STUDIES ON THE FUNGI OCCURRING AS LABORATORY CONTAMINANTS

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A routine subculture was continued to isolate the fungi occurring as laboratory contaminants. In all, nineteen species have been identified. Most of the isolated fungi belong to the genus *Aspergillus*. Two species of *Aspergillus*, viz , *Aspergillus nidulans* and *Aspergillus chevalieri* definitely belong to Ascomycetes in view of the presence of perithecia containing asci and ascospores. The two fungi of the genus *Aspergillus* and *Spicaria* which have been considered as varieties *de novo* are *Aspergillus chevalieri var prolitrans* and *Spicaria divaricata var. heterospora* owing to the proliferation phialides and the presence of two types of spores, respectively.

Introduction

It has been observed frequently that the indigenous materials and various intermediates and preparations therefrom on which work is carried out in the various divisions of these laboratories are attacked by fungi. It was therefore considered desirable to identify these fungi and study their life-history and accordingly, a systematic project has been taken up under the title 'Studies on the Fungi Occurring as Laboratory Contaminants'.

A systematic mycological study involves the collection of fungal species from the various sources of infected materials from the different sections of the Laboratories, followed by isolation, identification and observations on the cultural behaviour of the isolated fungi. Work done particularly on the fungi belonging to *Ascomycetes*, *Physcomycetes* and *Fungi imperfecti* has been described in this paper. The importance of the microbiology of these groups in the fermentation and other food and pharmaceutical industries is well-known. Hence the knowledge of the fungal flora of these groups is likely to be fruitfully utilized in the fermentation, food and pharmaceutical industries of East Pakistan. As a matter of fact, one of the isolates from *Aspergillus*, when studied further for their effect on fermentation, was found to be highly effective.[1]

Experimental

Source of Materials.—Fungi were isolated from laboratory contaminants. They were collected from different types of materials used in these laboratories like sugar solution, plant extract, glycosides and wood chips. Isolates were also obtained from a large number of bacterial and other contaminated culture plates.

Methods of Sampling and Isolation.—On getting a report of aerial fungal contaminations in the working materials of other research workers of the laboratories, the materials were collected and subcultures were made in Czapek's and Waksman's media.

The isolates obtained were purified in two ways. Fungi producing abundant spores were purified by single spore culture. For this purpose the dilution culture method was followed.[2] A dilute spore suspension was made by shaking vigorously a small mass of spore in a tube of sterile water and it was poured on a slide having a thin layer of water agar medium. On placing the slide under microscope, small blocks of agar were cut with the help of sterilized needle. Each block with one spore was transferred to Czapek's or Waksman's medium. Spore producing cultures were purified by pouring very dilute spore suspension in petridishes with culture media. After a period of incubation a small number of scattered colonies were seen to appear. Subcultures from these colonies were made.

To purify fungi which did not sporulate readily, hyphal tip culture method was followed by transferring single hyphal tip to culture tubes.

For isolations and cultures Czapek's and Waksman's media were used.

Organisms Isolated.—Nineteen species of fungi isolated from different materials as contaminants are dispersed in nine genera representing different groups, namely: *Phycomycetes*, *Ascomycetes* and *Fungi imperfecti* (Table 1.)

Among these isolates, *Syncephalastrum* and *Cunninghamella* come under *Phycomycetes*, while the others belong to *Fungi imperfecti* except for three, viz, two species of *Aspergillus* and one of *Chaetomium*.

Penicillium and *Aspergillus* were the most abundant organisms, the number being 5 and 8, respectively. No *Basidiomycetes* were isolated.

It is not necessary to give the description of all the species here, as they have already been fully described in the literature.[3–7] However, the six species which showed noticeable variation from the already described species are described and discussed here.

Discussion

Aspergillus chevalieri var. proliferans.—Colonies on Czapek's medium restricted, blue green in marginal area with heads and perithecia largely confined to central area. Reverse in yellow to brown. Conidial heads radiate. Conidiophores septate upto 496μ by 8μ (Fig. 1). Proliferation of phialides are found in most cases (Fig. 2), one

TABLE 1. FUNGI ISOLATED FROM DIFFERENT SOURCES.

Name	Sugar solution	Glycoside	Plant extract	Bacterial culture	Plate contamination	Wood chip
Aspergillus niger van Tieghem						
Aspergillus clavatus Desmazieres	+				+	
Aspergillus sachari Chaudhuri					+	
Aspergillus sydowi (Bainier and Sartory) Thom and Church					+	
Aspergillus nidulans (Eidam) Winter					+	
Aspergillus terreus Thom					+	
Aspergillus fumigatus Fresenius					+	
Aspergillus chevalieri (Mangin) Thom and Church					+	
Penicillium citrinum Thom	+					
Penicillium oxalicum Thom					+	
Penicillium piceum Raper and Fennell					+	
Penicillium lilacinum Thom		+				
Penicillium implicatum Biourge			+			
Syncephalastrum racemosus (Cohn) Schroeter						+
Curvularia lunata (Walker) Boedijn				+		
Cunninghamella verticillata Paine				+		
Spicaria divericata (Thom) Gilman and Abbott				+		
Chaetonium funicula Cooke					+	
Trichoderma viride Pers. ex Fr.						+

series phialides 8 to 10μ by 3 to 4μ. Conidia elliptical to subglobose 4.4 to 6μ roughened. Abundant perithecia have been found with conidiophores (Fig. 3) 64 to 152μ in diameter. Asci with 8 pores, ascospores lenticular, walls smooth with prominent crest 4 to 5.2μ by 3 to 3.6μ (Fig. 4).

This species of *Aspergillus* has the characteristics similar to those of the *Aspergillus glaucus* group[3] but it differs from the measurement of conidiophores of *Aspergillus chevalieri* which resembles more with this species than with others in this group.[3] This species also produces perithecia and ascospores like *Aspergillus chevalieri*. Some conidiophores show proliferations of phialides which terminate into separate heads.

This *Aspergillus* cannot be definitely identified as *Aspergillus chevalieri*, although they are alike in most cases. The main difference is in the proliferation of phialides which is not found in *Aspergillus chevalieri*. Due to this important variation the species may be considered as one of the varieties of *Aspergillus chevalieri* and named *Asp. che. var. pro.*

Aspergillus terreus.—Colonies on Czapek's medium floccose attaining a diameter of 2.7 cm. in six days at 25°C. Light buff to cinnamon brown in age, radiately furrowed. Reverse, yellow in colour. Conidiophores arising from substrutum smooth more or less flexuous (Fig. 1), 140 to 280μ by 4μ conidial heads columnar, vesicles hemispherical 12 to 16μ in diameter. Sterigmata in two series closely packed, primary 5 to 7μ by 2μ. Secondary 6 to 8μ by 1.6μ. Conidia globose, smooth 2 to 2.6μ in diameter.

Here it has been found that primary phialides are smaller than the secondary ones which resemble with the description of Thom and Raper, but Gilman[4] reported the primary phialides to be longer than the secondary phialides. Other characters have been found to be similar.

Aspergillus sachari.—Colonies on Czapek's medium floccose cream buff in colour. Reverse some shades of yellow to brown. Sclerotia begin to form in a week's time. They are white hard bodies in the beginning, then assume cinnamon colour scattered throughout the colony 2 mm in long axis with a depression in the centre. Conidiophores smooth 320 to 544μ by 8μ. Vesicles globose 30μ in diameter. Conidial heads radiating (Fig. 2) sterigma in two series primary 8 to 12μ by 2.8 to 4μ, secondary sterigma measuring 6 to 8μ by 2 to 2.8μ. Conidia smooth colourless globose 2 to 2.8μ in diameter.

Sclerotia have been found only in *Aspergillus sachari* which shows same characters as described by Gilman with slight variations in the measurement of conidiophores and primary sterigma.

Curvalaria lunata.—Colonies on Czapek's medium growing rapidly, olivaceous to black, reverse black. Conidiophores simple septate 48 to 116μ by 3 to 4μ. Conidia borne in a whorl at the tip

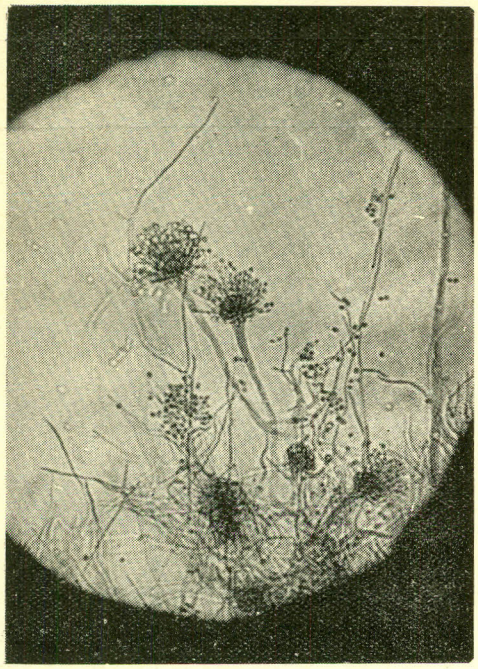

Fig. 1.—Conidiophores.

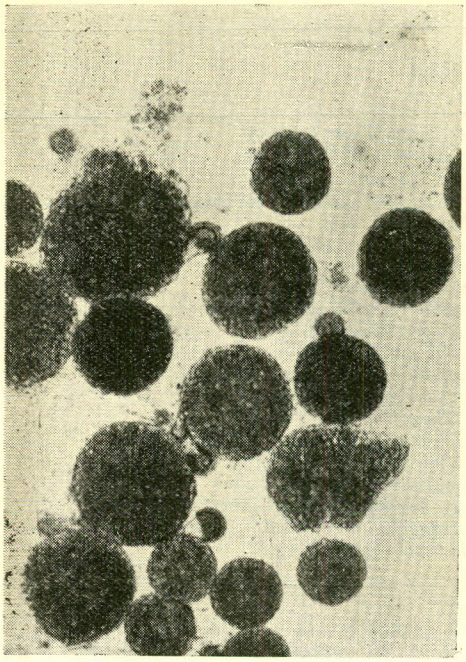

Fig. 3.—Perithecia.

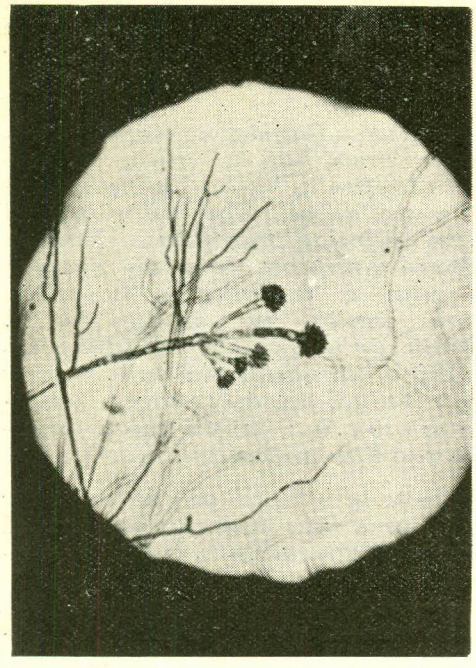

Fig. 2.—Proliferation of phialides.

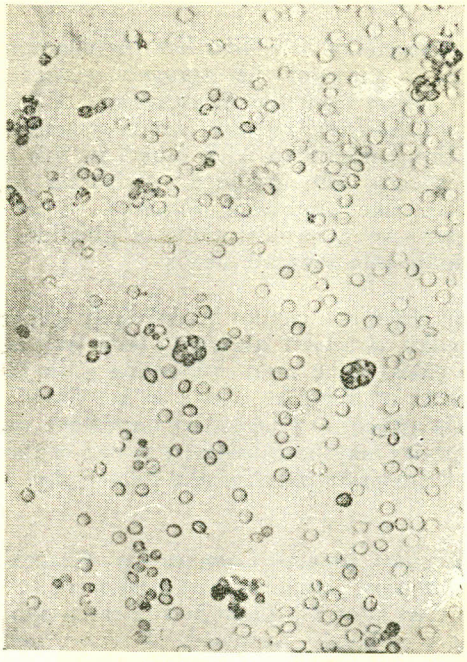

Fig. 4.—Asci and Ascospores.

PLATE I.-*Aspergillus chevalier* var. *proliferans*.

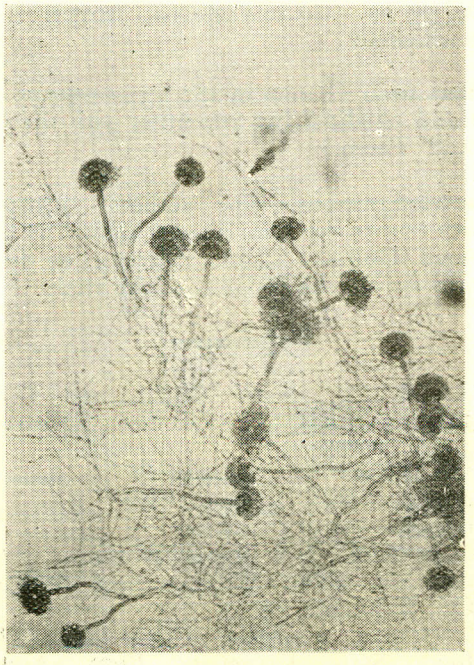

Fig. 1.—Conidiophores Aspergillus terreus.
mag. 20X

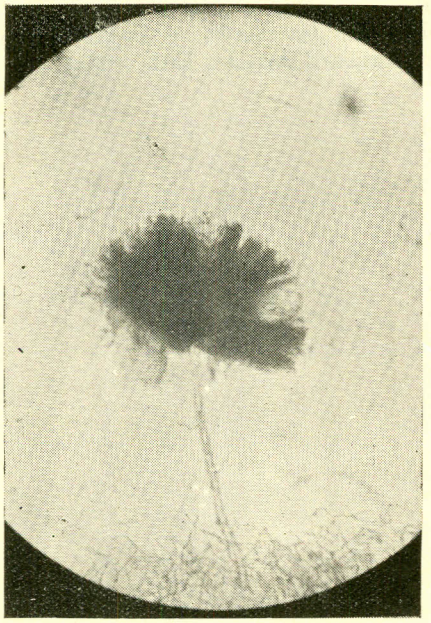

Fig. 2.—Conidiophore Aspergillus sachari
mag. 120X

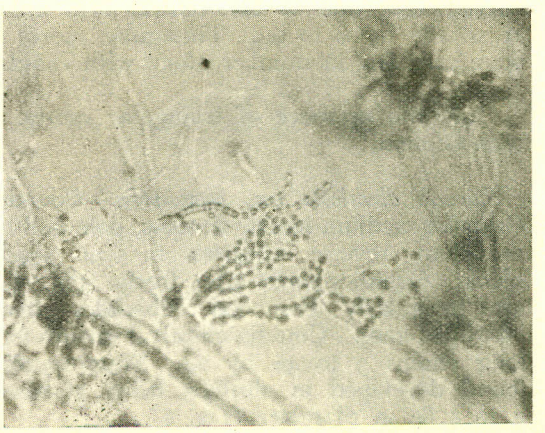

Fig. 3.—Conidiophores Spicaria divaricata Var. heterospora.
mag. ×400

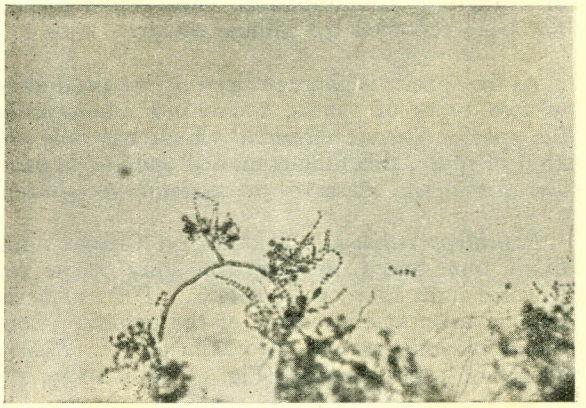

Fig. 4.—Conidiophores showing micro-and macrospores Spicaria divaricata Var. heterospora.
mag. 200X

Fig. 5.—Conidiophores Penicillium citrinum.
mag. 200X

PLATE II

of conidiophores, three septate. Conidia typically curved with one of the central cells enlarged, 16 to 28μ by 8 to 9μ. Triangular spores found were very few in number along with conidia.

Only the presence of triangular spores varies with Gilman.

Spicaria divaricata var. heterospora.—Colonies on Czapek's medium loosely floccose, pale dull brown in colour, reverse colourless to some shades of black. Conidiophores arise freely (Fig. 3) and are irregularly branched. Phialides divergent 16 to 32μ long. Conidia elliptical smooth forming long chains 3 to 4μ by 2μ. Macrospores also found developed in clusters, being globose and 4 to 4.8μ in diameter (Fig. 4).

There are two types of spores, found in this species. On the same conidiophore, develop both macro and microspores and also separately on different conidiophores. Macrospores have not been reported by Gilman so far.

As the species is characterized by the production of two types of spores, micro-and macro-unlike the species *Spicaria divaricata* which has only one kind of spore, this fungus named *Spicaria divaricata var. heterospora*, deserves a varietal recognition.

Penicillium citrinum.—Colonies on Czapek's medium blue green to brownish gray when old. Reverse light maroon in colour. Tufts of aerial hyphae arise at the centre of the colony. Conidiophores long septate upto 212μ by 2μ. *Penicilli* consist of 3 to 5 metulae (Fig. 5) 12 to 20 by 2 to 2.8 each producing a compact vertical of phialides 8 to 10μ by 2μ. Conidia globose smooth 2 to 2.4μ in diameter.

It differs from Gilman in the measurement of metulae and phialides but resembles with that of Raper and Thom.[5]

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