedure was done under aseptic condition.

PLATE I



Naturally infected diseased leaves

showing symptoms

After twelve days, the fungus growth started appearing from the affected spot. Initially very small growth was observed. Then sub-cultures were made on potato dextrose-agar. Organism was found to be extremely slow growing. Thus two isolates viz. Pune and Rahuri were obtained for further studies.

Pathogenicity :

Twenty-one day old culture having a good mycelial growth was used for inoculation for proving the pathogenicity. Potted plants of sapota obtained from the Regional Fruit Research Station, Gareshkhind, Pune were used for inoculation purpose. Mycelial suspension was prepared in sterilised water. The plants were inoculated by smearing the suspension on leaves with the help of a brush. Few leaves were injured by pinprick method. The plants sprayed with sterilised water served as a control. The pots with these plants were kept under moist chamber for 24 hours, before and after inoculation. The plants were kept under observation for a period of nearly two months. Symptoms did not develop on the inoculated leaves.

Sporulation :

Microscopic examination of the culture showed that there were no spores. It was only mycelium. Attempts were therefore made to sporulate the organism. The techniques and media used by Hande (1964) were followed for this purpose. The media tried were as below :

1. Potato dextrose agar + 1 per cent glucose.
2. Host-leaves + 1 per cent glucose.
3. Czapeck + 1 per cent yeast extract.
4. Czapeck + 0.5 % yeast extract.
5. Yeast mannitol extract.

These media were prepared by standard method, auto-claved at 15 lbs pressure for 15 minutes and the media slants were prepared in test-tubes. These slants were also sterilised and kept ready for inoculation. In case of host-leaf medium young to medium leaves (not old) were used. The leaves were cut into small pieces and filled in 250 ml Erlenmyer flask. The leaf pieces covered nearly 1/4 portion of the flask. Glucose solution (1 per cent) was then poured into the flask sufficient enough to keep the leaf pieces completely immersed into the solution. The pieces were allowed to be soaked for one hour and then the excess of glucose solution was drained off in such a way that the retained glucose solution covered only the bottom surface of the flask. These flasks were then sterilised for 15 lbs pressure for 15 minutes. In this way the media slants and flasks were kept ready for inoculation.

A very small piece of mycelium was lifted on a sterilised inoculating needle and aseptically transferred on the media slant to be inoculated. This mycelial piece was inverted in such a way that the top mycelial portion came in touch with the surface of the media slant. This piece was then very slowly moved on the surface of the media slant and finally placed on one side of the

slant in its original position. In case of host leaf pieces, the flasks were inoculated by placing 5-6 small pieces of mycelial bits on the leaf-pieces with the help of sterilised needle. The mycelial bits were then spread by shaking the flask. The observations on the appearence of the growth, its form, colour and sporulation were taken daily.

On media slants growth appeared on 4th day as small minute colonies which were round in shape and white in colour. The growth of this colony was very slow. It took nearly three week for the development of the culture to cover the entire surface of the slant. The growth was grey-white on potato-dextrose agar with 1 per cent glucose and Czapeck + yeast extract. It was white on yeast mannitol agar which turned slightly pink after about eleven days. In case of host leaf, the growth appeared after 5 days. This growth was white initially and after about twelve days turned pink. A black spot was developed on the leaf piece where there was mycelial growth.

There were no differences in the growth pattern among the two isolates i.e. Pune and Rahuri. Both the isolates were very slow growing, initially white in colour and become grey white. On yeast mannitol agar both the isolates showed pinkish growth. The microscopic examination did not reveal the development of spores. The attempts therefore to induce sporulation on these media proved futile.

The experiments on sporulation and pathogenicity were

repeated at Pune under the expertise and skill of Professor Y.K. Hande. These attempts also did not succeed to sporulate the organism nor to establish the pathogenicity.

Chinnappa (1968) reported Phaeophleospora indica as the species novo causing a new leaf spot of Chiku (Achras sapota, L.) . The symptoms described by him and as observed in the present investigations were similar. However, he observed black hemispherical pustular structures representing the fruiting bodies of the organism. Repeated examination of several specimen collected at Raburi and Pune did not reveal the presence of any fruiting body developed on the leaf-spots. Microscopic examination of the affected samples did not show the presence of spores. Sohi and Sridhar (1972) noted that this disease produced numerous, small, circular, pinkish to reddish brown conspicuous spots with whitish centres. They reported on the fungicidal control of the leaf-spots. Krishna Prasad et al. (1979) studied the varietal reaction of sapota to this organism. These workers however did not mention about the sporulation of this organism, which was very slow growing.

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