STUDIES ON

Corynespora cassiicola (Berk. & Curt) WEI.

BY

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THESIS

SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY) OF THE UNIVERSITY OF KERALA

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AGRICULTURAL COLLEGE & RESEARCH INSTITUTE,
VELLAYANI, TRIVANDRUM

AUGUST 1963

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 Shri N.Gopalan 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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16th August, 1963.

ACKNOWLEDGEMENTS.

I wish to express my sincere thanks and gratitude to Dr.J. Samraj, Professor of Plant Pathology for his guidance, encouragement and valuable suggestions both during the course of this investigation and during the preparation of the thesis.

I am extremely grateful to Dr.C.K.N.Nair,Principal for all the help rendered at every stage in the progress of the investigation.

I also thank Shri E.J.Thomas, Senior Lecturer in Statistics for suggesting suitable methods for the statistical analysis of the data collected during the studies.

Thanks are also due to the members of staff of the Plant Pathology Division for their generous assistance in various ways.

I am also grateful to the Government of Kerala for deputing me for the M.Sc. (Agriculture) Degree course which made this work possible.

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INTRODUCTION.

The genus Corynespora was first proposed by Güssow in 1906 to accommodate a fungus causing a disease of cucumber in glass house, which was previously named as Cercospora melonis. The same organism was reported to cause a disease of melon, cowpea, soybeans and a large number of other plants. Among the earlier workers some included the causal organism under Cercospora whereas others placed it under Helminthosporium. It was Wei (1950) who found all these organisms to be identical.

This fungus was similar to Cercospora in its slender shape and light colour of the conidia while its similarity to Helminthosporium could be found in the formation of the conidial chains and vesicles on the conidia. It could be distinguished from Cercospora, in the larger diameter and thicker exospore of the conidium and the lack of stromatic tubercles at the base of the conidiophores. At the same time, it differs from Helminthosporium in the pale colouration and slender shape of the conidium. Wei pointed out that in a typical Cercospora, though the young conidia may be terminal, the new growth of the conidiophore took place at the side of the tip, pushing the conidium to the side and that in the type species of Helminthosporium the conidia are formed in verticils. On the other hand, in the fungus under discussion, the terminal formation of conidia either

singly or in chains and the terminal proliferation of the conidiophore were constant characters. So Wei considered Corynespora as a good genus with the following characters: "It is a primary or secondary parasite, with mycelium either endophytic or sometimes superficial. The conidiophores arise from the hyphae penetrating through the epidermis perpendicular to, the surface of the substratum. The conidiophores may also develop from the aerial hyphae. They may or may not have a bulbous base, slightly or conspicuously swollen at the apex, simple, single or sometimes in tufts, proliferating terminally through the scar of the previous conidium or sometimes through the injured conidiophore or conidium, bearing a single conidium or conidial chain at the apex. Conidia are terminal, often connected with the conidiophore with a hyaline isthmus, under certain conditions, forming short chains in acropetal succession, pyriform, obclavate, or more rarely cylindrical, multiseptate, pale brown, becoming dark with age, with a thin, coloured pellicle, a thick, colourless exospore and a prominent, dark, annular basal hilum". Its conidial structure indicates a closer affinity to Helminthosporium. Wei proposed the name Corynespora cassiicola (Berk.&Curt.) Wei for the fungus causing the disease mentioned earlier on melon, cucumber, cowpea and soybean. This organism has been found to parasitise on a variety of plants both cultivated

and wild throughout the world.

In India the same organism has been found parasitic on jute (Corchorus capsularis), tomato (Lycopersicum esculentum), papaw (Carica papaya), gingelly (Sesamum indicum) and black gram (Phaseolus mungo), among cultivated plants.

In Kerala this fungus has been seen to attack Sesamum indicum and Lycopersicum esculentum causing severe spotting of leaves, stem and fruits in some seasons. It has also been seen to cause leaf spots on the hedge plant Dodonaea viscosa and on a dryland weed Ageratum conyzoides. In a saprophytic condition it occurred on Amorphophallus gigantea, Carica papaya, Hibiscus esculentus, Morinda tinctoria, and Solanum torvum.

Sesamum is one of the most important oilseed crops of Kerala and tomato an equally important vegetable crop. Due to the virulent nature of Corynespora cassiicola on these two crops and because it thrives on a wide variety of substrates it was thought fit to study the identity of the organism and its host range in Kerala. Another aspect of study that has been proposed is the variation in size of spores in different environmental conditions. An attempt has also been made to study the variation in the growth, sporulation and size of spores of the fungus in different substrates.

REVIEW OF LITERATURE.

Systematic position of Corynespora.

Wei (1950) has given an historical account and diagnosis of the genus *Corynespora*. He has traced the history of the genus from the earliest description of the diseases caused and the names by which the organisms were known till its final classification under the genus *Corynespora*.

The occurrence of a disease of melons in pit was first published in the "Gardener's Chronicle" by Cooke in 1896 caused by an undescribed species of *Cercospora* which he named *Cercospora melonis*. He later collected the same organism from cucumber in 1901. Güssow in 1906 while studying a glass house disease of cucumber came across the same fungus and was the first to erect a new genus *Corynespora* to include this fungus on account of its conidia being formed in chains and there being a hyaline isthmus between the conidiophore and conidium. He named the fungus *Corynespora mazei* in honour of Professor Maze who was working on the same fungus in France. Lindau (1910) treated *Cercospora melonis* and *Corynespora mazei* under one species *Corynespora melonis* (Cooke) Lindau. In 1931 Kawamura described a disease attacking cowpea (*Vigna catjang* var. sinensis) which he attributed to infection by a new species of *Cercospora vignicola*. Olive, Bain and Lefebvre (1945)

observed the leaves of cowpea severely infected with a leaf spot of a type hitherto unknown, which they found to be caused by a new species of Helminthosporium. They called this fungus Helminthosporium vignae sp.nov.

Liu (1948) observed a leaf spot on soybean which he considered to be due to Cercospora vignicola, which was also common on cowpea. The characters of the fungus both on the host plants and in culture were found to be identical with those of Helminthosporium vignae on soybean and cowpea described by Olive et.al. in 1945. The latter authors placed the fungus in the genus Helminthosporium because of the catenulate conidia and the presence of vesicles on conidia in culture. But Liu believed that these characters were influenced by environmental factors. Vesicles on conidia being not a constant structure and catenulate conidia being produced only under very moist conditions he considered the fungus to be of a type intermediate between the two genera Cercospora and Helminthosporium. He further stated that most of the conidial characters indicated this to be nearer to Cercospora and it seemed to him advisable to refer the fungus as C.vignicola with H.vignae as a synonym.

Olive (1949) did not agree with this view of Liu. He maintained that the fungus should be classified under Helminthosporium due to the fact that long and narrow conidia were produced only when diseased leaves of soybean and cowpea

were placed in a moist chamber and that in nature they were usually broader. Further the walls and septa of the conidia had the characteristic thickening which rightly made it possible to classify as Helminthosporium vignicola (Kawamura) Comb.nov.(syn.Cercospora vignicola Kawamura; Helminthosporium vignae Olive).

On a comparative study of the cowpea fungus and Corynespora melonis, Wei (1950) found the two to be similar and to agree with the descriptions given by Cooke, Güssow and Lindau excepting that he failed to find the conidia in chains in C.melonis. According to Güssow the catenulate condition was only a transitional one. Hughes pointed out that the conidiophore at any one time, bore only a single conidium or a chain of conidia at the apex and elongated by proliferating through the terminal conidial scar. The wall of the conidiophore thickened at its apex, but the abscission layer cutting off the conidium remained thin and through it new growth took place. Repetition of such a process gave the tip of conidiophores an appearance of successive constrictions. Earlier workers considered these as constrictions at the septa but what appeared to be a septum was actually only a scar. As the conidiophore proliferated, a new conidium was formed terminally which was a feature common to both C.melonis and the cowpea fungus. The hyaline isthmus was observed by Wei in C.melonis and by Olive et.al. in the case of the latter

fungus. The shape and structure of the conidia produced in both were quite similar. Hence it was concluded that the two were congeneric. Helminthosporium and Cercospora produced their succession of conidia in a different order. In Cercospora though the young conidia were terminal new growth of conidiophore took place at the side of the tip by pushing the previous conidium aside, while in Helminthosporium the conidia formed verticils. The catenulate formation of conidia pointed out as a character for Corynespora by Güssow was either transitional or non-typical. The formation of conidial chains was not quite common on specimens collected in the field and the hyaline isthmus was also not a common feature. But the terminal position of the single conidium or the single conidial chain and the terminal proliferation of the conidiophore was a constant and persistant feature. Hence Wei accepted Corynespora as a genus distinct from either Helminthosporium or Cercospora. He also made a detailed study of the type species Corynespora cassiicola and has provided the following detailed description of the type species.

"Parasitic on stems, leaves and fruits, but mostly on leaves, causing spots of variable sizes, from less than one millimetre to 1½ or even 2 cm. in diameter, pallid to yellowish brown with purplish-brown margin or zonations, often secondary. Conidiophores mostly hypophyllous, perpendicular to the surface of the substratum, from mycelium

emerging through the epidermis or occasionally from aerial hyphae, mostly single or sometimes in tufts, simple, straight, rather stiff, sparingly septate, dark brown, with or without a bulbous base, slightly or not at all swollen at the tip which is thick walled, proliferating terminally through the scar of the fallen conidia or some times through the injured conidiophore or conidium, reaching a length of 600 /u or more and 3.8 to 11.3 /u in diameter; proliferating joints variable in length, 10-100x5.2-11.4 /u lighter in colour towards the apex. Conidia borne singly at the apex, or under certain conditions, presumably under high humidity, forming chains of 2 to 6 spores, and some times connected by a hyaline isthmus to the conidiophore or to one conidium below, obclavate, sometimes cylindrical straight or often slightly curved, conspicuously tapering towards the apex which is usually thin-walled, pale olivaceous brown and darkening with age, 4-16, but sometimes 1-2 or upto 20 or more pseudo-septate, with a coloured pellicle and thick, hyaline exospore, and a conspicuous hilum 3.6 to 7.9 /u across, measuring 32-220x8.4-22.4 /u, commonly 65.8-181.6x13.6-20.0 /u, germinating by polar germ tubes".

The following have been proposed by Wei as synonymous with Corynespora cassiicola (Berk.& Curt.)Wei: Helminthosporium cassiicola Berk.& Curt.apud Berk.;

Cercospora melonis Cooke; Corynespora mazei Güssow; Helminthosporium papayae H.Sydow; Cercospora vignicola Kawamura; Helminthosporium vignae Olive apud Olive, Bain & Lefebvre. Ellis (1957) has included Helminthosporium warpuriae Wakefield also as synonymous.

In addition to the type species, seven others have been recognized by Ellis (1957). A further ten species were described by the same author (1960).

Ellis (1960) has given a standardized description of Corynespora cassiicola. "Colonies grey or pale brown, thinly hairy, effused. Mycelium mostly immersed in the substratum, composed of branched, septate, subhyaline to pale brown, smooth walled, 2-6 µ thick hyphae. No stromata. Conidiophores arising from immersed hyphae, erect, simple, or occasionally branched, straight or slightly flexuous, pale to mid brown, septate with up to nine successive cylindrical proliferations, 110-850 µ long, 4-11 µ thick. Conidia formed singly or in chains of 2-6 through a pore at the apex of the conidiophore which then often proliferates through the apical pore and forms another conidium at the apex of the proliferation; straight or curved, obclavate or cylindrical, smooth, subhyaline to pale olivaceous brown, with 4-20 pseudosepta, 40-220 µ long (upto 520 µ in culture), 9-22 µ thick, 4-8 µ wide at the truncate base".

Host Range and Distribution.

Corynespora cassiicola has been reported to be parasitic on a variety of host plants distributed over different families. Its cosmopolitan habit could be understood from the fact that it was even isolated from a human skin scraping (Ellis 1957). It has been mostly placed under the two genera Helminthosporium or Cercospora which later on Wei (1950) transferred to the genus Corynespora.

The fungus has been found by Berkely (1879) to be parasitic on Cassia alata on melon and cucumber by Cooke(1896 and 1901 respectively).Sydow (1921) reported this fungus to cause a disease on the leaves of Carica papaya in the Philippines and Mohanty and Behera (1960) found a severe leaf spot disease of papaya at Bhubaneswar in India caused by C.cassiicola. Deighton found the fruits attacked by the same organism in Sierra Leone. Simmonds (1956) recorded papaw fruit rot for the first time in Southern Queensland and tomato fruit rot in Northern Queensland caused by C.cassiicola. Choudhury found it on Corchorus capsularis in Assam. Hansford (1902) observed it on dying stems of Crotalaria juncea in Uganda. Liu (1947) in Nanking, China, Olive et.al. (1945) in Lousiana, Kurata (1960) in Japan and Stone and Jones (1960) in Mississippi found this fungus to attack Glycine max. Deighton found it in Sierra Leone on the leaves of seedlings of Hevea brasiliensis on leaves of

Hibiscus esculentus, Hibiscus sabdariffa, Impatiens balsamina and Tithonia speciosa, on leaves and fruits of Lycopersicum esculentus, and on pods of Phaseolus vulgaris. Martyn (1948) noted it on the leaves of Norantea guianensis. Kawamura (1931) in Fukuoka in Japan and Olive and his co-workers (1945) in Lousiana have reported a serious leaf spot disease caused by this fungus on cowpea (Vigna sinensis). Wallace (1933) from Tanganyika and Stone and Jones (1960) from Mississippi have reported severe spotting of stem and leaves of Sesamum caused by this fungus. Wallace and Wallace (1953) have recorded Corynespora cassiicola attacking Salvia leucantha in Tanganyika. Cifferi (1946) described a disease affecting cassava (Manihot esculenta) due to this fungus producing large, damp, diffuse, greyish or brownish spots of which only one was present, as a rule, on each leaf segment. These spots were observed only in the wet season. Ellis(1957) has given a list of host plants for Corynespora cassiicola, specimens of which were collected from Ceylon, China, Gold coast, India, Jamaica, Malaya, Nigeria, Philippines, Sierra Leone, Sudan, Tanganyika, Togoland, Trinidad and Uganda. The list contained the following plants the stems and leaves of which were attacked by this organism. Agave sisalana, Albizzia zygia, Aleurites montana, Ananas comosus, Bauhenia purpurea, Boehmeria nivea, Bridelia ferruginea, Capsicum frutescens, Carica papaya, Cassia spp., Clerodendron paniculatum, Corchorus olitorius, Crotalaria juncea, Crotalaria sericea,

Cucurbita pepo, Dalbergia sp., Desplatzia lutea, Dracaena sp., Elaeis guineensis, Euphorbia sp., Ficus sp., Gaillardia aristata, Glycine max, Hevea brasiliensis, Hibiscus esculentus, H.sabdariffa, Impatiens balsamina, Jacobinia sp., Lactuca sativa, Lagenaria siceraria, Lycopersicum esculentum, Manihot esculenta, Newboldia laevis, Norantea guianeensis, Petrea sp., Phaseolus aureus, P.lunatus, P.vulgaris, Plumaria acutifolia, Poinsettia sp., Psophocarpus tetragonolobus, Salacia senegalensis, Salvia leucantha, Sesamum indicum, S.orientale, Sida urens, Stachytarpheta angustifolia, Tacazzea apiculata, Tithonia speciosa, Tragia sp., Vigna sinensis, Vitex pubescens, Warpuria clandestina, Wedelia biflora and Xanthosoma sagittifolium.

Addy and Mohanty (1959) observed a leaf spot disease of Phaseolus mungo L. caused by C.cassiicola. Jones (1961) reported from U.S.A. a fungus attacking cotton which was identified as C.cassiicola. The fungus isolated from the affected tissues proved pathogenic to both Gossipium hirsutum, and G.barbadense and appeared identical with the pathogen attacking sesame and soybean in the same area and the fungus was found to overwinter on cotton stems in the field. Mohanty (1958) observed a leaf spot disease on Rauwolfia serpentina at Bhubaneswar in India caused by C.cassiicola. Creager (1961) isolated this fungus from hydrangea leaf spots.

Pathogenecity of the fungus.

There is considerable diversity of opinion in regard to the pathogenecity of C. cassiicola. While some are of opinion that it is highly pathogenic on a large number of hosts others consider it only as a weak pathogen and mostly a saprophyte rather than a parasite. Wei(1950) pointed out that during the early studies with Cercospora melonis it was reported to cause an epidemic disease on cucumber and melon in glass house. It was also said that at the beginning of the present century this organism was considered a limiting factor in growing cucumber in England, France, Holland, Denmark and Germany. In 1905 Willis reported on the failure to control the disease by any known methods of control including spraying with Bordeaux mixture, fumigation with sulphur and even sterilization of soil. Moore (1949) has included this among important plant diseases in Britain and has reported the introduction of a resistant variety to be responsible for the survival of cucumber culture in hot-house. But Deighton (1936 and 1949) was of opinion that it was only either a saprophyte or a secondary parasite and that the damage caused by other diseases was attributed to this due to its conspicuous fructification on the lesions. In this connection it is pointed out by Wei that its infection on cowpea was often mixed with that of Cercospora canescens.

SYMPTOMS OF THE DISEASE.

The disease manifests itself mainly on the leaf blade the symptoms varying slightly with the host.

(1) On sesamum.

The disease first makes its appearance on the leaves as minute brown spots which gradually increases in size to 1 cm. or much more. The lesions are irregular in outline and when in large numbers they coalesce forming large patches sometimes affecting a major portion of the lamina. The spots have a brown margin with concentric zonations inside, which impart to it the common name "target spot" (Fig. 1). The spots show on both surface of the leaf. Severe infection results in considerable defoliation. On the stem reddish purple dots appear which also enlarge in size to form elliptical purplish brown spots with a lighter centre. When the spots are numerous they run into each other to form purple streaks on the stem (Fig.2). When the plants have set fruit the capsules are also attacked by the fungus causing brown spots on them. The spots vary in size from less than a mm. to 2 to 3 mm. The spots have a lighter margin (Fig.3). Heavily infected plants are killed.

(2) On tomato.

The disease manifests itself initially on the leaves,

as minute yellow discolourations which turn brown. The circular to irregular brown spots (Fig.4) have concentric rings and are encircled by a yellowish halo. In cases of severe infection the whole leaf blade becomes diseased and the leaves wither and fall down. Brown spots appear on the stem in very large numbers (Fig.2). The fungus produces spots on the calyx lobes similar to that on the leaves in which case the calyx dries up. The fungus attacks the fruit causing brown lesions with concentric zonations inside(Fig.5) There is a lighter region all round the spots. The spots vary in size from 1 or 2 mm. to nearly 1 to 2 cm. The diseased area presents a rotted appearance.

(3) On dodonaea.

Scattered brown spots with concentric zonations appear on the leaf blade. They may be round to irregular in shape from 2 to 6 mm. in diameter. Rarely larger spots are also present. On a leaf the spots are smaller in number and extensive lesions are almost absent. Infection of the stem has also not been observed.

(4) On Ageratum.

The leaf spots on Ageratum very much resemble the target spot on tomato leaves (Fig.6). The brown spots 2 to 10 mm. in size have the characteristic zonations and a yellowish halo all round the spot. When the spots coalesce large areas of the leaf blade are involved and the leaves give a scorched appearance. When infection is heavy defoliation results.

MATERIALS AND METHODS.

1. Isolation of the organism and its maintenance in culture.

The isolates of Sesamum used in the studies were made from leaves of sesamum plants naturally infected in the garden attached to the Plant Pathology Division of the Agricultural College, Vellayani. The tomato isolates were similarly obtained from the leaves naturally infected with target spot in the same garden. The Dodonaea isolates were like wise single conidium isolates from leaves of Dodonaea viscosa plants infected by the fungus in nature. The isolates from the three hosts were separately maintained on potato dextrose agar in test tube slants.

2. Single spore isolation technique.

The method described by Riker and Riker (1936) with slight modification has been followed.

Two percent plain agar in test tubes is used 10 cc. per tube after sterilisation by autoclaving. A spore suspension is made in sterile water. With the aid of a platinum transfer needle with a ringed tip a drop of this suspension is transferred to one of the test tubes containing the agar medium which has sufficiently cooled but still in a liquid state (about 45° to 50°C.). The test tube is shaken well so that the spore suspension is well distributed inside the agar. The contents of the test tube is poured in a

Petri dish so that it spreads in a thin layer and is allowed to solidify. The well-isolated spores are then located by examining the inverted Petri dish under the low power of the microscope. The ocular of the microscope is next removed, the objective is raised and a small ink dot is placed just over the isolated spore, the tip of the pen being visible through the objective of the microscope. The tip of a nichrome wire slightly flattened is twisted to form a cylin,- drical "biscuit cutter" at the end of the wire. With this flamed "biscuit cutter" a disc of agar about the marked spore nearly 5 mm. in diameter is cut and transferred to another sterile Petri dish containing PDA.

3. Host Range.

In order to determine the host range, bits of plant material suspected to be infected were collected from the field, brought to the laboratory and kept in a humid chamber for forty eight hours. When species of Corynespora were detected on observing through a stereoscopic microscope, mounts of spores were made in water on microscope slides and spore measurements made. For each of the host material one hundred spores were measured for their length and diameter. The length was measured from tip to tip and the diameter at the widest part of the conidia viz., the bulbous base just above the hilum.

4. Variation of spore size under different environments.

The size of spores on the diseased plant material when brought into the laboratory directly from the field were compared with those produced on the material placed in a moist chamber and with those produced in culture on PDA. Comparisons were also made between the size of spores collected from the atmosphere with those from the infected tissues.

A simple spore trap was used to collect spores in the atmosphere in the immediate vicinity of tomato plants heavily infected with Corynespora cassiicola. This equipment (Fig.7) consists of a large conical flask with a side tube at the lower end of its neck. The mouth of the flask is fitted with a two holed rubber bung. Through one of the holes passes vertically a copper tubing 9 mm. in diameter, the end exposed to the atmosphere being bent at right angles. The tip of this tube inside the flask is bent at right angles in the opposite direction the opening being flattened to make a rectangular orifice 11 mm. x 2 mm.. A vertical metal rod tightly fits into the other hole in the bung to the free end of which inside the flask is attached a clip serving as the slide holder. A waxed glass slide is fixed in position so that the waxed surface faces the rectangular orifice of the copper tubing the two almost touching each other. The side tube of the

flask is connected to a suction pump through pressure tubing. When the pump is worked the air from the atmosphere is sucked in through the metal tube and strikes the slide through the rectangular orifice. Most of the particles in the air impinge on the waxed slide. As the flask is placed in the midst of heavily infected tomato plants, conidia of the fungus are also carried along with the air that is drawn in. After every two hours the slides were removed and examined under the microscope and the spores measured.

5. Studies on the germination of spores.

A circular filter paper is placed at the bottom of a clean Petri dish. An ordinary clean microscope slide is placed in this dish, each end of the slide resting on a short piece of glass rod. The dish containing the slide is then sterilized in a hot air sterilizer. After cooling, the filter paper is moistened with sterile water so as to maintain a high atmospheric humidity in the dish. A drop of spore suspension is then placed on the slide and the slide inverted and placed over the pieces of glass rod in such a manner that the suspension on the slide becomes a hanging drop. This is repeated with a number of slides. The cover is removed from the Petri dish and the drop examined periodically under the microscope to study

the mode of germination of the spores.

6. Pot culture studies.

(i) Pathogenecity tests:-

Seeds of the local cultivated variety of Sesamum were sown in a well prepared nursery bed. When the seedlings were about ten days old, healthy and vigorous seedlings were selected and transplanted in pots previously filled with a mixture of soil and farm yard manure. When the seedlings had established well in the pots, they were used for the inoculation studies. The inoculum for this purpose was prepared by scraping out the aerial growth in a ten day old Petri dish culture from a sesamum isolate with a sterile scalpel and transferring it into a clean beaker containing 100 cc. of sterile water. The contents were well mixed and the suspension examined under the microscope to see whether it contained an adequate number of spores. The plants selected for the experiment were then sprayed with the inoculum with the aid of an atomiser, until uniformly wet. These plants were then covered over by bell jars to provide a humid atmosphere. Five plants were treated with the inoculum each time while an equal number served as control.

(ii) Cross-inoculation studies.

Three plant species, sesamum, tomato and dodonaea were inoculated to compare the isolates from the same three

host plants. Inocula were prepared with Petri dish cultures of the fungus as in the previous case. In each inoculation series three plants of each species were inoculated with the respective isolates. The plants were twenty days old when inoculated. Control plants were provided for each of the treatments. The treated plants were placed in humid chambers and observed for the symptoms after forty eight hours.

7. Cultural studies.

The method consisted in growing the fungus in Petri dishes and measuring the radius of the colony, which represented the amount of growth, while the daily increase was the rate of growth. In order to find out the best medium for culturing Corynespora cassiicola, the fungus was grown on six different solid culture media and the rate of growth and sporulation compared. The following were the media used for the studies:

(i) Potato dextrose agar

Potato	200 gm.
Dextrose	20 gm.
Agar	20 gm.
Distilled water	1000 cc.

(ii) Oat meal agar

Rolled oats	50 gm.
Agar	20 gm.
Distilled water	1000 cc.

(iii) Czapek's solution agar

Magnesium sulphate	0.5 gm.
KH_2PO_4	1.0 gm.
KCl	0.5 gm.
$FeSO_4$	0.01gm.
$NaNO_3$	2.0 gm.
Sucrose	30 gm.
Agar	20 gm.
Distilled water	1000 cc.

(iv) Richard's solution agar

KNO_3	10 gm.
KH_2PO_4	5 gm.
$MgSO_4$	2.5 gm.
$FeCl_3$	0.02gm.
Cane sugar	50 gm.
Agar	20 gm.
Distilled water	1000 cc.

(v) Coon's medium

Saccharose	7.2 gm.
Dextrose	3.6 gm.
$MgSO_4$	1.23gm.
Potassium acid phosphate	2.72gm.
KNO_3	2.02gm.
Agar	20 gm.
Distilled water	1000 cc.

(vi) Crabill's medium

NH_4NO_3	10 gm.
K_2HPO_4	5 gm.
$MgSO_4$	2.5 gm.
Sucrose	50 gm.
Agar	20 gm.
Distilled water	1000 cc.

The different media were prepared as per the formula. The media were then poured into pyrex test tubes at 20 cc. per tube and autoclaved.

The media were next transferred to sterile Petri dishes of 9 cm. diameter. Circular discs of 5 mm. diameter were cut from a seven day old Petri dish culture of Corynespora cassiicola grown on potato dextrose agar. These were placed one in each of the Petri dishes containing the different media at its centre. The growth of the fungus was measured after every twenty four hours till the colony spread over the entire area of the Petri dish.

EXPERIMENTAL RESULTS.

Host Range.

When fresh leaves of Ageratum conyzoides, Dodonaea viscosa and Lycopersicum esculentum with target spot symptoms, dry leaves of Carica papaya, Amorphophallus gigantea and Solanum torvum and dead stems of Abelmoschus esculentus and Morinda tinctoria collected from the field were kept in a moist chamber for forty eight hours and then examined, conidiophores and conidia of the fungus Corynespora cassiicola could be observed in abundance. Measurements of conidiophores and conidia showed that the fungus on all the above hosts were identical though there were slight variations in the size and number of septa of the conidia (Table 1).

The conidiophores and conidia produced on the different hosts were similar in shape. The conidia were obclavate to cylindrical (Fig. 8). The length of the conidiophores ranged from a minimum of 70 µ to a maximum of 330 µ. The diameter varied from a minimum of 3.2 to a maximum of 7.9 µ. The number of septa ranged between two and eighteen. The length and width of the conidia were also highly variable, though the variability in the latter was within narrow limits. The mean length and width of conidia on the different hosts are presented in table 2 and the statistical analysis of the data, in table 3.

Table 1. MEASUREMENTS (in μ) OF CONIDIOPHORES AND CONIDIA OF *CORYNESPORA CASSIICOLA*.

Name of host.	Conidiophore.		Conidia.		
	Length.	Diameter.	Length.	Diameter	No.of septa.
Ageratum conyzoides	71-325	4.0-7.2	40-226	7.2-17.5	4-15
Amorphophallus gigantea.	79-253	3.6-7.2	45-295	7.2-16.2	3-11
Carica papaya.	86-305	3.6-7.2	32-285	5.4-15.4	6-15
Dodonaea viscosa.	73-330	3.2-7.2	22-208	7.2-15.4	2-8
Hibiscus esculentus.	70-252	5.2-7.9	64-190	10.7-17.9	8-12
Lycopersicum esculentum.	73-326	5.2-7.2	47-254	7.2-10.8	4-14
Morinda tinctoria.	78-259	4.5-7.7	46-171	7.2-90	5-12
Sesamum indicum.	124-272	3.6-7.8	43-253	5.4-9.5	4-18
Solanum torvum.	79-260	5.4-7.2	68-175	7.2-9.0	5-11

Table 2. VARIATION IN SPORE SIZE ON DIFFERENT HOST PLANTS: *C. cassiicola.*

	Host.	Mean length of conidia in µ.	Mean width of conidia µ.
A.	*Ageratum conyzoides.*	134	8.2
B.	*Amorphophallus gigantea.*	165	9.8
C.	*Carica papaya.*	168	9.8
D.	*Dodonaea viscosa.*	153	9.1
E.	*Hibiscus esculentus.*	109	14.0
F.	*Lycopersicum esculentum.*	155	8.8
G.	*Morinda tinctoria.*	156	8.7
H.	*Sesamum indicum.*	154	10.7
I.	*Solanum torvum.*	126	8.3

Table 3. STATISTICAL SIGNIFICANCE OF DATA OF TABLE 2.

	Degrees of freedom	Length of conidia			Width of conidia		
		Calculated value of t	Value of t from tables	Significant or not P=0.05	Calculated value of t	t from tables	Significant or not P=0.05
Between A and B	38	1.86	2.02	N.S.	2.29	2.02	S
Between A and C	38	2.35	2.02	S	2.79	2.02	S
Between A and D	38	1.09	2.02	N.S.	2.30	2.02	S
Between A and E	22	0.8	2.07	N.S	2.30	2.07	S
Between A and F	38	1.45	2.02	N.S.	2.20	2.02	S
Between A and G	24	0.93	2.06	N.S	1.38	2.06	N.S
Between A and H	38	1.32	2.02	N.S.	2.50	2.02	S
Between A and I	30	0.43	2.04	N.S.	0.06	2.04	N.S
Between B and C	38	0.22	2.02	N.S.	0.00	2.02	N.S
Between B and D	38	0.72	2.02	N.S.	0.94	2.02	N.S
Between B and E	22	2.02	2.07	N.S	2.53	2.07	S.
Between B and F	38	0.64	2.02	N.S	1.40	2.02	N.S.
Between B and G	24	0.42	2.06	N.S	0.42	2.06	N.S.
Between B and H	38	0.77	2.02	N.S	0.76	2.02	N.S
Between B and I	30	2.42	2.04	S	1.74	2.04	N.S

1.	2.	3.	4.	5.	6.	7.	8.
Between C and D	38	1.03	2.02	N.S	1.11	2.02	N.S
Between C and E	22	2.94	2.07	S	5.4	2.07	S
Between C and F	38	1.06	2.02	N.S	1.70	2.02	N.S
Between C and G	24	0.83	2.06	N.S	3.22	2.06	S
Between C and H	38	1.26	2.02	N.S	0.81	2.02	N.S
Between C and I	30	3.53	2.04	S	5.17	2.04	S
Between D and E	22	1.43	2.07	N.S	4.83	2.07	S
Between D and F	38	0.19	2.02	N.S	0.80	2.02	N.S
Between D and G	24	0.12	2.06	N.S	0.61	2.06	N.S
Between D and H	38	0.06	2.02	N.S	1.55	2.02	N.S
Between D and I	30	1.50	2.04	N.S	1.57	2.04	N.S
Between E and F	22	2.03	2.07	N.S	2.06	2.07	N.S
Between E and G	8	1.90	2.31	N.S	3.80	2.31	S
Between E and H	22	1.98	2.07	N.S	1.43	2.07	N.S
Between E and I	14	0.77	2.15	N.S	5.70	2.15	S
Between F and H	38	0.15	2.02	N.S	1.91	2.02	N.S
Between F and I	30	2.10	2.04	S	1.70	2.04	N.S.
Between G and H	24	0.12	2.06	N.S	1.08	2.06	N.S.
Between G and I	16	2.03	2.12	N.S	1.03	2.12	N.S
Between H and I	30	2.09	2.04	S	1.13	2.04	N.S

S=Significant
N.S=Not significant.

Variation of spore size in different environments and substrates.

The spores of the fungus collected with the spore trap from the atmosphere in the immediate vicinity of the heavily infected tomato plants resembled those of Helminthosporium in their shape, being broad at the base and gradually tapering towards the apex. They were straight or curved, the longer ones being mostly curved. A few cylindrical ones were also present. They measured 55-168x12-18 µ.

When the diseased tomato leaves collected from the field were brought to the laboratory and examined immediately the conidia present on the leaf spot were quite similar to those collected from the atmosphere measuring 47-161 x 11-21 µ (Fig.8). The conidiophores were dark brown while the conidia were lighter in colour than the former.

Infected tomato leaves placed in a humid chamber when examined after two days were found to produce conidiophores which were slightly longer. The conidia formed in the moist chamber were generally long, more slender than those on the leaves prior to putting in the humid chamber. They tapered only very gradually towards the apex so that they appeared to be more or less cylindrical. A very few had the typical Helminthosporium shape with a broad base perceptibly tapering towards the apex. The spores in either

case had the thickened walls common to the genus Corynespora. Under humid conditions the conidia were also formed in a catenulate fashion.

The fungus when cultured on potato dextrose agar sporulated within five days of starting the culture. The conidia were more slender than those on the host material. Most of them were cylindrical and formed in chains of two to five and its colour tended to be hyaline unlike that on the host material. The variation in size of conidiophores and conidia on the host material directly brought in from the field, when placed in a humid chamber and when produced in culture on potato dextrose agar could be seen from table 4.

From the statistical scrutiny of the above data it was found that there was significant difference in the length of conidia produced on the leaf spots on material placed in the humid chamber and those present on the leaf spots on material taken directly from the field. The difference in length of the conidia produced in the moist chamber and that in culture on potato dextrose agar was also statistically significant. The conidia on tissues placed in the humid chamber were always longer. But in regard to the length of conidia from material taken directly from the field and those in the culture there was no significant difference.

The width of the conidia was also similarly tested.

Table 4. MEASUREMENTS (MEAN VALUE IN µ) OF CONIDIA OF *Corynespora cassiicola* COLLECTED DIRECT FROM FIELD, FROM MATERIAL IN MOIST CHAMBER AND FROM PDA CULTURE.

	Direct from field. (A)		From moist chamber. (B)		From culture. (C)	
	Length	Width	Length	Width	Length	Width
Mean value	88.00	16.2	158.00	10.28	75.00	8.00
Standard deviation	24.15	2.65	46.04	4.05	36.00	1.2

The width was maximum in the case of conidia on material taken directly from the field and least in that produced in culture. The statistical analysis of the data are given in table 5.

Cultural characters.

A single spore isolate of the fungus from sesamum when cultured on potato dextrose agar in Petri dish grew and spread over the entire surface of the medium in about eight days. It also sporulated profusely on this medium. At first the mycelium was thin and hyaline forming a white flocculent mass on the surface of the medium. Gradually it assumed a light greyish tinge which progressively became darker ultimately forming an olivaceous black cushiony mat on the surface of the medium in the Petri dish. When the mycelium had spread over the entire surface the whole surface presented a blackish colouration. When the culture was examined from the underside of the Petri dish also the dark colouration could be perceived. But when the culture medium below the mycelium was scraped off and examined it was found that the medium did not take the colour of the mycelium. By the fifth day after the culture was started, conidia began to appear. Initially the conidiophores and conidia both appeared hyaline. As the growth progressed the conidiophores assumed a dusky brown colour while the conidia developed a pale brown shade. The conidia were produced terminally on the conidiophores either singly or

Table 5. STATISTICAL SIGNIFICANCE OF DATA IN TABLE 4.

	Degrees of freedom.	Calculated value of t	Value of t from tables	Whether significant or not (P=0.05)
		LENGTH OF CONIDIA.		
Between A and B	24	6.48	2.06	Significant.
Between A and O	24	1.45	2.06	Not significant.
Between B and C	24	6.86	2.06	Significant.
		DIAMETER OF CONIDIA.		
Between A and B	24	6.11	2.06	Significant.
Between A and C	24	13.60	2.06	Significant.
Between B and C	24	2.90	2.06	Significant

in a catenulate fashion (Fig.8). When borne in chains they were usually two to five in a chain. The conidia were produced acropetally, the oldest one being at the tip of the conidiophore, from the apex of which a secondary conidium was produced which in turn gave rise to the next one at its apex, the youngest being the terminal one. Quite often two or three conidia were found in a chain all of which were immature. Frequently they germinated even before they separated from one another and the developing germ tubes gave the impression of being connected by intercalary plugs. The terminal conidium was also seen to produce germ tubes before it had got itself detached from the chain.

In culture, hyaline vesicles were frequently observed in various positions on the conidia (Fig.9). Sometimes these were noticed at the basal portion of the conidium. In others they were seen to occur at the middle of its length enclosing one or two cells. This vesicle formation was observed only when the fungus was grown in culture and never on those produced on the lesions on the leaves, stem or capsule.

In culture, rarely a conidiophore was found to bear at its apex two conidia side by side. The wall of the conidiophore thickened at its apex with a characteristic bulging. At the tip of the conidiophore there were a

number of constrictions occurring in succession. At these constrictions were seen scars which gave the impression of septa. Some times the conidiophore had a bulbous base. The conidiophores developed either singly or in clusters (Fig.9) at the apex of each of which conidia were borne either singly or in chains. Frequently a hyaline isthmus was also found between the conidium and the conidiophore as well as between successive conidia. Conidiophores were simple, usually straight, brown in colour and mostly septate. They measured 73-325 by 3.6-7.8 /u.

The conidia were mostly five to nine septate, but those with no septa and upto fourteen septa were not uncommon. When cultured on potato dextrose agar they measured 14.4-216x5.4-10.8 /u. and were unicellular to fourteen septate. The shape of the conidia varied, some being obclavate, broader at the base and gradually tapering towards the apex while the others were cylindrical, this type being more common than the former. The conidia as soon as they were formed were hyaline but developed a pale brown colour as the culture grew old. After the culture had spread over the entire surface of the medium in the Petri dish, numerous thick walled chlamydospores made their appearance. (Fig.9). These were of various shapes most of them being irregularly spherical and measuring 14 to 20 /u in diameter. In old cultures these were found in large numbers

They were hyaline and were intercalary or terminal.

The cultural characteristics and measurements of conidiophores and conidia of tomato and Dodonaea isolates were similar to those of the sesamum isolate.

Growth and variation of the fungus in different media.

The radial growth of the fungus was measured after every twenty four hours after inoculation of the media in the Petri dishes. As the growth was not quite perceptible by the end of the first twenty four hours, the measurements were actually commenced only after forty eight hours. The measurements were continued till the entire surface of the Petri dish was covered by the mycelium. The growth in each of the six different media was measured in milli meters. The daily measurements are furnished in table 6. The comparative growth of the colonies in different media on the eighth day could be seen from (Fig.10).

Colonies on potato dextrose agar inoculated in the centre grew fairly rapidly attaining a diameter of 8 to 9 cm. in eight days at room temperature. (Table 7). At first a white woolly growth developed. On the third day a light olive grey colour appeared starting from the point of inoculation, the edge of the growing colony being always white. The aerial hyphae which was loose in the

Table 6. RADIAL GROWTH IN MILLI METRE (MEAN OF FIVE REPLICATIONS OF COLONY OF C.cassiicola IN DIFFERENT MEDIA.

Time	PDA (A)	Czapek's agar. (B)	Oat agar (C)	Coon's agar (D)	Richard's agar (E)	Crabill's agar. (F)
After 48 hours	5.7	4.9	2.8	2.9	2.8	1.7
After 72 hours	11.6	9.5	7.5	7.8	7.4	2.7
After 96 hours	17.6	16.1	13.8	13.5	14.0	4.6
After 120 hours	23.6	21.6	19.0	18.6	18.7	5.9
After 144 hours	30.2	26.8	24.6	22.7	21.4	7.6
After 168 hours	36.1	32.5	29.4	27.0	25.0	7.8
After 192 hours	40.7	35.6	34.3	30.5	29.1	8.1

Table 7. GROWTH MEASUREMENTS OF COLONY ON THE EIGHTH DAY AFTER STARTING THE CULTURE.

Replication Number.	PDA (A)	Czapek's agar. (B)	Oat agar (C)	Coon's agar. (D)	Richard's agar (E)	Crabill's agar. (F)
I.	41.0	35.0	34.0	28.0	28.0	8.5
II.	40.5	36.5	35.5	31.5	28.5	8.0
III.	40.5	36.5	36.0	31.5	30.0	8.5
IV.	41.5	35.0	32.5	30.0	29.0	8.0
V.	40.0	35.0	33.5	31.5	30.0	9.5
Mean	40.7	35.6	34.3	30.5	29.1	8.5

beginning, became dense and felt-like and formed an olivaceous mat at the surface of the medium. In the initial stages there were concentric circular zones in the culture which disappeared when the colony has spread uniformly and has taken a homogeneous dark olive colouration. On the reverse also the colour was dark.

Colonies on Czapek solution agar attained a diameter of 7 cm. in eight days with texture similar to that on PDA. The rate of growth was slower than in PDA and the colour of the colony on the upper and lower surfaces were just as in the former.

The growth of the colonies on oat-agar was almost as rapid as that on Czapek's solution agar reaching a diameter of 6.5 to 7 cm. in eight days. The other colony characteristics were quite akin to those on the previous two media.

Colonies on Coon's agar growing some what less rapidly than on the previous three reached a diameter of about 6.1 cm. in eight days. The aerial mycelium was lighter than in the former and the same colour could be seen on either surface.

On Richard's agar the growth was still lower and attained a diameter of 5.8 cm. in eight days. The colour of the colony was just as in the Coon's agar medium.

In Crabill's medium the rate of growth of the colony was least. The growth was abnormal and the colour of the colony was dark brown and darker than in all the other media. The fungus after producing this type of growth for a short time ceased growing altogether. The maximum diameter attained by the colony was only 1.9 cm. The fungus failed to sporulate at any stage in this medium. But chlamydospore-like swellings were present on the hyphae in large numbers. These were irregularly spherical in outline and measured 14 to 20 μ. (Fig. 9).

In all the other five media the fungus sporulated on the fifth day of starting the culture. The conidial characters were similar in all the media and agreed with the description already given under cultural characteristics in PDA. The fungus sporulated equally well in all these media. It was also observed that the agar media beneath the colony did not take any colour. So the fungus does not seem to produce any pigment when grown in any of these media. A statistical scrutiny of the radial growth measurements on the eighth day indicated that Potato dextrose agar was significantly superior to all the others for growing Corynespora cassiicola followed by Czapek's medium, oat-agar, Coon's medium, Richard's medium and Crabill's medium in the same serial order.

In all the media, to begin with the rate of growth

was slow, which increased at a rapid rate whereafter it again slowed down slightly as is generally the case with fungi in culture. This could be clearly seen from the growth curves drawn with growth in mm. on the Y axis and time on the X axis. (Fig. 11).

The structure of the conidia in all the media were uniformly the same. Measurements of length and width of conidia produced in the five different media were taken. For this purpose the conidia were selected at random. There was no variation in the diameter of the conidia in the different media. The diameter ranged from 7.2 to 9.0 µ. The length of the conidia produced in the different media appeared to show some differences as could be seen from table 9. So the measurements obtained were analysed statistically. The results are presented in table 10.

The statistical analysis of the data indicate that the length of the conidia produced in PDA culture is significantly more than those in all the other media excepting Coon's agar though the average length in the former is greater than that in the latter. There is no significant difference in the conidial length between PDA and Coon's agar. The conidia produced in Coon's agar were significantly longer than those in Czapek's and Oat-agar. In Richard's and Coon's media there was no significant difference in the length of conidia. The average length of conidia was maximum in PDA.

Table 8. ANALYSIS OF VARIANCE OF DATA OF TABLE 7.

Source.	Sum of squares.	Degrees of freedom	Variance.	F (calculated)	F (from tables) (P=0.05)	Whether significant or not.	C.D.
Total	3163.35	29					
Treatment.	3136.85	5	627.37	570.34	2.62	Significant	0.908
Error	26.50	24	1.10				

Conclusion: A B C D E F

Table 9. MEAN LENGTH OF CONIDIA (in μ) OF Corynespora cassiicola IN DIFFERENT MEDIA.

PDA (A)	Czapek's agar (B)	Oat agar (C)	Coon's agar (D)	Richard's agar (E)
61.9	35.6	36.7	52.6	40.5

Table 10. STATISTICAL SIGNIFICANCE OF DATA OF TABLE 9.

	Degrees of freedom	Calculated value of t	Value of t from tables	Whether significant or not (P=0.05)
Between A and B	9	3.08	2.26	Significant.
Between A and C	9	3.37	2.26	Significant.
Between A and D	9	1.15	2.26	Not significant.
Between A and E	9	2.80	2.26	Significant.
Between B and C	9	0.20	2.26	Not significant.
Between B and D	9	2.55	2.26	Significant.
Between B and E	9	0.82	2.26	Not significant.
Between C and D	9	3.10	2.26	Significant.
Between C and E	9	0.90	2.26	Not significant.
Between D and E	9	2.25	2.26	Not significant.

Germination of Spores.

When the conidia of Corynespora cassiicola mounted in hanging drops of distilled water were kept over-night in a moist chamber a good number were seen to have produced germ tubes from the end cells. In the basal cell the germ tube protruded through the centre of the hilum while in the apical cell it passed directly through the thin wall. Examination of the conidia a day later revealed that a few of them were found to produce germ-tubes laterally from an intermediate cell.

Pathogenecity of the fungus.

Healthy sesamum plants in pots were inoculated with a suspension of mycelial fragments and spores of the isolate from sesamum. The sesamum plants exhibited symptoms of infection three days after inoculation. Minute dark spots developed on the leaves. These spots gradually increased in size forming irregular light brown lesions with reddish brown margin and a lighter central portion. When the lesions on the leaves were numerous these coalesced resulting in a scorched appearance. The spots had the characteristic concentric zonations inside. The infection became severe after a fortnight when the necrosis spread over the entire leaf surface followed by withering and defoliation. On the stem the lesions appeared as small elongated purple spots with a lighter central region.

The spots gradually enlarged in size. When in large numbers these spots coalesced and gave the appearance of purplish streaks along the length of the stem. When the plants fruited dark brown circular spots appeared on the capsules. The fungus could be recovered from these infected plants.

Cross inoculation studies.

Cultures of the fungus isolated from diseased sesamum, tomato and Dodonaea leaves were used for preparing inocula. The inoculum consisting of a suspension of spores and mycelial bits in sterile water was sprayed on the test plants. Each of the isolates was tested on all the three host plants.

On sesamum and tomato the lesions appeared on the leaves on the third day after inoculation while on Dodonaea the lesions were apparent only on the fourth day. The sesamum, tomato and Dodonaea isolates were all pathogenic on the three hosts. As in nature the symptoms on Dodonaea were only mild.

Inoculation on cowpea and soybean.

As Corynespora cassiicola was found to be highly parasitic on cowpea and soybean in other countries inoculations with the isolates obtained from sesamum, tomato

and Dodonaea were made on potted two weeks old local variety of cowpea and Pocha's edible podded Bansei variety of soybean plants. Neither of the plants took infection. Further a few potted plants kept in the immediate vicinity of heavily infected sesamum and tomato did not develop any symptom of attack of this fungus.

DISCUSSION.

The results of the present studies indicate that Corynespora cassiicola has a wide host range in Kerala. This strengthens the views expressed earlier by Mohanty and Mohanty (1955) that in India the fungus probably has a wider host range than noted hitherto. In other countries this has been found on a very large number of host plants. In India it has so far been reported on Corchorus capsularis L. by Wei (1950), on tomato, Sesamum and Croton sparsiflorus by Mohanty and Mohanty (1955), on Carica papaya by Mohanty and Behera (1958), on Rauwolfia serpentina Benth. by Mohanty (1958) and on Phaseolus mungo L. by Addy and Mohanty (1959). A few more hosts for the fungus have been found in Kerala as a result of the present investigation. It occurs as a parasite on Ageratum conyzoides and Dodonaea viscosa causing leaf spots. In a saprophytic state it has been found on dead leaves of Amorphophallus gigantea and Solanum torvum and on dead stems of Hibiscus esculentus and Morinda tinctoria. The fungus also occurs on Carica papaya which host has already been reported from elsewhere.

The air in the vicinity of the infected sesamum and tomato crop is rich in spores of the fungus. These could be trapped in very large numbers on waxed glass slides with the help of a simple spore trap kept in the

neighbourhood of the diseased plants. The identity of these spores has been established by comparing them with those occurring on plant material directly taken from the field.

Wei (1950) has stated that the conidia produced on succulent host tissues were narrower than those on dry tissues and those formed on agar media were even more slender than those on the tissues. Olive et al. (1945) found the conidia of Helminthosporium vignae present on diseased leaves when first brought into the laboratory to measure 8-19x40-270 µ with an average of 17x120 µ while those formed when the same leaves were kept in the moist chamber measured 7-11x40-306 µ with an average of 10x150-250 µ. Welles (1924) found the fruiting structures of species of Cercospora on various hosts to be longer by 50 to 150 per cent when produced during the rainy season. He also showed by experiments with four species that the conidia on leaves exposed to a saturated atmosphere for three to four days were 30 to 80 per cent longer than those from undisturbed field lesions collected at the same time. Hence in the present studies the conidia obtained from diseased tomato leaves gathered directly from the field were compared with those produced on material kept in a humid chamber and with those produced in PDA culture. The average measurements of the conidia were 88x16 µ, 158x10 µ and 75x8 µ respectively. A statistical scrutiny of the

measurements of length indicated that the length of conidia on tissue kept in the moist chamber was more than that in the other two cases. With respect to the diameter, there was significant difference between all the three groups. It was maximum in the case of conidia obtained directly from material in the field and minimum in those produced in culture. The spores examined on the host taken directly from the field and those produced on the host tissue in the moist chamber were all formed on the same substrate viz., tomato leaf. The variation in the size of the spores in the above cases may be due to the environmental factors. In the humid chamber free moisture and high humidity will be available to the fungus for a longer period whereas in the field the operation of these factors is unsteady.

The significant difference in the size of the spores formed on PDA culture and on tomato tissues is a question of difference in substrate and environment. There is also significant difference in the size of spores produced by the fungus on different culture media. This may be due to the influence of the substrate. Variation in the size and septation of the conidia and conidiophores is also seen in the case of spores collected from other natural substrates on some of which the fungus occurs as a saprophyte (Table 1). The conidia produced on Amorphophallus gigantea and Carica papaya were significantly longer than the ones on the other

seven hosts. Though the mean length indicated significant differences between hosts, these could not be strictly grouped into distinct classes on the basis of their length. Wei (1950) tried to arrange his collections into two species depending upon the length of the spores, a short spored one and a long spored one but could not readily make such a division due to the range of one merging into that of the other and there being many intermediate types also. The width of the conidia is also a highly variable factor. On examining the data on spore measurements from the nine hosts the widest conidia (based on the mean width) were found on Hibiscus esculentus and Sesamum indicum. The statistical analysis of the data revealed that the conidia from Hibiscus esculentus was significantly broader than the rest. This variation in the size of spores on different hosts may be due to the difference in the nutritional status of the substrate. The above observations confirm the earlier findings of Stone and Jones (1959) that the host influences the size of conidia. They found that the sesamum and soybean isolates when inoculated on these plants produced wider conidia on sesamum than on soybean.

The growth studies were intended only to find out a suitable media for culturing the fungus. Lilly and Barnett (1951) and Cochrane (1958) have ruled out this method for conclusive growth studies as this method neglects the

thickness of the colony and ignores the mycelium buried in the agar. But as the growth of the fungus in the six media showed perceptible difference in the colony characters, the growth measurements were subjected to statistical scrutiny. The statistical analysis of the radial growth measurement data revealed that the rate of growth was uniformly better in PDA than in all the other media. The difference in growth was statistically significant. Judging from the rate of growth, nature and density of growth, pigmentation and sporulation, PDA was found to be the best medium followed by Czapek's agar, oat agar, Coon's medium, Richard's medium and Crabill's medium in the same order.

This fungus shows certain similarities to Helminthosporium. Possibly it was only on account of this that the organism was originally placed under the genus Helminthosporium.

In culture the conidia of the fungus arise in a catenulate fashion in acropetal succession which is in conformity with the earlier findings. The tendency towards catenulation also occurs in certain species of Helminthosporium viz., Helminthosporium catenarium Drechs., Helminthosporium carposaprum Pollack, H.papulosum Berg and H.phlei Scherif. Hyaline vesicles were formed in different positions of the conidia of C.cassiicola in

culture. Similar structures were observed by Berg.(1934) on the conidia of H.papulosum.

Germination of the spores of the fungus is bipolar which is a character of the genus Corynespora but then it is also a character of many of the graminicolous species of Helminthosporium which are included under the new genus Bipolaris.

In the light of these similarities it is worth while to re-examine the systematic position of the fungus. Though the other species of Corynespora described by Ellis (1957) show marked differences in structure from that of Helminthosporium, C.cassiicola resembles Helminthosporium in many respects. Possibly H.cassiicola an earlier name given to this fungus may be more appropriate.

Mohanty and Mohanty (1955) isolated the fungus from both tomato and sesamum but did not study the pathogenicity of the latter on either sesamum or tomato. In America Stone and Jones (1959) isolated the fungus from sesamum but did not test its effect on tomato. So cross inoculation and cultural studies with the isolates from the leaf spots of sesamum, tomato and dodonaea were made. Each of these isolates was found to be identical in its reaction to the three host plants.

Earlier workers strongly believed in the existence

of physiological races in the fungus. Olive and his co-workers (1945) concluded from their experiments on Helminthosporium vignae (the name by which Corynespora cassiicola was then known) that there are two parasitic races of the fungus. The one isolated from cowpea leaves was capable of causing severe infection of cowpea and light spotting of soybeans and the second which they designated as Race 2 isolated from leaves of soybean morphologically similar on the host tissue and in culture, but producing only light spotting of soybeans and few to many small spots on cowpea. The latter was also found to cause very little damage to both the host plants. Stone and Jones (1959) observed that his isolates from both sesamum and soybean caused severe infection of soybean and light infection of cowpea. Hence they suggested the possibility of a third race.

The isolates from sesamum, dodonaea and tomato did not infect cowpea or sesamum either when they were inoculated with these isolates or when potted plants were placed very close to highly diseased sesamum and tomato crops. This indicates the presence of a possible fourth race. Since the races tried by the earlier workers were not available for comparison and the varieties of cowpea and soybean used by them were also not available a definite conclusion could not be arrived at.

SUMMARY.

A study of the fungus Corynespora cassiicola (Berk.and Curt.)Wei causing target spot of Sesamum indicum, Lycopersicum esculentum, and Dodonaea viscosa has been made.

Two new host plants, Ageratum conyzoides and Dodonaea viscosa were found during the present investigations. The fungus has also been observed in a saprophytic state on Amorphophallus gigantea, Carica papaya, Hibiscus esculentus, Morinda tinctoria and Solanum torvum.

The air in the neighbour-hood of the diseased gingelly and tomato crops was rich in the spores of the fungus. The spores were similar in shape and size to the spores on the diseased plants in the field.

The organism was brought into pure culture and its pathogenecity on sesamum, tomato and dodonaea established.

A comparison of the size of conidia produced when the host material was kept in a humid chamber, the spores on the host in the field and those produced in PDA culture indicated that the conidia in the humid chamber are longer than in the other two cases and in culture the spores were more slender.

The organism grew and sporulated well in potato dextrose agar, Czapek's medium, oat agar, Coon's medium and

Richard's medium. The best growth and production of conidia were on PDA.

There was perceptible variation in the size of conidia produced on different substrates.

In morphological characters the fungus very much resembled certain species of Helminthosporium. The germination of the spores of the fungus was bipolar and rarely germ tubes were produced from intermediate cells. On hosts under high humidity and in culture the organism usually produced catenulate conidia.

The isolates from Sesamum indicum, Lycopersicum esculentum and Dodonaea viscosa were identical in their morphological characters and pathogenecity.

The isolates from these three hosts failed to infect cowpea and soybean indicating the presence of a new physiologic Race.

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* Originals not seen. Information taken either from Review of Applied Mycology or from other sources.

Fig. 1. Target spot symptoms on sesamum leaves caused by *C. cassiicola.*

Fig. 2. On the left are two bits of sesamum stem and on the right a bit of tomato stem showing lesions.

Fig. 1

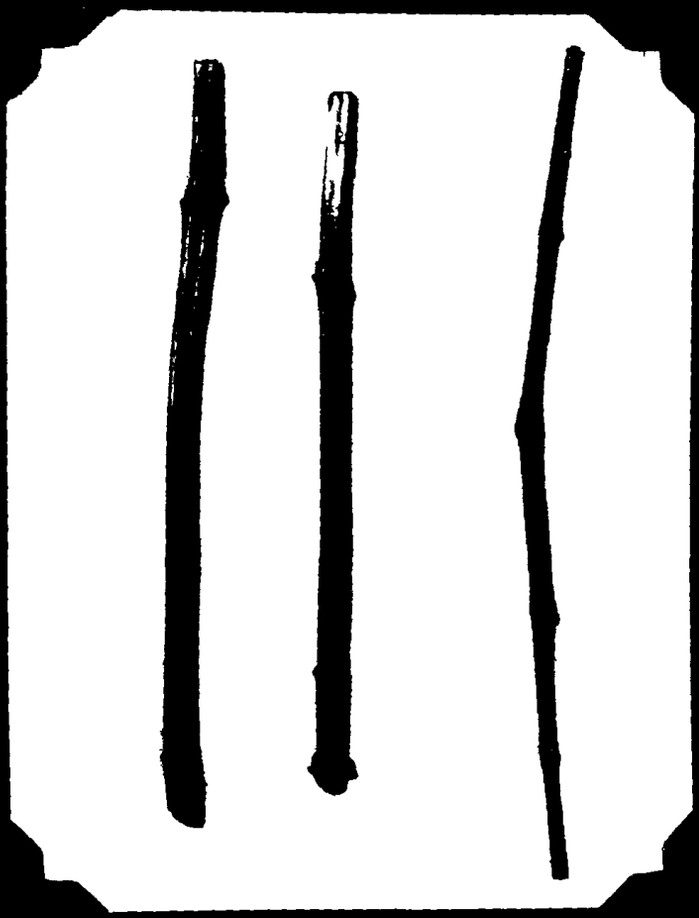


Fig. 2

Fig. 3. Sesamum capsules with symptoms of attack of the fungus.

Fig. 3

Fig. 4. Tomato leaves with target spot symptoms.

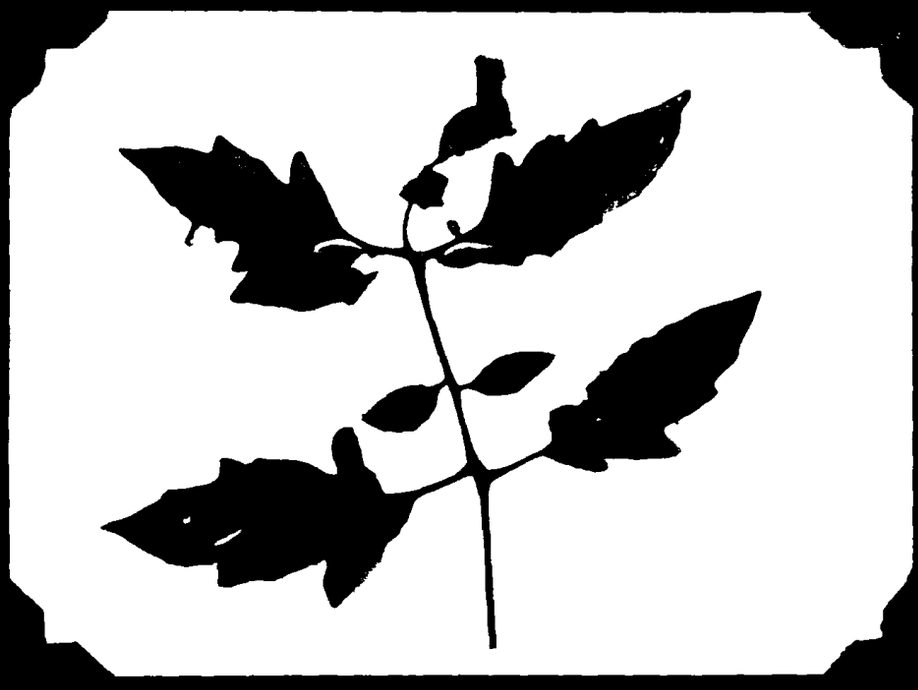


Fig. 4

Fig. 5 Tomato fruit with the characteristic zonate spot due to the attack of C. cassiicola.

Fig. 5a. Target spot symptoms on the leaves of Dodonaea viscosa.

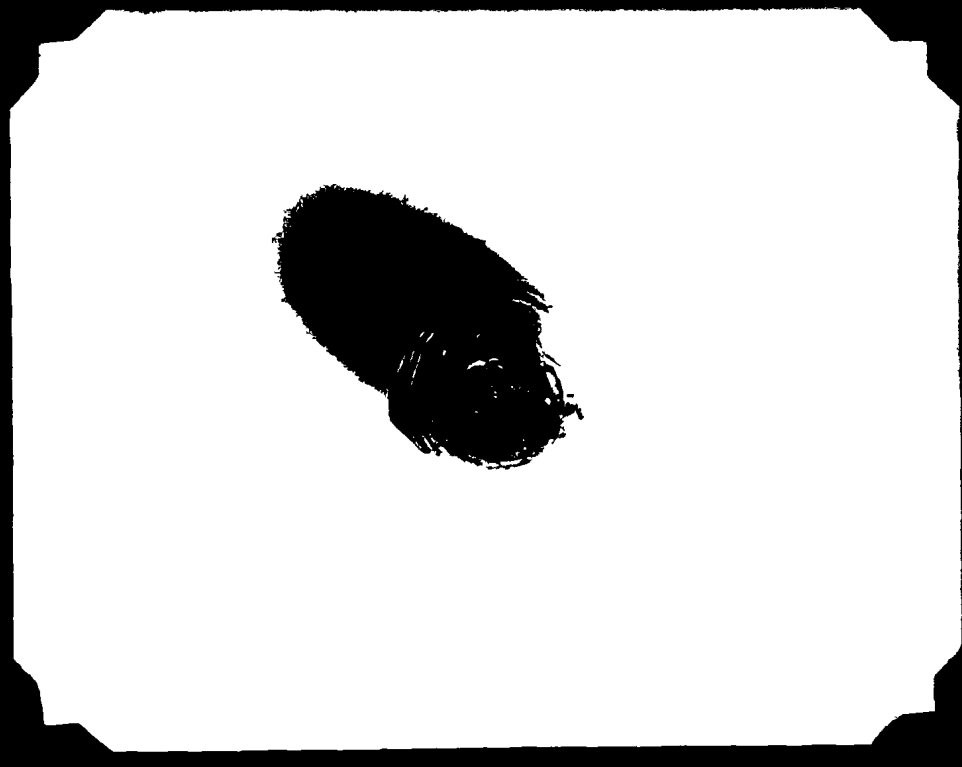


Fig. 5

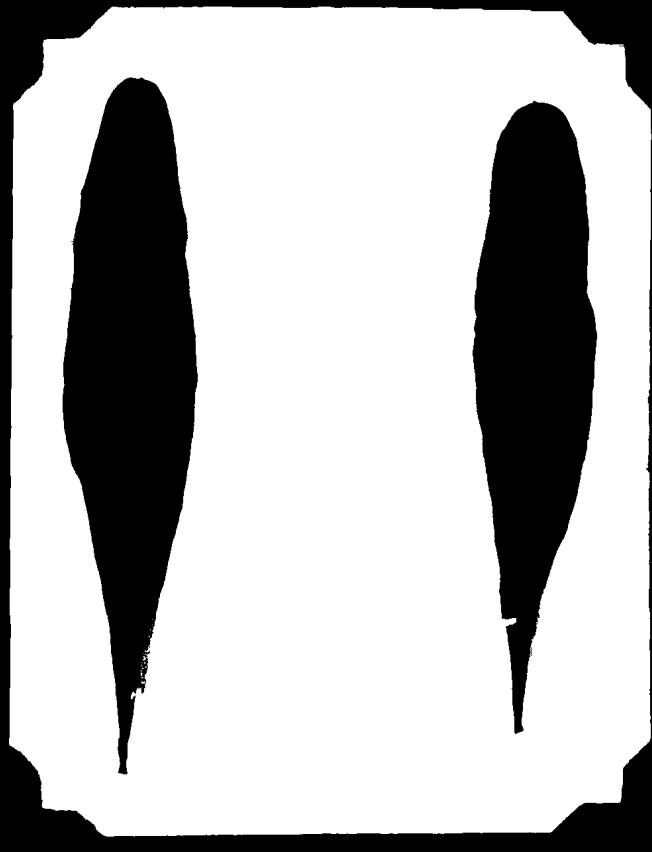


Fig. 5a.

Fig. 6. Spots due to the attack of *C. cassiicola* on the leaves of *Ageratum conyzoides*.

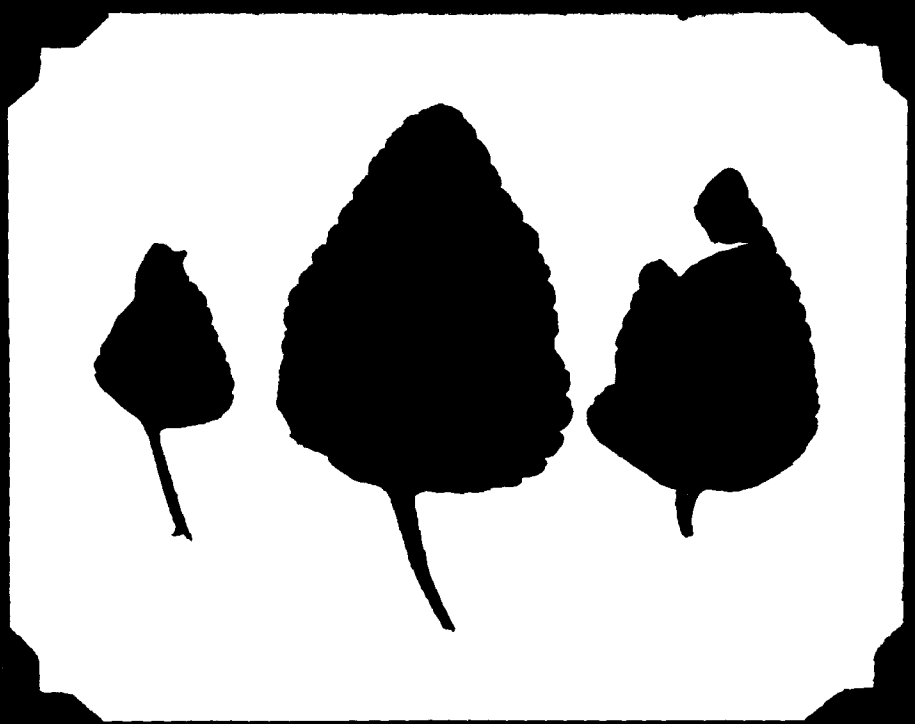
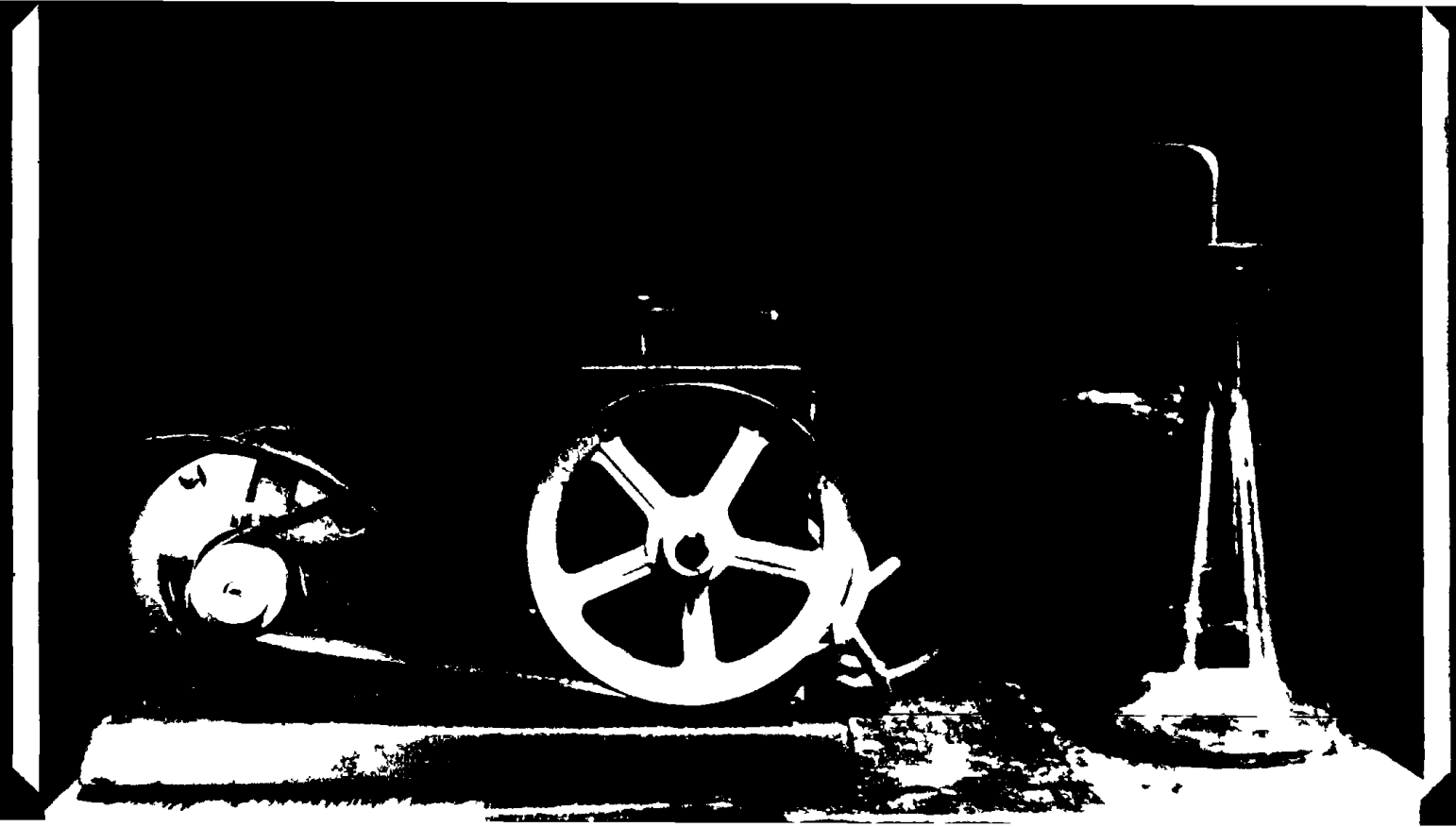


Fig. 6

Fig. 7. A simple spore-trap-connected to a suction pump. The waxed glass slide could be seen inside the flask.



GROWTH OF CORYNESPORA CASSIICOLA IN DIFFERENT MEDIA

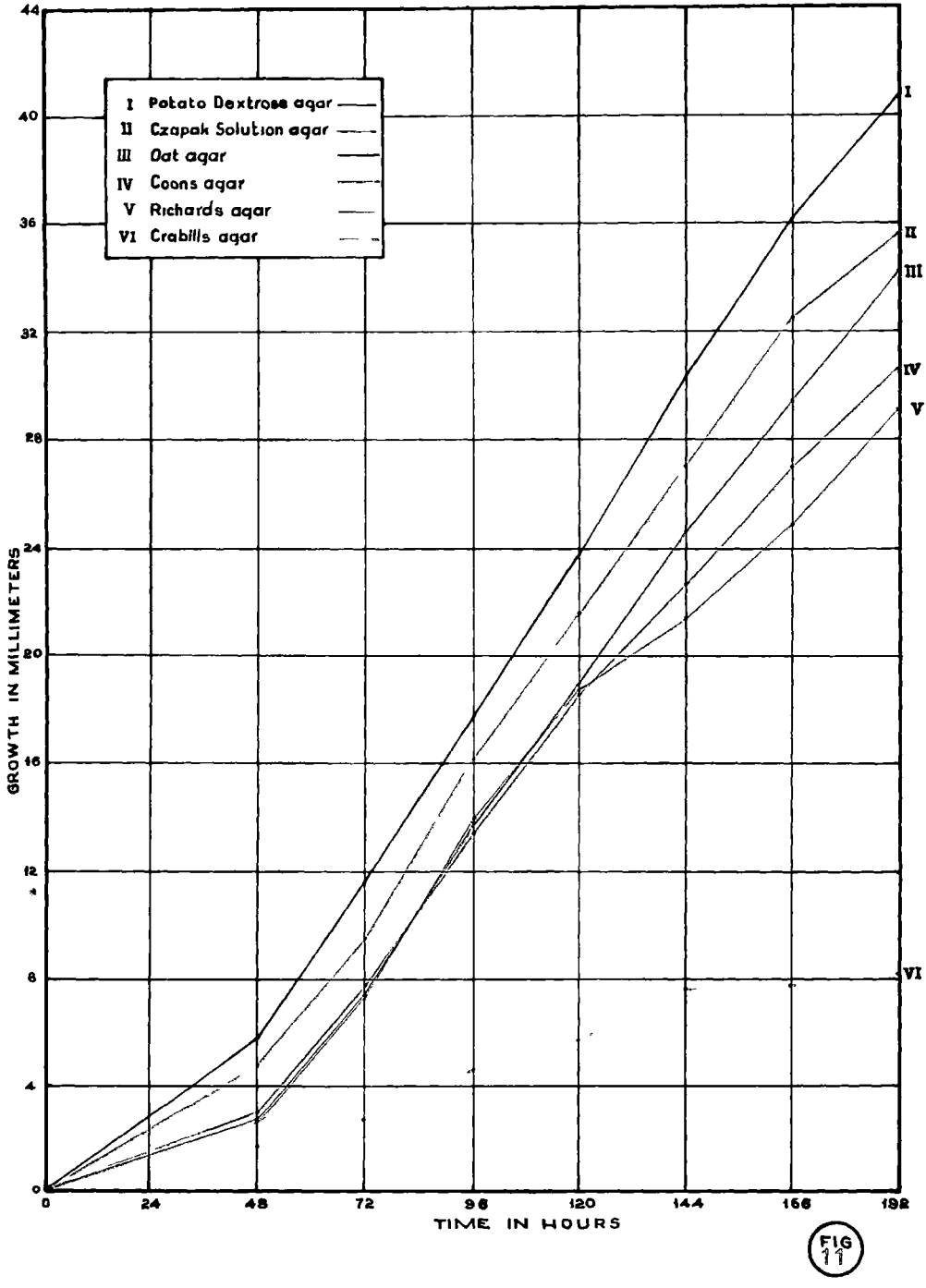
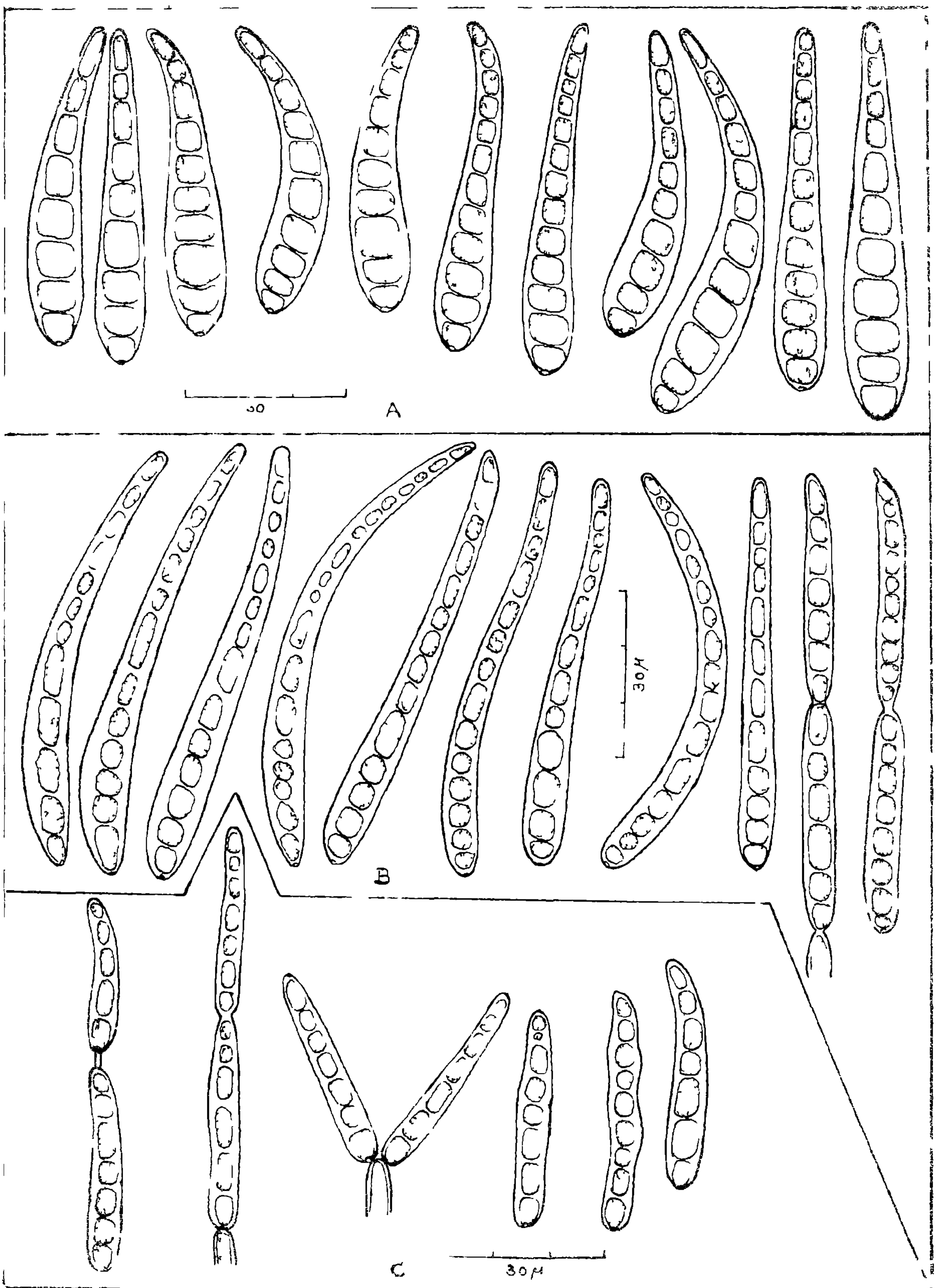


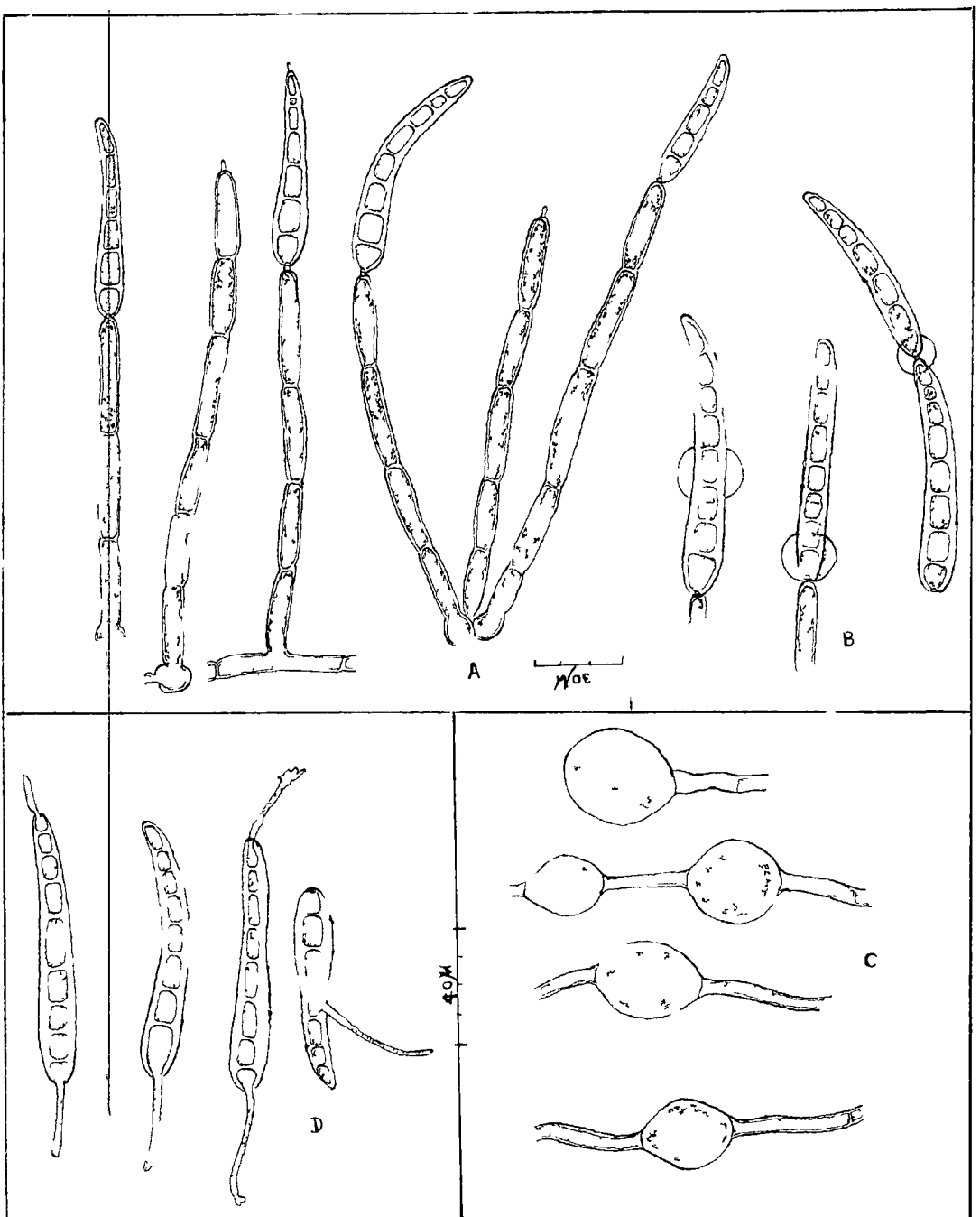
FIG 11

FIG 8



A ON HOST MATERIAL DIRECT FROM FIELD
B ON MATERIAL KEPT IN MOIST CHAMBER   C IN [illegible] CULTU[illegible]

FIG. 9



A CONIDIOPHORES

B VESICLES ON CONIDIA

C CHLAMYDOSPORES

D GERMINATING SPORES

Fig.10. Growth of the fungus in six different media.

a. PDA
b. Czapek's medium
c. Oat-meal agar
d. Coon's medium
e. Richard's medium
f. Crabill's medium

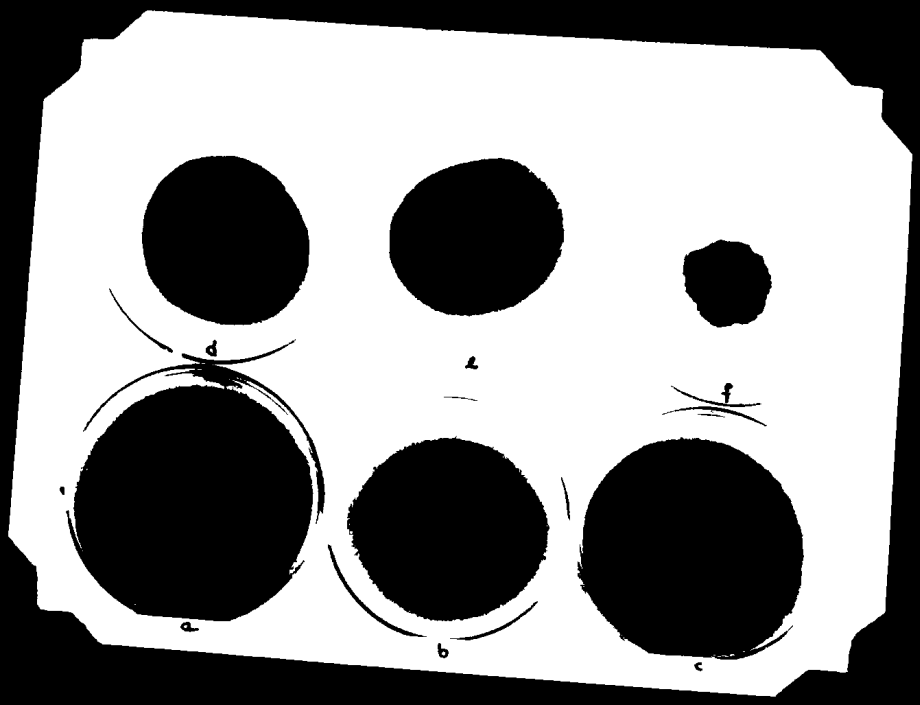


Fig. 10