# STUDIES ON THE PATHOGENICITY AND PHYSIOLOGY OF *Corynespora cassiicola* (Berk & Curt.) Wei.

By

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THESIS

Submitted in partial fulfilment of the requirements for the Degree of Master of Science in Agriculture (Plant Pathology) of the University of Kerala

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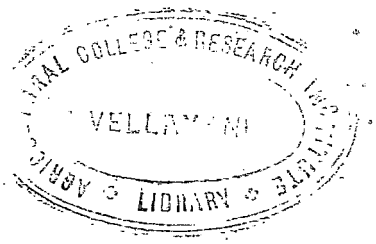
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
VELLAYANI, TRIVANDRUM.

1970

# C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Sri. P.V. George under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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# ACKNOWLEDGEMENTS

The author wishes to place on record his deep sense of gratitude and indebtedness to:

Shri. P.V. Pally, Lecturer in Plant Pathology for suggesting the problem, able guidance and supervision throughout the investigation and for his invaluable help in the preparation of the thesis,

Dr. J.Sam Raj, Principal for extending all facilities,

Sarvasree M. Ramanatha Menon, Dr. K.I.Wilson and C.K. Peethambaran of the Division of Plant Pathology for their valuable criticisms, suggestions and constant help,

Shri E.J. Thomas, Professor of Agricultural Statistics, Smt. L.Ramadevi, Smt. S.Sreedevi, Sri. K. Raveendran Nair and Sri. M.J. Thomas, Research Assistants of this college for the help rendered in various ways for successful completion of the work and

to the Government of Kerala for deputing him to undertake the Post-graduate course.

P.V. GEORGE.

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# INTRODUCTION

# INTRODUCTION

Corynespora cassiicola (Berk & Curt) Wei, the incitant of leaf spot diseases of a number of cultivated crops has been observed causing serious damage to crops like tomato, sesamum and rubber in Kerala. Its dry spores has been observed in large numbers in air spora studies conducted at Agricultural College, Vellayani (1964-1966), the maximum spore loads being observed during the period from November to April. This synchronizes with the occurrence of leaf spot diseases of tomato, sesamum and rubber caused by the fungus.

At the beginning of the present century, this organism was considered as a limiting factor in growing cucumber in England, France, Holland, Denmark and Germany (Willis 1905). The increase in spread of diseases caused by this organism which enjoy a wide spectrum of host plants like sesamum, tomato, rubber, tapioca, mentha and eucalyptus in Kerala poses as a potential threat to these crops as well as to summer vegetables.

C. cassiicola causes necrotic lesions on stem, leaves and fruits of tomato and sesamum while on leaves of rubber both in the nursery and main field it produces target spots. The spots are dark brown in colour and often coalesce into patches. There is a chlorotic halo around spots in the early stages of disease syndrome. As the disease advances, the spots eventually

differentiate into a holonecrotic area surrounded by a plesionecrotic zone that gradually merges into healthy tissue. The self expanding character of necrosis associated with coalescence and spread of spots which later dry up and fall off suggest involvement of some toxic principles.

Many plant pathogenic organisms are known to produce metabolites *in vitro* that are toxic to plants. These phytotoxic substances may play a decisive role in pathogenesis acting as pathotoxins causing typical disease symptoms. The *in vivo* production of these toxic substances in quantum sufficient to cause diseased condition confirms their role in a given disease syndrome. The concept of pathogenicity of a parasite in relation to its toxigenicity has opened new vistas in Plant Pathology not only in studying the mechanism of pathogenesis but also in finding out ways and means to prevent, eradicate and even to cure the disease.

Taking into consideration of the above aspects, the present investigations were undertaken on the following lines:

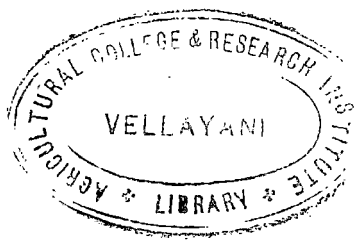
i) studies on the disease with reference to pathogenicity of isolates and mode of transmission of disease.

ii) studies on the pathogen with reference to its physiology in culture.

iii) studies on the pathogen with reference to its pathogenicity and toxigenicity and

iv) studies on the control of the pathogen.

# REVIEW OF LITERATURE



## REVIEW OF LITERATURE

### Host range and Pathogenicity.

Corynespora cassiicola (Berk & Curt.) Wei, has been reported from all parts of the world causing leaf spots and fruit rots of many cultivated crops. Deighton (1936) Thirumalachar and Lacy (1951) Simmonds (1956) and Mohanty and Behra (1960) found it causing fruit rots and leaf spots of papaya. Deighton (1936) Ramakrishnan (1960), Ramakrishnan and Radhakrishna Pillay (1961) and Ananth and Menon (1966) found it causing shot holes in leaves of rubber seedlings. Deighton (1936) Mohanty and Mohanty (1955) Chandrasekharan Nair and Samraj (1966) found it causing leaf spots and fruit rots of tomato, Wallace (1956) and Stone and Jones (1960) found it causing leaf spots on sesamum and Kawamura (1937, 1945), Olive et al (1945) and Spencer and Walters (1969) found it associated with leaf spots of cow pea. Besides these hosts it has been reported to cause leaf spot disease in tapioca (Cifferii 1945, Roy 1965), Soy-bean (Olive et al 1945 Boosalis & Hamilton 1957, Stone & Jones 1960, Spencer and Walters 1962, 1969 and Seaman et al 1965), Rauwolfia (Mohanty 1958), Cotton (Jones 1961, Massenot & Cassini 1967), Hydrangea (Sobers 1966) Eucalyptus (Wilson and Ramadevi 1966) Japanese Mentha (Abi Cheeran 1968) Brinjal (Asha Ram and Lele 1968), Betel-vine (Mohanty and Mahapatra 1968) and in a number of other plants like lady's finger

Ageratum sp., Dodonaea sp., Pouraria sp., Croton sp., jute, para rubber etc.

2. Pathogenicity of isolates.

Olive et al (1945) distinguished two races of Corynespora cassiicola (Berk & Curt) Wei, based on differential responses of cow pea (Vigna sinensis Torner) and soy-bean (Glycine max L.) to infection by the fungus. Race 1 caused severe leaf spots on cow pea, but only light spotting on soy-bean whereas Race 2 caused only light spotting on both. Stone and Jones (1960) reported that their isolates from Sesamum indicum L. and soy-bean did not fit the description of either race, thereby indicating the feasibility of an additional race. Jones (1961) found no difference in isolates from cotton (Gossypium hirsutum) sesamum, soy-bean and cow pea. Spencer and Walters (1962) reported that the cow pea and soy-bean isolates they got corresponded with the races 1 and 2 of Olive et al (1945). Later Spencer and Walters (1969) confirmed the existence of two distinct races of the organism by differential responses of cow pea and soy-bean to infection by Corynespora cassiicola isolates from plants grown in different parts of America.

Gopalan (1963) using isolates obtained from tomato, sesamum and Dodonaea studied their pathogenicity and found that the sesamum, tomato and Dodonaea isolates were pathogenic on all the three hosts. He also observed that the symptoms on Dodonaea were only mild.

5. Nature of disease transmission.

The nature of spread of the diseases caused by Corynespora cassiicola is not known much. Boosalis and Hamilton (1957) and Seaman et al (1965) reported a high inoculum potential in nursery plot soils at Nebraska and Canada respectively, causing root and stem rot of soy-bean plants. But Spencer and Walters (1969) obtained isolates from foliage only and not from soil.

Occurrence of spores of Corynespora cassiicola in air was reported by Sreeramulu and Ramalingam (1963). Chandrasekharan Nair (1964) and Prasannakumaran (1966) in their studies on air-spora observed a diurnal periodicity in spore liberation, the maximum number of spores occurring in the morning hours of the day. They also found a marked increase on the spore load on days succeeding rainfall.

B. Physiological studies

Since Raulin in 1869 first devised a synthetic medium (Lilly and Barnett 1951) various synthetic media are being used for in vitro studies of the physiology of fungi which vary in their ability to utilize the ingredients of the media. Besides growth, production of toxic metabolites which imparts pathogenicity to most fungi, also vary with the composition of the substrate on which they are grown.

(i) Effect of light on growth and sporulation

Light has profound effects on the morphogenetic or metabolic growth responses of many fungi.

Randas (1917) and Kunkel (1918) found that cultures of Alternaria solani and Macrosporium solani could be induced to sporulate by wounding the mycelium and exposing it to abundant light. Calpouzos and Stall Knecht (1965) found that conidiophores of Cercospora beticola in agar cultures bent toward continuous or diurnal light.

In the case of Alternaria porri, Fahim (1966) obtained good sporulation when the cultures were exposed to sunlight for a period of two hours followed by incubation of 48 hours.

(ii) Effect of humidity on growth and sporulation

Humidity in the environment has direct effect on growth, spore size and spore liberation of fungus.

Liberation of spores of dry spored fungi by hygroscopic movements have been demonstrated by Pinckard (1942) in the case of Peronospora tabacina and by trigger devices have been demonstrated by Meredith (1961, 1962, 1963) in the case of Deightoniella torulosa, Curvularia sp. Alternaria sp. and Corynespora sp. A change in the vapour pressure towards a decrease induced violent movements in the conidial apparatus resulting in the violent release of conidia from conidiophores has been observed in Helminthosporium turcicum and Corynespora cassiicola by Meredith (1965).

Chandrasekharan Nair and Samraj (1965) found the sporulation of Corynespora cassiicola high on days following rain

and suggests that the wetness of substrata as well as increase in the atmospheric humidity during night might have increased production of spores. Sreeramulu and Ramalingam (1964) obtained similar change in spore content of several fungi including Corynespora due to changes in weather conditions.

In the case of Helminthosporium sativum, Mishra and Singh (1965) found rapid increase in spore content of air in fields when atmospheric humidity was 75-77%. Padmanabhan (1965) observed that a minimum night temperature of 20-26°C in association with a high relative humidity of 90% and above lasting for a period of a week or more during any of the susceptible phase of growth of paddy crop viz., seedling stage, post transplanting and neck emergence favoured incidence of blast (Piricularia oryzae). Mishra and Nema (1969) found that Alternaria tenius developed better colonies and sporulated well when they were grown at 75% humidity.

(iii) Effect of carbon sources on growth and toxin production.

a) On growth

Different sources of carbon are utilized differently by fungi for their metabolic activities.

Out of the 19 carbon compounds studied, Grewal (1957) found that arabinose, rahmnose, lactose, sucrose, glucose and erytheritol supported good growth of Gloeosporium musarum, Gloeosporium papayae and Colletotrichum papayae.

Chandrasekharan Nair (1964) in studying the mycelial weight of Corynespora cassiicola in different sources of carbon found that sucrose, glucose and starch were equally good whereas mannitol was best. Kafi and Tarr (1966) found growth, sporulation and morphology, colour and dimension of conidia produced by Helminthosporium rostratum H. cynodontes, H.hawaiiense and H. australiense were significantly affected by culture medium, glucose concentration and carbon source. Dange and Patel (1968) found glucose supporting best growth of Cercospora beticola than maltose, dextrin, sucrose, lactose and starch. Of the different carbon sources studied for Pyrenochaeta dolichi, Mohanty and Mohanty (1969) found that sucrose supported the maximum growth followed by fructose, galactose, dextrose, starch lactose, glycerol and mannitol. Sporulation was excellent on sucrose, poor in dextrose and negligible in other carbohydrates and in the basal Richard's medium without carbon. Mirsha and Nema (1969) found glucose as best source of carbon for Alternaria tenius followed by starch, sucrose, lactose, maltose, mannose, dextrose and arabinose for both growth and sporulation. Without carbon, there was no growth and sporulation.

b) On toxin production

Brian et al (1951) showed that media containing high concentration (7.5% w/v or more) of sucrose proved to be best suited for the growth and activity of metabolic substances

produced by Alternaria solani. Ludwig (1957) found an increased toxicity with decrease in sugar content in the medium for growing Helminthosporium sativum and also that increased activity was not associated with greater growth. Fulton and Bollenbacher (1968) in their studies on the production of a chlorosis inducing agent by Alternaria tenius found that out of twelve sugars tested, medium with sucrose produced greatest amount of toxin followed by d-glucose, d-galactose, d-mannose, lactose and mannose whereas d-xylose, d-fructose, l-sorbose, maltose and cellobiose produced no toxin. Aspergillus niger and Sclerotinia sclerotiorum have increased pectinolytic activity when lactose was added to the medium with pectin (Astapov & Samtsevich 1968). The use of dextrose in the place of sucrose in Czapeck's-Dox medium was found to improve growth and toxin production of Trichaconis padwickii (Jayachandran Nair 1969).

iv) Effect of source of Nitrogen on growth and toxin production.

a) On growth.

Grewel (1955) in his studies on effect of nitrogen nutrition of Alternaria tenius using 28 nitrogen compounds found that growth was significantly improved by magnesium and calcium nitrates, ammonium acetate and oxalate, peptone, d-alanine, l-phenylalanine, glycine and acetamide while many ammonium salts, l-leucine and histidine were poor sources of nitrogen

and there was no growth when nitrogen was omitted from medium nor with sodium or potassium nitrite. Sporulation was best with peptone, l-aspartic acid and all nitrates except ammonium. Power and Patel (1957) found good growth of Alternaria ricini in organic nitrogen sources like asparagine and aspartic acid. Mirsa and Mukherjee (1962) reported that the maximum mycelial growth of Helminthosporium oryzae when peptone was used as nitrogen source. Good growth was also obtained when potassium and sodium nitrates were used as nitrogen sources. Rajderkar (1966) found that an isolate of Alternaria utilized a wide range of nitrogen compounds in culture medium giving a maximum growth in peptone medium. Dange and Patel (1969) found that Cercospora beticola give good mycelial growth in nitrates of calcium and potassium among nitrates, ammonium and organic sources of nitrogen. Garnier et al (1969) in their studies on the influence of nitrogen source in culture medium of Aspergillus niger found superior growth with organic nitrogen particularly asparagine. $NH_4^+$ was used more readily than $NO_3^-$. Mishra and Nema (1969) found $KNO_3$ and $KNO_2$ as best sources of nitrogen for Alternaria tenius than $Na\,NO_3$, $NaNO_2$, urea, $NH_4Cl$ and $(NH_4)_2\,SO_4$ for both growth and sporulation. Mohanty and Mohanty (1969) found that Pyrenochaeta dolichi was capable of utilizing nitrate, ammonium and organic forms of nitrogen and profered nitrate ion to ammonium so far as growth was concerned.

b) On toxin production.

The ability of pathogen to produce toxic metabolites in cultures varies with the nitrogen sources in the media. Brian et al (1951) supplied nitrogen as nitrate or casein hydrolysate for the production of alternaric acid by Alternaria solani whereas ammonia was found equally good when supplied in conjunction with a suitable organic acid, 0.25% (w/v) acetic acid being particularly favourable. Berry and Futrell (1961) in their studies on the different nitrogen sources in the production of toxins by Helminthosporium victoriae found that the primary toxin was produced in greater quantity in media containing ammonium nitrate followed in descending order by ammonium sulphate, phenylalanine, tryptophan, methionine, tyrosine and asparagine, using Almo for assay. When New cortex seedlings were used for assay, the secondary toxin was produced on highest amount in methionine followed by phenylalanine, ammonium sulphate, tryptophan, asparagine and ammonium nitrate. Musavea (1968) found activity of toxic metabolites more in media containing inorganic sources of nitrogen (sodium nitrate) than in media with organic sources of nitrogen when Aspergillus niger was cultured. Jayachandran Nair(1969) found that medium containing sodium nitrate as source of nitrogen was better for toxin production by Trichocenis padwickii than sodium nitrite,

peptone and ammonium sulphate.

v) Effect of vitamins on growth and production of toxin

a) On growth.

The requirement of vitamins for growth of fungus arises from the inability of particular fungi to synthesize the appropriate growth factor. Mathur, Burnett and Lilly (1950) have shown that Colletotrichum lindemuthianum is partially deficient in inositol and biotin.

Beckman et al (1955) found that a mixture of biotin, thiamine and inositol promoted growth of oak wilt fungus Chalara quercina. Gilpartik and Henry (1953) found that for Ophiobolus graminis biotin is necessary for its early growth and thiamine for its further mycelial development. Thiamine supplied alone depressed growth of Diaporthe phaseolorum but with biotin, inositol and pyridoxine and thiamine, Timnick et al (1951) found good mycelial growth. Mycelia of Memnoniella echinata can synthesize biotin from its precursor desthiobiotin, but the spores cannot, and so good growth and germination occurs when biotin is supplied (Perlman 1951). Though most fungi are heterotrophic for vitamins, some species of Leptographium associated with pole blight of western white pines are completely autotrophic in respect of vitamins or do

not require them for growth (Leahart 1956). Spores of Myrothecium verrucaria grow exceedingly slow in the absence of biotin but addition of vitamins just after germination results in an enormous growth rate, though the mycelium does not require exogenous biotin for normal growth (Mandels 1955).

The balance of mineral constituents of the medium determines a thiamine requirement of Pythium butleri according to Robbins and Kavanagh (1938) while the replacement of glucose by fructose as carbon source was found by Strigini and Morpugo (1961) to eliminate the heterotrophic requirement of biotin in Neurospora crassa. Burnett (1967) says that yeasts like Kloeckera brevis have a multiple vitamin requirement of thiamine, biotin, pyridoxine, nicotinic acid, Pantothenic acid, and inositol for growth.

b) On toxin production

Basappa et al (1968) found an increased rate of production of aflatoxins by Aspergillus oryzae when thiamine was added to medium.

vi) Effect of H-ion concentration of growth and toxin production

a) On growth.

Under a given set of conditions, a fungus will grow maximally over a certain range of pH values of the medium and

will fail to grow at high or low extremes. pH is also affected during growth by metabolic activities, raised by absorption of anions or production of ammonia from nitrogenous compounds and lowered by formation of acids or absorption of cations (Cochrane 1958). A double optimum is also sometimes reported for certain fungi.

Aoki (1937) reported that Pfricularia oryzae germinated and developed better at pH 5-6 and pH 8-9, with better mycelial weights at pH 4.6 and 9.6 whereas Ophiobolus miyabeanus germinated through a wide range of pH. Wolf at al (1950) found that Monosporium apiospermum grow within a pH range of 3.6 to 10.8 with an optimum at pH 7.0 to 7.8.

Karlinga (Rhizophlyctis) rosea has been reported to grow within a pH range of 3.4 to 8.0 maximum being at 6.8 - 7.0 (Haskins and Weston Jr. 1950). From an extremely acid side of pH 2.4 to an alkaline side of pH 9.6, growth of Sclerotinia sclerotiorum was found satisfactory by Tamrikut and Vaughan (1951). Chona and Mingorani (1951) found in three isolates of Colletotrichum falcatum that they differed in their requirement of pH for growth, isolate 78 required an acid range, isolate 3 required an alkaline range and isolate 29 was intermediate. Misra and Chatterjee (1963) observed in their study of two isolates of Helminthosporium oryzae that optimum pH for growth and sporulation was 6.0.

Subramonian (1964) found in his studies on sclerotial root rot of groundnut caused by Sclerotium rolfsii that it tolerated a pH range of 2-9 for growth, the optimum pH being 6.0. Growth of Alternaria tenius was found maximum when the pH of the medium was adjusted to 5.5 (Tandon and Chatruvedi 1964). Dange and Patel (1968) found that Cercospora beticola within a pH range of 3.1 to 9.1 prefer pH 6 for best growth and that the pH of the solution after 23 days of incubation ranged from 7.6 to 8.9. Mishra and Nema (1969) found best growth and sporulation of Alternaria tenius at pH 6.5 - 7.5. Mohanty and Mohanty (1969) found the growth of the fungus Pyrenochaeta dolichi was more satisfactory on the acidic side of the pH range than the alkaline side in pH range of 3 to 11, with an optimum pH of 5.0.

Narasimhan (1969) found that Sclerotium rolfsii exhibited a fixed pH optimum for maximum growth with $NaNO_3$, $KNO_3$ and $Ca(NO_3)_2$ 4. $H_2O$, being respectively pH 4.0, 2.0 and 3.0. With ammonium nitrate and ammonium chloride as N sources, the fungus exhibited good growth between pH 3.0 and 6.0. With $(NH_4)_2$ $HPO_4$ and $(NH_4)_2$ $SO_4$ growth was good over a wide pH range, exhibiting a double peak in its pH growth curve. The final pH in all media ranged between 1.6 to 2.8. He also found that Sclerotium oryzae had good growth in a wide range of pH 4-11, the final pH of the media becoming 7.5 to 8.0.

Rugunathan (1969) found two pH optima at 6.5 and 8.0 for Corcospora dolichi and Cercospora canescens, though there was good sporulation only at pH 6.5.

b) On production and activity of toxic metabolites

Production of metabolites by fungi in the medium changes the pH of the culture filtrate and the production of toxin and its action in most, if not all, cases are effected by the pH of the media.

Fahmy (1923) found an increase in alkalinity of culture filtrate as the age of culture of Fusarium solani increased. Oxalic acid is stated to be important for diseases caused by Sclerotium rolfsii which reduces rapidly the pH of the culture media. Plants are rapidly killed by filtrates of this fungus where oxalic acid is the only product formed and toxicity of filtrate increases directly with the increase in free acidity (Higgins 1927). Mehrotra (1949) in studying the enzyme action of 5 species of Phytophthora found that a reduction in the pH of the medium from 6.8 to 3.8 and 4.1. Akai (1951) found that the optimum pH for enzyme activity in Helminthosporium oryzae was between 5.5 to 6.0. Overall (1952) found oxalic acid as the thermostable toxin present in filtrates of Sclerotinia sclerotiorum, the pH of culture filtrate being on acid side. In his studies on parasitization of potato tubers by Pythium sp.

and Botrytis sp. Damle (1952) found that the optimum pH for enzyme production of P. debaryanum lay near 8, that of P. cinerea was 5.6 on medium that remained acid throughout and 8 when alkaline. Gentile (1952) found an increased production of toxin by Botrytis cinerea by lowering the pH of the medium.

Saksena et al (1953) found that various species of the genus Pythium are able to raise the pH of the medium after incubation due to accumulation of ammonia. In crown rot of groundnut seedlings caused by Aspergillus niger, Gibson (1953) found free acid production in cultures. Differences in pathogenicity of different isolates to seedlings are related to differences in ability to produce acid in a synthetic liquid medium. Endoconidiophora fagacearum, the oak wilt fungus had a maximum toxin titre with an initial pH of 3.4 to 4.2 in the media, and was completely inactivated at pH 8.0 (White 1955). The characteristic symptom of toxicity was noted in all the samples of culture filtrate after 42 hours independent of the values of pH to which they had been adjusted. It was concluded by White that the toxicity symptoms are not explained as a direct effect of the pH and that the toxic substance is active throughout the pH range tested. Pringle and Braun (1957, 1963) found that the toxin produced by Helminthosporium victoriae gets inactivated at pH above 4.0.

p-hydroxy phenyl acetic acid was isolated from culture filtrates of Rosellina necatrix causing white root rot of a wide range of plants (Chen 1958, 1960). Goodman (1960) found an increase in pH in the culture filtrates of Colletotrichum fuscum from 4.5 to 7 on the 17th day of incubation when the production of toxin became maximum. Aoki et al (1963) and Nishimura and Sabaki (1963) attribute toxicity of culture filtrates of Rhizoctonia solani to phenyl acetic acid and its p-and m-hydroxy derivatives. Leptosphaeria avenaria, a parasite of alfalfa forms in culture a thermostable toxin containing acid and amino groups having some features of a polypeptide according to Sondheim and Wilcoxson (1965). Tritica and Quimio (1966) found that culture filtrates of Helminthosporium maydis has maximum toxic activity after 18-22 days of growth and at pH 5.6 - 5.8 with an inverse relation between toxin production and growth. Mathur and Mathur (1967) found an increase in pH of culture filtrate of Fusarium oxysporium cumini, especially at pH 8.5 and over, related with the severity of wilting of seedlings and vascular browning.

Jayachandran Nair (1969) observed that the pH of the culture filtrate of Trichoconis padwikii changed from the initial pH of 6 to 7.6 on 21st day and 6.9 on 30th day.

vii) Effect of age of culture on toxin production

Since pathogenicity of an organism is related to its vegetative growth in most cases, toxigenicity is also likely to be influenced by age and growth of the culture. The increase in growth of the fungus with the age of culture was correlated with toxin production in the case of Fusarium solani (Falmy 1923) Ceratostomella ulmi (Zentmeyer 1941) Colletotrichum fuscum (Goodman 1960) Periconia circinata (Scheffer and Pringle 1961) Fusarium orthoceras f. lentia (Sharma and Agnihotri 1967) Penicillium restrictum (Sankhala 1967) Piricularia oryzae (Krishnaswamy et al 1969) etc.

In cases when the toxin is passed into the filtrate from mycelium due to autolysis, the concentration of toxic metabolites in filtrates will be maximum only in old cultures as seen in the case of Verticillium albo-atrum, V. dubuys and Fusarium solani (Picado 1924), and bacterium Pseudomonas mors-prunorum (Eriksson and Montogmery 1945).

According to Ludwig (1957) the greater activity of the culture filtrate was not associated with greater growth. His studies on Helminthosporium sativum suggested that toxins were produced until they limit further growth of the organism.

The activity of culture filtrates of Helminthosporium maydis was maximum after 18-22 days of seeding according to Tricita and Quimio (1966). Mathur and Mathur (1967) found that the age of culture of Fusarium oxysporium f. cumini had no effect on the ultimate severity of cumin cuttings.

Sharma and Sharma (1969) in their studies on Colletotricum gloeosporioides found that toxin production started after 8 days of growth, increased upto 22nd day and there after slowly decreased upto 30 days.

In the case of Trichocenis pedwickii, Jayachandran Nair (1969) found that the inhibitory property of culture filtrate increased with the age of culture attaining a maximum on 21st day.

viii) Effect of temperature on activity of culture filtrate.

Temperature may destroy the toxic properties of the culture filtrate if the toxin produced is a thermolabile one whereas thermostable toxins are not effected.

Thermostable toxins were observed in the culture filtrates of Fusarium solani (Fahmy 1925), Bacterium tabacum (Clayton 1933) Sclerotinia sclerotiorum (Demetriades 1950) Periconia circinata (Leukel 1948) Helminthosporium victoriae (Litzenberger 1949) Alternaria solani (Brian et al 1951) Fusarium culmorum

(Lendolt 1952) Phytophthora parasitica var nicotianae (Wolf 1953) Endoconidiophora fagacearum (White 1955) Colletotrichum nicotianae (Wolf & Flowers 1957) Fomes lignosus (Peries 1959) Colletotrichum fuscum (Goodman 1960) Pythium irregulare (Martin 1964) Alternaria tenius (Fulton et al 1965) Fusarium orthoceras f. lentis (Sharma and Agnihotri 1967) etc.

Narain and Om Prakash (1968) found that the culture filtrates of Aspergillus niger, even after autoclaving, inhibited the germination of onion seeds suggesting that the toxic principle is highly thermostable.

Culture filtrates of Colletotrichum gloeosporioides boiled at 100°C for 10 minutes was slightly less toxic than unboiled culture filtrates whereas sterilization at 10 lbs for 15 minutes resulted in significant reduction in toxicity (Sharma and Sharma 1969).

Jayachandran Nair (1969) found that the toxic principle in culture filtrate of Trichoconis padwickii was active even after boiling at 100°C for 20 minutes or autoclaving at 15 lbs for 20 minutes.

Thermolabile toxins were observed in Alternaria kikuchiana (Tanaka 1933) Septoria linicola (Covery 1962) etc.

The effect of heat is found dependent on pH of the culture filtrate in some cases. Clayton (1944) demonstrated that the activity of penicillin was not lost when the metabolic solution was kept at 80°C for 2 hours at a pH 7-8. But a solution of pH 5 when treated at 60°C for 4 hours lost all its activity. Brian et al (1951) found boiling of culture filtrates of Alternaria solani for 5 minutes in a pH range of 3-7.6 had no effect on loss of activity. Autoclaving at 15 lbs for 20 minutes also had no reduction in activity, except at pH 3 where there was a partial loss of activity.

ix) Specificity of toxic metabolite.

A phytotoxic substance produced by pathogenic fungi is called pathotoxin when it produces the characteristic symptoms of the disease on application to the host. Phyto-toxic substance produced by a fungus in vitro may sometimes vary in its activity to produce typical symptoms of pathogenesis produced by the pathogen. Further a toxin produced by the pathogen in vitro may be having a non-specific activity if it is toxic to plants other than the normal host. The in vivo production of toxin in quantum sufficient to produce symptoms confirm role of toxins in disease.

Cut shoots of host plants and others produced wilting

symptoms when placed in culture filtrates of Fusarium lycopersici (White 1937) Ceratostomella ulmi (Zentmeyer 1941) Sclerotinia sclerotiorum (Demetriades 1950) and Alternaria solani (Pound and Stahmann 1951, Brian et al 1951). Toxin produced by Helminthosporium victoriae is host specific and cause characteristic streaks when sprayed on disease susceptible varieties of oats (Litzenberger 1949).

Helminthosporium sativum produce a relatively non-specific toxin, affecting barley, oats and wheat in much the same way. Ludwig (1957) related the pathogenicity of various isolates to their capacity to produce the toxin in culture.

Braun (1959) found that strains of Pseudomonas tabaci which lose their ability to produce toxin in culture also do not cause typical wild fire symptoms in infected plants and concluded that the toxin is host specific.

Alternaria kikuchiana produced a host specific toxin which damages susceptible but not resistant varieties of Japanese pears (Hiroe et al 1958, Mori 1962). Periconia circinata causing milo disease of sorghum was not toxic to other species not parasitised by fungus as well as to new hosts like rye, barley, oats, radish, tomato, cabbage etc. (Scheffer & Pringle 1961, Pringle & Scheffer 1963). Tricita and Quimio (1966) found that Helminthosporium maydis produced a non-specific

phytotoxin in culture medium which inhibits root growth not only of corn but also of other non-suscept hosts like rice and wheat and also act on corn plants in a way not similar to the disease incited by the pathogen.

Pringle and Scheffer (1967) found a host specific toxin in culture filtrates of Helminthosporium (Cochliobolus) carbonum.

Fusarium orthoceras f. lentis produced toxins non-specific in effect according to Sharma and Agnihotri (1967). White and Starratt (1967) found that zinniol, a phyto-toxic substance produced by Alternaria zinniae caused wilting of cut seedlings not only of the host zinnia but also of watermelon, squash, spinach, beet, tomato, oat, corn, pea and bean. Sharma and Sharma (1969) reported that Colletotrichum gloeosporioides produced a non host specific toxin in Richard's solution. Jayachandran Nair (1969) found that culture filtrates of Trichoconis padwickii inhibited both germination and elongation of plumule and radicle of the host seeds of paddy and other seeds like tomato, brinjal, cowpea, cucumber and bhindi.

x) Effect of culture filtrate on inhibition of germination of of seeds and elongation of radicle and plumule

Boosalis (1950) found that the filtrates of Rhizoctonia solani reduces germination of soybean seeds and growth of roots

of seedlings. Wheeler and Luke (1954) used the inhibitory effect of toxin on root growth of oats as a method of bioassay of toxin produced by Helminthosporium victoriae. Ludwig (1957) used seed inhibition as a method of bioassay for testing the toxicity of the culture filtrates of Helminthosporium sativum. Helminthosporium oryzae reduces germination of rice seed and causes seedlings to grow abnormally. Growth of roots and coleoptiles are greatly reduced when plants are placed in solutions containing as little as 30 ppm of the toxin (Orsenigo 1957). Gangully and Padmanabhan (1962) found that soaking of seeds of susceptible varieties of rice in culture filtrates of Helminthosporium oryzae at concentrations of 1/1000 markedly brought down the infection in the susceptible varieties and also increased the yield.

Scheffer and Pringle (1961) demonstrated that radicale growth of sorghum was inhibited upto a dilution of 1:5200 of culture filtrate of Periconia circinata. Brian et al (1961) found that Fusarium equiseti produces toxic metabolites that inhibits elongation of stem at concentrations as low as 1 ppm. Govindan (1965) found that culture filtrates of Piricularia oryzae inhibited the germination and plumule elongation of blast susceptible varieties of paddy.

Pythium irregulare produces a toxin that reduces growth of roots of Beta vulgaris, Brassica oleracea and Avena sativa and also gets translocated to leaves from roots (Martin 1964).

Culture filtrates of Helminthosporium carbonum inhibited the growth of roots of hybrid maize to 50% at 0.5 µg/ml concentrations (Scheffer and Pringle 1967).

White and Starratt (1967) found that zinniol produced by Alternaria zinniae inhibited the germination of zinnia, tomato, lettuce and watermelon at 500 ppm strength and above.

Narain and Omprakash (1968) found that culture filtrates of Aspergillus niger reduced seed germination and disorganised the succulent scales and leaves of onion.

Plumule and radicle inhibition was found as a bioassay for testing toxin productivity of Piricularia oryzae by Krishnaswamy et al (1969).

Inhibition of germination of paddy seeds and elongation of radicle and plumule were observed by Jayachandran Nair (1969) with the culture filtrates of Trichoconis padwickii.

xi) Effect of culture filtrate on other microorganisms.

Brian et al (1949, 1952) found alternaric acid produced by Alternaria solani as not particularly antibacterial, but

produce symptoms that may be typical of the disease or sometimes entirely different.

When cut ends of plants were kept in culture filtrate, wilting was observed by many workers as in the culture filtrates of Leptosphaeria herpotrichoides (Marsh 1926) Fusarium vasinfectum (Marsh 1926) Fusarium lycopersici (White 1927) Alternaria soloni (Brien et al 1949, 1951) Helminthosporium sativum (Sayed 1961) Fusarium oxysporum f. cumini (Mathur and Mathur 1967) Alternaria zinniae (White and Starratt 1967) Colletotrichum gloeosporioides (Sharma and Sharma 1969) etc.

Destruction of chlorophyll tissues and production of halo lesions were found when culture solution of Bacterium tabacum was pricked into leaves of tobacco (Clayton 1934).

Chlorosis and necrosis typical to disease was found when filtrates of Alternaria soloni was introduced into lower petiole of tomato plant (Pound and Stoehmann 1952). Goodman (1960) obtained sunken or pitted areas on tomato foliage 24 hours after 6 hour exposure of toxic substance produced by Colletotrichum fuscum. Application of culture filtrates of Gloeosporium fructigenum resulted in necrosis on tomato and bean plants (Radha Menon 1961). Culture filtrates of Helminthosporium sativum produced necrotic spots on leaves of barley (Sayed 1961).

Cochliobolin, the toxic principle of Helminthosporium oryzae produced abnormalities in rice seedlings and it inhibited the root and coleoptile growth (Orsenigo 1956).

Injection of culture filtrate of Ceratostomella ulmi into healthy elm seedlings produced typical symptoms of the dutch elm disease (Zentmeyer 1941). Zinniol produced by Alternaria zinniae caused chlorosis of areas round veins, curling of leaf tips and withering of leaves of zinnia (White & Starrett 1967). Samaddar and Scheffer (1968) described the effect of specific toxin of Helminthosporium victoriae on host cell membrane and Heneby et al (1968) produced various changes in oat roots of susceptible variety with victorin. Aspergillus niger produced a culture filtrate that disorganised fleshy scales and green leaves of onion (Narain & Om Prakash 1968). Culture filtrates of Colletotrichum gloeosporioides reduced the sclrenchymatous tissue both in number of cells and thickening of walls as occured during wilting of citrus plants due to pathogen (Sharma and Sharma 1969). Culture filtrates of Trichocomis paludicld caused stunted growth of seedlings when irrigated with and produced necrotic spots when placed on wounded lamina (Jayachandran Nair 1969).

C. Control of the disease.

Falck (1907) showed that fungicidal value of a toxicant could be measured by comparing the growth of a fungus on treated media with that untreated media, and Prasannakumari et al (1961) adopted the poisoned food technique of Flack for assaying the effect of various copper fungicides on the growth of Helminthosporium halodes.

Chandrasekharan Nair (1964) got complete inhibition of mycelial growth of Corynespora cassiicola in treatments with 0.5% Bordeaux mixture and 0.5% Dithane whereas Fytolan 1% was not effective to inhibit growth completely.

In the studies conducted at Rubber Research Institute of India (Anonymous 1966 a) suppression of growth of Corynespora cassiicola in artificial culture by fungicides was assessed using filter paper discs dipped in fungicides as treatments and discs dipped in sterile water as control. Ferbam (0.4%) was found best followed by 3% Bordeaux mixture, 1% Bordeaux mixture, 0.2% Bresten and sterile water. However in the field trials no satisfactory control of disease was obtained by any of these treatments.

Singh et al (1969) observed complete inhibition of mycelial growth of C. cassiicola with ceresan wet and antracol at 2000 ppm and with cuprous oxide 5000 ppm (copper sandoz and fungimor). All the other chemicals used were not satisfactory. Though duter (1000 ppm) karthane (3000 ppm) blitox 50 (5000 ppm) dithane-Z 78 (2000 ppm) and bisdithane (2000 ppm) were better than control in that order.

# MATERIALS AND METHODS

## MATERIALS AND METHODS

### Isolation of the organism

Isolates of Corynespora cassiicola used for the studies were obtained from the following plants.

Tomato isolates: from leaves of tomato plants (Lycopersicon esculentum Mill.) grown at Agricultural College Farm, Vellayani.

Sesamum isolate: from leaves of gingelly plants (Sesamum indicum.L) grown at Oil Seed Research Station, Kayamkulam.

Rubber isolate: from leaves of rubber seedlings (Hevea brasiliensis Mull. Arg) grown at Rubber Research Institute of India, Kottayam.

Single spore isolation technique described by Riker and Riker (1936) with some modifications was followed to isolate, purify and maintain the cultures. Infected bits of leaves were kept in a moist chamber for 5 days to sporulate. Spore suspensions from this were made in sterile water. A droplet of this spore suspension was added to 10 c.c. of 2% plain agar in distilled water (D.W.A.) cooled to 45-50°C to retain its liquid nature and by means of a flamed transfer needle few drops of 0.1% streptomycin

added to this, and then agitated well and plated on sterile petridishes. The dishes were incubated at room temperature (28-30°C) for four hours, then inverted and observed under low power of microscope for single, isolated, germinating spores. The position of these spores were located by dot method and using a flamed cylindrical 'biscuit cutter' of a nichrome wire, a disc of agar about the dot (5 mm diameter) was transferred to petridishes and slants containing potato dextrose agar.

Sub-culturing of the organisms were made on P.D.A. at an interval of 15 days.

Pathogenicity of isolates.

Pathogenicity of the three isolates was studied by cross inoculation studies between the three plants. 30-45 day old sesamum (Onattukara local) and tomato (Pusa Ruby) and 50-70 day old rubber (TJIR) seedlings were used for cross inoculations.

Spore suspensions of the isolated cultures on potato dextrose agar were made in sterile water (50-100 spores per low power view) and sprayed on both sides of leaves using an atomizer. Inoculations were done on leaves with and without injury, injury being made by gently rubbing with a cotton wool dipped in carborandum powder. The plants were covered with humid chambers made of polythene sheets 24 hours before and 48 hours after

inoculations. Observations were made for a period of 15 days.

Five plants each were inoculated and five plants were uninoculated but given a spray of distilled water.

Both soybean and cowpea leaves were artificially inoculated by these three isolates to study whether these three isolates produce symptoms similar to the isolate of soybean and cowpea described by Spencer and Walters (1962). Hedonaea viscosa plants were also used as differential hosts.

Host range

Plants which were suspected to be infected by the organism were collected, leaf bits kept in humid chamber and spores observed. Length and breadth of spores, as well as number of septa were recorded for at least 100 spores, length being measured from tip to tip and breadth at the widest part of conidia, ie. the bulbous base just above the hilum.

Eventhough a number of plants were found to be infected by Corynespora cassiicola, the tomato and rubber isolates were taken for further studies since these two have shown differences in pathogenicity on cross inoculations between tomato, sesamum and rubber. Sesamum isolate resembled almost the tomato isolate both in pathogenicity and spore measurements.

Nature of transmission of disease

1) Seed borne nature

a) Seed mycoflora

The seed mycoflora of tomato and sesamum seeds obtained from naturally infected fruits was studied by using the "standard blotter technique" and by modifying the "agar plate method" described by Chandler and Kilpatrick (1967). Hundred seeds each of tomato and sesamum were spread on acidified potato dextrose agar in petridishes or on moist blotting paper in petridishes @ 20 each and incubated at room temperature of 26-30°C. Five days after planting, the petridishes were examined for fungal spores and bacteria and sub-cultured on PDA slants for fungi and on nutrient agar for bacteria. Observations were made on the number of seeds uncontaminated and contaminated. The contaminated seeds were further grouped for the type of organism present.

Surface sterilised seeds (with 0.1% mercury chloride in sterile water for 1 minute) were similarly spread and observed.

b) Effect of Corynespora cassiicola on emergence and vigour of seedlings.

Surface sterilized seeds and seeds treated with hot water (52°C for 10 minutes) were inoculated with a spore suspension from 15 days old culture of the organism by keeping it in spore

suspension for 30 minutes and then spread on moist filter papers kept in petridishes @ 20 each (5 x 20 = 100 seeds) and then after 5 days transferred by hair brushes to sterilized soils. Observations were on the 5th and 10th day for pre-emergence and post emergence seedling rots. Controls with sterilised or hot water treated seeds with no artificial inoculations were also maintained.

c) Effect of seed treatment.

Tomato and sesamum seeds (100 each) were artificially inoculated with the fungal spores and were then treated with seed protectants like 0.1% mercuric chloride, 0.2% Agrosan G.N. and 0.2% Captan or by hot water at 52°C for 10 minutes. The seeds were then transferred to petridishes containing acidified P.D.A. and observed for the fungal growth if any for 15 days. Artificially inoculated seeds without any treatment served as control.

2) Soil borne nature of disease.

a) Infection from soil.

The method followed by Rao and Rao (1966) for study of Fusarium wilt of cotton was followed with some modifications.

An oat meal-sand culture was prepared (Oat meal 15 grams., river sand 500 gms, distilled water 100 ml) in conical flasks, sterilized, and inoculated with tomato isolate of the organism

and incubated at room temperature of 28-30°C for 20 days. This source was used to artificially inoculate the soil. Sieved garden soil, free of root debris and other organic matter was taken in card board boxes of 6" x 5" x 4", and sterilized in an autoclave.

Seeds of tomato and sesamum surface sterilized with 0.1% $HgCl_2$ were sown in sterilized soil containing inoculum at different levels viz. 5 and 10% (w/w of inoculum and sterilized soil). Surface sterilized seeds sown in sterilized but uninoculated soils served as checks. Seeds were sown @ 5 seeds per boxes of soil kept in polythene chambers. Fifty seeds were tested at each level. Observations on pre-emergence damping off, post emergence damping off and development of leaf spots for a period of 30 days were made.

3) Air borne nature of the disease.

One month old tomato and sesamum seedlings were artificially inoculated with a heavy spore suspension of the fungus. After 10 days when the symptoms were clearly developed, these seedlings were used as the source of infection. These seedlings along with healthy seedlings of one month old, were placed in rows at a distance of 30 cms., and covered with a single cloth cage in order to study the air borne nature of the fungus. The cloth cover of the cages and the ground were wetted as to provide a moist

atmosphere for 25 days. Number of plants that have developed spots were recorded. Ten plants each were observed. Another set of ten uninoculated plants were kept under similar humid conditions to serve as check.

Growth of fungus in culture.

1) Effect of light.

The method described by Fahim (1966) for Alternaria porri was followed with some modifications.

Three treatments were made, one without light by covering in an opaque black paper and keeping in a dark place, another with diffused day light throughout, and the last with exposure to direct day light for 3 hours a day and then covering by an opaque black paper. Petridishes containing P.D.A. were inoculated with 15 day old culture of the fungus by transferring a 5 mm size disc of actively growing culture and then they were incubated at room temperature. Mycelial growth measurements and colony characters were noted after 8 days. The amount of sporulation was judged empirically by obtaining a spore suspension by transferring a 5 mm disc into 1 ml of sterile water and then counting the spores present in droplets drawn at random. Each treatment was replicated four times.

11) Effect of humidity.

Three petridishes each containing P.D.A. were inoculated with a 5 mm disc taken from an actively growing 15 day old culture and inculated at varying humidities. The following humidities were tried using suitable humidity controlling solutions in sealed vessels.

| Humidity controlling solution | Relative Humidity (%) |
|---|---|
| 3.4 c.c. con $H_2SO_4$ in 100 ml water | 97.5 |
| 8.6 c.c. " " | 93.9 |
| 22.6 c.c. " " | 80.6 |
| 30.6 c.c. " " | 70.4 |
| Saturated solution of Na $NO_2$ | 61.4 |
| Ca $(NO_3)_2$ $4H_2O$ (Saturated solution) | 51.0 |
| $K_2$ $CO_3$ $2H_2O$ ( " ) | 42.8 |
| Ca $Cl_2$ 6 $H_2O$ | 31.0 |

Under each humidities, four petridishes with the inoculated media were kept.

Observations on radial growth of mycelium was taken on 8th day. Sporulation was adjudged empirically by obtaining a spore suspension from a 5 mm disc of the culture in 1 ml sterile water and observing the spore concentration of droplets.

iii) Growth in different media.

Tomato and rubber isolates of the organism were grown in different media, both solid and liquid. In solid media the radial growth was measured from tip of the transferred 5 mm disc to edge of the colony at an interval of 24 hours for 9 days. The mycelial dry weight at intervals of 4 days for 20 days were noted in liquid media. Averages of four replications were made for each observation.

Solid media tried were (1) potato dextrose agar (Potato 200 gm., Dextrose 20 gm., Agar agar 15 gm., distilled water 1000 ml.) (2) Czapeḳk's solution agar (Mg. sulphate 0.5 gm., Dipotassium hydrogen phosphate 1.0 gm., potassium chloride 0.5 gm., Ferrous sulphate 0.01 gm., Sodium nitrate 2.0 gm., Sucrose 30 gm., agar 20 gm., Distilled water 1000 ml), (3) Czapeḳk's-Dox agar. Ingredients same as that of Czapeḳk's solution agar but with 3 gms. of sodium nitrate). (4) Richard's solution agar (Potassium nitrate 10 gm., Potassium dihydrogen phosphate 5.0 gm., Magnesium sulphate 2.5 gm., Ferric chloride 0.02 gm, Cane sugar 50 gm., agar 20.0 gm and distilled water 1000 ml) (5) Coon's medium (Saccharose 7.2 gm., dextrose 3.6 gm., Mg. sulphate 1.23 gm., Potassium acid Phosphate 2.72 gm., Potassium nitrate 2.02 gm., agar 20.0 gm., Distilled water 1000 ml) (6) Crabill's medium (Ammonium nitrate 10 gm., Dipotassium

hydrogen phosphate 5.0 gm., Magnesium sulphate 2.5 gm., Sucrose 50.0 gm., Agar 20.0 gm., Distilled water 1000 ml) and (7) Barne's medium (Potassium phosphate 1.0 gm., Ammonium nitrate 1.0 gm., Potassium nitrate 1.0 gm., Glucose 1.0 gm., agar 20 gms., distilled water 1000 ml). Host leaf extract agars with 200 gm., host leaf, 15 gm agar and 1000 ml distilled water were also made, using the chopped respective host leaves.

In the preparation of solid media, 500 ml of distilled water with the agar and 500 ml of distilled water with the other ingredients except carbon sources were boiled separately and added together to which the carbon source was then added and plugged in test tubes and sterilized by autoclaving. 15 ml of molten media (47-50°C) was poured out aseptically into sterile petridishes to which 5 mm discs of an actively growing 15 day old culture were transferred.

For preparation of liquid media, the same ingredients without agar was taken and poured into 250 ml. Erlenmeyer flasks at the rate of 35 ml per flask and sterilized by autoclaving at 15 lbs for 20 minutes. Five mm discs were marked by a flamed cork borer from 15 day old cultures and were transferred aspetically into the conical flask containing the liquid media and incubated at room temperature for different periods. After

the required periods of growth, the liquid cultures were filtered through filter papers (Whatman No.41) and filtrates were bio-assayed for its toxic activity by keeping 20-30 day old tomato and sesamum seedlings dipping their roots only in the filtrates. The time taken for wilting and severity of symptoms produced were observed. The following notations were used as a gradations of severity of wilting. 0 = no wilting; + curling of lower leaves; ++ curling of all leaves and bending of lower leaves; +++, wilting of all leaves; ++++ wilting of the entire plant. For further studies Czapek's media was selected unless otherwise specified, since the growth of the fungus and activity of culture filtrate were satisfactory in this medium. Since the expression of wilting on tomato seedlings by the two isolates were more or less similar, tomato isolates were selected for further studies unless otherwise specified.

(v) Effect of different carbon sources on growth and activity culture filtrate.

Using Czapek's media as basal, different carbon sources, both mono, di, and poly saccharides were used as sources of carbon in quantum equivalent to that contained in 30 grams of sucrose per litre of basal media. Treatments included were as follows:-

| Source of carbon. | Wt. in gm/litre. |
|---|---|
| No carbon | 0 |
| Monosaccharides ($C_6H_{12}O_6$) | |
| D-glucose | 31.575 |
| D-fructose | 31.575 |
| Disaccharides ($C_{12}H_{22}O_{11}$) | |
| Sucrose | 30.00 |
| Maltose | 30.00 |
| Lactose | 30.00 |
| Poly saccharides | |
| Starch | 14.360 |
| Mannitol | 14.360 |

35 ml of media was poured into 250 ml Erlenmeyer flasks, sterilised by autoclaving at 15 lbs pressure for 20 minutes and inoculated with 5 mm discs of actively growing cultures. Each treatment was replicated three times and mycelial dry weights and activity of culture filtrate on tomato seedlings were recorded.

Radial growth in different carbon sources was recorded on 2nd, 4th, 6th and 8th day by using solid media with different carbon sources.

v) Effect of different sources of nitrogen on growth and activity of culture filtrate.

Inorganic and organic forms of nitrogen in quantum equivalent to 2 gms sodium nitrate of basal media (Czapek's solution) was used in solid and liquid media; radial growth in solid media and mycelial growth and activity of culture filtrate in liquid media were observed with 5 replications for each treatment. Different sources of nitrogen used are given below:-

| Sources of nitrogen | Gms/litre |
|---|---|
| No nitrogen | 0 |
| **Organic nitrogen** | |
| Peptone | 2.190 |
| Asparagine | 1.775 |
| Urea | 0.710 |
| **Inorganic Nitrogen** | |
| Sodium nitrate | 2.000 |
| Potassium nitrate | 2.570 |
| Ammonium sulphate | 1.564 |
| Ammonium carbonate | 1.150 |
| Ammonium nitrate | 0.943 |
| Sodium nitrite | 1.150 |
| Ammonium chloride | 1.164 |

vi) Effect of vitamins on growth of fungus and activity of culture filtrates

To the basal media of Czapek's solution, vitamins were added @ 2µgm per litre, the vitamins being prepared to the concentration by dilution technique. The following vitamins were tried. Pyridoxine, Thiamine, Inositol, Calcium pantothenate, Lactoflavin, Ascorbic acid, Para-amino benzoic acid, Nicotinic acid, Folic acid, Cyanocobalamine and Biotin. They were compared with no vitaminized basal medium. There were two replicates for each treatment and the mycelial dry weight and activity of culture filtrate on tomato seedlings were observed.

vii) Effect of age of culture on activity of culture filtrate and its change in pH

Activity of culture filtrates at different ages of the culture was determined by its ability to produce wilting symptoms in tomato seedlings kept in the culture filtrates after 4, 8, 12, 16, 20 and 24 days of growth. Czapek-Dox medium with a pH of 7.2 was used for these studies.

The dry weight of the mycelium and the pH of the filtrate at different ages of cultures were observed, using two replicates for each observation.

viii) Effect of H-ion concentration of media on growth and activity of culture filtrate.

Using Czapek's-Dox solution with pH 7.2 as the basal medium, media with different levels of hydrogen ion concentrations were made with 1 N, NaOH and 1 N HCl. The pH readings were recorded with the help of a Trombay pH meter. Readings were taken before and after autoclaving and the inoculated cultures were incubated at room temperature (27-30°C) for twenty days. Both tomato and rubber isolates were inoculated, with three replications for each treatment. Dry weight of the mycelium after 20 days of seedling, drift in pH of culture filtrate and activity of culture filtrate on tomato seedlings as well as inhibition on elongation of radicle length were observed.

ix) Effect of change of pH of culture filtrate on its activity.

The pH of the culture filtrates in most cases were brought to 4.2 in a range of 4.0 to 5.0. In order to study whether the effect of the filtrate will change if its pH is adjusted to different hydrogen-ion concentrations this experiment was taken up.

Culture filtrates of the fungus after 20 days of growth in Czapek's-Dox medium, attaining a pH of 4.2 was adjusted with 1 N NaOH and 1 N HCl to different pH values viz. pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The adjustment of pH was done with

a Trombay pH meter. The adjusted culture filtrates were used to study the effect on wilting of 20-30 day old tomato seedlings when placed in the filtrate for a period of 72 hours and also for its effect on inhibition of radicle length elongation of tomato seeds.

The activity of orginal culture filtrate of pH 4.2, media at pH 4.2 and sterile water were also studied for comparison.

The effect of the filtrate on inhibition of elongation of radicle length of tomato seeds was carried out by following the method described by Luke & Wheeler (1955) with some modifications. Tomato seeds were placed for germination in sterile filter papers kept in sterilized petridishes and moistened with sterile water. After 72 hours, 25 seeds that have got a radicle length of 10 mm were transferred on a filter paper kept in a sterilized petridish moistened with 5 ml of toxin. As control germinated seeds were placed in uninoculated autoclaved media and kept for further observations. Each treatment was replicated four times. Further elongation of radicle after 48 hours was observed.

x) Effect of temperature on activity of culture filtrate.

Culture filtrates of Corynespora cassiicola after 20 days

of growth in Czapeck's-Dox medium were taken in 250 ml Erlenmeyer flasks and placed in water baths for 20 minutes at 50, 60, 70, 80, 90 and 100°C, using thermostatically controlled water baths, and assayed for their activity on 20-30 day old tomato seedlings when placed in test tubes with roots immersed in the filtrate. Effects of autoclaving culture filtrates at 10 lbs pressure for 15 minutes as well as keeping filtrates in refrigerators for 24 hours at 5°C were also studied. Uninoculated media with the same treatments and sterile water were used as checks.

xi) Effect of storage.

The effect of storage of toxin at different storage conditions at 5°C and at 27-30°C with and without autoclaving was studied by bioassaying the toxin on tomato seedlings. Tomato seedlings (20-30 day old) were kept in culture filtrates taken at 5, 10, 15 and 20 days of storage under the three different conditions, and its effect on wilting was noticed.

xii) Effect of dilution.

Different concentrations of filtrate in sterile water, namely 6.25%, 12.5%, 25%, 50% and 100% were prepared and 20-30 day old tomato seedlings were placed in it with their root portions immersed in test tubes containing these solutions. Observations

on wilting symptoms at 6, 12, 18, 24, 48 and 72 hours were taken.

Similar concentrations of uninoculated media and sterile water were used for comparing the effects.

xiii) Effect of culture filtrate on germination of host seeds.

Hundred healthy seeds each of tomato (Pusa ruby) and sesamum (Onattukara local) were taken, immersed in 10 ml of culture filtrate of tomato isolate for 15 minutes and then spread on sterile filter papers (What No.1) kept in sterile petridishes @ 25 each and were then again wetted with 5 ml of culture filtrate. Controls were run with sterile water and uninoculated media. Observations on germination were taken after 24, 48, 72 and 96 hours.

xiv) Effect of culture filtrate on plumule elongation.

Healthy tomato and sesamum seeds were planted in sterile filter papers kept in sterilized petridishes and moistened with sterile water. Germinated seeds with a plumule length of 5 mm were transferred on sterilized filter papers kept in sterilized petridishes at the rate of 25 each and then irrigated with 5 ml of culture filtrate. Each treatment was replicated four times and 100 seeds in each variety were tested. Suitable controls with uninoculated medium and sterile water were also run.

made into spore suspension in culture filtrate. The concentration of spores was so adjusted that there will be 60-80 spores in a field under low power. Hanging drops of this suspension were prepared and kept in a humid chamber for 12 hours after which they were observed for germination. Average of four replications were taken.

Spore suspensions in sterile water and uninoculated media served as checks.

xviii) Purification of toxin

The method adopted by Sathiabalan Samuel (1969) in his studies on toxin production of Alternaria sesami was followed for the purification of toxin.

Corynespora cassiicola was cultured in Czapek-Dox medium for 20 days and the filtrates obtained were mixed with different solvents. The solvents used were ether, chloroform, ethyl alcohol (100%), benzene, butanol, methanol and acetone.

30 ml of filtrate and 30 ml of solvent were mixed well in a separating funnel, shaken well, and the solvent layer was separated out, and kept for evaporation. The residue left after evaporation was dissolved in 2 ml of double sterilised water.

These solutions were used to study their effect on wilting at concentrations of 2 parts in 30 parts (v/v) of sterile water on 20-30 day old tomato seedlings to determine the best solvent.

The purified toxin dissolved in sterile water (2:30) was sprayed on tomato and sesamum leaves kept in moist chambers for 24 hours before the treatment. After spraying, the plants were again kept in moist chambers and observations were made after 48 hours.

Laboratory evaluation of fungicides on control of Corynespora cassiicola.

1) Poisoned Food technique (Flack 1907)

Fifty, hundred and one hundred and fifty ugms of fungicides were weighed and incorporated well to 50 ml of melted potato dextrose. Fifteen ml of the poisoned media was poured aseptically into sterile petridishes. Circular discs of 5 mm diameter of actively growing 15 day old cultures of the organism were cut with flamed cork borer and transferred to the centre of the poisoned media, using a flamed inoculation needle. Each treatment was replicated four times. Controls were made with non-poisoned media.

Observations on radial growth measured from the tip of the disc to the edge of the mycelial spread was made on 6th day of seeding. Colony characters were also observed.

2) Inhibition of spore germination.

Method followed by Akinrefon (1967) in his studies on fungi-toxicity of cycloheximide towards Alternaria brassicicola

was followed, with some modifications. Spore suspensions (100 spores per low power view) from 2 weeks old cultures prepared in sterile water was mixed immediately with equal quantities of poisons in sterile water at double the strength (25 gms, 50 gms, 100 gms and 150 gms per 25 ml). Hanging drop suspensions of these spores in fungicides were prepared and kept in moist chambers. After 6 hours, these were observed for number of spores germinated, for number of spores germinated with malformed germ tubes and for number of spores not germinated. Spore suspensions in sterile water served as check. Each treatment was replicated four times and average taken.

The chemicals used for the studies, were:-

| | |
|---|---|
| 1) Fytolan | (Copper oxychloride 50% metallic copper) |
| 2) Blitox | (50% metallic copper as copper oxychloride) |
| 3) Dithane-Z 78. | (75% Zinc ethylene bisdithio carbamate) |
| 4) Dithane-M 45. | (Zinc ion and manganese ethylene bisdithio carbamate 75%) |
| 5) Zirido | (80% Ziram) (Zinc ethylene dithiocarbamate) |
| 6) Cuman | (Zinc dimethyl dithiocarbamate) |
| 7) Duter | (Triphenyl tin hydroxide) |
| 8) Difolten | C-S-N (C 1, 1, 2,2,-tetrachloro ethyl thio)-4 cyclohexane- 1,2 dicarboximide. |

9) Captan (N-trichloro methyl mercapto-4-cyclo hexyl- 1,2 dicarboximide)

10) Mercury chloride

11) Thiovit (Wettable sulphur 80%)

# RESULTS

Observations on plumule length over 5 mm length were taken after 24, 48 and 72 hours.

xv) Effect of culture filtrate on cut shoots.

Culture filtrates of the two isolates from tomato and rubber after 20 days growth in Czapek's-Dox solution were taken in test tubes and cut shoots of 50-60 day old tomato, sesamum and rubber were placed in them. Observations after 24, 48 and 72 hours were taken. Control with uninoculated media, and sterile water were also kept.

xvi) Effect of culture filtrate on other seeds.

Seeds of cow-pea, cluster beans, kidney beans, lima beans, pole beans, soybeans, peas, dodonea, bhindi and papaya were kept in culture filtrates for 30 minutes, and then spread on sterilized filter papers kept in sterilized petridishes and moistened with 5 ml of culture filtrates. Hundred seeds each were treated with culture filtrate and an equal number was treated with sterile water and uninoculated media. Germination percentage after 72 hours was taken.

xvii) Effect of culture filtrates on microorganisms.

Freshly collected spores of Alternaria sesami, Cercospora cassiicola, Helminthosporium halodes and Sphacelotheca sp. were

# RESULTS

Corynespora cassiicola (Berk and Curt.) Wie, was easily isolated from naturally infected leaves of tomato (Vellayani) sesamum (Kayamkulam) and rubber (Kottayam) by single spore isolation technique of Riker and Riker (1936).

The morphological characters of the three isolates were similar with gray or pale brown mycelium, thinly hairy, effused and immersed in the medium. Hypha septate, sub-hyaline to pale brown; conidiophores arise from immersed hypha, simple and sometimes branched, 4-11 μ thick and 110-950 μ long with septations. Conidia formed singly or in chains of 2 to 6, are obclavate or cylindrical, pale olivaceous in colour., 45-250 μ long, 6.5-18.0 μ broad at broadest base, and with 2-16 pseudo-septa, Conidiophores in chain show an isthmus. Sporulation starts by the 5th day of inoculation and is abundant during 12-15 days, where-after, it decreases. In the early stages (upto 3 days) concentric rings and zonations were found towards periphery which were non-detectable by the 8th day.

## Symptoms of infection

In tomato, sesamum, and rubber leaves symptoms of infection on inoculations with cultures were observed. Symptoms

started to appear on leaves in 3-5 days, as small brown spots of about one mm size. The spots with irregular margins enlarged with an yellowish chlorotic area around it and in about 10 days the spots became more than 5 mm in diameter with brownish margins surrounded by a chlorotic halo and with grayish necrotic centres. As the disease advanced, several spots coalesced together and the leaf surface got necrotic on both sides. The necrotic centres of spots often fall off leaving shot holes on leaves. Severely affected leaves dry and fall off.

On veins and midrib, the lesions appeared as brownish discolourations which enlarged lengthwise.

On stems of tomato and seasamum, lesions appeared as reddish spots which enlarged elliptically with light coloured centres and reddish margins. As infection advanced the entire stem got discoloured and dried up.

On fruits of tomato and capsules of sesamum the symptoms developed as brownish spots which enlarged in concentric zonations. The centre became grayish with an yellow halo. Severely affected fruits and capsules crinkled and got shrivelled.

Pathogenicity of isolates:

Results of cross inoculation of the three different isolates of the organism isolated from tomato, sesamum and rubber with the three host plants and also cow pea, soy-bean

TABLE I

Pathogenicity of isolates

I. Effect of wounding on leaf

| Isolate from | Observations after 10 days of inoculation | | | | | |
|---|---|---|---|---|---|---|
| | un-injured leaves of | | | injured leaves of | | |
| | Tomato | Sesamum | Rubber | Tomato | Sesamum | Rubber |
| Tomato | ++ | + + | — | +++ | +++ | + |
| Sesamum | ++ | + + | — | +++ | +++ | + |
| Rubber | ++ | + + | + + | +++ | +++ | +++ |
| Control (distilled water spray) | — | — | — | — | — | — |

— no symptons

\+ Spots of 1 mm diameter

++ Spots of 2 - 5 mm diameter

+++ More than 5 mm diameter, but no coalescence

++++ Coalescence of necrotic spots.

TABLE II

Pathogenicity of isolates II: Difference in infectivity between isolates.

| Isolate from | Inoculation on wounded leaves of | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tomato | | | | | Sesamum | | | | | Rubber | | | | | Cowpea | | | | | Soybean | | | | | Dolichos | | | | |
| | 3 | 5 | 7 | 10 | 15 | 3 | 5 | 7 | 10 | 15 | 3 | 5 | 7 | 10 | 15 | 3 | 5 | 7 | 10 | 15 | 3 | 5 | 7 | 10 | 15 | 3 | 5 | 7 | 10 | 15 |
| Tomato | + | ++ | ++ | +++ | ++++ | + | ++ | ++ | +++ | ++++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ++ | ++ | ++ |
| Sesamum | + | ++ | ++ | +++ | ++++ | + | ++ | ++ | +++ | ++++ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ++ | ++ |
| Rubber | + | ++ | ++ | +++ | ++++ | + | ++ | ++ | +++ | ++++ | + | ++ | + | +++ | ++++ | ± | + | + | + | + | ± | + | + | + | + | ++ | ++ | +++ | +++ | +++ |
| Distilled water spray (control) | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

- No symptoms
+ Lesions of 1 mm size
++ Lesions of 2-5 mm size
+++ More than 6 mm but no coalescence
++++ Coalescence of spots

and Dodonaea are given in Table I & II.

The isolates obtained from tomato and sesamum were infectious, to both on cross inoculations, producing similar symptoms whereas they failed to produce typical symptoms in rubber leaves in 3 days. The isolate from rubber produced symptoms on all the three host plants in 3 days. The intensity of symptoms produced by rubber isolate on all the three hosts were similar and resulted in spots of size 5 mm in 10 days and coalescence of spots in 15 days. Conversely, the tomato and sesamum isolates which produced typical symptoms on tomato and sesamum leaves (spots of 5 mm size and coalescence in 15 days) produced only spots of 1 mm size on rubber which failed to develop further. The rubber isolate, infectious to tomato and sesamum was reisolated from tomato and sesamum and it was able to produce typical symptoms on rubber. Rubbing with a sterile cotton wool dipped in carborandum powder before spraying spore suspension was found favouring earliness in symptom expression in the hosts (Table I). Plates i, ii,iii and IV show infected tomato, sesamum and rubber leaves and capsules.

Inoculations of tomato, sesamum and rubber isolates failed to produce spots that enlarge giving a target effect on cow pea and soy-bean, but caused small purple flecks which later turned brown. On Dodonaea viscosa the tomato and sesamum isolates

TABLE III

Mycoflora of Tomato and Sesamum seeds

| Name of variety | No.of seeds taken | No.of uncontaminated seeds | No.of seeds contaminated with | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Corynespora sp | Alternaria sp | Rhizopus sp | Aspergillus sp | Penicillium sp | Fusarium sp | Cercospora sp | Helminthosporium sp | Unidentified: Septate | Unidentified: aseptate | Bacteria |
| **Agar plate method** | | | | | | | | | | | | | |
| Sesamum | | | | | | | | | | | | | |
| a) Onattukara local | 100 | 28 | 6 | 16 | 23 | 20 | 12 | 8 | 9 | 5 | 4 | 11 | 29 |
| b) TMV-3 | 100 | 39 | 13 | 21 | 16 | 8 | 9 | 6 | 4 | 9 | 9 | 10 | 14 |
| Tomato | | | | | | | | | | | | | |
| a) Local | 100 | 19 | 13 | 19 | 13 | 2 | 16 | 20 | 1 | - | 6 | 14 | 16 |
| b) Pusa ruby | 100 | 26 | 2 | 14 | 18 | 1 | 19 | 16 | - | 1 | 5 | 11 | 7 |
| **Blotter method** | | | | | | | | | | | | | |
| Sesamum | | | | | | | | | | | | | |
| a) Onattukara local | 100 | 20 | 18 | 13 | 6 | 29 | 3 | 3 | 2 | 1 | 9 | 14 | 16 |
| b) TMV-3 | 100 | 18 | 11 | 12 | 2 | 16 | 7 | 2 | 6 | 2 | 11 | 11 | 13 |
| Tomato | | | | | | | | | | | | | |
| a) Local | 100 | 24 | 13 | 14 | 3 | 31 | 9 | 13 | - | 1 | 6 | 10 | 9 |
| b) Pusa ruby | 100 | 21 | 6 | 19 | 2 | 19 | 3 | 9 | - | 1 | 12 | 12 | 14 |

TABLE IV

Effect of *Corynespora cassiicola* on germination and post emergence vigour of Sesamum and Tomato

| Seeds | Treatment | No.of seeds | Germination and vigour | | | Remarks |
|---|---|---|---|---|---|---|
| | | | pre emergence damping off (5th day) | Post emergence damping off (10th day) | No.of seeds survived | |
| Sesamum (Onattukara local) | Surface sterilized and inoculated | 100 | 6 | 3 | 91 | 6 weak |
| | Surface sterilized | 100 | 3 | 3 | 94 | All Healthy |
| | Hot water treated and inoculated | 100 | 17 | 5 | 78 | 2 weak |
| | Hot water treated | 100 | 6 | 4 | 90 | All Healthy |
| Tomato (local) | Surface sterilised and inoculated | 100 | 3 | 4 | 93 | 11 weak |
| | Surface sterilised | 100 | 2 | 3 | 95 | All Healthy |
| | Hot water treated and inoculated | 100 | 9 | 2 | 89 | 14 weak |
| | Hot water treated | 100 | 1 | 1 | 98 | All Healthy |

produced similar symptoms. Rubber isolates produced comparatively bigger spots on these leaves (Table II). Plate V shows spot development in Dodonaea leaves by isolates of tomato, sesamum, rubber and dodonaea.

Nature of transmission of disease

A. Seed borne nature

i) Seed mycoflora of tomato and sesamum

Seeds obtained from naturally infected fruits of tomato (Vellayani) and Sesamum (Kayamkulam) by "agar plate method" and "standard blotter technique" studies revealed that a number of fungi are associated with seeds, including the test fungus Corynespora cassiicola. The results of seed mycoflora studies are given in Table III which shows that 61 to 82 seeds out of every 100 seeds of tomato and sesamum contain some micorabial contaminants of which 8.5 - 11.50% are due to C. cassiicola.

2) Effect of inoculation on treated seeds

Seeds of tomato and sesamum treated chemically with $HgCl_2$ or physically by hot water took infection on immersion in heavy spore suspensions of Corynespora cassiicola. The preemergence and post emergence damping off (Table IV) were higher in seeds inoculated with spore suspension whereas the corresponding data for uninoculated seeds were much lower.

TABLE V

Effect of seed treatment on control of seed borne fungus

(Agar Plate method)

| Sl. No. | Chemical used | Tomato No. of seeds in the trial | | | Sesamum No. of seeds in the trial | | |
|---|---|---|---|---|---|---|---|
| | | Total | Contaminated | Uncontaminated | Total | Contaminated | Uncontaminated |
| 1. | Mercuric chloride .1% | 100 | 2 | 98 | 100 | - | 100 |
| 2. | Agrosan G.N. 0.2% | 100 | 5 | 95 | 100 | 3 | 97 |
| 3. | Captan 0.2% | 100 | 9 | 91 | 100 | 6 | 94 |
| 4. | Hot water treatment | 100 | 2 | 98 | 100 | 8 | 92 |
| 5. | Control (no treatment) | 100 | 81 | 19 | 100 | 68 | 32 |

TABLE VI

Soil borne nature of disease

I-Effect of artificially inoculating soil

| Variety | Inoculum level. | No.of seeds sown | Pre-emergence damping off in 5 days | Post emergence damping off in 10 days. | Seedlings survived | | No.of seedlings that developed spots in 15 days | No.of seedlings that developed spots in 30 days | % of seedlings that developed spots | Total % of affected seeds and seedlings | Total % of seeds and seedlings unaffected |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. | % | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Sesamum | 5% | 50 | 6 | 4 | 40 | 80 | 1 | 3 | 7.5 | 26 | 74 |
| | 10% | 50 | 18 | 12 | 20 | 40 | 2 | 4 | 2.0 | 48 | 52 |
| | 0 | 50 | 2 | 1 | 47 | 94 | . | . | | 6 | 94 |
| Tomato | 5% | 50 | 4 | 3 | 43 | 86 | 1 | 4 | 9.3 | 22 | 78 |
| | 10% | 50 | 9 | 6 | 35 | 70 | 3 | 5 | 14.3 | 36 | 64 |
| | 0 | 50 | 3 | 2 | 45 | 90 | . | . | | 10 | 90 |

TABLE VII

Air borne nature of disease

| Crop/treatment | | Observations on 10th day | Observations on 15th day |
|---|---|---|---|
| Tomato | Previously infected plants | ++++ | ++++ |
| | with infected plants | + | ++ |
| | without infected plants | - | - |
| Sesamum | Previously infected plants | +++ | ++++ |
| | with infected plants | + | ++ |
| | without infected plants | - | - |

| | |
|---|---|
| - | no spots |
| + | few spots on some leaves (10% or less) |
| ++ | Spots on more than 10 - 25% leaves |
| +++ | Bigger spots in more than 25% leaves |
| ++++ | Coalescence of spots on leaves, stem, etc. (more than 60%) |

3) Effect of seed treatment on control of seed borne fungus

Table V shows the data on number of seeds contaminated with different microbes after the seeds were treated with chemicals (mercury chloride, agrosan G N and captan) or by hot water. For both tomato and sesamum seeds all the treatments reduced the number of seeds contaminated, than the untreated seeds which showed a very high degree of contamination.

B. Soil borne nature

Artificially inoculating the fungus to sterilized soil and then using it for growing tomato and sesamum seeds resulted in higher percentage of infection both at 5 and 10% of inoculum level in both the seeds. The percentage of unaffected seedlings was high in sterilized soils without inoculum (Table VI). Sesamum seeds were found more damaged by higher inoculum levels then tomato seeds.

C. Air borne nature of disease.

Data on air borne nature of the disease (Table VII) indicate that previously infected plants kept near uninfected plants caused disease manifestation in 80% seedings while there was no infections when two sets of uninfected plants were kept in cages for 25 days.

TABLE VIII

Effect of light on growth and sporulation

| No. | Treatment | Radial growth on 6th day of seeding | | | | | Sporulation | Colony characters |
|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Mean | | |
| 1 | Diffused light throughout | 30 | 30 | 32 | 36 | 32 | +++ | Mycelium whitish grey with olivaceous border, fluffy, under side black, concentric rings present. |
| 2 | Direct light for 3 hours a day | 28 | 32 | 31 | 29 | 30 | ++ | Mycelium greyish white, wholley, concentric rings present more towards periphery, margins definite under side slightly blackish. |
| 3 | No light | 18 | 20 | 20 | 25 | 21 | + | Mycelium whitish centre button like and greyish, fluffy no concentric rings, margins definite. |

\- no or sparse sporulation less than 5 spores
\+ Sporulation poor 5 - 20
++ Sporulation good 20 - 50
+++ sporulation abundant > 50

TABLE IX

Effect of humidity on growth and sporulation

| Treatment | Nominal humidity | Radial growth on 8th day in mm. | | | | | Sporulation | Colony characters |
|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Mean | | |
| 1 | 97.5% | 28 | 36 | 35 | 33 | 33 | ++ | mycelium greyish white, fluffy wooley boarder olivaceous. |
| 2 | 93.9% | 30 | 36 | 35 | 35 | 34 | ++ | --do-- |
| 3 | 80.6% | 43 | 41 | 38 | 42 | 41 | +++ | --do-- |
| 4 | 70.4% | 36 | 34 | 37 | 37 | 36 | +++ | --do-- |
| 5 | 61.4% | 29 | 32 | 27 | 36 | 31 | ++ | --do-- |
| 6 | 51.0% | 31 | 29 | 30 | 30 | 30 | + | mycelium greyish, wooley, boarder definite |
| 7 | 42.8% | 23 | 36 | 36 | 37 | 33 | + | Greyish white mycelium, not wooly margins olivaceous |
| 8 | 31.0% | 29 | 31 | 29 | 25 | 28 | ++ | --do-- |

+++ Sporulation abundant 50
++ good 20-50
+ poor

CR - 1065

Table IX

Analysis of variance table (Effect of humidity)

| Source. | Sum of squares | D.F. | Variance | F Calculated | F (0.01 level) | Whether significant or not | C.D. |
|---|---|---|---|---|---|---|---|
| Total | 706.88 | 31 | -- | -- | - | - | - |
| Treatment | 425.88 | 7 | 60.840 | 5.196 | 3.50 | Significant | 4.99 |
| Error | 281.00 | 24 | 11.708 | -- | - | - | - |

Mean of treatment and Ranking

| $S_3$ | $S_4$ | $S_2$ | $S_1$ | $S_7$ | $S_5$ | $S_6$ | $S_8$ |
|---|---|---|---|---|---|---|---|
| 42 | 36 | 34 | 33 | 33 | 31 | 30 | 28 |

Physiological studies

1) Effect of light

Data on effect of light on sporulation and growth of the fungus carried out are presented in Table VIII. The radial growth and sporulation of fungus were more in cultures kept at diffused day light and partial exposure to light, whereas it was poor when cultures were kept under dark conditions for 8 days.

2) Effect of humidity

Humidity has shown varying effects on radial growth of the fungus and its sporulation. Radial growth was more at humidity levels 70.4 & 80.6; medium at 93.9, 97.5, 42.8 and 61.4 and low at 51.0 and 31.0. Sporulation was abundant at humidity ranges 70.4, and 80.6 good at 97.5, 93.9, 61.4 and 42.8 and poor at 51.0 and 31.0 (Table IX).

Humidity levels 70.4 and 80.6 were most favourable for both growth and sporulation.

3) Growth in different media.

a) Radial growth

Radial growth measurements of the two isolates from tomato and rubber for a period of 8 days showed that P.D.A. was the best solid media followed by Czapeḳ-Dox media, Czapeḳ's solution

TABLE X-(A)

Radial growth measurements of colony on 8th day of seeding in mm

| Sl. No. | Treatment (media) | $R_1$ | $R_2$ (Tomato isolate) | $R_3$ | $R_4$ | Mean | $R_1$ | $R_2$ (Rubber isolate) | $R_3$ | $R_4$ | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Potato dextrose agar | 42.5 | 44.5 | 43.0 | 42.0 | 43.00 | 44.5 | 45.0 | 43.5 | 45.0 | 44.5 |
| 2. | Czapecks' medium | 35.5 | 37.0 | 35.0 | 36.5 | 36.0 | 42.0 | 44.0 | 45.0 | 43.0 | 43.5 |
| 3. | Czapeck-Dox medium | 38.0 | 39.0 | 38.5 | 38.5 | 38.0 | 44.0 | 44.5 | 44.0 | 43.5 | 44.0 |
| 4. | Richards' medium | 30.5 | 31.5 | 31.0 | 31.0 | 31.0 | 43.0 | 44.5 | 44.5 | 44.0 | 44.0 |
| 5 | Coons' medium | 31.0 | 30.5 | 30.5 | 30.0 | 30.5 | 37.0 | 40.0 | 40.5 | 38.5 | 39.0 |
| 6 | Barnes' medium | 14.0 | 10.5 | 16.0 | 11.5 | 13.0 | 39.0 | 37.5 | 36.5 | 35.0 | 37.0 |
| 7 | Crabills' medium | 14.0 | 15.0 | 16.0 | 13.0 | 14.5 | 38.0 | 40.5 | 41.5 | 40.0 | 40.0 |

TABLE X (B)

Analysis of variance Table (Radial growth in different media)

| Source | Sum of squares | DF | Variance | F calculated | F 0.01 level | Whether significant or not | C.D. |
|---|---|---|---|---|---|---|---|
| Total | 5582.72 | 55 | - | - | - | - | - |
| Treatment | 5517.72 | 13 | 424.44 | 273.83 | 2.59 | Significant | |
| I (Isolates) | 2298.85 | 1 | 2298.85 | 1547.60 | 7.27 | " | 0.6696 |
| S (Media) | 2393.72 | 6 | 398.95 | 257.36 | 3.23 | " | 1.7602 |
| Error | 65.00 | 42 | 1.55 | - | - | - | - |

Mean of Isolates and Ranking

| $I_2$ | $I_1$ |
|---|---|
| 41.71 | 29.42 |

Mean of treatments in $I_1$ and Ranking.

| $S_1$ | $S_3$ | $S_2$ | $S_4$ | $S_5$ | $S_7$ | $S_6$ |
|---|---|---|---|---|---|---|
| 43.0 | 38.0 | 36.0 | 31.0 | 30.5 | 14.5 | 14.0 |

Mean of treatments in $I_2$ and Ranking.

| $S_1$ | $S_3$ | $S_4$ | $S_2$ | $S_7$ | $S_5$ | $S_6$ |
|---|---|---|---|---|---|---|
| 44.5 | 44.0 | 44.0 | 43.5 | 40.0 | 39.0 | 37.0 |

TABLE XI

Radial growth of *Corynespora cassiicola* in different media (Tomato isolate)

(Average of four replications each)

| Trt No. | Treatment | Radial growth in millimeters in | | | | | | | | Average radial growth for 24 hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 48 hrs | 72 hrs | 96 hrs | 120 hrs | 144 hrs | 168 hrs | 192 hrs | 216 hrs | |
| 1 | Potato Dextrose Agar | 6.0 | 10.0 | 16.5 | 21.0 | 29.5 | 36.0 | 43.0 | 45.0 | 5.00 |
| 2 | Czapecks' medium | 4.2 | 9.5 | 14.5 | 20.5 | 26.0 | 29.0 | 36.0 | 40.5 | 4.50 |
| 3. | Czapeck Dox medium | 4.5 | 10.0 | 16.0 | 22.0 | 26.0 | 32.0 | 38.0 | 43.5 | 4.83 |
| 4 | Richards' medium | 3.0 | 7.0 | 13.0 | 19.0 | 23.0 | 26.0 | 31.0 | 35.5 | 3.94 |
| 5 | Coons' medium | 2.5 | 6.5 | 9.0 | 13.0 | 19.5 | 25.5 | 30.5 | 35.0 | 3.90 |
| 6 | Barnes' medium | 3.0 | 4.0 | 5.0 | 6.0 | 9.0 | 11.0 | 13.0 | 16.0 | 1.80 |
| 7 | Crabills' medium | 2.0 | 3.5 | 4.8 | 5.5 | 8.0 | 11.0 | 14.5 | 18.0 | 2.00 |

TABLE XII

Radial growth of the three isolates in different host leave extract agar media (in mm) after 6 days (average of 4 replication)

| Sl.No. | Isolate from | Host leaf extract agar (pH of leaf extract) | | |
|---|---|---|---|---|
| | | Tomato (5.9) | Sesamum (5.6) | Rubber (6.3) |
| 1. | Tomato | 25.0 | 23.5 | 26.5 |
| 2. | Sesamum | 31.5 | 36.0 | 39.5 |
| 3. | Rubber | 21.5 | 24.0 | 28.0 |

TABLE XIII

Dry weight of mycelium in different media in mgms
(Average of 4 replicates in 35 cc. of culture in 250 ml flasks)

| Mycelial weights on | Media | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Potato Dextrose solution | | Czapecks' medium | | Czapeck-Dox medium | | Richards' medium | | Coons' medium | | Barnes medium | | Crabills' medium | |
| | T | R | T | R | T | R | T | R | T | R | T | R | T | R |
| 4th day | 20 | 30 | 25 | 20 | 35 | 40 | 35 | 40 | 30 | 25 | 10 | 20 | 30 | 25 |
| 8th day | 75 | 95 | 70 | 75 | 85 | 90 | 80 | 95 | 75 | 95 | 35 | 40 | 75 | 80 |
| 12th day | 145 | 160 | 190 | 185 | 140 | 180 | 136 | 160 | 120 | 140 | 55 | 65 | 110 | 140 |
| 16th day | 175 | 205 | 175 | 220 | 210 | 225 | 180 | 210 | 145 | 160 | 70 | 80 | 155 | 175 |
| 20th day | 185 | 210 | 180 | 225 | 220 | 235 | 190 | 215 | 160 | 165 | 90 | 85 | 165 | 195 |
| Mean growth per day | 9.25 | 10.5 | 9.0 | 11.25 | 11.0 | 11.75 | 9.5 | 10.75 | 8.0 | 8.25 | 4.5 | 4.25 | 9.25 | 9.75 |

T - Tomato isolate of C. cassiicola
R - Rubber isolate of C. cassiicola

agar and Richard's media for both the isolates. While both Coon's and Barne's media recorded poor growth, Crabill's medium gave a satisfactory growth for rubber isolate and poor growth for tomato isolate. (Table X-A). Table XI depicts the daily growth measurements of tomato isolate for 9 days and it can be seen that the average radial growth for 24 hours was highest in P.D.A. (5.00) followed by Czapeck-Dox medium, Czapek's solution agar, Richard's medium, Coon's medium, Barne's medium and Carbill's medium. The data has been analysed statistically and presented in Table X(B).

Radial growth of the three isolates in host leaf extract agar

Host leaf extract agar media used for growth of the three isolates from tomato, sesamum and rubber gave different degree of growth. Tomato isolate grew best in rubber extract and poor in sesamum., sesamum isolate grew best in rubber extract and poor in tomato whereas rubber isolate has grown best only in rubber extract and the growth was poorest in tomato extract. (Table XII).

b) Mycelial growth in liquid media

Observations on dry weights of mycelium taken on 4th, 8th, 12th, 16th and 20th days of growth in different liquid media are summarised in Table XIII. The maximum dry weight in 20 days was obtained in Czapeck-Dox media for both the isolates, followed by

## Table XIV

Effect of culture filtrates of 20 day old *Corynespora cassiicola* cultures (tomato isolate) in 20-30 day old tomato and sesamum seedlings.

| Sl. No. | Treatment | Wilting symptoms in 20-30 day old tomato seedlings in | | | | | | | Wilting symptoms in 20-30 day old sesamum seedlings in | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 hrs. | 6 hrs. | 12 hrs. | 18 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 3 hrs. | 6 hrs. | 12 hrs. | 18 hrs. | 24 hrs. | 48 hrs. | 72 hrs. |
| 1. | Sterile water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. | Uninoculated media (Czapek's solution) | 0 | 0 | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | + |
| 3. | Culture filtrate | 0 | 0 | + | ++ | +++ | ++++ | ++++ | 0 | 0 | + | ++ | +++ | ++++ | ++++ |

0 - No symptoms

\+ - Curling of lower leaves

++ - Drooping of lower leaves and curling of upper leaves

+++ - Drooping of 75% leaves

++++ - Drooping and wilting of entire plant.

TABLE XV

Effect of 20 day old culture filtrates of Corynespora cassiicola isolates on 20-30 days old Tomato seedlings

| Treatment No. | Media | Tomato isolate | | | | Rubber isolate | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12 hrs | 24 hrs | 48 hrs | 72 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| 1. | Potato dextrose solution. | + | + | +++ | ++++ | + | ++ | +++ | ++++ |
| 2. | Czapecks' medium | + | ++ | +++ | ++++ | + | ++ | +++ | ++++ |
| 3. | Czapeck-Dox medium | + | ++ | +++ | ++++ | + | ++ | +++ | ++++ |
| 4. | Richards' medium | + | + | +++ | ++++ | + | + | +++ | ++++ |
| 5. | Coons' medium | 0 | + | ++ | +++ | + | ++ | ++ | +++ |
| 6. | Barnes' medium | 0 | + | ++ | ++ | 0 | + | ++ | ++ |
| 7. | Crabills' medium | + | + | +++ | ++++ | + | + | +++ | +++ |
| 8. | Sterile water | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

0 no symptoms
\+ Curling of lower leaves
++ Curling of leaves and drooping of lower leaves
+++ Drooping of 75% of leaves
++++ Drooping and wilting of entire plant
+++++ Drying of leaves and shedding.

Czapeḳk's media, potato dextrose solution, Richard's media, and Crabill's media. In Coon's media and Barne's media, the growth obtained was poor.

4) Effect of culture filtrate on seedlings.

Using culture filtrates of the fungus in Czapeck Dox media after 20 days growth, and using U.I.M. (uninoculated media) and sterile water as controls the activity on 20-30 days old tomato and sesamum seedlings when the roots were immersed and kept in the filtrates for 6, 12, 18, 24, 48 and 72 hours was observed as a preliminary study and the results are presented in Table XIV. The results indicate that the culture filtrate causes wilting of plants whereas U.I.M. showed only very light wilting in 72 hours and sterile water no wilting. Plates VI & VII show wilting of tomato and sesamum seedlings when kept in culture filtrate.

Effect of the 20 days old culture filtrate of the two isolates of C. cassiicola from rubber and tomato on 20-30 day old tomato seedlings were studied and the different grades of wilting symptoms produced by the culture filtrates on plants after different periods of immersion of their roots are given in Table XV. Except Barne's and Coon's medium which gave poor mycelial growth, all others served as good sources for increased activity of culture filtrates and the activity was more in potato dextrose solution, Czapeḳk's medium, Czapeḳ-Dox medium and

TABLE XVI

Radial growth in different carbon sources (Czapek's media-basal) in mm.

Average of three observations)

| Source | On 2nd day | | 4th day | | 6th day | | 8th day | | Mean growth/day. | | Sporulation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | R | T | R | T | R | T | R | T | R | T | R |
| No carbon | 0.5 | 0.3 | 2.5 | 2.0 | 4.5 | 5.5 | 8.0 | 7.5 | 1.00 | 0.94 | — | — |
| D-glucose | 5.5 | 6.5 | 16.5 | 18.5 | 29.5 | 32.0 | 40.5 | 40.0 | 5.06 | 5.00 | ++ | ++ |
| D-fructose | 4.5 | 6.0 | 18.0 | 19.0 | 30.5 | 31.0 | 45.0 | 44.0 | 5.62 | 5.50 | +++ | +++ |
| Sucrose | 4.5 | 4.0 | 13.5 | 12.0 | 26.5 | 27.5 | 42.0 | 43.0 | 5.25 | 5.38 | +++ | +++ |
| Maltose | 4.5 | 3.0 | 14.0 | 15.5 | 29.5 | 31.5 | 44.0 | 44.5 | 5.50 | 5.56 | +++ | +++ |
| Lactose | 3.5 | 3.5 | 11.5 | 13.0 | 28.5 | 29.0 | 44.5 | 45.0 | 5.56 | 5.62 | +++ | +++ |
| Starch | 3.0 | 2.0 | 9.5 | 8.5 | 17.5 | 17.5 | 30.5 | 32.5 | 3.81 | 4.06 | +++ | +++ |
| Mannitol | 3.5 | 3.0 | 9.5 | 9.5 | 22.5 | 21.5 | 37.0 | 36.5 | 4.62 | 4.56 | ++ | ++ |

\- no or scarse sporulation 5

\+ Poor sporulation >5, but < 20

++ Good sporulation >20, but < 50

+++ Abundant sporulation > 50

T Tomato isolate

R Rubber isolate

TABLE XVII A

Mycelial weight after 20 days of growth in different carbon sources in gm

(Cooper's modin basal)

| No. | Carbon source | Tomato isolate (gm) | | | | Rubber isolate (gm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean | $R_1$ | $R_2$ | $R_3$ | Mean |
| 1 | No carbon | 0.040 | 0.050 | 0.056 | 042 | 0.020 | 0.040 | 0.050 | 050 |
| 2 | D-glucose | 1.025 | 0.845 | 0.895 | 922 | 1.010 | 0.740 | 0.660 | 810 |
| 3 | D-fructose | 0.410 | 0.220 | 0.290 | 333 | 0.350 | 0.240 | 0.220 | 203 |
| 4 | Sucrose | 0.395 | 0.370 | 0.470 | 412 | 0.400 | 0.410 | 0.380 | 397 |
| 5 | Maltose | 1.015 | 1.170 | 0.650 | 945 | 0.850 | 0.940 | 0.620 | 803 |
| 6 | Lactose | 0.360 | 0.210 | 0.290 | 287 | 0.340 | 0.220 | 0.280 | 280 |
| 7 | Starch | 0.360 | 0.340 | 0.360 | 365 | 0.360 | 0.360 | 0.340 | 353 |
| 8 | Mannitol | 0.433 | 0.340 | 0.360 | 384 | 0.410 | 0.360 | 0.240 | 337 |

TABLE XVII (B)

Analysis of variance Table (Effect of carbon sources on mycelial weight)

| Source | Sum of squares | D.F. | Variance | F Calculated | F 0.01 level | Whether significant or not | C.D |
|---|---|---|---|---|---|---|---|
| Total | 12587.60 | 47 | - | - | - | - | - |
| Treatment | 9558.71 | 15 | 636.51 | 6.705 | 2.740 | Significant | - |
| I (Isolates) | 669.62 | 1 | 669.62 | 7.520 | 7.500 | Significant | 1.79 |
| S (Carbon source) | 3425.80 | 7 | 488.71 | 5.142 | 3.250 | Significant | 16.18 |
| Error | 3028.89 | 32 | 95.00 | - | - | - | - |

Mean of I

$I_1$ = 466.51

$I_2$ = 410.62

Ranking

$I_1$ $I_2$

Mean of treatments in $I_1$ and Ranking.

| $S_5$ | $S_2$ | $S_4$ | $S_8$ | $S_7$ | $S_3$ | $S_6$ | $S_1$ |
|---|---|---|---|---|---|---|---|
| 945 | 922 | 412 | 384 | 363 | 333 | 287 | 42 |

Mean of treatments in $I_2$ and Ranking

| $S_2$ | $S_5$ | $S_4$ | $S_7$ | $S_8$ | $S_3$ | $S_6$ | $S_1$ |
|---|---|---|---|---|---|---|---|
| 810 | 802 | 397 | 353 | 337 | 283 | 280 | 30 |

$I_1$ - Tomato isolate

$I_2$ - Rubber isolate

TABLE XVIII

Effect of carbon sources on activity of culture filtrate

| No. | Treatment (Sources of carbon) | Wilting symptoms on tomato leaves | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 hours | 6 hours | 12 hours | 18 hours | 24 hours | 48 hours | 72 hours. |
| 1 | No carbon | 0 | 0 | 0 | 0 | 0 | 0 | + |
| 2 | D-glucose | 0 | + | + | ++ | +++ | ++++ | ++++ |
| 3 | D-fructose | 0 | + | ++ | +++ | +++ | ++++ | ++++ |
| 4 | Sucrose | 0 | 0 | + | ++ | +++ | ++++ | ++++ |
| 5 | Maltose | 0 | + | ++ | +++ | +++ | ++++ | +++++ |
| 6 | Lactose | 0 | + | + | ++ | ++ | +++ | +++ |
| 7 | Starch | 0 | 0 | + | ++ | +++ | +++ | ++++ |
| 8 | Mannitol | 0 | 0 | + | + | ++ | ++ | +++ |

0 No wilting
\+ Curling of lower leaves
++ Curling of upper leaves and drooping of lower leaves.
+++ drooping and curling of 75% leaves
++++ drooping and wilting of entire plant
+++++ drying of leaves.

(Tomato isolate) grown under different sources of carbon was assayed by its effect in producing wilting symptoms when root portions of 20-30 day old tomato seedlings were placed in the filtrate. The results are furnished in Table XVIII. In 6 hours, lactose, maltose D-fructose and D-glucose produced light symptoms and curling of lower leaves. In 18 hours culture filtrates from all carbon sources showed varying degrees of wilting, D-fructose and maltose showing more severe symptoms than D-glucose, sucrose, lactose or starch. In 24 hours D-glucose, D-fructose, sucrose maltose and starch have shown severe symptoms followed by lactose and mannitol. In 48 hours the entire plant wilted in filtrates from monosaccarides, disaccharide sucrose and maltose. The disaccharide lactose and polysaccharide starch also showed severe symptoms and polysaccharide mannitol caused dropping of lower leaves and curling of upper leaves. There was no wilting signs in filtrates from 'no carbon' medium. In 72 hours the plants kept in filtrates from no carbon media showed drooping of lower leaves, mannitol and starch showed severe symptoms, starch sucrose, D-fructose and D-glucose caused complete wilting and maltose caused wilting and drying of leaves.

Maltose was found the best source of carbon for toxic activity of the culture filtrate and lactose and mannitol as poor sources.

TABLE XIX

Radial Growth in different Nitrogen sources in m.m

(Average of 3 replications)

N = 2 gms of $NaNO_3$ of Czapeck's media.

| Nitrogen source. | 2nd day | | 4th day | | 6th day | | 8th day | | Average growth/day. | | Sporulation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | R | T | R | T | R | T | R | T | R | T | R |
| No Nitrogen | 0.5 | 0.5 | 3.5 | 4.0 | 8.0 | 8.5 | 10.0 | 10.5 | 1.25 | 1.31 | - | - |
| Peptone | 4.5 | 6.0 | 14.5 | 16.0 | 28.5 | 30.0 | 43.0 | 44.5 | 5.38 | 5.56 | +++ | +++ |
| Asparagine | 4.5 | 4.0 | 13.0 | 14.5 | 26.0 | 26.0 | 37.0 | 38.0 | 4.62 | 4.75 | +++ | +++ |
| Urea | 4.0 | 4.5 | 12.5 | 13.0 | 22.0 | 23.5 | 28.5 | 29.0 | 3.56 | 3.62 | ++ | ++ |
| Sodium nitrate | 4.0 | 4.0 | 13.5 | 14.5 | 26.5 | 26.0 | 42.5 | 43.0 | 5.31 | 5.37 | +++ | +++ |
| Potassium nitrate | 3.5 | 3.0 | 11.0 | 10.5 | 20.0 | 21.0 | 23.5 | 24.0 | 2.94 | 3.00 | ++ | ++ |
| Ammonium sulphate | 6.5 | 6.0 | 19.0 | 20.0 | 34.0 | 36.0 | 45.0 | 44.5 | 5.62 | 5.56 | ++ | ++ |
| Ammonium carbonate | 1.5 | 2.0 | 8.0 | 8.0 | 12.0 | 14.0 | 18.5 | 21.0 | 2.31 | 2.62 | + | + |
| Ammonium nitrate | 5.0 | 4.5 | 14.0 | 15.0 | 29.0 | 31.0 | 38.5 | 39.5 | 4.81 | 4.92 | +++ | +++ |
| Sodium nitrite | 1.5 | 2.0 | 10.5 | 11.0 | 21.0 | 20.0 | 29.5 | 30.0 | 3.70 | 3.75 | + | + |
| Ammonium chloride | 0.5 | 0.5 | 4.5 | 6.0 | 8.5 | 10.5 | 16.5 | 20.0 | 2.06 | 2.50 | + | + |

\- no or sparse sporulation $< 5$

\+ $> 5, < 20$

++ $> 20, < 50$

+++ $> 50$

T Tomato isolate

R Rubber isolate

TABLE XX A

Mycelial Dry weight in mgm after 20 days of growth in different N-sources

(N = 2 gms $NaNO_3$ of Czapek's medium)

| No. | Source | Tomato isolate | | | | Rubber isolate | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean | $R_1$ | $R_2$ | $R_3$ | Mean |
| 1 | No nitrogen | 60 | 50 | 45 | 51.7 | 45 | 65 | 50 | 53.3 |
| 2 | Peptone | 280 | 230 | 250 | 255.3 | 270 | 245 | 220 | 245.0 |
| 3 | Asparagine | 285 | 190 | 260 | 245.0 | 265 | 190 | 280 | 245.0 |
| 4 | Urea | 85 | 60 | 75 | 66.6 | 90 | 65 | 70 | 75.0 |
| 5 | Sodium nitrate | 180 | 210 | 220 | 206.3 | 220 | 200 | 210 | 210.0 |
| 6 | Potassium nitrate | 130 | 100 | 90 | 106.6 | 125 | 150 | 110 | 121.7 |
| 7 | Ammonium sulphate | 210 | 185 | 200 | 198.3 | 220 | 180 | 210 | 203.3 |
| 8 | Ammonium carbonate | 105 | 95 | 110 | 103.3 | 105 | 100 | 100 | 101.7 |
| 9 | Ammonium nitrate | 165 | 180 | 175 | 173.3 | 175 | 165 | 180 | 173.3 |
| 10 | Sodium nitrite | 125 | 100 | 115 | 113.3 | 120 | 115 | 120 | 118.3 |
| 11 | Ammonium chloride | 105 | 80 | 100 | 95.0 | 100 | 75 | 90 | 88.3 |

TABLE XX (B)

Analysis of variance table (Effect of N-sources on mycelial weight)

| Source | Sum of squares | D.F. | Variance | F calculated | F 0.01 level | Whether significant or not | Critical difference |
|---|---|---|---|---|---|---|---|
| Total | 309456.50 | 65 | - | - | - | - | - |
| Treatment | 291739.80 | 21 | 13892.40 | 34.498 | 2.30 | Significant | |
| I | 4.58 | 1 | 4.58 | < 1 | 7.24 | Not significant | |
| S | 291076.50 | 10 | 29107.65 | 72.21 | 2.75 | Significant | 32.88 |
| Error | 17716.70 | 44 | 402.70 | - | - | - | - |

Mean of treatments in $I_1$ and Ranking (Tomato isolate)

| $S_2$ | $S_3$ | $S_5$ | $S_7$ | $S_9$ | $S_{10}$ | $S_8$ | $S_6$ | $S_{11}$ | $S_4$ | $S_1$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 253.3 | 245.0 | 205.3 | 198.3 | 175.3 | 115.3 | 106.6 | 105.3 | 95.0 | 66.6 | 51.7 |

Mean of treatments in $I_2$ and Ranking (Rubber isolate)

| $S_2$ | $S_3$ | $S_5$ | $S_7$ | $S_9$ | $S_8$ | $S_{10}$ | $S_6$ | $S_{11}$ | $S_4$ | $S_1$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 245 | 245 | 210 | 203.3 | 175.3 | 121.7 | 118.3 | 101.7 | 88.3 | 75.0 | 53.3 |

7) Effect of Nitrogen sources on growth

a) Radial growth

Table XIX depicts the effect of different sources of nitrogen on radial growth and sporulation of both tomato and rubber isolates of C. cassiicola for 2nd, 4th, 6th and 8th days, along with a medium having no nitrogen as control.

The data reveals that for both the isolates, ammonium sulphate was best for growth followed by peptone, sodium nitrate, ammonium nitrate, asparagine, sodium nitrite, urea, potassium nitrate, ammonium carbonate and ammonium chloride. Highest sporulations were obtained in peptone, asparagine, sodium nitrate and ammonium sulphate, and poor in others. Growth and sporulation in no nitrogen medium were poor.

b) Mycelial dry weight

After 20 days of growth in different nitrogen sources both isolates have shown highest mycelial weights in peptone and asparagine, followed by sodium nitrate, ammonium sulphate and ammonium nitrate. The lowest growth was in media without nitrogen (51.7 mgm & 53.3 mgm). Among the sources of nitrogen, urea was found to give the lowest weight (66.6 mgm and 75.0 mgm) for tomato and rubber isolates. (Table XX(A)). The data analysed statistically is presented in Table XX(B).

TABLE XXI

Effect of Nitrogen sources on activity of culture filtrate

| No. | Source of Nitrogen | Wilting symptoms in 20-30 day old tomato seedlings kept in culture filtrates for | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 hour | 6 hour | 12 hour | 18 hour | 24 hour | 48 hour | 72 hour |
| 1 | No nitrogen | 0 | 0 | 0 | 0 | 0 | + | + |
| 2 | Peptone | 0 | + | ++ | ++ | +++ | +++ | +++ |
| 3 | Asparagine | 0 | + | ++ | ++ | +++ | +++ | ++++ |
| 4 | Urea | 0 | + | ++ | +++ | +++ | ++++ | ++++ |
| 5 | Sodium nitrate | 0 | + | ++ | ++ | ++ | +++ | ++++ |
| 6 | Potassium nitrate | 0 | + | ++ | ++ | ++ | +++ | ++++ |
| 7 | Ammonium sulphate | 0 | ++ | ++++ | ++++ | ++++ | +++++ | +++++ |
| 8 | Ammonium carbonate | 0 | ++ | +++ | +++ | +++ | ++++ | ++++ |
| 9 | Ammonium nitrate | 0 | ++ | ++++ | ++++ | ++++ | +++++ | +++++ |
| 10 | Sodium nitrite | 0 | + | +++ | +++ | +++ | +++ | +++ |
| 11 | Ammonium chloride | + | ++ | +++ | +++ | +++ | ++++ | ++++ |

0 no wilting
\+ Curling of lower leaves
++ drooping lower leaves, curling of upper leaves.
+++ drooping & curling of 75% leaves
++++ drooping and wilting of entire plant
+++++ drying of leaves

8) Effect of sources of nitrogen on activity of culture filtrate

All the culture filtrates from ammoniacal sources of nitrogen produced curling of lower leaves within six hours of immersion, and in twelve hours all the filtrates from different nitrogen sources caused curling while the ammoniacal sources showed wilting. The wilting symptoms progressed with time, in more or less the same manner with ammoniacal salts showing more wilting especially ammonium sulphate and ammonium nitrate in 18 hours and 24 hours. At 48 hours, the seedlings kept in no nitrogen source showed curling of lower leaves, ammoniacal N sources showed drying symptoms while all others showed wilting of more than 75% of leaves. In 72 hours, plants in filtrates from ammonium sulphate and ammonium nitrate sources dried, those from sodium nitrate, asparagine urea, ammonium carbonate, sodium nitrite and ammonium chloride wilted completely while the other two, peptone and potassium nitrate showed 75% of leaves wilted (Table XXI).

Among organic forms both peptone and asparagine were superior to urea as source of nitrogen for growth, and also for activity of culture filtrate. Sodium nitrate, ammonium sulphate and ammonium nitrate were best inorganic sources of nitrogen for growth as well as activity of culture filtrate.

TABLE XXII A

Mycelial dry weight in different vitamins after 20 days growth in Czapek's medium

Vitamins @ 2 λ gms/lit.

| No. | Vitamin | Tomato isolate mgm | | | Rubber isolate mgm | | |
|---|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | Mean | $R_1$ | $R_2$ | Mean |
| 1 | No vitamins | 215 | 245 | 230.0 | 230 | 225 | 227.5 |
| 2 | Pyridoxine | 220 | 250 | 235.0 | 240 | 220 | 230.0 |
| 3 | Thiamine | 240 | 265 | 252.5 | 260 | 255 | 257.5 |
| 4 | Inositol | 210 | 225 | 217.5 | 220 | 230 | 225.0 |
| 5 | Calcium pantothenate | 225 | 210 | 217.5 | 220 | 220 | 220.0 |
| 6 | Lactoflavin | 200 | 210 | 205.0 | 210 | 200 | 202.5 |
| 7 | Ascorbic acid | 205 | 200 | 202.5 | 190 | 220 | 205.0 |
| 8 | Para amino benzoic acid | 215 | 220 | 217.5 | 220 | 210 | 215.0 |
| 9 | Nicotinic acid | 205 | 245 | 225.0 | 210 | 250 | 230.0 |
| 10 | Folic acid | 215 | 250 | 232.5 | 240 | 230 | 235.0 |
| 11 | Cyanocobalamine | 210 | 205 | 202.5 | 220 | 190 | 205.0 |
| 12 | Biotin | 225 | 240 | 232.5 | 245 | 240 | 242.5 |

TABLE XXII (D)

Analysis of variance table (Effect of vitamins on mycelial weight)

| Source | Sum of squares | D.F. | Variance | F (calculated) | F (0.01) level | Whether Significant or not | C.D. |
|---|---|---|---|---|---|---|---|
| Total | 15582.00 | 47 | - | - | - | - | - |
| Treatment | 10374.50 | 23 | 451.065 | 1.727 | - | - | - |
| I (isolates) | 42.20 | 1 | 42.200 | < 1 | 7.82 | Not significant | |
| S (vitamins) | 10105.00 | 11 | 918.640 | 4.2 | 3.09 | Significant | 30.134 |
| Error | 5187.50 | 24 | 216.145 | - | - | - | - |

Mean treatments in $I_1$ and Ranking

| $S_5$ | $S_2$ | $S_{10}$ | $S_{12}$ | $S_1$ | $S_9$ | $S_4$ | $S_5$ | $S_8$ | $S_6$ | $S_7$ | $S_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 252.5 | 235.0 | 232.5 | 232.5 | 230.0 | 225.0 | 217.5 | 217.5 | 217.5 | 205.0 | 202.5 | 202.5 |

Mean of treatments in $I_2$ and Ranking.

| $S_3$ | $S_{12}$ | $S_{10}$ | $S_2$ | $S_9$ | $S_1$ | $S_4$ | $S_5$ | $S_8$ | $S_{11}$ | $S_7$ | $S_6$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 257.5 | 242.5 | 235.0 | 230.0 | 230.0 | 227.5 | 225.0 | 220.0 | 215.0 | 205.0 | 205.0 | 202.5 |

TABLE XXIII

Effect of vitamins on activity of filtrates on 20-30 day old tomato seedlings

| No. | Source of vitamin | Wilting symptoms of tomato plants kept in culture filtrates for | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 hours | 6 hours | 12 hours | 18 hours | 24 hours | 48 hours | 72 hours |
| 1 | No vitamins | 0 | 0 | + | ++ | ++ | +++ | ++++ |
| 2 | Pyridoxine | 0 | 0 | + | ++ | ++ | +++ | ++++ |
| 3 | Thiamine | 0 | 0 | + | ++ | +++ | ++++ | ++++ |
| 4 | Inositol | 0 | 0 | 0 | + | ++ | +++ | ++++ |
| 5 | Calcium pantothenate | 0 | 0 | 0 | + | + | ++ | +++ |
| 6 | Lacto flavin | 0 | 0 | 0 | + | + | ++ | ++ |
| 7 | Ascorbic acid | 0 | 0 | 0 | 0 | + | ++ | ++ |
| 8 | Para amino benzoic acid | 0 | 0 | 0 | 0 | + | ++ | +++ |
| 9 | Nicotinic acid | 0 | 0 | + | + | ++ | +++ | ++++ |
| 10 | Folic acid | 0 | 0 | 0 | 0 | + | ++ | ++ |
| 11 | Cyanocobalamine | 0 | 0 | 0 | 0 | + | ++ | ++ |
| 12 | Biotin | 0 | 0 | + | ++ | ++ | +++ | ++++ |

(Notations same as in previous tables)

9) Effect of different vitamins

Eleven vitamins were tried individually along with a control (Czapek's medium without vitamins). Their mycelial weight varied as some of them increased the weight while some others reduced it. For tomato isolates, thiamine, pyridoxine biotin and folic acid increased mycelial weight while cyanocobalamine, ascorbic acid, lactoflavin, calcium pantothenate, and inositol reduced growth (Table XXII-(A).

For rubber isolate, better growths were obtained with thiamine, biotin, folic acid and pyridoxine than non vitaminized medium while lactoflavin, ascorbic acid, cyanocobalamine and para amino benzoic acid reduced mycelial weight. In Table XXII-(B), statistical analysis of the data is presented.

10) Effect of vitamins on activity of culture filtrates

The culture filtrates from the vitaminized medium varied in their ability to cause wilting symptoms in 20-30 days old tomato seedlings when their roots were kept in test tubes containing these filtrates. Culture filtrates from control, pyridoxine, thiamine nicotinic acid and biotin alone produced light wilting in 12 hours and the symptoms became more severe with the increase in time. Inositol, calcium pantothenate, lacto-flavin and nicotinic acid produced symptoms in 18 hours whereas ascorbic acid para amino benzoic acid, folic acid and cyanocobalamine produced first symptoms in 24 hours only.

TABLE XXIV

Growth and drift in pH in Czapek's-Dox medium with pH 7.2

| Sl. No. | Observations on | Tomato isolate | | | | | | | | Rubber isolate | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $R_1$ | | $R_2$ | | $R_3$ | | Mean | | $R_1$ | | $R_2$ | | $R_3$ | | Mean | |
| | | pH | Dry wt. | pH | Dry wt. | pH | Dry wt. | pH | Dry wt(g-) | pH | Dry wt. | pH | Dry wt. | pH | Dry wt | pH | Dry wt.(g-) |
| 1 | 1st day | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 | 7.2 | 0 |
| 2 | 4th day | 6.7 | 0.040 | 6.8 | 0.035 | 6.9 | 0.030 | 6.8 | 0.035 | 7.0 | 0.045 | 6.9 | 0.040 | 7.0 | 0.050 | 7.0 | 0.45 |
| 3 | 8th day | 5.9 | 0.090 | 6.0 | 0.100 | 5.9 | 0.095 | 5.9 | 0.095 | 6.9 | 0.095 | 6.0 | 0.100 | 6.8 | 0.120 | 6.6 | 0.105 |
| 4 | 12th day | 5.0 | 0.140 | 4.5 | 0.155 | 4.9 | 0.110 | 4.8 | 0.135 | 5.2 | 0.180 | 6.0 | 0.210 | 6.4 | 0.200 | 6.2 | 0.197 |
| 5 | 16th day | 4.5 | 0.220 | 4.0 | 0.230 | 4.4 | 0.215 | 4.2 | 0.222 | 4.9 | 0.230 | 5.5 | 0.230 | 5.2 | 0.240 | 5.2 | 0.233 |
| 6 | 20th day | 4.0 | 0.230 | 3.8 | 0.240 | 4.3 | 0.235 | 4.0 | 0.235 | 4.8 | 0.240 | 4.7 | 0.245 | 4.8 | 0.255 | 4.8 | 0.247 |
| 7 | 24th day | 4.0 | 0.225 | 3.9 | 0.245 | 4.2 | 0.230 | 4.0 | 0.233 | 4.7 | 0.240 | 4.7 | 0.240 | 4.7 | 0.250 | 4.7 | 0.243 |

TABLE XXV

Effect of age of culture on activity of culture filtrate on 20-30 day old tomato seedlings.

| Sl. No. | Observations on | Change of pH from 7.2 | Wilting symptoms on 20-30 day old tomato seedlings when kept in culture filtrates for | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 3 hours | 6 hours | 12 hours | 18 hours | 24 hours | 48 hours | 72 hours |
| 1 | 1st day | 7.2 | 0 | 0 | 0 | 0 | + | + | + |
| 2 | 4th day | 6.8 | 0 | 0 | 0 | 0 | + | + | + |
| 3 | 8th day | 5.9 | 0 | 0 | 0 | 0 | + | + | + + |
| 4 | 12th day | 4.8 | 0 | 0 | 0 | + | ++ | ++ | ++ |
| 5 | 16th day | 4.2 | 0 | 0 | 0 | + | +++ | +++ | +++ |
| 6 | 20th day | 4.0 | 0 | 0 | + | ++ | +++ | +++ | ++++ |
| 7 | 24th day | 4.0 | 0 | 0 | + | ++ | +++ | +++ | ++++ |

Remarks: Seedlings kept in sterile water developed no symptoms even after 72 hours and those in uninoculated medium produced light wilting (+) in 24, 48 and 72 hours.

EFFECT OF AGE OF CULTURE ON MYCELIAL WEIGHT AND DRIFT IN pH

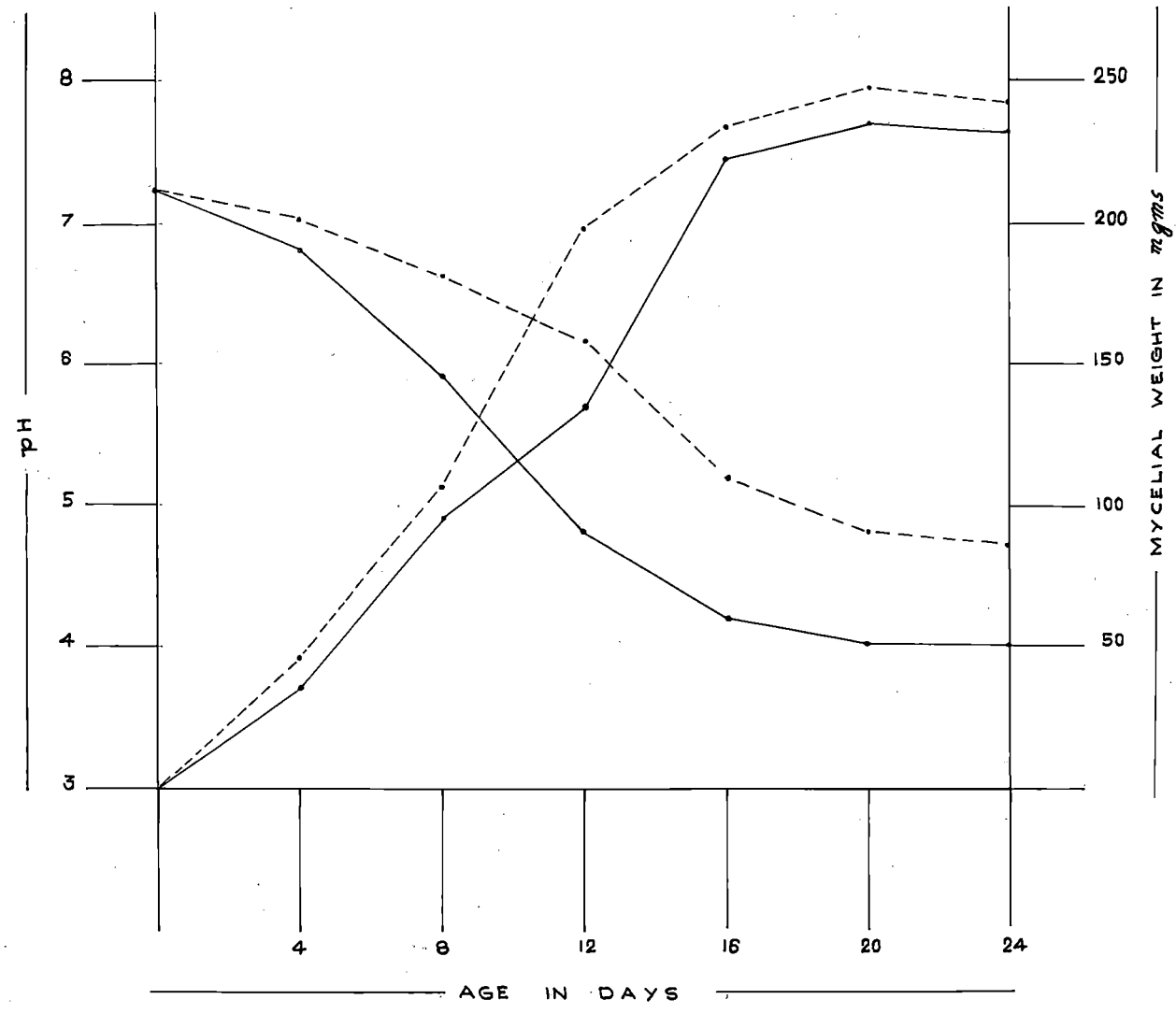



FIG 1

None of the vitamins was found to enhance the activity of culture filtrates where as some have actually decreased their activity (Table XXIII).

11) Age of culture and drift in pH

Cultures of Corynespora cassiicola in Czapek-Dox medium with pH 7.2 have recorded gradual increase in mycelial dry weights with a corresponding decrease in hydrogen ion concentration, for both tomato and rubber isolates. The maximum growth and minimum pH occured on 20th day of seeding for tomato isolates whereas maximum growth for rubber isolate occurred on 20th day but minimum pH was on 24th day of seeding (Table XXIV and figure 1).

12) Effect of age of culture on activity of culture filtrate

When 20-30 days old tomato seedlings were kept in test tubes with their root portions immersed in culture filtrates obtained from different periods of growth, there was clear difference in wilting symptoms. 20 and 24 day old culture filtrates produced light wilting signs in 12 hours. There after the symptoms gradually became severe and the plants completely wilted in 72 hours (Table XXV). Twelve and 16 day old culture filtrates produced light wilting signs only after 18 hours and in 72 hours 75% of the leaves of seedlings kept in 16 day old culture filtrate wilted. Culture filtrates from 1st, 4th and

TABLE NO. XXVI

Effect of pH medium on growth of culture and activity of culture filtrate of Tomato isolate
(Average of 3 replications)

| Sl. No. | H-ion concentrations | | | Mycelial weight after 20 days (mgms) | Wilt signs on 20-30 day old tomato seedlings. | | | | | | Inhibition of radical length over 10 mm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before anto-clav-ing. | After anto-clav-ing | After 20 days growth | | 3 hrs | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs | in U.I.M | in cul-ture filtrate | Percentage inhibition over U.I.M |
| 1 | 2.0 | 2.3 | 2.3 | Ø25 | + | ++ | ++ | +++ | ++++ | +++++ | No growth all discoloured and died. | | |
| 2 | 3.0 | 3.1 | 3.0 | 130 | 0 | + | + | ++ | +++ | ++++ | 1.5 | 1.5 | No inhibition |
| 3 | 3.5 | 3.6 | 3.7 | 160 | 0 | 0 | + | ++ | +++ | ++++ | 2.0 | 1.9 | 5.0 |
| 4 | 4.0 | 3.9 | 4.0 | 260 | 0 | 0 | + | ++ | +++ | ++++ | 2.4 | 2.2 | 8.3 |
| 5 | 4.5 | 4.6 | 4.1 | 265 | 0 | 0 | + | ++ | +++ | ++++ | 2.8 | 2.2 | 21.4 |
| 6 | 5.0 | 5.1 | 4.1 | 250 | 0 | 0 | + | ++ | +++ | ++++ | 4.9 | 2.7 | 45.3 |
| 7 | 6.0 | 6.2 | 4.2 | 260 | 0 | 0 | + | ++ | +++ | ++++ | 5.0 | 2.6 | 48.0 |
| 8 | 7.0 | 6.9 | 4.2 | 265 | 0 | 0 | + | ++ | +++ | ++++ | 7.2 | 2.6 | 63.8 |
| 9 | 7.5 | 7.4 | 5.0 | 265 | 0 | 0 | + | ++ | ++++ | ++++ | 7.6 | 3.0 | 51.5 |
| 10 | 8.0 | 8.0 | 6.3 | 230 | 0 | 0 | + | + | ++ | +++ | 6.8 | 5.0 | 25.3 |
| 11 | 8.5 | 8.4 | 7.2 | 220 | 0 | 0 | 0 | 0 | + | ++ | 6.5 | 6.1 | 6.1 |
| 12 | 9.0 | 9.1 | 7.3 | 200 | 0 | 0 | 0 | 0 | + | ++ | 7.8 | 6.3 | 7.2 |

0 No wilting
\+ Curling of lower leaves
++ Curling of upper leaves & dr oping of lower leaves
+++ Drooping & curling of 75% leaves
++++ Drooping and wilting of complete plant
+++++ Drying of lower leaves.

TABLE XXVII

Effect of pH of medium on growth of culture and activity of culture filtrate:Rubber isolate

Average 3 replications.

| Sl. No. | H-ion concentration | | | mycelial weight after 20 days growth in mgms | Wilting symptoms on 20-30 day old tomato seedlings. | | | | | | Radicle length elongation of seeds over 10 mm of original after 48 hrs in tomato | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH before autoclaving. | pH after autoclaving. | pH after 20 days growth | | 3 hr | 6 hr | 12 hr | 24 hr | 48 hr | 72 hr | Uninoculated medium (average of 100 seeds) | Culture filtrate | Percentage of inhibition over U.I.M |
| 1 | 2.0 | 2.3 | 2.3 | Ø20 | + | ++ | ++ | +++ | ++++ | +++++ | No growth | No growth | all died in both (100%) |
| 2 | 3.0 | 3.1 | 3.0 | Ø90 | 0 | 0 | + | ++ | +++ | ++++ | 1.5 | 1.5 | no inhibitio |
| 3 | 3.5 | 3.6 | 3.2 | 190 | 0 | 0 | + | ++ | +++ | ++++ | 2.0 | 1.9 | 5.0 |
| 4 | 4.0 | 3.9 | 4.0 | 280 | 0 | 0 | + | ++ | +++ | ++++ | 2.4 | 2.0 | 16.6 |
| 5 | 4.5 | 4.6 | 4.2 | 285 | 0 | + | ++ | ++ | +++ | ++++ | 2.8 | 2.0 | 32.1 |
| 6 | 5.0 | 5.1 | 4.3 | 260 | 0 | 0 | + | ++ | +++ | ++++ | 4.9 | 2.2 | 55.1 |
| 7 | 6.0 | 6.2 | 4.3 | 265 | 0 | 0 | + | ++ | +++ | ++++ | 5.0 | 2.2 | 56.0 |
| 8 | 7.0 | 6.9 | 5.0 | 260 | 0 | 0 | + | ++ | +++ | ++++ | 7.2 | 4.4 | 38.9 |
| 9 | 7.5 | 7.4 | 6.9 | 265 | 0 | 0 | 0 | + | ++ | +++ | 7.6 | 5.8 | 23.7 |
| 10 | 8.0 | 8.0 | 7.8 | 270 | 0 | 0 | 0 | + | ++ | +++ | 6.8 | 6.0 | 11.7 |
| 11 | 8.5 | 8.4 | 8.2 | 285 | 0 | 0 | 0 | + | ++ | +++ | 6.5 | 6.2 | 4.6 |
| 12 | 9.0 | 9.1 | 8.3 | 230 | 0 | 0 | 0 | 0 | + | ++ | 6.8 | 6.4 | 5.8 |

0 No wilting
\+ Curling of lower leaves
++ Curling of upper leaves & drooping of lower leaves
+++ Drooping & curling of 75% leaves
++++ Drooping and wilting of complete plant
+++++ Drying of lower leaves.

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8th day old cultures caused light signs only in 24, 48 and 72 hours, of which the culture filtrate of 8th day culture caused drooping of lower leaves and curling of upper leaves in 72 hours.

13) Effect of pH of medium on growth of the fungus

Tables XXVI and XXVII and figure 2 show the data on growth of the two isolates, tomato and rubber of C. cassiicola when the media was adjusted to different hydrogen ion concentrations. Autoclaving of the pH adjusted media has caused slight drift in pH of the medium.

a) Drift in pH

Tomato isolate caused a drift in pH of the culture filtrate from 4.0 to 5.0 when they were grown in media with pH 4 to 7.5 . Media with pH 2 to 3.5 showed a tendency to remain in the same pH whereas cultures in pH 9, 8.5 and 8.0 decreased the pH (Table XXVI).

Rubber isolate caused a drift of pH of medium after 20 days of growth towards 4.0 - 5.0 when grown in media with pH 4.0, 4.5, 5.0, 6.0 and 7.0. The pH of media 2.0, 3.0 and 3.5 was not altered even after 20 days of growth whereas media with pH 7.5 was changed to pH 6.9 in 20 days of growth. There was also a decrease in pH from 8.0 to 7.8 and 8.5 to 8.2 and from pH 9.0 to

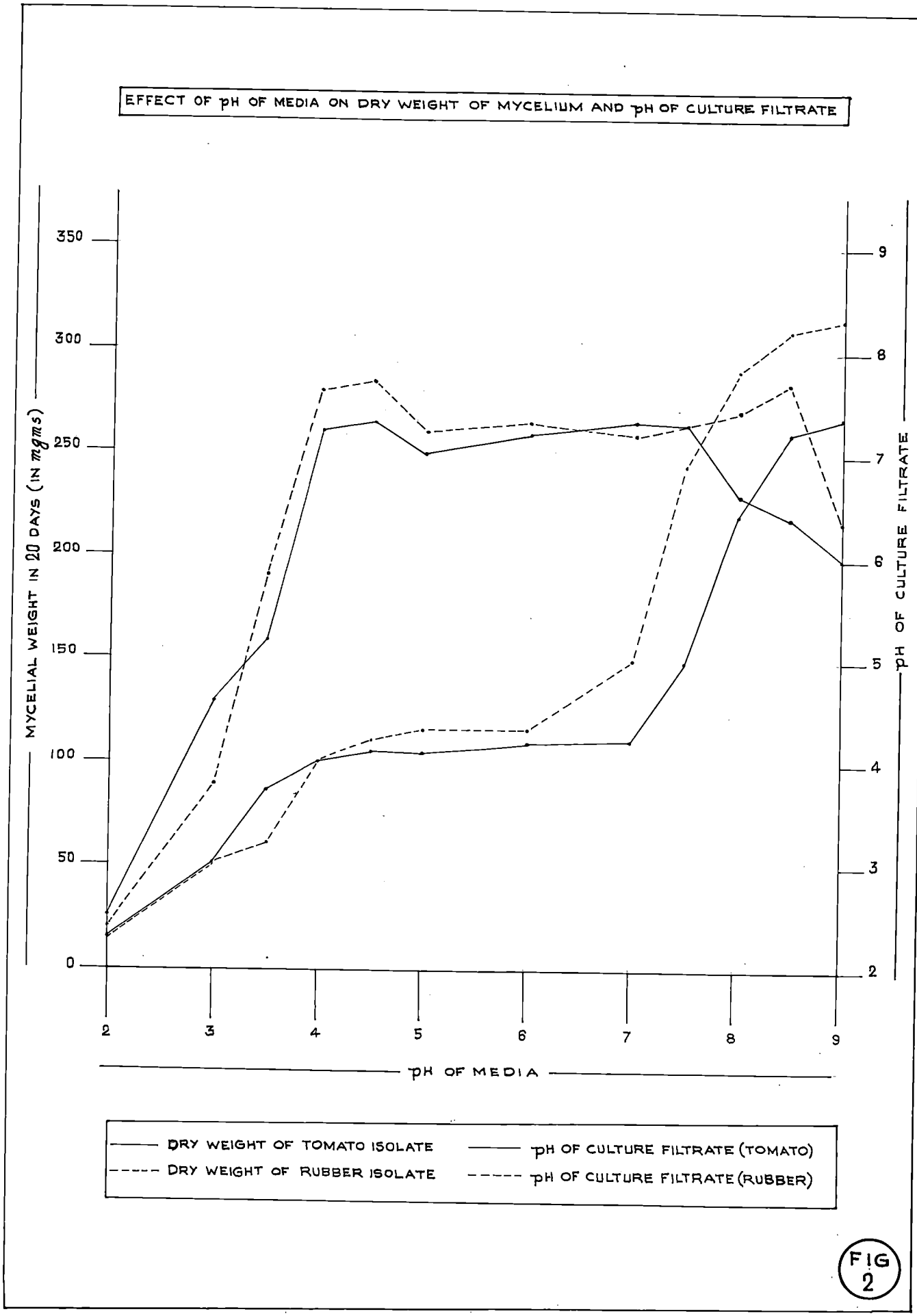
EFFECT OF pH OF MEDIA ON DRY WEIGHT OF MYCELIUM AND pH OF CULTURE FILTRATE
MYCELIAL WEIGHT IN 20 DAYS (IN mgms)
350
300
250
200
150
100
50
0
pH OF CULTURE FILTRATE
9
8
7
6
5
4
3
2
2
3
4
5
6
7
8
9
pH OF MEDIA
DRY WEIGHT OF TOMATO ISOLATE
DRY WEIGHT OF RUBBER ISOLATE
pH OF CULTURE FILTRATE (TOMATO)
pH OF CULTURE FILTRATE (RUBBER)

FIG 2

8.5 by 20 days of growth of fungus (Table XXVII).

b) Dry weight of mycelium

Dry weight of mycelium at different pH of the medium for tomato isolate (Table XXVI) and rubber isolate (Table XXVII) also show similar preference of pH. Growth of both the isolates were very poor in media with pH 2.0. Tomato isolate have yielded maximum dry weight of mycelium in media with pH 4.5 and 7.0 moderate in media with pH 6.0, 5.0, 9.0, 4.0 and 8.5 whereas the growth was only satisfactory at pH 9.0, 3.5 and 3.0 in decreasing order.

Growth of rubber isolate was maximum in media with pH 8.5 and 4.5 followed by moderate growth in 4.0, 8.0, 6.0, 7.0, 5.0, 9.0 and satisfactory growth at pH 3.5 whereas the growth at pH 3.0 and 2.0 were very poor.

14) Effect of pH of media on activity of culture filtrate

Activity of the 20 day old culture filtrates of tomato and rubber isolates grown in Czapek-Dox media at different hydrogen ion concentrations was measured by its effect on wilting signs on 20-30 day old tomato seedlings and also by inhibition of radicle elongation of tomato seeds over 10 mm before treatment. Data are given in Table XXVI for tomato isolate and in Table XXVII for rubber isolate.

a) Wilting of seedlings

Wilting of seedlings in culture filtrates from media with pH 2.0 was severe, showing symptoms within 5 hours of placement and gradually increasing, the plants getting completely wilted in 48 hours and dried in 72 hours for both isolates.

In 6 hours, filtrates from media at pH 3.0 and 4.0 showed symptoms of wilting in the case of tomato isolates and at pH 4.5 in the case of rubber isolate, the symptoms became severe as time passed and plants completely wilted in 72 hours.

In 12 hours, all except those in culture filtrates from media at 8.5 and 9.0 for tomato isolate and at 7.5, 8.0, 8.5 and 9.0 for rubber isolate, showed wilting symptoms that progressed in 24 hours and 48 hours. In 72 hours, plants kept in filtrates of cultures of tomato isolates grown at pH 3.0 to 7.5 wilted completely while those in filtrates of cultures grown at pH 8.0 produced only 75% wilting of leaves. In the case of rubber isolates grown at different pH, all those that have shown wilting symptoms in 12 hours, completely wilted in 72 hours. At pH 7.0, only 75% of leaves wilted.

Wilting started only at 48 hours, in the case of plants kept in culture filtrates from cultures of tomato isolate grown at pH 8.5 and 9.0 and from cultures of rubber isolates grown

at pH 9.0. But in both cases, in 72 hours wilting progressed showing drooping of lower leaves and curling of upper leaves while uninoculated media at pH 7.2 remained the same with curling of lower leaves only at 48 and 72 hours.

b) Inhibition of radicle elonation

When germinated tomato seeds with radicle length of 10 mm were placed in petridishes and irrigated with culture filtrates of the tomato and rubber isolates grown at different hydrogen ion concentrations, there was a marked inhibition of elongation of radicle (Tables XXVI & XXVII).

Culture filtrates of both the isolates grown at pH 2.0 caused discolouration, shrivelling and death of the radicle, showing 100% of inhibition, by both media and filtrate. Growth at pH 3.0 and 5.5 were very poor for both isolates. Marked radicle elongation inhibitions were shown by culture filtrates of tomato isolates grown at pH 4.5 to 7.5. Those grown at pH 9.0 and 8.5 showed poor rates of inhibition (7.2% and 6.1%).

In the case of rubber isolates also a similar pattern of inhibition was observed. Media at pH 2.0, caused death of seeds. At 3.0 and 5.5 there was very poor growth. Media at 4.0,—7.5 were was superior to culture filtrate and favoured elongation of radicle. The growth of radicle at 8.0, 9.0 were not much different with that of the culture filtrates.

TABLE XXVIII

Stability of toxin at various H-ion concentrations

Culture filtrate of tomato isolate in Czapeck-Dox medium

| Sl. No. | Treatment | Wilting signs in 20-30 day old tomato seedlings kept for | | | | | | | Radical inhibition in 48 hrs over 10 mm of original. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 hrs | 6 hrs | 12 hrs | 18 hrs | 24 hrs | 48 hrs | 72 hrs | Over 10 mm | % over sterile water | % over U.I.M. | % over filtrate at pH.3 |
| 1 | Sterile water | 0 | 0 | 0 | - | - | - | - | 10.1 | - | - | - |
| 2 | Uninoculated media (pH 4.2) | 0 | 0 | 0 | - | + | + | ++ | 2.8 | 73.0 | - | - |
| 3 | Culture filtrate (not adjusted) pH.4.2 | 0 | 0 | + | ++ | +++ | +++ | ++++ | 2.4 | 77.0 | 14.3 | - |
| 4 | Adjusted to pH.3 | 0 | + | + | ++ | +++ | ++++ | +++++ | 2.0 | 81.0 | 28.5 | 16.6 |
| 5 | " " 4 | 0 | 0 | + | ++ | +++ | +++ | ++++ | 2.3 | 78.0 | 17.9 | 4.1 |
| 6 | " " 5 | 0 | 0 | + | ++ | ++ | +++ | ++++ | 2.9 | 72.0 | 103.5 | 119.9 |
| 7 | " " 6 | 0 | 0 | + | + | ++ | +++ | +++ | 6.0 | 41.0 | 224.0 | 240.0 |
| 8 | " " 7 | 0 | 0 | 0 | + | ++ | +++ | +++ | 6.1 | 40.0 | 227.0 | 244.0 |
| 9 | " " 8 | 0 | 0 | 0 | 0 | + | ++ | ++ | 7.0 | 31.0 | 260.0 | 292.0 |
| 10 | " " 9 | 0 | 0 | 0 | 0 | 0 | + | ++ | 9.0 | 11.0 | 321.0 | 375.0 |

15) Stability of toxin at various H-ion concentrations

Tomato isolate grown in Czapeck-Dox media for 20 days gave a culture filtrate of pH 4.2. The pH of this filtrate was changed to different levels ranging from 3 to 9 and, the effect of this culture filtrate on tomato seedlings as well as on radicle elongation are given in Table XXVIII.

a) Wilting symptoms in tomato seedlings

Wilting symptoms were produced by plants kept in culture filtrates at pH 3 in 6 hours, progressing with time and resulted in drying of plant in 72 hours. Symptoms of wilting developed in 12 hours which progressed and by 72 hours the entire plant wilted in culture filtrates unaltered and those altered to pH 4 and 5. In filtrate changed to pH 6, 75% of the leaves wilted in 72 hours.

Culture filtrates altered to pH 7 produced light symptoms in 18 hours, and those at 8 in 24 hours and at pH 9 in 48 hours. In 72 hours, 75% leaves wilted in the case of pH 7, lower leaves drooped and upper leaves curled in the case of pH 8 and lower leaves remained curled in the case of pH 9.0.

Sterile water caused no wilting even after 72 hours Uninoculated liquid medium (pH 4.2) caused light wilting in 24 hours and drooping of lower leaves and curling of upper leaves in 72 hours.

TABLE XXIX

Effect of temperature on activity of culture filtrate

| Sl. No. | Treatment | Wilting symptoms on 20-30 day old tomato seedlings after 48 hours of keeping in culture filtrate heated at | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50°C | 60°C | 70°C | 80°C | 90°C | 100°C | Autoclaved | 0°C |
| 1 | Culture filtrate | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| 2 | Uninoculated media | + | + | + | + | + | + | + | + |
| 3 | Sterile water | - | - | - | - | - | - | - | - |

0 No symptoms
\+ Curling of lower leaves
\+++ Curling and wilting of 75% leaves
\+++ Complete wilting

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b) Inhibition of radicle length

Data on inhibition of elongation of radicle of tomato seeds are given in Table XXVIII. Sterile water gave maximum growth of radicle (10.1 mm). Uninoculated media at pH 4.2 caused inhibition of 58% over sterile water, unadjusted culture filtrate at pH 4.2 gave 76% inhibition over sterile water and 14.4% inhibition over media. Growth of radicle at pH 3.0 and 4.0 were poor compared to sterile water and media at pH 4.2 and culture filtrate at pH 4.2. As the pH of culture filtrate was adjusted to pH 5, 6, 7, 8 and 9 there was a marked increase in elongation of radicle over U.I.M. and culture filtrate at pH 4.2. But it was not equal to growth in sterile water.

Adjustment of culture filtrate towards acid side increased both wilting and radicle inhibition activity whereas towards alkaline side, it decreased the activity.

16) Effect of temperature on the culture filtrate

Effect of different heat treatments on activity of culture filtrate, uninoculated media and sterile water are given in Table XXIX. The culture filtrate was thermostable since its activity was not lost by any of the treatments.

17) Effect of storage on the culture filtrate

Results on activity of culture filtrate by different types of storage namely at 5°C and at room temperature (28-30°C) with

TABLE XXX

Effect of storage of culture filtrate

| Observation on | Symptoms on 20-30 day old tomato seedlings when kept in filtrates kept | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | at 5°C | | | room temperature | | | Autoclaved kept at room temperature | | |
| | 24 hrs. | 48 hrs. | 72 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 24 hrs. | 48 hrs. | 72 hrs. |
| 1st day | ++ | +++ | ++++ | ++ | +++ | ++++ | ++ | +++ | ++++ |
| 5th day | ++ | +++ | ++++ | ++ | +++ | ++++ | ++ | +++ | ++++ |
| 10th day | ++ | +++ | ++++ | ++ | +++ | ++++ | ++ | +++ | ++++ |
| 15th day | ++ | ++ | +++ | + | ++ | +++ | ++ | +++ | ++++ |
| 20th day | ++ | ++ | +++ | + | ++ | +++ | ++ | +++ | ++++ |

0 No symptoms

\+ Curling of lower leaves

\+++ Curling and wilting of 75% leaves

++++ Complete wilting

TABLE XXXI

Effect of dilution of culture filtrate on activity.

| Concentration | Treatment concentration of culture filtrate or U.I. media. | Observation for wilting symptoms of tomato plants after | | | | | |
|---|---|---|---|---|---|---|---|
| | | 6 .hr. | 12.hr. | 18.hr. | 24.hr. | 48.hr. | 72.hr. |
| 0 | Sterile water | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.25 (1:16) | Culture filtrate | 0 | 0 | 0 | 0 | + | + |
| | U.I.M | 0 | 0 | 0 | 0 | 0 | + |
| 12.50 (1:8) | Culture filtrate | 0 | 0 | 0 | + | ++ | ++ |
| | U.I.M | 0 | 0 | 0 | 0 | 0 | + |
| 25.00 (1 :4) | Culture filtrate | 0 | + | + | ++ | ++ | +++ |
| | U.I.M. | 0 | 0 | 0 | 0 | 0 | + |
| 50.0 (1:1) | Culture filtrate | 0 | + | + | ++ | +++ | +++ |
| | U.I.M. | 0 | 0 | 0 | 0 | + | + |
| 100.0 | Culture filtrate | 0 | + | ++ | +++ | +++ | ++++ |
| | U.I.M. | 0 | 0 | 0 | 0 | + | + |

0 no symptoms
\+ curling of lower leaves
++ Curling of upper leaves, drooping of lower leaves
+++ Drooping & curling of 75% leaves
++++ Wilting of complete plant.

TABLE XXXII

Germination of Tomato and sesamum seeds treated with culture filtrates

| Treatment | Tomato seeds (100 nos) | | | | Sesamum seeds (100 nos) | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 96 hrs. | |
| 1. Culture filtrate | - | - | 8 | 15 | 28 | 74 | 80 | 90 | 60% sesamum seeds with discoloured and rotting radicles. |
| 2. Uninoculated media | - | 38 | 69 | 82 | 30 | 76 | 85 | 92 | Radicles unaffected. |
| 3. Sterile water | - | 40 | 86 | 99 | 45 | 80 | 100 | 100 | Radicles unaffected. |

and without autoclaving are given in Table XXX. The toxin is active upto a period of 20 days (the period studied) in storage at room temperature when kept after autoclaving. The activity was decreased with time when stored at 5°C and at room temperature without autoclaving.

18) Effect of dilution on activity

Effect of diluting the culture filtrate and uninoculated medium to cause wilting symptoms was studied and the data are given in Table XXXI.

When the concentration of the culture filtrates were 100%, 50% and 25%, wilting symptoms started in 12 hours but varied in intensity in 18 hours, and at 24 hours undiluted culture filtrate was more active than the rest. At 12.5% concentration wilting signs started only at 24 hours. At 6.25% concentrations the filtrate acted similar to uninoculated media, showing light symptoms in 48 hours.

19) Effect of culture filtrate on germination of seeds

Data on germination of sesamum and tomato seeds in culture filtrates of tomato isolates are given in Table XXXII.

In the case of tomato seeds germination percentage was very low, only 15% germinated in 96 hours. In uninoculated media and sterile water germination percentages were 82 and 99, respectively.

TABLE XXXIII

Effect of culture filtrate on elongation of plumule

Average of 100 seeds each

| Sl. No. | Treatment | Tomato: Elongation of plumule over 5 mm after | | | | | Sesamum: Elongation of plumule over 5 mm after | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | % of inhibition over | | | | | % of inhibition over | |
| | | 24 hrs. | 48 hrs. | 72 hrs. | over sterile water | over u.i.m. | 24 hrs. | 48 hrs. | 72 hrs. | sterile water | u.i.m. |
| 1 | Sterile water. | 5 | 13 | 18 | - | - | 9 | 21 | 30 | - | - |
| 2 | Uninoculated medium | 5 | 11 | 15 | 16.7 | - | 8 | 20 | 28 | 6.6 | - |
| 3 | Culture filtrate | 4 | 9 | 12 | 33.3 | 20.0 | 6 | 18 | 24 | 20.0 | 14.3 |

In the case of sesamum seeds treatment with the filtrates gave 90% germination in 96 hours while uninoculated media and sterile water gave 92 and 100% germination respectively. But out of the 90 seeds germinated, 60% seeds (54 Nos) showed discolouration and rotting of radicles.

20) Effect of culture filtrate on plumule elongation

Table XXXIII depicts the effect of culture filtrate on inhibition of plumule elongations of tomato and sesamum seeds over 5 mm length.

Uninoculated medium caused 16.7% inhibition of tomato seeds over sterile water in 72 hours while culture filtrate caused 33.3% inhibition over sterile water and 20.0% over uninoculated medium.

Percentage of inhibition of plumule length in sesamum seeds is less marked compared to tomato seeds but culture filtrates cause inhibitions of 3 times more than that of uninoculated medium when both are compared for their inhibitory action over sterile water. There was 14.3% more inhibition due to culture filtrate than uninoculated medium.

21) Effect of culture filtrate on cut shoots

Culture filtrates of both tomato and rubber isolates caused curling, drooping of leaves and wilting in 72 hours, when cut shoots of tomato and sesamum were placed in test tubes

TABLE XXXIV

Effect of culture filtrate on cut shoots of host plants

| Sl. No. | Treatment | Cut shoots of tomato | | | Cut shoots of sesamum | | | Cut shoots of rubber | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 hrs. | 48 hrs. | 72 hrs. | 24 hrs. | 48 hrs. | 72 hrs. | 24 hrs. | 48 hrs. | 72 hrs. |
| 1 | Sterile water | - | - | - | - | - | - | - | - | - |
| 2 | Uninoculated media | - | + | + | - | + | + | - | - | + |
| 3 | Culture filtrate of tomato isolate | +++ | ++++ | +++++ | +++ | ++++ | +++++ | ++ | +++ | ++++ |
| 4 | Culture filtrate of rubber isolate | +++ | ++++ | +++++ | +++ | ++++ | +++++ | ++ | +++ | ++++ |

0 No symptoms
\+ Curling of lower leaves
++ Drooping of lower leaves and curling of upper leaves
+++ Curling and drooping of 75% leaves
++++ Wilting of entire plant
+++++ Drying of leaves

TABLE XXXV

Effect of culture filtrate on germination of other vegetable seeds.

| Variety | Germination after 72 hours. | | | Inhibition % over | |
|---|---|---|---|---|---|
| | In sterile water | in U.I.M | In culture filtrate | Sterile water | U.I.M. |
| Cow pea | 85 | 82 | 71 | 16.5 | 13.4 |
| Culster bean | 79 | 70 | 58 | 26.6 | 17.1 |
| Kidney beans | 60 | 56 | 54 | 10.0 | 3.8 |
| Lima beans | 90 | 88 | 86 | 4.4 | 2.0 |
| Polo beans | 65 | 60 | 56 | 13.8 | 6.7 |
| Soya beans | 80 | 75 | 60 | 25.0 | 20.0 |
| Peas | 90 | 84 | 80 | 11.1 | 4.1 |
| Pepaya | 65 | 62 | 41 | 36.9 | 33.8 |
| Bhindi | 100 | 90 | 69 | 31.0 | 22.2 |
| Dodonaea | 80 | 80 | 65 | 18.8 | 18.8 |

TABLE XXXVI

Effect of culture filtrate on germination of fungal spores.

(Average of 4 replications)

| No. | Name of fungal spore | Germination percentage after 12 hrs. | | | % inhibition over U.I.M. |
|---|---|---|---|---|---|
| | | Sterile water | U.I.M. | in filtrate | |
| 1. | Corynespora cassiicola | 96 | 94 | 94 | - |
| 2. | Alternaria sesami | 95 | 93 | 92 | 1.1 |
| 3. | Helminthosporium halodes | 100 | 99 | 98 | 1.1 |
| 4. | Sphaceloïtheca sp. | 100 | 98 | 84 | 14.3 |

containing culture filtrates. There was only very light symptoms in uninoculated media and no symptoms in sterile water. Cut shoots of rubber also showed similar symptoms, but the intensity was less when compared to tomato and sesamum which are more succulent (Table XXXIV).

22) Effect of culture filtrate on germination of seeds

Results of studies on effect of culture filtrate on germination of seeds are given in Table XXXV. The germination of all the seeds tried were inhibited at varying degrees. The percentage of inhibition of germination over uninoculated media and over sterile water was highest in papaya (33.8 and 56.9) followed by bhindi (22.2 and 31.0) soy-bean (20.0 & 25.0) dodonaea (18.8 and 16.8) cluster beans (17-1 and 26.6) and cow pea (15.4 and 16.5) while those of pole beans (6.7 and 13.8) peas (4.1 and 11.1) kidney beans (3.8 and 10.0) and lima beans (2.0 and 4.4) were not significant.

23) Effect of culture filtrate on germination of fungal spores

Results on the germination of fungal spores in culture filtrates are given in Table XXXVI. Germination of the fungal spores tried were not inhibited but slight inhibition of germination accompanied with malformation of germ tubes was found in spores of Sphacelotheca sp (14.3%).

TABLE XXXVII

Effect of Purified toxin at strength of 2:30 (v/v) with sterile water on 20--30 day old tomato seedlings)

| Sl. No. | Solvent | Effect on tomato seedlings kept at 2:30 strengths for | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 hrs. | 6 hrs. | 12 hrs | 18 hrs. | 24 hrs. | 48 hrs. | 72 hrs |
| 1 | Acetone | + | ++ | +++ | +++ | ++++ | ++++ | +++++ |
| 2 | Benzene | + | + | ++ | +++ | +++ | ++++ | +++++ |
| 3 | Cholorofrm | 0 | + | + | ++ | +++ | +++ | ++++ |
| | Crude culture filtrate | 0 | 0 | + | + | ++ | +++ | ++++ |

24) Purification of toxin

Of the seven solvents used to extract and purify the toxin from culture filtrates, clear layers were obtained with acetone, chloroform and benzene. So purification was done only from these three solvents. A brown viscous material was obtained on evaporation of the solvents which was dissolved in 2 ml sterile water. Unlike the culture filtrates which produced first sign of wilting in 12 hours, the purified material produced symptoms in 3 hours when solvents were acetone, chloroform and benzene. Acetone purified toxic substance was more active (Table XXXVII).

The acetone purified toxic principle on spraying to leaves of 30-45 day old sesamum and tomato seedlings caused crinkling, discolouration (greyish brown colouration in tomato and dark brown colouration in sesamum) and drying of tissues, in about 48 hours. These discoloured leaf lamina later disintegrated and fall off. (Plates VIII & IX).

Laboratory studies on control of the organism

a) Poisoned food technique

Results on the evaluation of different fungicides using poisoned food technique are given in Table XXXVIII & XXXIX. Mercury chloride and Dithane - M-45 gave complete inhibition of growth at all concentrations tried. In Cumin, there was a

Effect of fungicides on the mycelial growth of *Rhizoctonia solani*

| Sl. No. (s) | Fungicide | | Radial growth in mm after 6 days of seeding. $R_1$ | $R_2$ | $R_3$ | Mean | Percentage inhibition over control | Colony characters |
|---|---|---|---|---|---|---|---|---|
| 1 | Control | | 19 | 19 | 22 | 20 | -- | Mycelium whitish gray with olivaceous boarder; underside black, concentric rings present. |
| 2 | | 1000 ppm | 9 | 10 | 8 | 9 | 55 | Greyish white, wooly cushiony; margin definite. |
| | Fytolan | 2000 ppm | 7 | 9 | 8 | 8 | 60 | -do- |
| | | 3000 ppm | 7 | 8 | 6 | 7 | 65 | -do- |
| 3 | | 1000 ppm | 14 | 15 | 13 | 14 | 30 | Ashy grey, partly submerged |
| | Blitox | 2000 ppm | 14 | 12 | 13 | 13 | 35 | Gray, partly submerged |
| | | 3000 ppm | 12 | 11 | 10 | 11 | 45 | Gray, partly submerged |
| 4 | | 1000 ppm | 6 | 5 | 7 | 6 | 70 | Whitish grey, cottony, cushion like with dark green centre. |
| | Dithane Z-78 | 2000 ppm | 2 | 3 | 1 | 2 | 90 | -do- |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| 5 | | 1000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| | Dithane M-45 | 2000 ppm | 0 | 0 | 0 | 0 | 100 | -do- |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | -do- |
| 6 | | 1000 ppm | 1 | 3 | 2 | 2 | 90 | Ashy grey, button like poor growth |
| | Zirido | 2000 ppm | 2 | 0 | 1 | 1 | 95 | -do- |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| 7 | | 1000 ppm | 1 | 1 | 1 | 1 | 95 | Ashy grey, button like poor growth |
| | Cuman | 2000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | -do- |
| 8 | | 1000 ppm | 6 | 5 | 4 | 5 | 75 | Ashy gray mycelium; button like poor growth. |
| | Dutar | 2000 ppm | 3 | 4 | 5 | 4 | 80 | -do- |
| | | 3000 ppm | 3 | 3 | 3 | 3 | 85 | -do- |
| 9 | Difolatan | 1000 ppm | 5 | 4 | 6 | 5 | 75 | Ashy gray mycelium; button like poor growth |
| | | 2000 ppm | 5 | 5 | 5 | 5 | 75 | -do- |
| | | 3000 ppm | 2 | 4 | 3 | 3 | 85 | -do- |
| 10 | | 1000 ppm | 4 | 3 | 2 | 3 | 85 | Ashy grey, button like poor growth margins irregular |
| | Captan | 2000 ppm | 3 | 1 | 2 | 2 | 90 | -do- |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| 11 | | 1000 ppm | 0 | 0 | 0 | 0 | 100 | No growth |
| | Mercuric chloride | 2000 ppm | 0 | 0 | 0 | 0 | 100 | -do- |
| | | 3000 ppm | 0 | 0 | 0 | 0 | 100 | -do- |
| 12 | | 1000 ppm | 14 | 15 | 16 | 15 | 25 | Whitish grey, mycelium, wooly margins definite. |
| | Thiovit | 2000 ppm | 13 | 11 | 12 | 12 | 40 | -do- |
| | | 3000 ppm | 10 | 12 | 11 | 11 | 45 | -do- with concentric rings. |

TABLE XXXIX

Analysis of variance Table (Effect of fungicides)

| Source | Sum of squares | D.F. | Variance | F calculated | F (0.01 level) | Whether significant or not. | C.D. |
|---|---|---|---|---|---|---|---|
| Total | 2661.573 | 101 | - | - | - | - | - |
| Treatment | 1929.150 | 33 | 58.46 | 5.423 | 1.98 | Significant | |
| Control vs fungicides | 719.292 | 1 | 719.292 | 66.725 | 7.04 | Significant | |
| Between fungicides | 2009.858 | 10 | 200.986 | 18.660 | 2.61 | Significant | 2.56 |
| Error | 736.223 | 66 | 10.780 | - | - | - | - |

Mean of treatments and Ranking.

| $S_3$ | $S_{12}$ | $S_2$ | $S_9$ | $S_8$ | $S_4$ | $S_{10}$ | $S_6$ | $S_7$ | $S_5$ | $S_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 12.67 | 12.67 | 8.0 | 4.33 | 4.0 | 2.67 | 1.67 | 0.89 | 0.33 | 0 | 0 |

Conclusion:- Treatment $S_{10}$ $S_6$ $S_7$ $S_5$ & $S_{11}$ gave poorest growth, ie., their more effective in controlling the fungus.

very poor growth in 6 days at 1000 ppm, while at higher concentrations there was no growth. There was also very poor growth in Ziride 1% and 2%, Dithane-Z 78 1% and 2% and Captan 1% and 2%. Though there was little growth in 1%, 2% and 3% of Duter and Difolton, the growth was markedly inhibited. Among Blitox and Fytolan, the latter inhibited growth of the organism better than the former. Thiovit was not effective to inhibit growth even at 3% level.

b) Inhibition of spore germination

Results of studies on inhibition of germination of spores of C. cassiicola in suspensions of different fungicides at different strengths are given in Table XXXX. At 500 ppm more than 75% of spores failed to germinate or germinated with malformed germ tubes in the case of Dithane M-45, Dithane -Z-78 and mercury chloride.

At 1000 ppm, complete inhibition of spore germination was found in Mercury chloride, Cuman, and Ziride. Dithane-Z 78 gave 96%, Dithane M-45 and Captan gave 95% inhibition and Duter gave 80% inhibition.

At 2000 ppm Dithane-Z 78, Dithane M-45, Ziride, Cuman, Captan and Mercury chloride gave 100% inhibition, Duter gave 96% and Difoltan 88%.

TABLE XXXIX

INHIBITION OF SPORE GERMINATION BY FUNGICIDES

Average of 4 replications.

| Sl. No. | Fungicede | Inhibition % of spores germination at 500 ppm | at 1000 ppm | at 2000 ppm | at 3000 ppm |
|---|---|---|---|---|---|
| 1 | Fytolan | 29 | 61 | 80 | 92 |
| 2 | Blitox | 14 | 55 | 75 | 84 |
| 3 | Dithane Z-78 | 78 | 96 | 100 | 100 |
| 4 | Dithane-m-45 | 83 | 95 | 100 | 100 |
| 5 | Ziride | 69 | 100 | 100 | 100 |
| 6 | Cumen | 70 | 100 | 100 | 100 |
| 7 | Duter | 46 | 80 | 96 | 100 |
| 8 | Difoltan | 32 | 66 | 88 | 100 |
| 9 | Captan | 71 | 95 | 100 | 100 |
| 10 | Mercury chloride | 99 | 100 | 100 | 100 |
| 11 | Thiovit | 6 | 18 | 36 | 42 |
| 12 | Control (sterile water) | 4 | 4 | 4 | 4 |

At 3000 ppm Dithane Z-78, Dithane M-45, Ziride, Cuman, Duter, Difoltan captan and mercury chloride gave 100% inhibition, Fytolan 92% and Blitox 84% while thiovit inhibited germination of only 42% spores.

# DISCUSSION

# DISCUSSION

## Isolation of the organism

The isolates of Corynespora cassiicola obtained from tomato, sesamum and rubber agree to the description of the organism by Wei (1950) later modified by Ellis (1959). The conidia of rubber isolate were comparatively bigger in size more obclavate (mean length 185 $\mu$, width 14.0 $\mu$ and pseudosepta 9) and were darker in colour than the tomato isolate (150 $\mu$ x 9.0 $\mu$ with 11 pseudosepta) and sesamum isolate (150 $\mu$ x 9.5 $\mu$ with 11 pseudosepta) which were paler. Similar variations in spore measurements of Corynespora cassiicola have been reported by Spencer and Walters (1969) in the case of isolates obtained from Nebraska and Canada on cowpea and soy-bean. Nebraska isolates had very dark spore while Canadian isolates were lighter in colour.

## Symptoms of infection

Symptoms of infection on leaves were more or less similar in the three plants; however, it was noticed that concentric rings were formed more profoundly in leaves of mature rubber trees than in tomato and sesamum leaves. Eventhough concentric rings on infected rubber, pueraria, and aralia leaves were reported previously (Anonymous 1966a) such spots are not so far reported

in tomato and sesamum. On sesamum leaves, the spread and enlargement of spots were quicker with necrotic and blightened appearance of leaves. Brown to purplish spots with yellowish halo were seen on infected capsules of sesamum in which the infection was more severe causing complete drying up of the plant.

Mechanical injury to the plants favoured infection and expression of symptoms (Table I and II). The increased infectivity probably depends on the fact that on the abraded surfaces of leaves spores germinate better and germ tubes grow more strongly as stated by Wood (1967).

Pathogenicity of isolates

Cross inoculation studies using isolates of the fungus obtained from tomato, sesamum and rubber revealed differences in the production of symptoms on leaves. Rubber isolate produced typical target spots which enlarged in size with brownish margins and grey centres in tomato, sesamum and rubber. The reisolates also infected rubber similarly. But the tomato and sesamum isolates, which were infectious to both hosts on cross inoculation failed to produce typical shot hole symptoms on rubber leaf. The reisolates were also infectious to both sesamum and tomato. They produced small brownish flecks which never enlarged beyond 1 mm in diameter on rubber leaves.

Gopalan (1963) found mild symptoms on Dodonaea viscosa leaves by the tomato and sesamum isolates of the fungus. In the present studies also the symptoms on dodonaea leaves were mild on inoculation with tomato and sesamum isolates, but rubber isolate caused more severe symptoms, with spots enlarging more than 5 mm in diameter. All the three isolates caused only mild spotting in cowpea and soy-bean leaves.

Similar differences in pathogenicity has been observed in Corynespora cassiicola by earlier workers. Olive et al (1945) found two races, race 1 causing severe infection of cowpea (Vigna sinensis Torner) and light spotting of soy-bean (Glycine max. L) whereas race 2 causing light spotting of both. Stones and Jones (1960) suggested feasibility of an additional race since the isolates they got from Sesamum indicum L. and soy-bean did not fit the description of race I or II. But, later Jones (1961) found no difference between isolates from cotton, soy-bean, cowpea and sesamum. Spencer and Walters (1962,1969) got isolates from cowpea and soy-bean that behaved similar to race 1 and 2 of Olive et al (1945).

In the present studies, when soy-bean and cowpea plants, the differential hosts used by Olive et al (1945), Stones and Jones (1960), Jones (1961) and Spencer and Walters (1962, 1969), were used for inoculations with tomato, sesamum and rubber

isolates, in all cases only mild spotting was observed. The varieties used were Pocha's bush cowpea and Pocha's soybean seeds. This indicates that the organism found in these hosts in different from race 1 and 2 of Olive et al. Gopalan (1963) reported that isolates from tomato, sesamum and dodonaea failed to produce symptoms on local variety of cowpea and Pocha's edible podded Bansei variety of soybean.

Among the three isolates, rubber isolate was more virulent producing characteristic symptoms on tomato, sesamum, rubber and dodonaea (spots of >5 mm size). Tomato and sesamum isolates caused only mild spotting in rubber leaves (spots of 1 mm diameter) and moderate spots (2-5 mm) on dodonaea and bigger sized spots (>5 mm) on tomato and sesamum. This indicates involvement of variation in pathogenicity of the isolates. The tomato and rubber isolates obtained in the present studies can be considered as two additional races to the already reported races of C. cassiicola.

Nature of transmission of the disease

A characteristic feature of the occurrence of the disease observed in Kerala is the prevalence of the disease in greater magnitude during the period from November to March - April. During these months rubber nurseries and sesamum plants get

serious infection by C. cassiicola. Chandrasekharan Nair (1964) and Prasannakumaran (1966) observed greater spore load of the fungus during this period and correlated it with a low night temperature and high relative humidity. These observations indicate that the disease is mostly air borne. But occurrence of the disease spread from soils has also been reported. Boosalis and Hamilton (1957) and Seaman et al (1965) reported a high inoculum potential in nursery plot soils at Nebraska and Canada, causing even root rots of soybean plants. Hence, the mode of transmission of the disease was investigated in detail.

Studies on seed mycoflora

Results of studies on seed mycoflora using standard blotter method and "agar plate technique" indicate that tomato and sesamum seeds from infected fruits carry with them the test organism C. cassiicola and several other fungi like Rhizopus sp. Penicillium sp. Aspergillus sp. Alternaria sp. etc. More than 60% of seeds were contaminated with these fungi. Sesamum seeds showed more infection by C. cassiicola (11.5%) than tomato seeds (8.5%). Similar seed borne nature of leaf spot producing fungi has been reported by Jain and Patel (1967) and Khandelwal and Prasada (1970).

Artificial inoculation with spores of C. cassiicola on sterilised seeds of tomato and sesamum showed that the infection

by the organism could cause poor germination as well as reduced vigour of seedlings. Baker (1948) Mathur and Sehgal (1964), Grewal and Mahendra Pal (1967) and Khandelwal and Prasada (1970) observed that mycoflora present on or in the seeds may result in prolonged dormancy and reduced emergence and vigour of the seedlings.

Studies on the effect of different seed treatment on sesamum and tomato seeds revealed that all the treatments were superior to the control. More than 90% of seeds were free of contamination by fungi in both tomato and sesamum seeds when treated with hot water or by different fungicides. This indicates that infection may be seed borne and that infected seeds could also serve as a source of inoculum to fresh soils.

Soil borne nature of the disease

Surface sterilized seeds of tomato and sesamum when sown in sterilized soil containing the inoculum at 5% and 10% levels, showed varying degrees of both pre and post-emergence damping off. For sesamum seeds, the pre and post-emergence damping off were 12 and 8% respectively at 5% level of inoculum, and 36% and 24% at 10% level, while the corresponding data for surface sterilized seeds in sterilized soils without inoculum was 4 and 2% respectively. A similar pattern was observed in tomato seeds also.

When planted in inoculated soil, leaf spot production was noticed on tomato and sesamum plants after thirty days. The leaf spot production was more in 10% inoculum level than in 5%. In control no leaf spots were produced.

The results indicate that the organisms from soil can cause infection and that the inoculum level influences the intensity of infection. These results confirm the views of Boosalis and Hamilton (1957) and Seaman et al (1965) that the fungus is soil borne. Hence, in nature use of infected seeds and incorporation of infected plant materials may serve as sources of inoculum. Longivity of the survival of the organism in soil needs further investigation.

Air borne nature of the disease

The result of investigations on the air borne nature of the disease using diseased plants as source of inoculum indicates that the organism can be spread through air also. Eight out of the ten tomato and sesamum plants kept along with disease affected plants in a cloth covered cage developed symptoms in 15-20 days while the controls were free from infection. The dry spores of the fungus have been found in the air by Sreeramulu and Ramalingam (1963) Gopalan (1963) Chandrasekharan Nair (1964) and Prasannakumaran (1966). The occurrence of maximum spore loads observed by Chandrasekharan Nair (1964) and Prasannakumaran (1966)

during the period from November-April and the prevalence of the disease during these months on the different hosts indicate the air borne nature of the disease.

In short, the disease could be spread through seed, soil, and air, wind being the major agency.

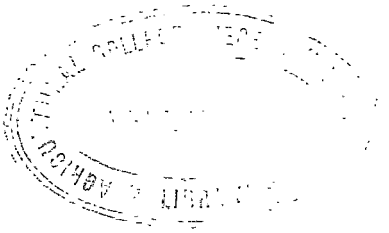
Physiology of the organism

Effect of light

Diffused day light and partial exposure to day light for 3 hours (09 hours to 12 hours) favoured more growth (32 mm and 30 mm radial growth in 8 days) and sporulation, while those kept in darkness showed limited growth (21 mm) and poor sporulation. Similar effects were found in the case of Alternaria solani and Macrosporium solani by Rendas (1917) and Kunkel (1918), Cercospora beticola by Calpouzos and Stallknecht (1965) and Alternaria porri by Fahim (1966).

Effect of relative humidity

The growth of the fungus and its sporulation at different levels of humidity were found to show varying results. Growth and sporulation were moderate at relative humidity levels of 97.5, 93.9, 80.6, 70.4 and 61.4. Growth at 51.0 and 51.0% relative humidity levels was poor. The relative humidity obtained in closed dessicators may not be correct since the agar media



kept in them may also affect the percentage of moisture in the closed vessels.

Fahim (1963) found that sporulation and growth of Alternaria porri was influenced by relative humidity. Sporulation was highest at 90%, moderate at 93%, 95%, 98% and 100% and poor at 73% and 52% R.H. levels. Mishra and Nema (1969) got better growth and sporulation of Alternaria tenius when grown at 75% humidity, whereas growth and sporulation were poor at 100% and moderate at 25% levels of humidity.

Growth of the fungus in different media

Of the different solid media used for tomato and rubber isolates, maximum radial growth was found in potato dextrose agar in 8 days, spreading in the entire surface area of the medium followed by Czapek-Dox medium. Czapek's agar and Richard's medium were satisfactory for both. Coon's agar, Crabill's medium and Barne's medium in that order favoured growth of tomato isolate while Crabill's medium and Coon's medium were better than Barne's medium for rubber isolate.

Gopalan (1963) found maximum radial growth of the organism in P.D.A. followed by Czapek's agar, Oat agar, Coon's medium, Richard's medium and Carbill's medium in that order for the tomato isolate.

When the three isolates were grown in the different host leaf extract agar media, rubber leaf extract favoured maximum growth for all the three isolates. This indicates that the rubber leaf may contain more nutrients favouring the growth of the fungus.

When the mycelial dry weight was taken as a measure of growth, it was found that Czapek-Dox medium was best for both tomato and rubber isolates giving a mean growth of 11.0 and 11.75 mgms in 24 hours respectively. This was followed by Czapek's solution Richard's medium, potato dextrose solution, Crabill's medium, Coon's medium and lastly Barne's medium. Chandrasekharan Nair ( 1964) got best growth in Crabill's medium followed by Czapek-Dox medium, Richard's medium, P.D.A., Czapek's medium, Coon's medium and Barne's medium. In the present studies Czapek's-Dox solution was found to be the best while Crabill's medium gave only a moderate growth.

Effect of culture filtrate on seedlings

Culture filtrates of both tomato and rubber isolates of C. gossilicola when applied to leaves of tomato, sesamum and rubber did not produce the typical symptoms of the disease, but caused a slight discolouration of the leaf lamina. When leaves were wounded and then sprayed with the filtrate, grayish brown discolourations developed. The discolourations were prominent in tomato and sesamum leaves, but not in rubber leaves. This may

be due to the more succulent and soft nature of the leaves of tomato and sesamum.

When cut shoots of 40-60 day old tomato, sesamum and rubber were placed in culture filtrates, wilting symptoms were exhibited in about 3 hours and the shoots wilted completely in 48 hours. When 20-30 day old seedlings of tomato and sesamum were placed with the root portion immersed in the culture filtrates, wilting started in about 12 hours and the plants wilted completely in 72 hours. When these wilted plants were kept in sterile water, they never recovered.

These observations indicate that the fungus produces certain toxic metabolites in the culture that are phytotoxic. These toxic metabolites may be involved in pathogenesis. Similar wilting symptoms, not characteristic of the disease, have been found in the culture filtrates of many organisms especially in leaf spot diseases caused by Alternaria sp., like A. solani (Brian et al 1951) A. kikuchiana (Hiroe et al 1956) A. zinniae (White and Starratt 1967) A. tenius (Fulton and Bollenbacher 1968) etc. Similar symptoms were reported when plants were kept in culture filtrates of necrosis causing fungi like Periconia circinata (Leukel 1948) Helminthosporium victoriae (Litzenberger 1949) Endothia parasitica (Razzigher 1953, Boller et al 1957, Gauman and Naef Roth 1957) Colletotrichum nicotianae (Wolf and Flowers 1957)

Colletotrichum fuscum (Goodman 1960) Colletotrichum atramentarium (Wagner 1961), etc.

The medium used for the culture of a fungus may sometimes affect in vitro production of toxic metabolites by the organism. Usually the medium that gives maximum growth may also favour maximum production of toxic metabolites. Since Czapek-Dox medium and Czapek's medium were found more or less equally good for both growth and toxin production, these were used for further studies. Both tomato and rubber isolates behaved similarly in their production of toxic principles as judged from wilting induced.

Effect of carbon sources

Studies on the effect of the different carbon sources on growth revealed that disaccharides lactose and maltose and monosaccharide D-fructose were increasing the mean radial growth per day for both tomato and rubber isolates of C. cassiicola. Sporulation was abundant in D-fructose, sucrose, maltose, lactose and starch after 8 days of growth. Growth and sporulation was sparse in treatment without carbon. Dry weight of mycelium after 20 days of growth was highest in maltose and D-glucose than sucrose while all other carbon sources gave lesser weights than sucrose. Taking into consideration of radial growth, sporulation

and dry weight of the mycelium, maltose was better than all other sugars tried.

Maltose was found as a good source of carbon for growth of Venturia inaequalis (Curt et al 1948) Ophiobolus graminis (Kilpatrick and Henry 1950) Chalara quercina (Beckmen et al 1953) and Cochliobolus miyabeanus (Tanaka 1956). But Chandrasekharan Nair (1964) got mannitol as best source of carbon for the tomato isolate of C. cassiicola than sucrose and maltose when mycelial weights were taken after 10 days growth in Czapek's medium. In the present studies mannitol was found inferior to maltose, D-glucose and sucrose when mycelial weights were taken after 20 days growth.

The activity of the culture filtrate on tomato seedlings after 12 hours was more in those filtrates obtained from D-fructose, maltose, D-glucose and lactose as indicated by curling of lower leaves. In 12 hours all except the control produced symptoms. However, in 72 hours symptoms were most severe in maltose, D-fructose, D-glucose, sucrose and starch causing 100% wilting, while mannitol and lactose caused only 75% wilting. Maltose was found as the best source of carbon for growth as well as for toxic activity of culture filtrates.

Effect of nitrogen sources

Studies on the effect of both organic and inorganic sources

of nitrogen revealed that ammonium sulphate followed by peptone, sodium nitrate and ammonium nitrate favoured radial growth in 8 days, while sporulation was highest in peptone, asparagine, ammonium nitrate and sodium nitrate. Dry weight of mycelium in 20 days growth was highest in peptone and asparagine followed by sodium nitrate, ammonium sulphate and ammonium nitrate (Table XXI). Both tomato and rubber isolates showed a more or less similar nitrogen preference.

The activity of culture filtrate as assayed by its effect in tomato seedlings was best for ammoniacal sources of nitrogen in 6 hours and progressed well till the end of observations in 72 hours. By that time, ammonium nitrate and ammonium sulphate caused wilting and drying of leaves whereas all other sources of nitrogen except potassium nitrate and sodium nitrite caused wilting only. Slight curling of lower leaves was observed in no nitrogen treatment after 72 hours.

Growth of fungi vary in different sources of nitrogen, both in the organic and inorganic forms. Ammoniacal forms of nitrogen was utilized best by Aspergillus niger (Steinberg 1939) Boletus elegans (How 1940) Rhizophlyctis (Karlingia) rosea (Haskins and Weston Jr. 1950) Cercospora beticola (Dange & Patel 1968) while organic nitrogen as peptone was used best by Ophiobolus graminis (Gilpatrick and Henry 1950) Helminthosporium oryzae

(Misra and Mukherjee 1962) and Alternaria sp. (Rajderkar 1966). Chandrasekharan Nair (1964) found peptone as the best source of nitrogen and ammoniacal salts were inferior to peptone for growth of C. cassiicola.

Ammoniacal forms of nitrogen was found best sources of nitrogen for toxic activities of Helminthosporium victoriae (Berry and Futrell 1962) while peptone was found best for Rhizoctonia solani (Edwards Newton 1937).

In the present studies ammonium nitrate and ammonium sulphate were the best inorganic sources of nitrogen for growth, sporulation and toxic activity. Among the different organic sources of nitrogen peptone was found best.

Effect of vitamins

Vitamins thiamine, pyridoxine, biotin and folic acid increased mycelial weights than the media without vitamins while cyanocobalamine, ascorbic acid, lactoflavin, calcium pantothenate and inositol reduced growth (Table XXII). Only pyridoxine, thiamine, nicotinic acid and biotin produced symptoms similar to unvitaminized culture filtrate in 12 hours while the others delayed activity (Table XXIII). Similar inhibiting action of vitamins has also been reported earlier. Inositol and lactoflavin were found to inhibit growth of Alternaria brassicae while nicotinic acid, pyridoxine and ascorbic acid stimulated growth. (Prasada et al. 1970).

Ascorbic acid and para-amino benzoic acid were found inhibiting growth of Curvularia pellescense while thiamine, pantothenic acid, pyridoxine, riboflavin, inositol, nicotinic acid, folic acid and choline were stimulatory in effect (Reis et al 1970). Mathur, Burnett and Lilly (1950) found inositol and biotin good for growth of Colletotrichum lindemuthianum, biotin, thiamine and inositol for Chalara quercina by Beckman et al (1953), biotin and thiamine for Ophiobolus graminis by Gilpatrick and Henry (1953). Basappa et al (1968) found increased rate of production of aflatoxins by Aspergillus oryzae in the presence of thiamine.

Effect of age of culture

An increase in mycelial weight with a decrease in pH values of culture filtrates was noticed when C. cassiicola was grown for 24 days in Czapek-Dox medium. The maximum growth and minimum pH occurred on 20th day of seeding for both tomato and rubber isolates. The activity of the culture filtrate was found increasing with age, maximum activity being on the 20th day. After 4 days growth the activity was practically nil. From 8 day old cultures it was mild, moderate from 12 and 16 days old cultures and maximum from 20 day old cultures. The activity of culture filtrate from 24 day old culture remained the same as that of 20th day culture filtrates, indicating that the activity does not increase after 20 days.

The maximum mycelial weight of 247 mgs was achieved in 20 days by rubber isolate with a drift in pH from 7.2 to 4.7 while the corresponding values for tomato isolate were 235 mgs and pH 4.0. The rate of change in pH was also slow in rubber isolate. However, this difference in pH has not affected the activity of culture filtrates of the two isolates.

Similar reduction of pH of culture filtrates from alkaline to acid side has been observed in Helminthosporium victoriae (Pringle and Brian 1957, 1963) Rhizoctonia solani (Aoki et al 1963, Nishimura and Sabaki 1963) Helminthosporium maydis (Tritica and Quimio 1966) etc.

Increase in toxin production with age of culture has also been noted by many workers as in Colletotrichum fuscum (Goodman 1960) Periconia circinata (Scheffer and Pringle 1961) Helminthosporium maydis (Tritica and Quimio 1966) Fusarium orthoceras f. lentis (Sharma and Agnihotri 1967) Alternaria solani (King 1967) Penicillium restrictum (Sankhala 1967) Piriqularia oryzae (Kirshnaswamy et al 1969) Trichocomis paduichii (Jayachandran Nair 1969) etc.

The results obtained indicate that both isolates of Corynespora cassiicola change the pH of culture medium from 7.2 to 4.0 – 4.7 in 20 days growth. The activity of the culture

filtrate in causing wilting symptoms in tomato seedlings started as the pH of filtrate changed to an acid aside in 12-16 days after seeding.

Effect of Hydrogen ion concentration of medium

The mycelial dry weights of tomato and rubber isolates of C. gassiicola show two pH optima for growth at acid and alkaline ranges. The mycelial dry weight of tomato isolate was maximum when the pH of the media were 4.5, 7.0 and 7.5 (265 mgms in 20 days) while that of rubber isolate was maximum at 4.5 and 8.5 (285 mgms in 20 days). Eventhough the two isolates have shown different pH optima for growth, it could be seen from figure 2 that the organism can grow well in both acidic and alkaline conditions ranging from pH 4.0 to 8.5. Growth at pH 9.0 was poor. At very low pH values of 3.5, 3.0 and 2.0, the growth was extremely poor for both isolates.

Different pH optima for growth on acidic and alkaline sides as observed in present studies have been reported in several fungi. Aoki (1937) found two pH optima at 4.6 and 9.6 for Piricularia oryzae, Nagunathan (1969) at 6.5 and 8.0 for both Cercospora dolichi and Cercospora canescens while a number of fungi have been reported to grow well in a wide pH range, like 5.8 to 10.0 for Helminthosporium nodulosum (Mitra and Mehta 1934),

pH 5.6 10.8 for Monosporium aniosporum (Wolf et al 1950), 3.4 - 8.0 for Karlinga rosea (Haskins and Weston Jr. 1950), 2.4 - 9.6 for Sclerotinia sclerotiorum (Tanrikut and Vaughan 1951), pH 5.6 in acid side and 8.0 in alkaline side for Botrytis cinerea (Damle 1951), pH 2.0 - 9.0 for Sclerotium rolfsii (Subramonian 1964) and 4.0 - 11.0 for Sclerotium oryzae (Narasimhan 1969). Between isolates difference in pH optima for growth in acidic and alkaline media have been reported in some fungi. Isolates of Colletotrichum falcatum were reported to show different pH optima for growth by Chona and Mingorlani (1951). Hodge et al (1970) found that two isolates of Helminthosporium nodulosum grew well at different pH (6.0 & 7.6). In the present studies, the two isolates of C. capsicola have shown two different pH optima for growth. Both grew well at pH 4.5 in acid side. But they differed in alkaline side. Tomato isolate preferred pH 7.0 - 7.5 whereas rubber isolate grew well at pH 8.5.

It could be seen from figure 2 that growth of the fungus in different hydrogen ion concentrations of media has been found to influence the final pH of the filtrate after 20 days of growth. For both tomato and rubber isolates there was no marked drift in pH of the medium when grown at and below pH 4.0. But both the isolates when grown at pH 4.5, 5.0, 6.0 and 7.0 lowered the pH after 20 days growth to 4.1 - 5.0. At pH 7.5 tomato isolate lowered the pH to 5.0 and rubber isolate to 6.9. At

higher pH values of media (8.0, 8.5 and 9.0) both the isolates lowered the pH but never crossed the acid range. In general both the isolates lowered the pH of the media when grown at pH values of 4.5 to 9.0.

Alternaria soleni (Brien et al 1951) Fusarium oxysporium f. cumini (Mathur and Mathur 1967) and Colletotrichum gloeosporioides (Sharma and Sharma 1969) are reported to change the original pH of the media when grown at different hydrogen ion concentrations.

Wilting symptoms produced by the culture filtrates of the organism grown under different pH levels indicate that lowering of pH of the media after 20 days growth caused toxicity of culture filtrates. All culture filtrates with a final pH below 5 caused complete wilting in 72 hours. Those culture filtrates with higher pH values were also toxic, but only to a lesser degree. The same pattern was exhibited in the inhibition of radicle length of tomato seeds treated with the culture filtrate.

All the above observations indicate that pH of the medium influences growth of the fungus as well as the toxicity of the culture filtrate.

As the fungus grows, certain acidic metabolites are released by it, which might have lowered the pH of the culture

filtrate. Growth of the tomato and rubber isolates at pH 5.5, 5.0 and 2.0 was poor. The culture filtrates from these and the uninoculated media of the same pH values were equal in inhibiting radicle elongation of tomato seeds. This shows that in these pH range no toxin production takes place. When grown at pH 4.5, 5.0, 6.0, 7.0 and 7.5 both the isolates produced good mycelial growth. The radicle inhibition by the culture filtrate was also higher over uninoculated media at these pH values. This indicate that a toxic metabolite is produced by the fungus at this range of pH. At higher pH values of 8.0 to 9.0 though growth was satisfactory the toxic activity decreases. These results suggest that the fungus requires a pH range of 4.5 to 7.5 for good growth and for production of toxic metabolites.

Wilting and poor radicle elongation of tomato seedlings in media below pH 3.5 were merely due to the low pH of the media. Wilting and inhibition of radicle elongation caused by culture filtrates of the fungus grown at pH 4.5 - 7.5 could be attributed to the acidic toxic metabolites produced by the organism.

Production of acidic toxic metabolites, similar to that of C. *capsici* has been reported in many other fungi like *Sclerotium rolfsii* (Higgins 1927) *Phytophthora* sp. (Mehrotra 1949) *Sclerotinia sclerotiorum* (Overall 1952) *Alternaria solani* (Nelson et al 1951) *Helminthosporium victoriae* (Pringle and

Braun 1957) Rhizoctonia solani (Aoki et al 1965) Leptosphaeria avenaria (Wilcoxson 1955) Helminthosporium maydis (Tritica and Quimso 1966) etc.

Stability of toxin at different H-ion concentrations

Results of change of pH of the culture filtrate from 4.2 to different levels in a range of 3-9 indicate (Table XXVIII) that the toxin is stable in a range of 3 to 5, while it decreased its activity on conversion to higher pH values of 6 to 9. The wilting symptoms produced by adjusting the Hydrogen-ion concentration to pH 3.6 were almost similar to that produced by the culture filtrate at pH 4.2. But increasing the pH to 6 and 7 reduced severity and at pH 8 and 9 the culture filtrates almost lost their activity. Similarly the culture filtrate inhibited elongation of radicle of tomato seeds almost uniformly when the pH was adjusted to 3 to 5. When adjusted to higher hydrogen ion concentrations (6-9) there was no inhibition. Similar loss in toxicity of culture filtrates when their pH was adjusted to different hydrogen ion concentrations have been observed in Fusarium oxysporum f. cumini (Mathur and Mathur 1967) and Alternaria sesami (Sathiabalan Samuel 1969).

Stability of toxin at various temperature

The toxic activity of culture filtrate was not affected by heat treatment including autoclaving at 10 lbs for 15 minutes

indicating that the toxic metabolite produced is a thermostable one. Similar thermostable toxins have been reported in the case of Alternaria solani (Brian et al 1951) Helminthosporium victoriae (Litzen-berger 1949) Periconia circinata (Leukel 1948) Colletotrichum nicotianae (Wolf and Flowers 1957) Colletotrichum fuscum (Goodman 1960) Alternaria tenius (Fulton et al 1965) Aspergillus niger (Narain and Om Prakash 1968) Colletotrichum gloeosporioides (Sharma and Sharma 1969) and Trichocenis maduiehii (Jayachandran Nair 1969).

Stability of culture filtrate on storage

Storage of culture filtrate at 5°C and at room temperature did not cause any loss of activity till the 15th day. There appeared a reduction in the severity of wilting symptoms at 72 hours by storing the culture filtrate for 15 days. The culture filtrate autoclaved and kept at room temperature retained its full activity throughout the period of observation indicating that the reduction in activity noticed in 15 days in the former cases is probably due to some contamination.

Effect of dilution

The potency of the culture filtrate and its probable in vivo nature studied by diluting the culture filtrate indicate that the activity decreases with dilution. The activity sharply

declined below 25% concentration. The above findings suggest that the metabolites produced by the organism is a toxic one. Similar decrease in activity of culture filtrates was observed by Mathur and Mathur (1967) in Fusarium oxysporum f. cumini at 1:4 dilutions with water, Sharma and Sharma (1969) in Colletotrichum gloeosporioides at 1:9 dilutions and Sathyabalan Samuel (1969) in Alternaria sesami at 1:5 dilutions.

Effect of culture filtrate on germination of seeds

The culture filtrate inhibited germination of tomato seeds and caused discolouration and disintegration of radicles (60%) of germinated sesamum seeds. Inhibition of germination by the activity of culture filtrates has been reported in many cases like Helminthosporium sativum (Ludvig 1957), Helminthosporium oryzae (Groenigo 1957) Piricularia oryzae (Govindan 1965) Alternaria zinniae (White and Starratt (1967) Aspergillus niger (Narain and Om Prakash 1968) and Trichoconis padwickii (Jayachandran Nair 1969). Disorganisation of the succulent scales followed by discolouration similar to the one observed in the case of sesamum seeds has been reported in onion infected by Aspergillus niger (Narain and Om Prakash 1968).

Effect of culture filtrate on radicle and plumule inhibition

Culture filtrate of Corynespora cassiicola is found to

inhibit both radicle and plumule elongation of tomato and sesamum seeds. Similar inhibition of radicle growth in various seeds has been found by the culture filtrates of Helminthosporium victoriae (Wheeler and Luke 1954) Periconia circinata (Scheffer and Pringle 1967) Helminthosporium carbonum (Scheffer and Pringle 1967) Pirieularia oryzae (Krishnaswamy et al 1969) Trichocenis padwickii (Jayachandran Nair 1969) etc. while plumule inhibition was reported by the activity of culture filtrates of Fusarium semiceti (Brian et al 1961) Pirieularia oryzae (Govindan 1965 and Krishnaswamy et al 1969) Trichocenis padwickii (Jayachandran Nair 1969) etc.

Effect of culture filtrate on cut shoots

Cut shoots of 50-60 days old rubber, sesamum and tomato exhibited wilting symptoms within 6 hours of treatment (cut ends kept in culture filtrate) and the wilting was more severe in succulent tomato and sesamum shoots. Similar wilting of cut shoots due to the toxic activity of culture filtrates have been reported in the case of Alternaria solani (Brian et al 1951) Helminthosporium sativum (Sayed 1961) Fusarium oxysporum f. cumini (Mathur and Mathur 1967) Alternaria zinniae (White and Starratt 1967) and Colletotrichum gloeosporioides (Sharma and Sharma 1969).

Effect of culture filtrate on other seeds

Besides tomato and sesamum seeds, culture filtrate of Corynespora cassiicola inhibited germination of a number of seeds like cowpea, papaya, bhindi, dolonaea, soybean, cluster beans, kidney beans, lima beans and pole beans at varying levels. The lowest inhibition was in lima beans (4.4% over sterile water) and the highest in papaya (36.9%).

Effect of culture filtrate on fungal spores

The culture filtrate of C. cassiicola did not show much inhibitory effect on germination of spores of Alternaria sp. Helminthosporium halodes, Sphacelotheca sp. and C. cassiicola. The highest inhibition of germination was found in Sphacelotheca sp. with 14.3% while in others it was only about 1%.

Purification of toxin

Acetone, chloroform and benzene gave an orange yellow viscous material on extraction and vacuum evaporation of the culture filtrate. The chloroform and benzene extracts showed equal activity on tomato and sesamum seedlings. Acetone was the best solvent as the symptoms were more severe when it was used. Purified toxin caused wilting symptoms in 3 hours whereas the crude culture filtrate produced similar symptoms only after 12 hours. In 18 hours drying of plants commenced. Spraying

of the extracts on leaves caused discolouration and necrosis followed by drying up and falling off of affected area. Extraction and purification of toxin using acetone, chloroform etc. has been employed by Brian et al (1951) for Alternaria solani and Sathiabalan Samuel (1969) for Alternaria sesami.

Control aspects

In the spore germination inhibition method, mercuric chloride even at 500 ppm brought complete inhibition. At 1000 p.p.m more than 95% inhibition of germination was obtained with Dithane-Z 78, Zirde, Cuman, Captan and Dithane-M 45. All these gave complete inhibition at 2000 p.p.m. At 2000 p.p.m Duter gave 96% inhibition. At 3000 p.p.m all except Fytolan (92%) Blitox (84%) and Thiovit (42%) gave 100% inhibition. Chandrasekharan Nair (1964) obtained 100% inhibition of spores by 0.5% Dithane-Z 78 and 0.5% Bordeaux mixture while Fytolan was not effective. In the present studies also, Fytolan and Blitox were found poor in inhibiting germination of spores.

In poisoned food technique also, more or less similar results were obtained. Mercuric chloride and Dithane-M 45 at 1000 p.p.m. and Dithane-Z 78, Zirde, Cuman and Captan at 3000 p.p.m completely inhibited mycelial growth. Duter and Difoltan at 3000 p.p.m caused 85% inhibition and Thiovit 3000 p.p.m inhibited 45% only. Fytolan and Blitox brought about only 45%

and 65% growth inhibition at 3000 p.p.m. For C. cassiicola Singh et al (1969) got complete inhibition with Ceresan wet and Antracol at 2000 p.p.m. Cuprous oxide brought complete control at 5000 p.p.m and Duter (1000 p.p.m) Karathane (5000 p.p.m) Blitox (5000 p.p.m) Dithane-Z 78 (2000 p.p.m) and Blasticidin (2000 p.p.m) were found effective in that order. In the present studies also a more or less similar pattern was observed.

Willis (1905) reported the failure to control the disease caused by C. cassiicola on cucumbers (then known as Cercospora melongis) by any known methods of control including spraying with Bordeaux mixture, fumigation with sulphur and by soil sterilization. In the field trials conducted at Rubber Research Institute of India, Kottayam, 1% Bordeaux mixture was not effective. Eventhough a complete inhibition of the organism in laboratory trials may not bring forth a similar effect in the field, it gives an indication on the efficacy of the different fungicides that can be used. The present studies suggest that proprietory fungicides like Dithane-M 45, Dithane-Z 78, Cuman and Ziride may be useful for controlling C. cassiicola on crops like sesamum, tomato and rubber.

# SUMMARY AND CONCLUSIONS

# SUMMARY

Corynespora cassiicola (Berk & Curt) Wei, the incitant of leaf spot diseases of tomato, sesamum and rubber has got a wide spectrum of host plants in Kerala and occurs in greater magnitude during the summer months of November to April.

The isolates of the fungus obtained from rubber, tomato and sesamum vary in their virulence, the former being more active producing characteristic spots which enlarge to more than 5 mm in diameter in all the three hosts while the tomato and sesamum isolates which were pathogenic to tomato and sesamum produced only mild spotting on rubber leaves. This suggests the existence of two pathogenic races.

The organism can be spread through seed, soil and air, the principal agency being wind. Seed treatment with mercuric chloride 0.1% and hot water treatment at 52°C for 10 minutes reduced the incidence of the disease in tomato and sesamum.

Diffused day light and partial exposure to light for 3 hours from 09.00 - 12.00 hours for 8 days favoured better growth and sporulation than those kept under dark conditions.

Humidity showed no conclusive results. However at 70.4% and 80.6% relative humidity levels both growth and sporulation of the fungus were good.

Czapek-Dox medium, Czapek's solution, Richard's media and Potato dextrose solution in that order was best suited for laboratory culture as judged by the radial growth and mycelial weight.

Corynespora cassiicola produces toxic metabolites in vitro that cause wilting of cut shoots of tomato, sesamum and rubber as well as in seedlings of tomato and sesamum.

Maltose and D-fructose as sources of carbon and ammonium nitrate, ammonium sulphate and sodium nitrate as sources of nitrogen were best for over-all growth, sporulation and toxic activity of culture filtrate. Vitamins showed varied effects on the fungus. Growth of the organism and the activity of culture filtrate were accelerated to a slight extent by thiamine, pyridoxine, biotin and folic acid at 2 Ug/lit. Inositol, ascorbic acid, lactoflavin and calcium pantothenate on the other hand retarded the growth.

The age of the culture influenced mycelial growth of the fungus and activity of culture filtrate. Maximum mycelial growth and activity of culture filtrate were obtained after 20 days of seeding. The pH of the Czapek-Dox medium was brought from 7.2 to a range of 4.0 - 4.7 by both tomato and rubber isolates in 20 days growth.

The tomato isolate favoured an acid medium for growth and activity, with a pH optima of 4.5 in the acid range and 7.5 in the alkaline range. The rubber isolate showed a pH optima of 4.5 in the acid side and 8.5 in the alkaline side. The activity of culture filtrate was more for both isolates when grown in acid range. Both isolates in different media at a range of pH 4.5 to 7.5 brought the pH of culture filtrate to 4.0 - 5.0 in 20 days.

The culture filtrate was found active in a pH range of 3 to 5, but lost its activity at higher pH values.

The toxicity of culture filtrate was not affected by heat. Even autoclaving at 10 lbs for 15 minutes could not alter it indicating that the toxic meatbolite is thermostable.

The culture filtrate was stable for a period of 20 days when kept at room temperature after sterilization.

The activity of culture filtrate decreased on dilution with sterile water and it lost its toxicity when the dilution ratio exceeded 1:4.

Culture filtrate inhibited germination of tomato seeds and caused discolouration and disintegration of germinating sesamum seeds. It also inhibited radicle and plumule elongation of both tomato and sesamum seeds. Besides tomato and sesamum, it inhibited germination of a number of other vegetable seeds at varying degrees. The culture filtrate was not active against

germination of spores of Alternaria sp. Helminthosporium halodes, and Corynespora cassiicola while it caused 14.3% inhibition of spores of Sphaerotheca sp.

Purification of toxin is possible by extraction with acetone, chloroform or benzene followed by vacuum evaporation. The purified toxin caused discolouration, necrosis and drying of leaves of tomato and sesamum and could produce severe wilting symptoms on tomato and sesamum seedlings.

Complete inhibition of growth of C. cassiicola was obtained by treatment with 0.3% of mercuric chloride, Dithane M-45, Dithane Z-78, Cuman and Ziride. The former two also caused 100% inhibition even at 0.1% strength.

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* Original not seen. Taken from Review of Applied mycology and from other sources.

PLATE I

Tomato leaf infected by *C. cassiicola*

PLATE II

Sesamum leaf infected by *C. cassiicola*

PLATE III

Target spots on Rubber leaves

PLATE IV

Infection on capsules and stem of sesamum

PLATE III

Target spots on Rubber leaves

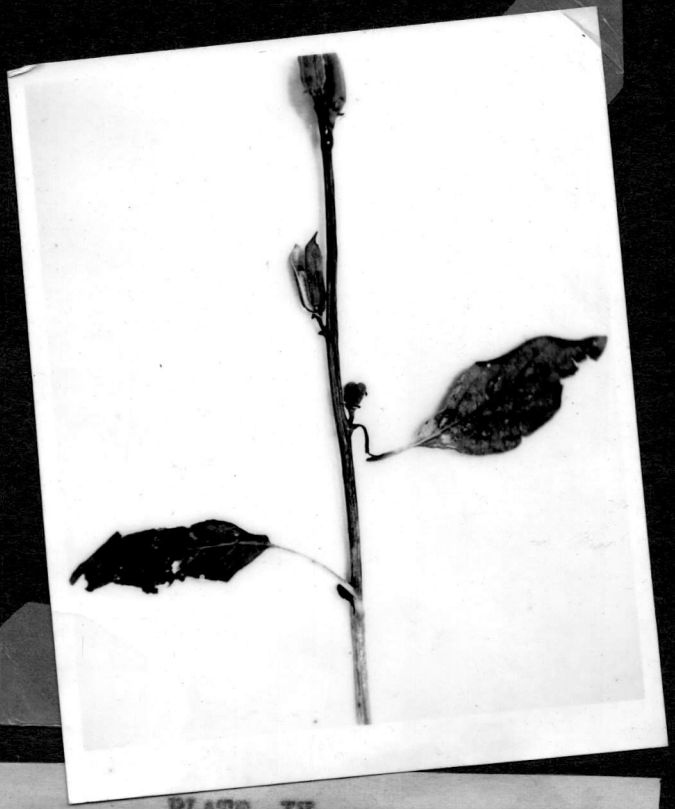

PLATE IV

Infection on capsules and stem of sesamum

PLATE III

Target spots on Rubber leaves

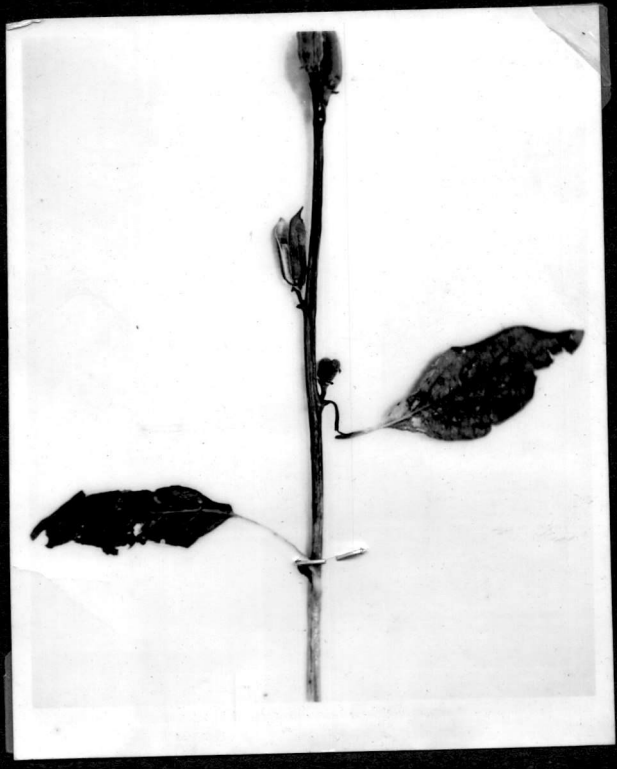

PLATE IV

Infection on capsules and stem of sesamum

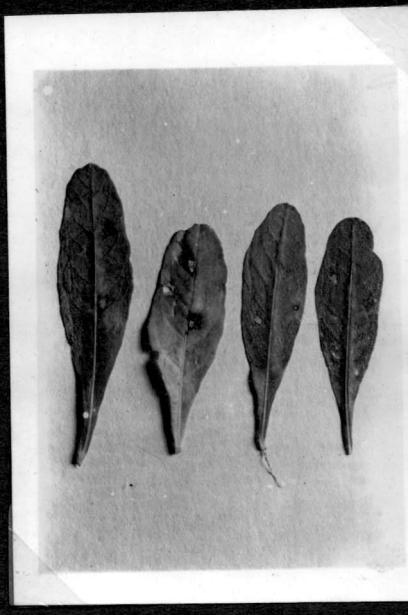

PLATE V

Symptoms on *Dolichos* leave

1. Natural infection
2. by Rubber isolate.
3. by Tomato isolate.
4. by Sesamum isolate.


PLATE VI

Effect of culture filtrate on 20-30 day old tomato seedlings.

1. Sterile water.
2. uninoculated media.
3. culture filtrate.


PLATE VII

Effect of culture filtrate on 20-30 day old sesamum seedlings.

1. Sterile water
2. uninoculated media
3. culture filtrate

PLATE VIII

Effect of purified toxin on tomato leaves.

PLATE IX

Effect of purified toxin on sesamum leaves.