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STUDIES IN THE GENUS *CERCOSPORA**

BY

UDAI BHAN SING, M. Sc.,

Research Scholar, Department of Botany, University of Allahabad.

Contents.

I. Introduction.

A large number of plant diseases are caused by species of *Cercospora*, and some of the diseases caused are of considerable economic importance, such as the "Celery Early blight", "Pomegra-

*This work was done under the kind guidance of Dr. J. H. Mitter, Professor of Botany, University of Allahabad, to whom my grateful acknowledgment is due for the help rendered.

nate Blotch ", " Leaf spot of sugar beet ", " Frogeye leaf spot of Soy bean ", " Brown leaf spot of Pecans ", " Leaf spot of Bur clover ". The species of *Cercospora* are parasitic on herbaceous plant parts, specially leaves, more rarely on pedicel, petioles, stems, fruits and bracts, usually forming definitely amphigenous necrotic spots which may become confluent and involve large areas of the leaf.

In this investigation the following four species were collected in the neighbourhood of Allahabad.

(i) *Cercospora Patouillardi* Sacc. et D. Sacc, on *Calotropis procera*. (CP)*

(ii) *Cercospora Feuilleauboisii* Sacc. on *Solanum nigrum*. (CF).

(iii) „ *leocosticta* E. et Ever, on *Melia Azedarach*. (Cl).

(iv) „ sp on *Cajanus indicus*. (CS).

II. Symptoms of the Disease.

(i) (CP) Spots formed are rounded or irregular, grayish black and are on the under-surface of the leaf in the early stages of the disease, in advance stage both sides of the leaves get infected. Infection of stem as well as petiole takes place. Leaves dry ultimately and fall. In advance stages several spots coalesce to form irregular patches.

(ii) (CF) Irregular small patches of smoky brown colour on the lower surface of the leaves, very rarely on the upper surface of the leaf. No infection of stem or petiole is found. Infected leaves become brown, curled up and ultimately fall off.

(iii) (Cl) The spots on the lower surface of the leaf are concentric, the centre being occupied by black dots of conidiophores surrounded by a more or less grayish brown ring. No infection of petiole or stalk. Here the attack is severe and the whole infected spot ultimately dries and falls, leaving a conspicuous shot hole in the leaf. In advance stages leaf curls and falls.

(iv) (CS) The spots are irregular in outline and raised above the surface of the leaf. Very rarely the upper surface is infected. The spots are discrete usually, but occasionally several coalesce, forming diseased areas (15mm. × 5mm). The spots in early stages are brownish drab in colour. Petiole as well as stem are sometimes attacked.

The diseased areas arising from single spot do not cross the midrib or the primary veins of the leaflets.

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* For simplicity these species will be referred to in the paper by these symbols.

III. Morbid Anatomy.

The mycelium of the fungus is septate, hyaline in the early stages but gradually acquiring a smoky brown colour. The mycelium is both intercellular and intracellular. (Text-Fig. 2).

Text-Fig. 1. A portion of T. S. of leaf of *Cajanus indicus* infected with *Cercospora* spp., showing the emergence of conidiophore through the stoma.

Hyphae collect in the airspaces underneath the stoma and form stromatic masses, which are sometimes found projecting out of stomata. (Text-Fig. No. 1 & 3). From this conidiophores originate and come out through the stoma. The conidiophores are simple, rarely branched septate and yellow in early stages and dark brown later on, the tips sometimes presenting the knee-joint appearance. At the tip of a mature conidiophore are noticed distinct scars circular in outline and with a small circular dot occupying the centre which indicates the point of attachment of spores previously borne. Older conidiophores have along their surfaces from 2 to 14 of these scars, with a bend or geniculation at each scar. In section of the leaves they sometimes present a corymboid appearance, all of them coming to the same level, and the outer ones being bent at the base. These conidiophores and even bits of them readily germinate in water. The length of the conidiophores is variable and depends on the humidity. The germ-tubes are given off from both ends as well as from the sides near the septa. Conidia are borne at the apices of the conidiophores and are much elongated and slender. They are clavate or obclavate in form, the proximal end is greater in diameter than the distal end. The length of the conidia is also variable and depends on humidity. The conidia are commonly constricted at the septa. The distal end from one fourth to one half is usually reduced in transverse diameter and cells are longer than those of the proximal portion. Towards the base of the conidia the cells are often distended at the equator. This gives the basal half a slightly undulating outline from septa to septa.

The conidia are from hyaline to light yellow. The cell contents are of a clear yellowish colour and finely granular. When the conidium

Text-Fig. 2. A portion of transverse section of leaf of *Cajanus indicus* infected with *Cercospora* spp., showing both intra- and intercellular mycelium.

has been in water for a few hours the cell contents become more distinct, and what seem like small oil drops appear and become

Text-Fig. 3. A portion of T. S. of leaf of *Cajanus indicus* infected with *Cercospora* spp., showing stroma-like structure formed in the air space by conidiophores emerging through the stomata.

aggregated at or near the ends of the cell. This is the first step in the process of germination. Germination takes place within four hours.

IV. Study of the Various Species under Conditions of Artificial Culture.

Single spore cultures of above-mentioned four species were grown on the following four media with a view to find out the range of variability under different environmental condition.

(i) Potato-glucose agar.
(ii) Brown's-starch.
(iii) Prune juice agar.
(iv) Malt agar.

1. Macroscopic Characters.

All the plates were inoculated on one and the same day and were examined after eight and sixteen days. The fungi were cultivated both in tubes and on plates. The inoculated plates and tubes were kept at the room temperature of (24°c–33°c). The fungus showed slow growth in all the four media tried. The characters are noted down in Table I.

Table I.

Fungus.	Media.	Cultural characters.
CP.	Potato-glucose agar.	Grayish white loose aerial mycelium covering grayish black compact growth. Substratum, centre grayish black, edge light blue, non-staling colony.
	Brown's-starch medium.	Dull white loose aerial mycelium covering bluish black compact growth. Substratum, centre black, edge olive green, later on becoming bluish black, staling colony.
	Prune juice agar.	White loose aerial mycelium covering grayish black compact growth. Substratum, centre greenish black, edge bluish surrounded by fungi of white mycelium, later on becoming bluish black with slightly grayish edge, non-staling colony.
	Malt agar.	Brownish grey loose aerial mycelium covering grayish black compact growth, later becoming grayish green. Substratum, centre bluish gray, later becoming grayish black, edge light blue, non-staling colony.

Table I—(*contd.*).

Fungus.	Media.	Cultural characters.
CF.	Potato-glucose agar.	Grayish white aerial mycelium covering grayish black compact growth. Substratum, grayish black, later on becoming grayish black in the centre, and bluish black on the edge, non-staling colony.
	Brown's-starch medium.	Loose white aerial mycelium covering black compact growth. Substratum, centre grayish black, edge blue, later on becoming bluish gray, staling colony.
	Prune juice agar	Dull white loose aerial mycelium covering grayish black compact growth. Substratum, centre bluish gray, edge light blue, non-staling colony.
	Malt agar.	White loose aerial mycelium, later becoming grayish black covering grayish black compact growth tinged with blue. Substratum, centre grayish black, edge grayish black, staling colony.
Cl.	Potato-glucose agar.	Grayish white loose aerial mycelium covering grayish black compact growth. Substratum, greenish centre, later becoming bluish black, edge greenish black, non-staling colony.
	Brown's-starch medium.	Dull white loose aerial mycelium, later becoming grayish white covering black compact growth. Substratum, centre grayish black, edge greenish. Outside the colony the medium became pink in colour, staling colony.
	Prune juice agar.	Loose aerial mycelium smoky gray in the centre gray at the periphery covering grayish black compact growth. Substratum, bluish green later on becoming grayish black in the centre, non-staling colony.
	Malt agar.	Grayish white loose aerial mycelium covering grayish black compact growth. Substratum, centre grayish black, margin light blue, non-staling colony. A saltant formed after a fortnight.

Table I—(*concld.*).

Fungus.	Media.	Cultural character.
CS.	Potato-glucose agar.	Dull white loose aerial mycelium covering, later becoming grayish white covering grayish black compact growth. Substratum, greenish blue at first, later becoming bluish black, non-staling colony.
	Brown's-starch medium.	Grayish white loose aerial mycelium covering bluish black compact growth. Substratum bluish black, staling colony.
	Prune juice agar.	Grayish white loose aerial mycelium covering grayish black compact growth. Substratum, bluish black with slightly bluish edge, non-staling colony.
	Malt agar.	White loose aerial mycelium covering greenish gray, later becoming grayish black compact growth. Substratum, centre grayish black, edge light green, later becoming blue, non-staling colony.

2. Microscopic Characters.

These species when grown on different culture media presented distinctive characters. CP, CI and CS respectively all formed conidia on all the four media. CF, however, failed to form conidia on any of the media tried. Even six months old culture of this species did not show conidia. Chlamydospores were abundant in all the four species and were seen even in a week-old cultures. Older hyphae sometimes develop swollen rounded thick-walled intercalary cells dark in colour. These contain highly refractive rounded granules. Rarely such cells develop at the tip of a hypha or are formed in chains along the length of hypha. Probably these function as Chlamydospores. Lehman, S. G. (?) has observed certain structures comparable to these in *Cercospora diazu* in the older mycelium on potato dextrose agar medium. H-connections are very common in the submerged mycelium. Sclerotial bodies submerged are found in old cultures.

Table II.

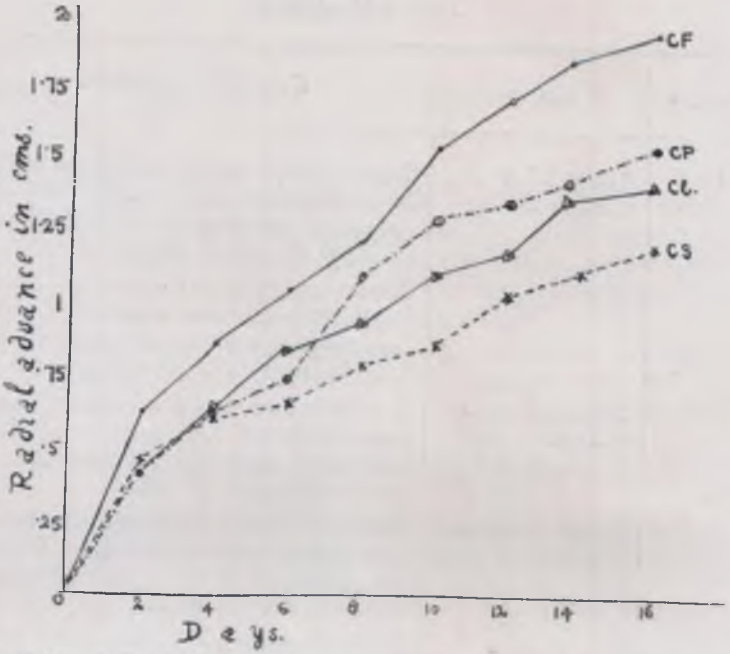
Fungus	Media.	Cultural characters.
CP.	Potato-glucose agar.	Thin, sterile, even loose mycelium along with bluish gray mycelium bearing conidiophores and conidia. Chlamydospores present in abundance. Submerged mycelium, highly granular and dark brown in colour.
	Brown's-starch agar.	Smoky brown mycelium, in the centre bearing conidiophores and conidia. Submerged mycelium bearing chlamydospores present in abundance. Submerged mycelium thick, highly granular and brown in colour.
	Prune juice agar.	Thin, sterile, even mycelium present in abundance, along with grayish brown aerial mycelium bearing conidiophores and conidia. Chlamydospores present in abundance. Same as in other media.
	Malt agar.	Similar to the culture on Prune juice agar.
CF.	Potato-glucose agar.	Thin, sterile, even loose pale yellow mycelium. Chlamydospores abundant, greenish brown in colour.
	Brown's-starch agar.	Grayish blue aerial mycelium. Chlamydospores abundant. Submerged mycelium, black.
	Prune juice agar	Smoky brown aerial mycelium. Chlamydospores abundant. Submerged mycelium grayish black.
	Malt agar.	Brownish gray aerial mycelium. Chlamydospores abundant. Submerged mycelium compact greenish black.
Cl.	Potato-glucose agar.	Thin, sterile, moderately dense mycelium along with smoky brown aerial mycelium, bearing conidiophores and conidia. Chlamydospores rare. Submerged mycelium greenish gray.
	Brown's-starch agar.	Greenish gray aerial mycelium bearing conidiophores and conidia. Chlamydospores rare. Submerged mycelium brownish gray.

Table II—(*contd.*)

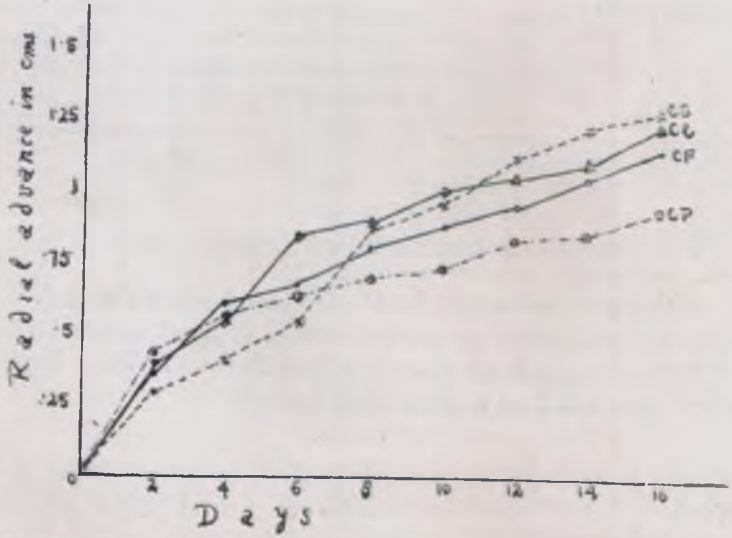
Fungus.	Media.	Cultural characters.
Cl.	Prune juice agar.	Smoky brown aerial, mycelium bearing conidiophores and conidia. Chlamydospores present in abundance. Submerged mycelium grayish black.
	Malt agar.	Smoky brown aerial mycelium bearing conidiophores and conidia. Chlamydospores present in abundance. Submerged mycelium brownish black.
CS.	Potato-glucose agar.	Smoky brown aerial mycelium bearing conidiophores and conidia. Chlamydospores abundant. Submerged mycelium brownish black.
	Brown's-starch agar.	Brownish gray aerial mycelium bearing conidiophores and conidia. Chlamydospores abundant. Submerged mycelium grayish black.
	Prune juice agar.	Smoky brown aerial mycelium bearing conidiophores and conidia. Chlamydospores numerous. Submerged mycelium brownish black.
	Malt agar.	Grayish green aerial mycelium bearing conidiophores and conidia. Chlamydospores abundant. Submerged mycelium grayish black.

3. Linear Growth-Rate.

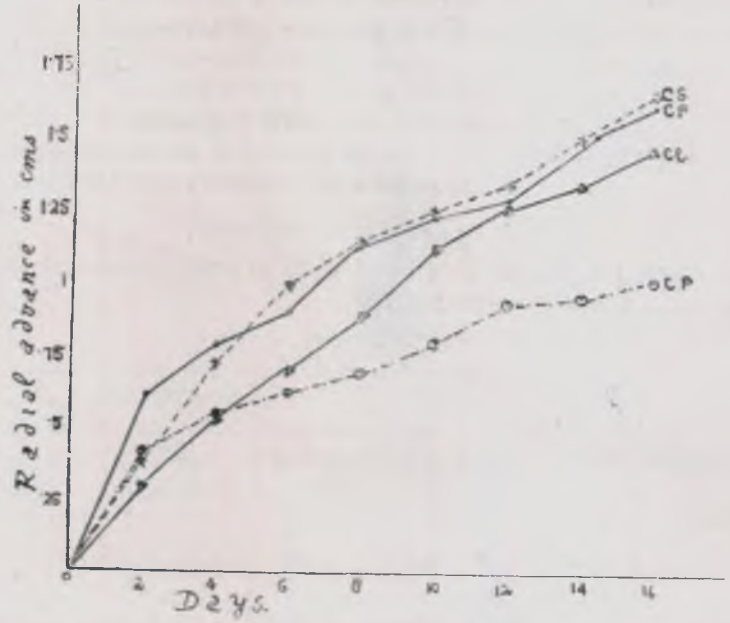
A study of the comparative linear rate of growth of each of the four species was made on the four media, at room temperature, and the figures obtained are shown in Text Figs. IV, VII. Depth of the medium was kept uniform as far as possible.



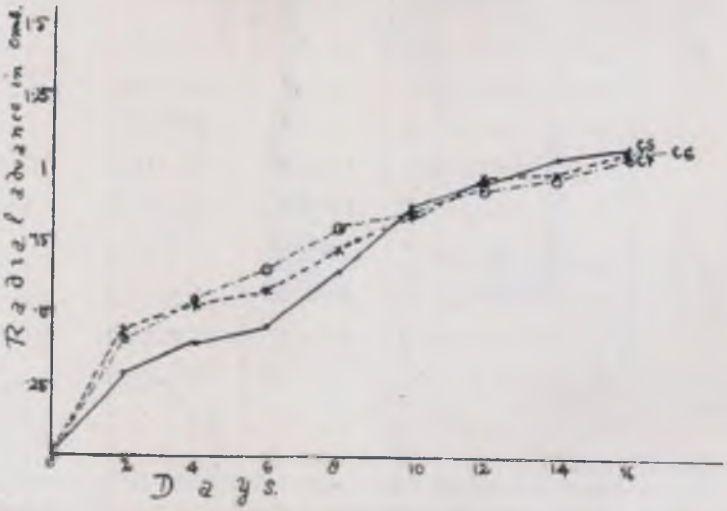
Text-Fig. 4. Graph showing the rate of growth of CP, CF, CI and CS, on Potato-glucose agar.



Text-Fig. 5. Graph showing the rate of growth of CP, CF, CI and CS of Brown's-starch medium.



Text-Fig. 6. Graph showing the rate of growth of CP, CF, Cl and CS on Prune juice agar.



Text-Fig. 7. Graph showing the rate of growth of CP, CF and CS on Malt agar.

From figures of linear growth rates it is seen that :—

(i) On PG*, the rate of growth of CF is greatest.
(ii) On BS, the rate of growth of CS is greatest.
(iii) On PR, the rate of growth of CS is greatest.
(iv) On M, the rate of growth of CF is greatest.
(v) The different species of Cercospora when grown on the same culture media show different rates of growth.

4. Spore Measurements.

Table III gives the length and width of conidiospores and conidia which are based on a count of 100.

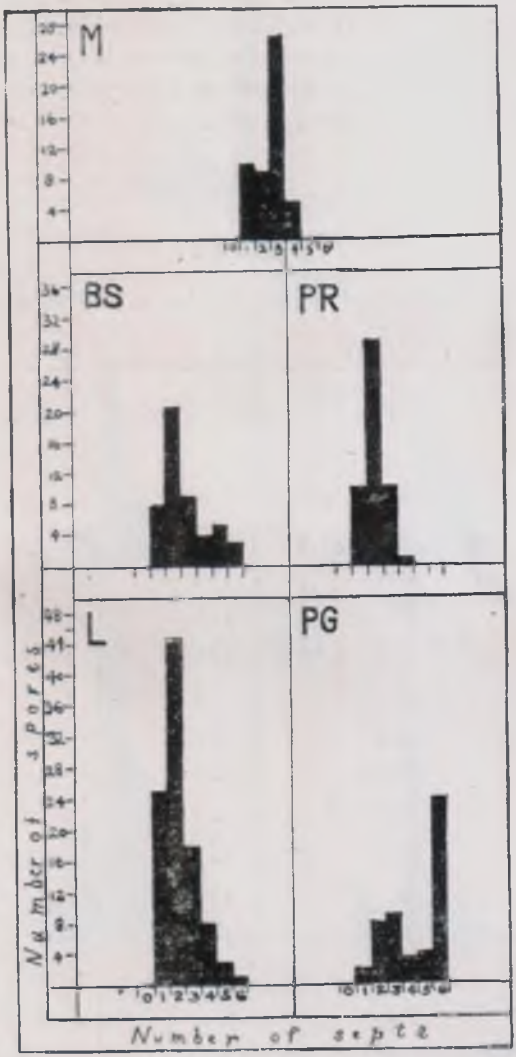
Table III.

Fungus.	Media.	Conidia.		Conidiophores.	
		Range of length	Range of width.	Range of length.	Range of width.
CP.	Leaf	31.2–124.0	3–4.0	33.6–82.0	4.8–7.2
	PG.	24.0–86.0	3.0–4.8	24.0–79.4	4.8–7.2
	BS.	21.6–81.2	3.0–4.8	24.0–55.2	4.8–7.2
	PG.	26.4–50.4	3.0–4.8	28.8–86.4	4.8–7.2
	M.	36.0–52.8	3.0–4.8	24.0–84.0	4.8–7.2
CF.	Leaf	19.2–76.8	3.0–4.8	14.4–50.0	3.0–4.8
Cl.	Leaf	26.4–88.8	2–2.4	19.2–64.8	3.0–4.8
	PG.	19.2–96.0	1.5–2.4	25.0–84.0	3.0–4.8
	BS.	24.0–86.0	1.5–2.4	36.0–84.0	3.0–4.8
	PR.	24.0–60.0	1.5–2.4	24.0–48.0	3.0–4.8
	M.	24.0–86.0	1.5–2.4	36.0–45.6	3.0–4.8
CS.	Leaf	21.6–86.4	3.0–7.0	19.2–40.8	3.0–7.0
	PG.	16.8–88.8	3.0–4.8	40.8–72.0	3.0–7.0
	BS.	36.0–108.4	3.0–7.0	45.6–70.0	3.0–8.5
	PR.	31.0–98.6	3.0–5.8	48.0–68.0	3.0–7.0
	M.	40.0–90.3	3.0–4.8	44.8–72.0	3.0–4.8

* For the sake of simplicity the following symbols are used for various media, Potato-glucose agar (PG), Brown's-starch medium (BS), Prune juice agar (PR) and Malt agar (M).

5. Septation Mode.

According to the cultural treatment the spores of a fungus may show hyaline, granular or vaculated contents. In the first case the



Text-Fig. 8. Graphical representation of the septation as shown by CP. when grown on different culture media. (L-Leaf).

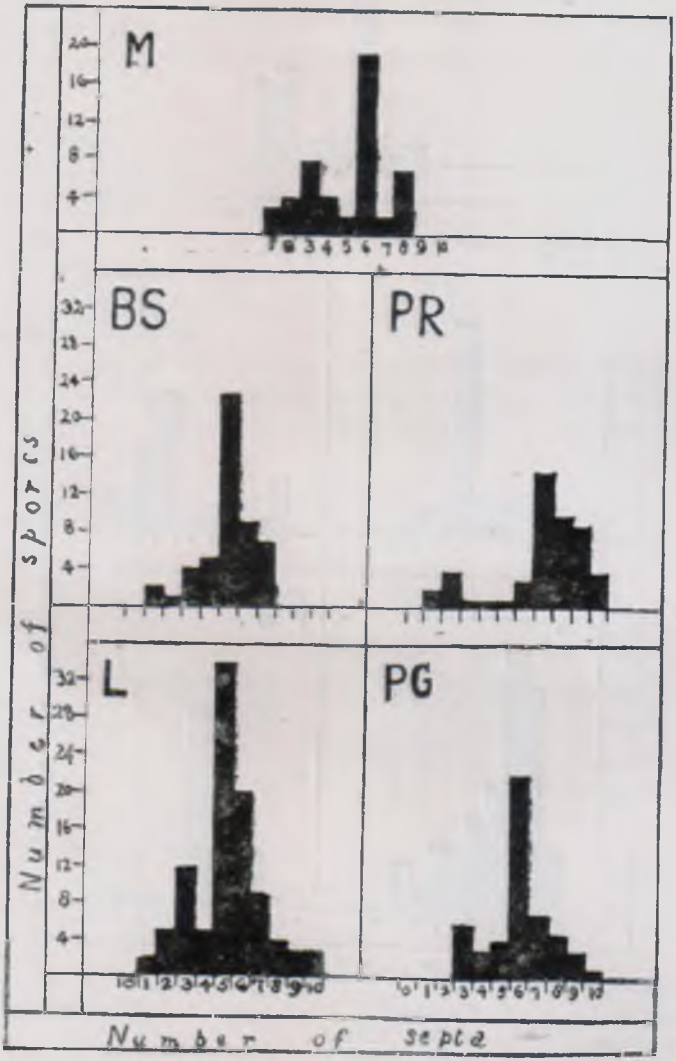
septa show up quite clearly when the spores are mounted simply in water. With the granular type (which was the prevailing type in all the Cercospora cultures used), the dense nature of the cell contents in

many cases responsible for completely obscuring the septa, so that staining becomes necessary. For this purpose an aqueous solution of Ruthenium red, proved to be of great value, the spores being mounted directly in a drop of the stain. Staining is almost instantaneous and is practically confined to the spore walls and septa. In order to determine the septation mode, a count of hundred spores was made when the spores were numerous, a count of fifty spores was made in those cases where the spores were less numerous.

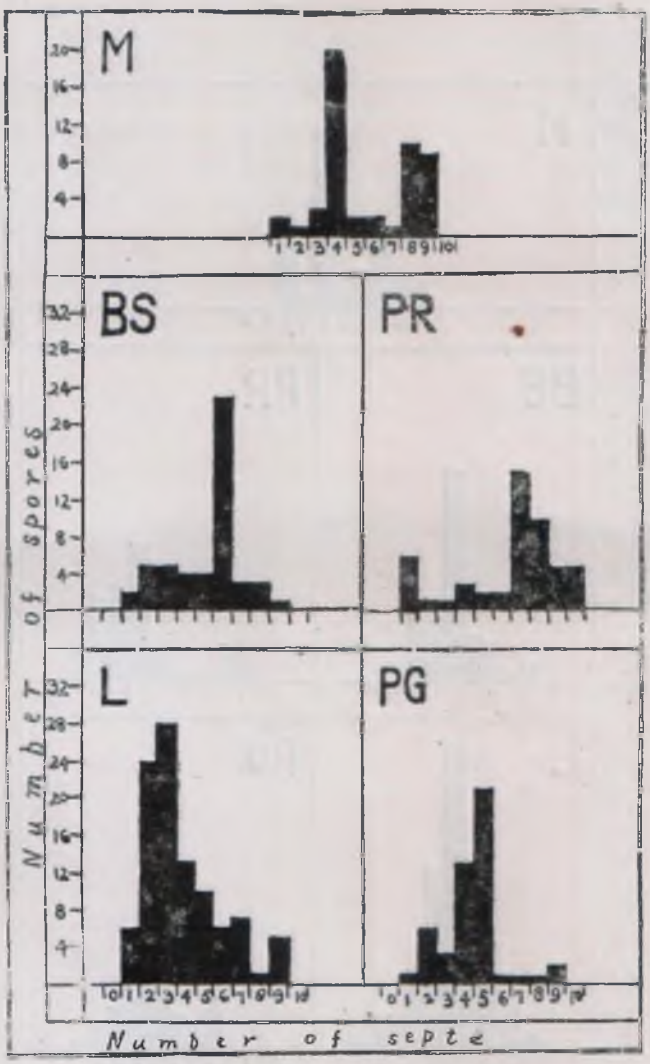
Table IV.

Septation mode of different species of *Cercospora* on different culture media.

Fungus.	Media.	Septation.	Septation mode.
CP.	Leaf	1–6	2
	PG.	1–7	6
	BS.	1–6	2
	PR.	1–5	2
	M.	1–5	3
CF.	Leaf	1–9	...
Cl.	Leaf	1–10	5
	PG.	1–9	6
	BS.	1–7	5
	PR.	1–10	7
	M.	1–8	6
CS.	Leaf.	1–9	3
	PG.	1–9	5
	BS.	1–8	6
	P.R.	1–10	7
	M.	1–7	4



Text-Fig. 9. Graphical representation of the septation as shown by Cl when grown on different culture media. (L-Leaf).



Text-Fig. 10. Graphical representation of septation as shown by CS when grown on different culture media. (L-Leaf).

From figures of the septation mode it is seen that :—

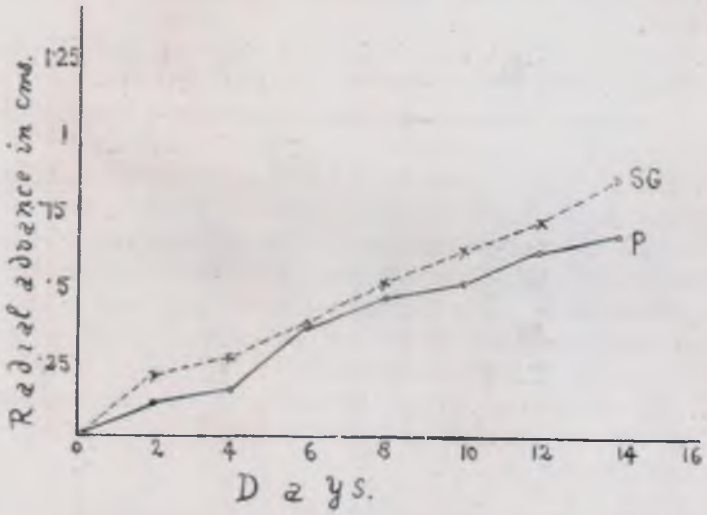
(i) The septation mode of all the species of *Cercospora* either remains the same or increases when grown on different artificial culture media.

(ii) There is a strong correlation between the septation mode and the degree of the staiing shown by the colony itself in different culture media. Non-staling type of growth is

associated with high septation mode, while staling type of growth is associated with low septation mode. This has been observed not only as regards different species on the same medium, but also as regards the same species when grown on different culture media.

V. Saltation.

During the course of this study, a search was made concerning the saltants produced. Cl, when grown on malt agar produced after a fortnight a saltant which was sterile and bore no conidiophores or conidia. It has been noticed by myself and previously by other workers that the changes produced by growing a fungus on artificial culture media are of two distinct types. The first type of change belongs to modifications, these are of temporary nature, so that when the fungus is restored to its original environment, its characters likewise revert to the original condition. Changes of more lasting nature may be conceived of as arising gradually in response to or an



Text-Fig. 11. Graph showing the rate of growth of the parent and the saltant (S-Saltant, P-Parent).

adaptation to certain growth conditions, or by sudden jumps. The later type of changes are termed Mutation Saltation in fungi may perhaps be regarded as a case of mutation. This is, however, a vexed question. Only one saltant was obtained from Cl, when grown on malt agar. *Cercospora* does not appear to form saltants so readily as certain other genera, e.g. Fusarium. *Parent*-Lower mycelium compact grayish black. Upper mycelium smoky brown in the centre

bearing conidiophores and conidia substratum grayish black with a bluish tinge. Staling colony.

Saltant-Loose white mycelium covering chocolate colour compact submerged mycelium. Substratum chocolate brown in colour. Non-staling colony.

VI. Summary.

1. Species of *Cercospora* cause leaf spot disease in plants. This fungus is a hemiparasite and permeates the tissues of the leaves both between and across the cells. The length of the conidiophores and conidia is variable and to some extent dependent on humidity relations

2. A study of the various species of *Cercospora* under conditions of artificial culture is given (as to Macroscopic characters, microscopic characters, linear growth rates, spore measurements and septation mode).

3. CP, CF show highest rate of growth on Potato-glucose agar; while Cl and CS show the highest rate of growth on Prune juice agar.

4. CF, failed to form conidiophores and conidia on any of the media used.

5. Only one saltant was obtained and that was from Cl, grown on malt agar. The saltant was sterile and its rate of growth was greater than that of the parent. *Cercospora* does not appear to form saltants so readily as certain other genera, e.g. *Fusarium*.

6. There is a strong correlation between the septation mode of the spore and the degree of staling shown by them in the culture media used. The non-staling type of growth is associated with high septation mode; while the staling type of growth is associated with low septation mode. This has been observed not only as regards different species on the same medium, but also as regards different species grown on different culture media.

7. The septation mode of all the species of *Cercospora* used either remained the same on some media and showed an increase on others. In no case the mode lowered.

VII. References.

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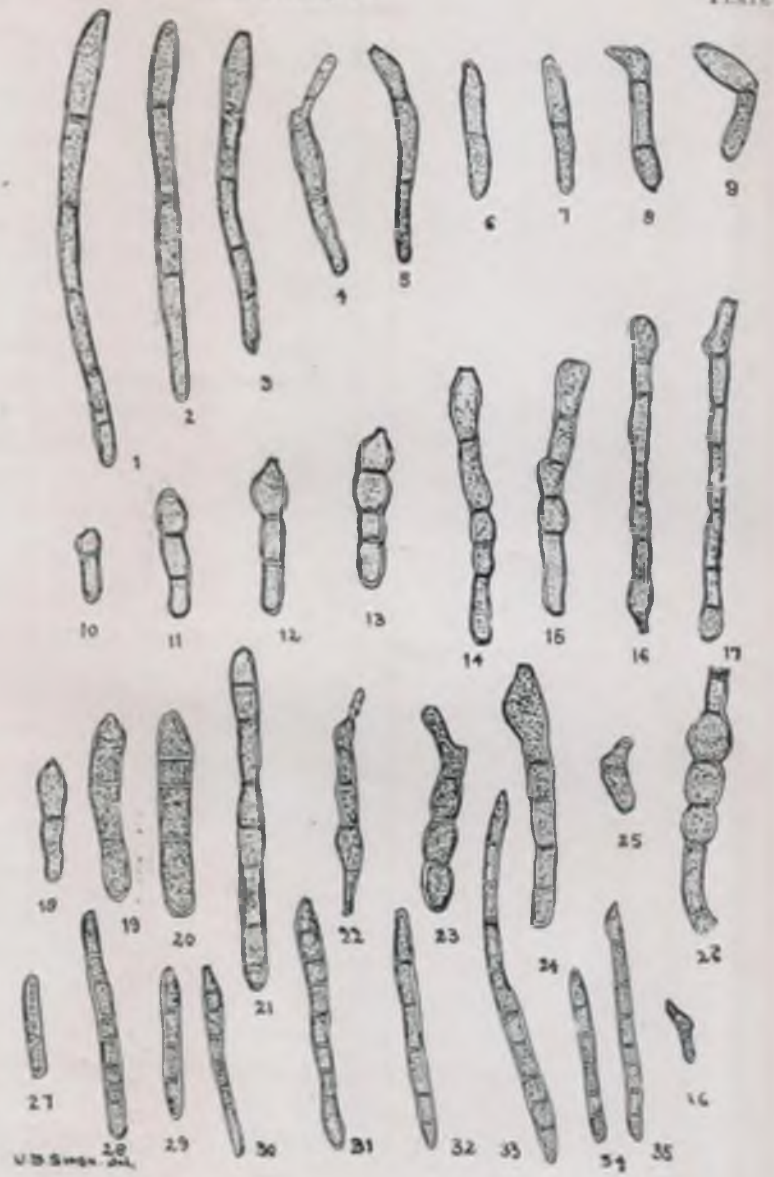

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VIII. Explanation of Plates I and II.

Illustrating U. B. Singh's paper on studies in the genus *Cercospora*.

All the figures were drawn with camera lucida at an enlargement of 800 diameter, and have been reduced to three-fourth.

Plate I.

Figs. 1–26.	C. Patouillardi Sacc. et D. Sacc, on *Calotropi-procera* (Sixteen days old culture).
Figs. 1–7.	Conidia from leaf.
Figs. 8 & 9.	Conidiophores from leaf.
Figs. 10–13.	Conidia from Potato-glucose agar.
Figs. 14–17.	Conidia from Prune juice agar.
Figs. 18–22.	Conidiophores from Prune juice agar.
Figs. 23–25.	Conidiophores from Prune juice agar.
Fig. 26.	Chlamydospore from Prune juice agar.
Figs. 27–36.	*C. Feuilleauboisii* Sacc, on *Solanum nigrum* (Six-teen days old culture).
Figs. 27–34.	Conidia from leaf.
Figs. 35 & 36.	Conidiophore from leaf agar.

Plate II.

Figs. 37–51.	*C. leucosticta* E. et Ever, on *Melia Azedarach*. (Sixteen days old culture).
Figs. 37–45.	Conidia from leaf.
Figs. 46–50.	Conidia from Prune juice agar.
Fig. 51.	Conidiophores from Prune juice agar.
Figs. 52–67.	*C. Cajani* on *Cajanus indicus*. (Sixteen days old culture).
Fig. 52.	Conidiophore from leaf.
Figs. 53–57.	Conidia from leaf.
Figs. 58–61.	Conidia from Potato-glucose agar.
Fig. 62.	Conidiophore from Potato-glucose agar.
Fig. 63.	Condiophore from Brown's-starch medium.
Figs. 64–67.	Conidia from Brown's-starch medium.