STUDIES ON THE SURVIVAL OF CERTAIN FUNGI IN INDIAN ALKALINE SOILS

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ABSTRACT

The paper deals with the survival and development of 2 dark-spored fungi—*Curvularia penniseti* (Mitra) Boedijn and *Helminthosporium hawaiiense* Bougn. that occur commonly in the Indian *Usar* soils. The development of the mycelium in *Usar* soil is remarkably slow. Occasional germination of conidia occurs by the formation of short and stumpy germ tubes. The hyphae and conidia tend to be wider, the former often presenting a beaded appearance while the latter sometimes become deformed in shape. Long incubation results in the development of chlamydospores in the hyphae as well as in the cells of conidia of both the fungi.

INTRODUCTION

The soil is in a state of dynamic equilibrium with its environment and the microbial population of a particular soil is governed by various edaphic factors that influence its growth and survival. The microflora occurring in *Usar* (alkaline) soils is subjected to rather extreme conditions of high pH, high osmotic concentration, low moisture content, high degree of solar radiation and relatively higher temperatures during summer months making their active life and survival an extremely difficult proposition (RAI *et al.*, 1970). Most of these micro-organisms particularly fungi do not occur in an active state in such soils for most part of the year, and experiments conducted so far in this laboratory have shown that active fungal life occurs only in soils with high moisture content, pH obviously playing no major role within certain limits. However, in most of the *Usar* soils which have very low moisture content coupled with other extreme conditions, survival of such forms appeared to be difficult and the present paper embodies results of some experiments planned to study how exactly such forms survive the unusual conditions present in these soils.

MATERIALS AND METHODS

Two dark-spored fungi, commonly isolated from *Usar* soils viz., *Curvularia penniseti* (Mitra) Boedijn and *Helminthosporium hawaiiense* Bougn. were selected for this study. BYTHER AND POWELSON'S (1966) method, with slight modification, was employed for this purpose. It consisted of spreading (i) the conidial and mycelial suspension from a fully mature culture of the fungus and, (ii) active young (non-sporulating) mycelium taken from a 2-3 day old culture on sterilized glass slides and allowed to air dry. Hyphal and conidial characters of the control set were noted from such preparations. The slide ends carrying the fungus were buried vertically down in 4″ earthen pots containing sterilized *Usar* soil and were incubated at controlled temperature of 30 and 45°C. (± 1°C.). One set of pots was periodically supplied with 10-15% moisture while, the other was left dry. Slides for examination were taken out after 15, 30, 60, 90, 135, 180, 240 and 270 days. Those

for microscopic examination were washed in water and stained with lactophenol-cotton blue while, others meant for the study of viability were rinsed well in sterilized water and a thin film of Czapek-Dox medium was spread over them in sterilized Petri plates.

OBSERVATIONS

Observations, for each set of experiment, were taken periodically. However, not many remarkable changes were observed in the slides taken out after 30 and 60 days of incubation.

The young mycelium of *Helminthosporium hawaiiense* Bougn. in *Usar* soil generally, showed no development up to 90 days after which a few hyphae turned light brown and these after 150 days developed a few 3-4 celled conidia. The chlamydospore formation was noticed only after an incubation period of 240 days at 45°C. in the absence of moisture (Text-fig. 3). No marked changes were, however, noticed in the young mycelium of *Curvularia penniseti* (Mitra) Boedijn.

Observations with regard to mature mycelium and conidia of both the fungi have been summarised in the Table-1. The detailed results are given below :

Curvularia penniseti (Mitra) Boedijn:

(i) Deformation in the shape of conidia was observed after 30 days of incubation (Text-fig. 2, C) followed by further changes in the hyphae and conidia that tend to be wider; the former presenting a beaded appearance after 135 days of incubation (Text-fig. 1, A-C). (ii) The conidial germination, although uncommon, occurs only by short tubes or bulbous protrusions from their narrow end after 90 days (Text-fig. 2, A-B). (iii) The chlamydospore formation in the hyphae initiates after 135 days in absence of moisture at 45°C., while, mature chlamydospores in the hyphae (Text-fig. 1, C-E) as well as in the large cells of conidium (Text-fig. 2, D-F) develop only after 270 days of incubation.

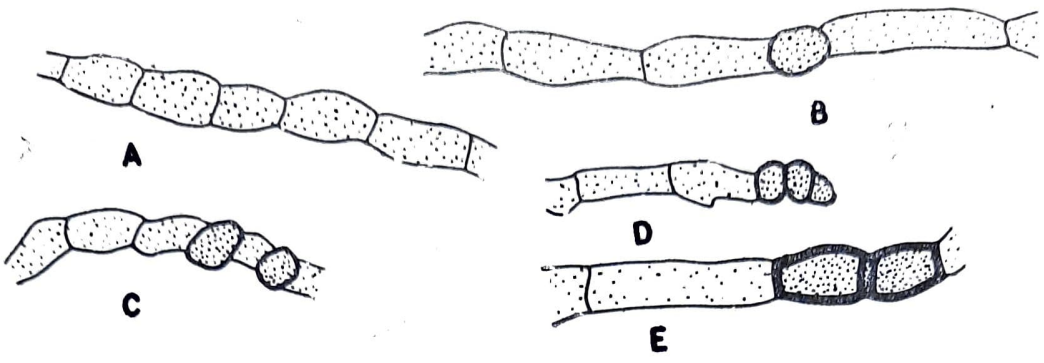
Helminthosporium hawaiiense Bougn. :

(i) At higher temperature of 45°C. after 90 days, the hyphae form hyphal tangles with indistinct walls which, in the absence of moisture, turn lighter in colour becoming light olivaceous. After 180 days of incubation, the hyphae become closely septate and beaded with a few swollen cells (Text-fig. 3, A-B). (ii) The conidial germination, in presence of moisture, occurs after 90 days—generally by short or long tubes usually from one pole but sometimes bipolar also (Text-fig. 4, A-C). The percentage of germination exhibits a considerable fall in absence of moisture becoming infrequent at 45°C. and when present, it is only by short stumpy tubes. (iii) The conidia tend to be wider after 270 days of incubation. (iv) The degeneration of some or all the inner cells in some conidia resulting in appearance of conidia with unequal septation or at times in the development of apparently single-celled conidia was also noticed after 90 days of incubation at 45°C. (Text-fig. 4, G; I-K). (v) The chlamydospore initiation in hyphae and also within the conidial cells takes place after 135 days which may be intercalary or terminal. In the hyphae, they are formed singly as well as in chains (Text-fig. 3, B-F, Text-fig. 4, H and J). Mature chlamydospores were seen after 240 days.

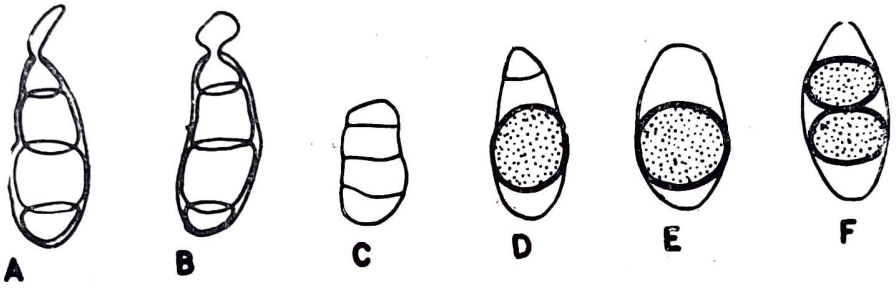
The hyphae and conidia of both *C. penniseti* and *H. hawaiiense* remained viable up to 180 days beyond which they appeared to loose their viability fast.

Table 1—Survival and development of mature mycelium and conidia of *C. penniseti* and *H. hawaiiense* in *Usar* (alkaline) soil.

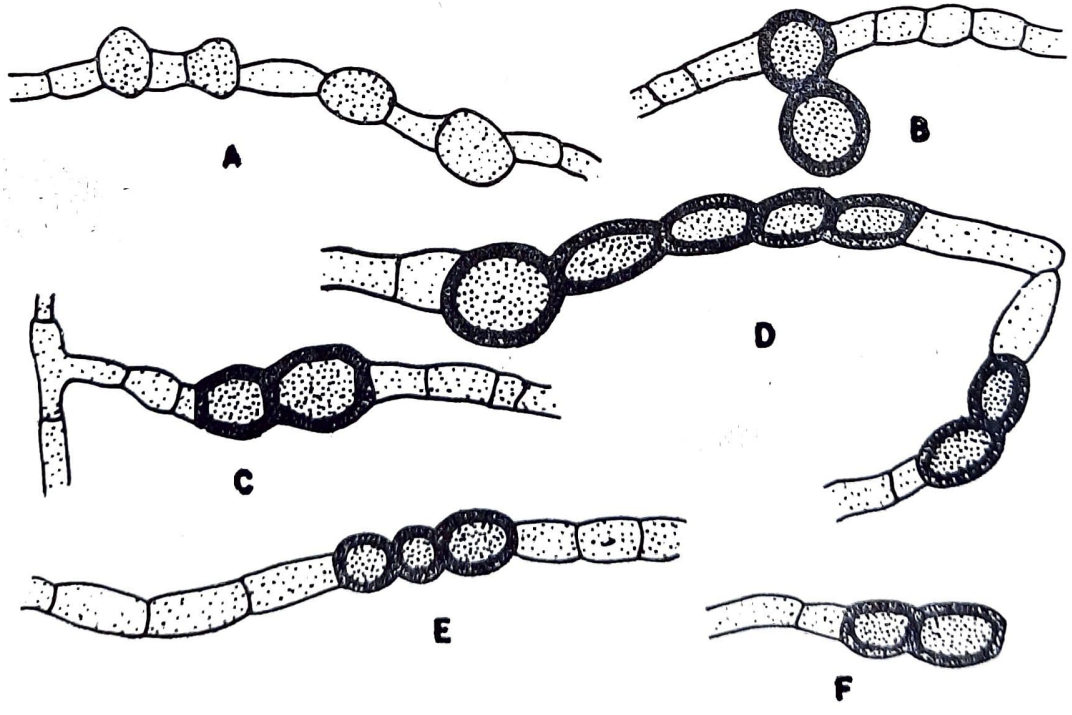
FUNGUS	Number of Days							
	15	30	60	90	135	180	240	270
1. *CURVULARIA PENNISETI*	..	Deformation of conidia.	Widening of hyphae and conidia.	Occasional germination of conidia.	Development of beaded hyphae. Chlamydospore initiation.	Chlamydospore development continues.		Formation of mature chlamydospores in hyphae and in conidia.
2. *HELMINTHOSPORIUM HAWAIIENSE*	..	..	..	Degeneration of septa in hyphae and conidia. Development of hyphal tangles. Occasional germination of conidia.	Chlamydospore initiation.	Chlamydospore development continues. Development of closely septate and beaded hyphae.	Formation of mature chlamydospores in hyphae and in conidia.	More chlamydospore formation. Widening of conidia.



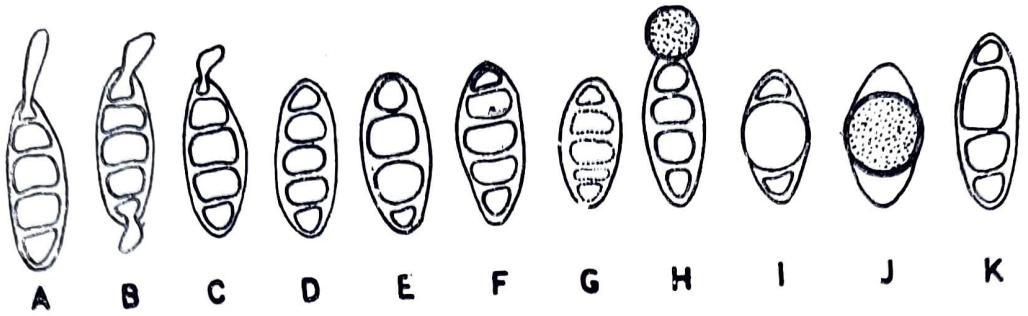
Text-fig. 1. *Curvularia penniseti* (Mitra) Boedijn (A-C)—Beaded hyphae with swollen cells. (C-E)—Developing and mature chlamydospores in the hyphal cells.



Text-fig. 2. *Curvularia penniseti* (Mitra) Boedijn (A-B)—Unipolar germination of conidia from their narrow end by a short tube and bulbous protrusion. (C)—Deformed conidium. (D)—Large cell of the conidium turned into chlamydospores. (E)—Single celled conidium with central chlamydospore (F)—Conidium with two chlamydospores formed in successive cells.



Text-fig. 3. *Helminthosporium hawaiiense* Bougn. (A)—Beaded hypha with a few swollen cells. (B)—Intercalary and lateral chlamydospores in a closely septate hypha. (C-E)—Developing and mature intercalary chlamydospores. (F)—Terminal chlamydospores.



Text-fig. 4. *Helminthosporium hawaiiense* Bougn. (A and C)—Unipolar germination by long and short tubes. (B)—Bipolar germination by short tubes. (D-F)—Rounding of the inner walls of conidia with thickening of a terminal cell in F. (G, I and K)—Apparent dissolution of the inner walls of conidia and development of unequal cells. (H)—Terminal cell of the conidium forming the chlamydospore. (J)—Single celled conidium with a central chlamydospore.

DISCUSSION

The mechanism of survival of soil micro-organisms is greatly influenced by the soil environment coupled with the biological interaction of other soil microbes. The picture becomes still more complex under drastic conditions of *Usar* soils where high surface temperature accompanied by other extremes including low organic matter provide a real hazard to the microbial life in its active state as also in their survival.

Information on the survival of microbial plant pathogens in soil is available through the work of KATZNELSON (1940 a & b), ANWAR (1949), CHONA AND NARIANI (1952), STOVER (1954), NASH *et al.* (1961), OLD (1967), SCHREIBER AND GREEN (1962) and a few others. The fungal pathogens are known to generally form resting spores and sclerotia to survive the diverse factors present in the soil (MENZIES, 1963). PARK (1954) reported the survival of certain soil fungi e.g., *Botrytis*, *Stemphylium* and *Trichoderma* spp. by the formation of chlamydospores. The survival of macroconidia of *Fusarium solani* f. *phaseoli* by the formation of chlamydospores either directly or in their germ tubes has been studied by NASH *et al.* (1961). Recently, there has been some work to show the survival of *Helminthosporium turcicum* Pass. and *H. sativum* Pam., King and Bakke in soil by the formation of chlamydospores within the cells of conidia and also with regard to their viability in different kinds of soils (BOOSALIS, 1962; BOOSALIS *et al.*, 1967; MERONUCK & PEPPER, 1968).

The work done here relates to the survival studies of *Curvularia penniseti* (Mitra) Boedijn and *Helminthosporium hawaiiense* Bougn. in *Usar* (alkaline) soils. The results indicate that the inner wall of the large cell of conidia in case of the former while, any of the cells or a single terminal cell or several cells of the conidia in *H. hawaiiense* becomes thick and gets converted into the chlamydospore. The initiation of chlamydospore development was observed after a period of 135 days at an incubation temperature of 45° C. They were, however, formed abundantly after 270 days. BOOSALIS *et al.* (1967) and MERONUCK AND PEPPER (1968) have also reported the formation of chlamydospores within the conidia of *H. turcicum* and *H. sativum* respectively when they were examined after having been buried in the soil for 98 to 159 days. The degeneration of the inner cells of conidia has also been observed in *H. sativum* by MERONUCK AND PEPPER (1968). Results obtained with regard to young hyaline mycelium of *H. hawaiiense* revealed that, if incubated in *Usar* soils, the mycelium usually does not develop beyond its young hyaline state except forming a few light brown hyphal fragments and few 2-4 celled conidia that may take a period of 150 days or more. In the set incubated at 45° C. without moisture, a few hyphae indicated the initiation of

chlamydospore formation also. This shows that their formation may be associated to some extent with high pH and temperature coupled with absence of moisture. The young hyaline mycelium of *Curvularia penniseti*, under similar conditions, did not exhibit any development whatsoever.

The viability of fungal spores and hyphae is variable depending upon the soil and environmental conditions. Boosalis (1962) has shown survival of the conidia of *H. sativum* for more than 490 days in soil from one part of Nebraska while, their viability declined rapidly after only 90 days in soils from another area. However, in *C. penniseti* and *H. hawaiiense* the conidia and the hyphae showed viability up to 180 days of incubation beyond which they appeared to loose their viability fast. Development of the mycelium in *Usar* soil conditions is rather very slow and occasional germination of the conidia occurs after 90 days by development of unipolar or bipolar short and stumpy germ tubes.

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