A STUDY OF THE FUNGAL ENDOPHYTE OF SOME *ANTHOCEROS ERECTUS* KASHYAP

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Received November 5, 1940

The fungal endophytes of certain liverworts have been studied in detail from this laboratory. Chaudhuri and Rajaram (1925) described a case of symbiosis between the fungal endophyte and *Marchantia nepalensis*. Chaudhuri (1935) also isolated and made a comparative study of the fungal endophytes from *Petalophyllum indicum*, *Athalamia pinguis*, *Aitchisoniella himalayensis* and *Marchantia nepalensis*. In all these, the fungus was never absent from the thallus of the host tissue and the presence of the fungus produced no untoward effect on the host, though definite symbiosis has not been proved except in the last case. The fungal endophyte of *Anthoceros erectus*, however, is not universally found and when present the growth of the host is affected adversely. So in this case, the fungus behaves as a parasite, though it does not kill the host. It may be looked upon as a disease causing organism. The infected plants were collected from Mussoorie by Mr. P. N. Mehra in September 1935, and the authors, Chaudhuri and Quraishi, read a short note on a " New disease of *Anthoceros erectus* Kashyap " at the 24th Indian Science Congress Meeting in 1937.

The host plant.—Anthoceros erectus Kashyap is synonymous with *Anthoceros Butleri* Stephani. Kashyap (1929) found that there was practically no difference between the two species and he combined the two species under *Anthoceros erectus* Kashyap as Stephani established his species a year later than Kashyap.

This species is one of the very few annual liverworts and occurs in outer and Kumaon Himalayas from 5,000 to 8,000 feet, in Mussoorie, Kulu, Manali and also in Madras, Travancore and other places.

The infected plants were found growing on an exposed ridge with stony and gravelly soil. The diseased plants become stunted and develop a reddish tint, the healthy plants having bright green colour (Figs. 1 and 2).

The normal healthy plants grow upto 10 millimetres in diameter but measurements of twenty-five diseased specimens showed that none of them

exceeded 6 millimetres in diameter and the average plant measured only 4·5 millimetres. The sporogonia are formed but they do not grow to the normal size. The number of sporogonia produced on each thallus of *Anthoceros* was also reduced. More than three sporogonia on the diseased gametophyte were not observed, while the healthy plants produced normally five or even more sporogonia. Measurements of the length of the capsule exhibited the same phenomenon. Average length of the capsule in the diseased plants for twenty-five measurements was found to be only 10·2 mm., while the capsules of the healthy plants may be upto 30 mm. long. The viability of these spores could not be determined as neither these nor the bigger spores from the healthy plants germinated under local conditions.

The infected region of the plant shows hypertrophy—a tubercle-like structure is formed. This is reddish in tinge. Presence of fungal mycelium is seen in this region. *Nostoc* colonies which are universally present in these plants, become very conspicuous and bigger in the infected plants than in the normal plants (Fig. 5). It seems that the *Nostoc* plants, whatever their relation may be with the normal *Anthoceros* plants, try to take more than their due share, when the host plant itself is affected by the parasitic fungus.

Isolation of the fungus.—After the diseased plants were carefully washed in several changes of distilled water, they were treated with dilute mercuric chloride solution (1 in 1,000) for 30 minutes and washed again in several changes of distilled water. Then the plants were placed between dry sterilised blotting papers to remove the water from the surface. Now small bits were cut aseptically from the swollen region and these bits were dipped in alcohol and flamed before planting on agar plates. In 4–5 days, fungal hyphæ were seen growing out. Bits of these hyphæ were removed and pure cultures were thus obtained.

Description of the fungus.—The morphology of the fungus was studied from the diseased material as well as from the cultures. The infected plants were fixed in formalin-acetic acid solution in 70% alcohol. Later paraffin blocks of the fixed material were prepared. Microtomic sections of these blocks, 12, 14 and 16 μ thick were cut and stained in light green, safranin, cotton blue, alcoholic eosin and erythrosin.

The fungus is confined within the tissue of the thallus. The fungus has not been found in the sporophyte. Examination of the sections, shows that the mycelium is intracellular. The hyphæ are found in all parts of the thallus but occur more generally in the basal region and conspicuously in some of the chambers. In the teased material and in some of the sections hyphæ were also observed in the rhizoids. This fact combined with the general

absence of hyphæ in the dorsal region of the gametophyte makes it probable that the hypha makes its entry into the plant tissue through the rhizoids. The hyphæ in the thallus tissue were of two kinds:—

(1) Branched, hyaline and thin-walled in which the septa were not distinct (Fig. 3). The diameter of these hyphæ is variable, ranging from $3 \cdot 5 \mu$ to $5 \cdot 4 \mu$.

(2) Branched septate hyphæ (Fig. 4) with thick contents and an average diameter of 6μ, occasionally forming knots in the cavities, or cells of the thallus. They often show considerable swellings at their tips. These hyphæ appear to be a later stage in the life of the fungus in the host tissue.

The effect of the fungus on the gametophyte is very well marked. Cells of healthy plants and of those parts of the infected thallus which escape the attention of the fungus possess a single large chloroplast and well-marked protoplasmic contents. In the infected region the bright green colour fades into reddish brown and generally the chloroplast and the protoplasmic contents suffer disintegration. It has also been noted how the infected plants remain dwarf and fail to produce healthy sporogonia of normal size. These facts leave little doubt regarding the parasitic nature of the infection.

Growth of the Fungal Endophyte in Culture

The fungal endophyte grows slowly in potato-glucose-agar. The hyphæ are hyaline when young but change to light brown when fully mature. Mature hyphæ are branched and septate and the cells are thick-walled. The breadth of the hyphæ varies from $2 \cdot 8 \mu$ to $8 \cdot 5 \mu$, although the hyphæ with the average diameter of $4 \cdot 8 \mu$ are most common. The length of the cells varies greatly. The terminal cells have the greatest length and are correspondingly very narrow. Septa in the young hyphæ are very indistinct. The older hyphæ become closely septate and their cells are practically square shaped. Fully mature hyphæ form abundant chlamydospores which may be intercalary or lateral (Figs. 6, 7). Sometimes all the cells of the hyphæ become converted into chlamydospore-like structures. The dimensions of the chlamydospores are extremely variable, average being $7–9 \mu$.

Thick dark coloured sclerotia (Fig. 8) are formed in abundance in all media and are a prominent feature of the endophyte in culture media. The size of the sclerotia varies and an average for 25 counts in potato-glucose-agar media is $260 \mu \times 310 \mu$.

Examination of the sections of sclerotia shows only meshes of interwoven hyphæ with thick walls of black or dark brown colour. No conidia were

observed. The sclerotia on germination put out hyphæ which are at fi very narrow, hyaline and without septa (Fig. 9). The septa appear lat

Growth characters of the endophyte were studied in different synthe and other media containing vegetable extracts. Effects of different factors the growth of the fungus were studied in cultures. Inoculations were do throughout by placing a single sclerotium from a four-weeks old culture the endophyte (in potato-glucose-agar) in the centre of the Petri-di containing the medium. Petri-dishes of uniform size were employed, a for each Petri-dish, a measured quantity of the medium, about 20 c.c. w poured. Measurements in millimetres were taken after every 24 hou Two readings at right angles were taken for each Petri-dish.

Seven different media were used. The daily spread of the fungus and t cultural characteristics have been studied and are as follows:—

(1) The rate of daily spread varies widely in different media. It is lea in Wort-agar, and in Nutrient agar the rate of linear growth is better o to that of Wort-agar. Growth is greatest in Purple lactose agar. In t remaining synthetic as well as in other media containing vegetable extrac the growth in general is good. It flourishes well in sugary than in starchy med

(2) In Wort-agar, where the rate of spread is least, luxuriant aer growth is obtained, while in general the aerial growth is very moderate in the other media and is least in Nutrient agar and in media containing sugar.

(3) In all sugary media the colony is fairly compact, while it is loose those lacking this ingredient. Sugary media are favourable for the form tion of chlamydospores.

(4) Sclerotia are formed in all media, although their formation is mu delayed in Nutrient agar and very few are formed in Czapek's mediu

(5) The size of the hyphæ varies in different media; the length of the ce and the dimensions of sclerotia also vary.

For studying the effect of different temperatures on growth, the rate spread of the fungus on potato-glucose-agar was followed. All the Pet dishes were inoculated at the same time and the experiment in each of t eight sets were run in triplicate. The data obtained are given below.

There was no growth at 37° C., even after 7 days, but the fungus w not dead: when subsequently the cultures were transferred to lower temp rature growth resumed, though slowly. The optimum temperature was fou to be 22° C. At 15° C. the rate of growth slowed down considerably, un

here was no growth at 6° C.; when the Petri-dishes incubated at 6° C. were ransferred to 22° C., growth almost resumed at once.

The colony was loose and thin at lower temperatures, gradually becoming compact at higher temperatures. Aerial growth was scanty at low temperatures and only very moderate at higher ones. The size of the hyphæ nd length of the cells also decreased with the rise of temperature. The rownish tinge of the hyphæ became more evident at higher temperatures. he chlamydospores were formed much earlier at higher temperature. The clerotial formation was slow at lower temperature.

Effect of hydrogen-ion concentration was studied using eight grades of 'zapek's medium with pH values ranging from less than 3·4 to more than H 9·5.

The fungus could not grow in media with pH values less than 3·4 or reater than 9·5. While the growth was very slow in pH 3·4 or pH 9·5, aximum growth was obtained with pH 5·2 and the rate of spread gradually ecreased in either direction. There was very little growth in alkaline media nd sclerotial formation was scanty. In acid media the colony was more ompact, there was abundant sclerotial formation and some aerial growth. he size of the hyphæ did not vary much. The chlamydospore formation as earlier in neutral media and did not take place in more alkaline or acidic edia. The dimensions of the sclerotia was not very different.

Response to light was studied with three sets of potato-glucose-agar lates. One set was completely wrapped with black paper; the second set as left unwrapped. Both sets of Petri-dishes were incubated at the room mperature under a bell jar. A third set was left unwrapped in the dark oom under a constant source of artificial light (sixty candle power) at a istance of eight feet. It was found that light had no perceptible influence n the spread of the fungus. The rate of spread and growth characters ere same in all cases.

Though the fungus has been brown in various culture media under ifferent conditions, apart from chlamydospore and sclerotia formation, no ther spore form has been found. So, it has not been possible to name the ungus.

Summary

A fungal endophyte was noticed in the thallus of certain *Anthoceros rectus* from Mussoorie. These plants are stunted in growth and take up reddish tinge, and the sporogonia on these plants are smaller in size. Healthy lants do not have any fungal endophyte. *Nostoc* colonies which are universally present, are more conspicuous in the infected plants. The endophyte

has been isolated and grown in culture in various media under different environmental conditions. Apart from formation of chlamydospores and sclerotia no other spore forms were produced.

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EXPLANATION OF PLATE XVI

FIG. 1.—Normal plant of *Anthoceros erectus*. × 3.

FIG. 2.—Infected plant of the above. × 3.

FIG. 3.—Part of a vertical section through the thallus of the infected plant showing branched hyphæ. Hyphæ unseptate. × 250.

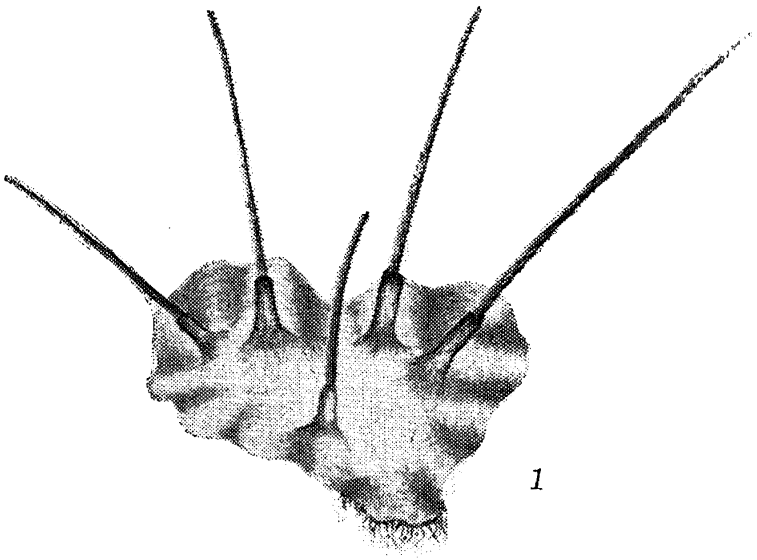
FIG. 4.—Part of thallus showing swollen branched hyphæ. Hyphæ septate. × 170.

FIG. 5.—Vertical section of the thallus through the *Nostoc* colony and showing the fungal endophyte. × 40.

FIGS. 6 & 7.—Chlamydospores on branched hyphæ. × 350.

FIG. 8.—Sclerotium with mature hyphæ. × 170.

FIG. 9.—Germination of sclerotium. × 170.



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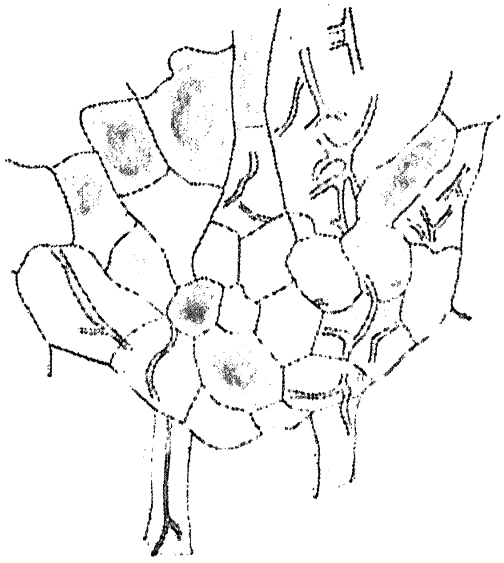

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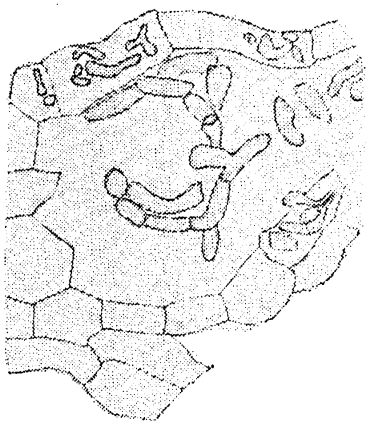

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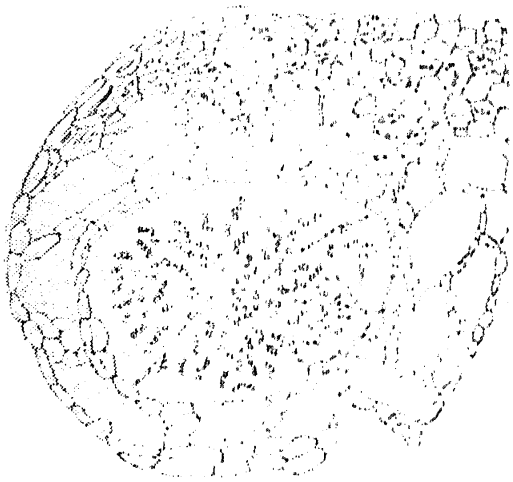

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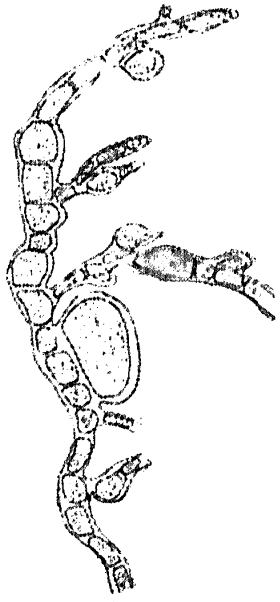

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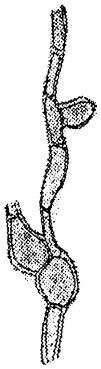

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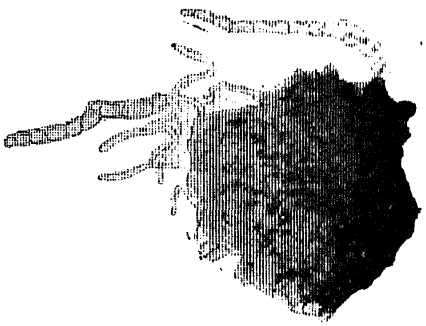

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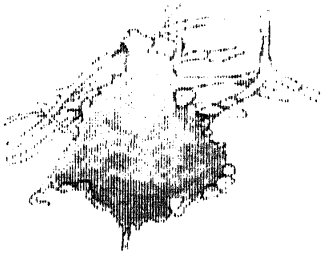

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