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GROWTH AND SPORULATION OF THREE COMMON SPECIES OF *CERCOSPORA* IN CULTURE

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Though *Cercospora* is a large genus with species distributed throughout the world, only a few of them have thus far been isolated and studied in culture. This is possibly so because a proper technique for the easy isolation and maintenance of these fungi in a sporulating condition is not available. Though, some of the earlier workers like Welles (1921), Nagel (1934), Shanta (1956, and Chandrasekharan and Rangaswamy (1960) have isolated a few species of *Cercospora*, we have only limited information about their cultural characters, nutritional requirements and sporulation. It has been suggested that these fungi are very exacting in their nutritional requirements and this may possibly explain why they have not been studied in any detail.

In the present studies, an attempt was made to bring into culture three common species of *Cercospora* and to evolve a suitable medium for their growth and sporulation. The species so selected were *C. henningsii* Allescher, occurring on tapioca (*Manihot utilissima*) *C. hibisci* Tracy & Earle, occurring on bhindi (*Abelmoschus esculentus*) and *C. personata* (Berk. & Curt.) Ellis & Everheart, occurring on groundnut (*Arachis hypogaea*).

Material and methods

The three species of *Cercospora* were isolated by the single spore method as well as by tissue culturing. For single spore isolation, fresh diseased spots were washed in several changes of distilled water and then placed in sterile petri-dish moist chambers for sporulation. The spores were removed after twentyfour hours. For tissue culturing very young spots were used.

The organisms were cultured in the in the following media.

1. Potato-dextrose agar (Potato 200g, dextrose 20g, agar 20g, distilled water 1000 ml)
2. Host leaf extract agar (Host leaf 200g, agar 20g, distilled water 1000 ml
3. Potato-dextrose agar enriched with extract from host leaf (Potato 200g, leaf from the respective hosts, viz , groundnut, tapioca or bhindi 200g, dextrose 20g, agar 20g, distilled water 1000 ml)
4. Carrot leaf extract agar (Carrot leaf 200g, agar 20g, distilled water 1000 ml)
5. Czapek's agar with yeast extract ($NaNo_3$ 2·00g, KH_2PO_4 1·00g, KCl 0·50g, $MgSO_4$ 0.5g, $FeSO_4$ 0·01g, sucrose 30g, yeast extract 1·00 g, agar 2° g, distilled water 1000 ml).

The media used for determining the dry weight of the mycelium had the same composition as above except that agar was omitted.

The pH of the media was adjusted between 6 and 6·5 before autoclaving. Inoculation in the media was done with 3 mm culture discs, cut out of young cultures of the organism on enriched potato dextrose agar. The cultures were incubated at room temperature.

Results

Isolation of *Cercospora henningsii*, *C. hibisci* and *C. personata* was possible both by the single spore method and by tissue culturing. Growth of these organisms was generally slow in all the media used. Best growth as determined by radial growth and dry weight of the mycelium was, however, obtained in potato-dextrose medium enriched with host leaf extract as well as in Czapek's medium enriched with yeast extract (Table 1).

All the species sporulated well in potato-dextrose medium enriched with host leaf extract. A certain amount of sporulation was noted in host leaf extract and carrot leaf extract media but growth of the organisms in these media was poor. Sporulation in Czapek's agar was invariably poor. There was no sporulation in potato-dextrose medium eventhough growth of the organisms in this medium was fairly good (Table 1).

The colour and growth characters of the individual species in different media were more or less similar with only slight variations. The aerial mycelium of *C. hibisci* was dark grey in all the media, while those of C. *personata* and C. *henningsii* were light grey in colour. In solid media, the colony of *C. hibisci* was compact and velvetty with undulating surface and wavy margins, while those of the other two species were leathery with irregular undulating hollow ridges and wavy margins.

In liquid media, colonies of all the species initially developed as numerous dark grey pellet-like structures submerged in the medium. These structures came to the surface in about eight to ten days. They were small and cushiony and had a velvetty surface. These structures aggregated to form larger colonies in *C. personata* aad *C. henningsii*, while they remained independant in *C. hibisci*.

The organisms showed a tendency to produce a pinkish brown pigment which diffused into the medium. This pigment was noted in all the media used except in potato-dextrose agar.

Discussion

Sporulation in *C. henningsii*, *C. hibisci* and *personata* was induced by the presence in the medium of extracts either from host leaf or from carrot leaf. Potato-dextrose agar with host leaf extract was found to be better than the other media, in so far as it promoted vegetative growth as well as sporulation. Vegetative growth in Czapek's medium was equal to or even better than that in the above medium but sporulation in this medium was very poor. Potato-dextrose agar also gave satisfactory growth but sporulation was absent in this medium. A certain amount of sporulation was obtained in carrot leaf extract agar and also in host leaf extract agar eventhough vegetative growth was very poor in these media. It would therefore appear that host leaf extract and also carrot leaf extract contain certain undetermined factors which are essential for the sporulation of the organisms.

Table 1

Growth and sporulation of *Cercospora* spp. on different media after 30 days of inoculation

Media	*C. henningsii*			*C. hibisci*			*C. personata*		
	Sporulation	Radial growth (mm)	Dry Wt. (mg)	Sporulation	Radial growth (mm)	Dry Wt. (mg)	Sporulation	Radial growth (mm)	Dry Wt. (mg)
Potato-dextrose	Nil	25·00	205·75	Nil	20·25	321·20	Nil	18·00	170·25
Host leaf extract	Moderate	18·75	72·50	Moderate	7·25	81·75	Moderate	11·00	40·00
Enriched Potato-dextrose	Good	24·75	218·00	Good	15·00	400·00	Good	20·75	253·75
Carrot leaf extract	Moderate	22·00	125·25	Moderate	8·75	147·20	Moderate	18·00	109·75
Czapek's with yeast extract	Very poor	26·00	299·50	Very poor	17·25	409·20	Very poor	23·50	319·00

Note: Radial growth was determined on agar media while dry weight was determined on liquid media.

Potato-dextrose agar enriched with the extract from host leaf can therefore be considered as a satisfactory medium for the growth and sporulation of the three species of *Cercospora* studied. This medium cannot, however, be considered an ideal one, since for each species the extract from the leaf of its own host has to be incorporated into the medium. What is possibly required with regard to *Cercospora* is a more or less universal medium which is suitable for a larger number of species so that a comparative study of the different species in a common substratum will be possible.

Summary

Three species of *Cercospora* viz. *C. henningsii*, fl. *hibisci* and *C. personata* were successfully isolated and grown in different culture media. Good growth and sporulation of all the three organisms were obtained in potato-dextrose agar enriched with extract from the leaf of the respective hosts. A certain amount of sporulation was obtained in carrot leaf extract agar and host leaf extract asar also but the growth in these media was poor. Growth in Czapek's medium was good but sporulation in this medium was very poor. There was no sporulation in potato-dextrose agar,

In the absence of other more suitable media, potato dextrose agar enriched with host leaf extract is considered a satisfactory medium for the growth and sporulation of species of *Cercospora*.

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