PRELIMINARY STUDY OF FUNGAL FLORA ON STORED FEEDS

HSI-YEN TSENG(1) and ZUEI-CHING CHEN(2)

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Abstract: A mycological survey on stored feeds was performed. By dilution method, total of 17 genera, 37 species of fungi was detected. The majority of the species belonged to the genera *Aspergillus* and *Penicillium*. The dominant species of fungi isolated were *Aspergillus vesicolor* and *Aspergillus oryzae* var. *microsporus*.

INTRODUCTION

Storage fungi playing a decisive role in the deterioration of stored grains have been discussed (Christensen, 1957). Tung *et al.* (1967) showed that 16 strains of *Aspergillus flavus* isolated out of 106 foodstuffs were capable of producing aflatoxin. Ling *et al.* (1967) proposed that the contaminated aflatoxin might explain partly the high incidence of primary liver carcinoma in Taiwan. Efforts also had been concentrated on aflatoxin contamination of rice, peanut oil, peanut products, herbal medicines, imported cereals, corn and feedstuff (Ling *et al.*, 1968; Chung *et al.*, 1971; Cheng & Wu, 1972; Tseng, 1975; Koh & Tseng, 1975; Tseng *et al.*, 1977; Tseng & Yuan, 1978). Tseng & Yuan (1978) indicated that feeds of swine and chicken exhibited more severely contaminated with aflatoxins. Tzean *et al.* (1979) conducted a survey upon the population dynamics of fungi on stored rice, and isolated numerous storage fungi. However, the mycoflora on feedstuff was little known. This study was dealing with fungal flora in the stored feeds of swine and chicken.

MATERIALS AND METHODS

1. Sources of feeds

Various feeds were randomly sampled from Taipei, Taoyuan, Taichung, Chiayi, Tainan, Kaohsiung, respectively. Status of these samples were shown in Table 1.

2. Isolation and identification of fungal flora on feeds

By dilution method, 1 g of each sample was put into a sterile tube containing 9 ml sterile water, stirred and then transferred 1 ml of this dilution to a second tube containing 9 ml sterile water to reach the final 1/100 dilution. One ml of the dilutions were pipetted into plates of Czapek's solution agar containing streptomycin (20 mg/l). Five plates were used for each sample. After the plates were incubated at 25°C for 3 days, two single-hypha tips growing out from each colony were cut with flame-sterilized knife, and transferred to two individual PDA slants to established pure cultures. The plates were subsequently kept for 7 days to re-examine slow growing colonies. Identification was undertaken by gross morphology, microscopic examination and slide culture, and was made by following the key references: Arx, 1974; Booth, 1966; Ellis, 1971; Morton & Smith, 1963; Raper & Fennell, 1973; Raper & Thom, 1968; Zycha & Siepmann, 1969; and by the reference cultures supplied by IFO, Osaka, Japan.

(1) 曾禧燕, Instructor, Department of Botany, National Taiwan University.
(2) 陳瑞青, Professor, Department of Botany, National Taiwan University.

Table 1. Status of feeds sampled from Taipei, Taoyuan, Taichung, Chiayi, Tainan, Kaohsiung areas, respectively

Sampling locality	Sampling date	No. of samples	Storage periods (days)	Storage location	Antiseptic (propionic acid)	Feeds for	Note
Taipei	8. 22, 1980	3	105	feed mill (warehouse)	−	chicken	high calorie
Taipei	8. 22, 1980	3	30	feed store	−	chicken	high calorie
Taoyuan	9. 25, 1980	1	21	chicken farm (barn)	−	chicken	high calorie
Taichung	8. 20, 1980	1	90	feed mill (warehouse)	+	swine	antibiotics (+)
Taichung	8. 20, 1980	1	45	pig farm (barn)	+	swine	sugar
Taichung	8. 20, 1980	1	30	pig farm (barn)	+	swine	high sugar
Taichung	8. 20, 1980	2	60	pig farm (barn)	+	swine	high sugar
Chiayi	8. 13, 1980	2	19	pig farm (barn)	−	swine	antibiotics (+)
Chiayi	8. 13, 1980	2	4	pig farm (barn)	−	swine	high sugar
Chiayi	8. 14, 1980	4	45	chicken farm (barn); open storage	+	chicken	low calorie
Tainan	8. 14, 1980	4	105	chicken farm (barn)	+	chicken	high calorie
Tainan	8. 14, 1980	1	150	pig farm (barn); open storage	+	swine	sugar
Kaohsiung	8. 16, 1980	2	15	chicken farm (barn)	−	chicken	high calorie
Kaohsiung	8. 16, 1980	2	13	chicken farm (barn)	−	chicken	high calorie

* All the samples were supplied by AGRIASIA DEVELOPMENT CORP. Taipei, Taiwan, ROC.

RESULTS

Fungal flora and their prevalence of different locality were given in Table 2. A total of 17 genera, 37 species of fungi were isolated from stored feeds. The majority of the species belonged to the genera *Aspergillus* (29.7%) and *Penicillium* (21.6%). Some other genera such as *Mucor*, *Rhizopus* and *Absidia* also isolated frequently. *Aspergillus oryzae* var. *microsporus* and *Aspergillus versicolor* were found to be the most prevalent among the Aspergilli isolated. *Penicillium martensii* occurred as the most widely distributed species in the Penicilli isolated. Storage fungi as mentioned above were dominant in all samples, while field fungi only occurred rarely, such as *Fusarium*, *Alternaria*, *Curvularia*, *Phoma* and *Trichoderma*. Some of the isolated fungi with economic or unique value in fungal flora of Taiwan were selected and photographed as illustrating in Plates I-IX.

DISCUSSION

This survey was a preliminary study on fungal flora of feeds. At the times of sampling, these feeds were not subjected to any practice of quantitative survey, therefore only a dilution method was applied. The most prevalent fungi in this study were found to be the Aspergilli and Penicilli groups, which agreed with the results reported by several other surveys on fungal flora in stored cereals (Sakabe & Kurata, 1973; Ichinoe *et al.*, 1973; Tzean *et al.*, 1979). It is almost axiomatic in microbiological work that any culture medium used to isolate microorganisms from materials in which they are present is to a certain extent selective. Christensen (1957) indicated that acid potato dextrose agar, as well as Czapek-Dox agar, were unsuitable to detect the presence of some of the common subspecies of *Aspergillus glaucus* that were most

Table 2. The total fungal flora on stored feeds sampled from Taipei, Taoyuan, Chiayi, Tainan, Kaohsiung areas, respectively

Species of fungus	Districts of fungus recorded					
	Taipei	Taoyuan	Taichung	Chiayi	Tainan	Kaohsiung
**,* *Absidia corymbifera* (Cohn) Saccardo & Trotter				+	+	
Absidia hyalospora (Saito) Lendner	+	+				
Alternaria alternata (Fr.) Keissler					+	
**,* *Aspergillus aculeatus* Iizuka			+			
Aspergillus candidus Link	+			+	+	+
Aspergillus clavatus Desmazieres	+					
Aspergillus flavus Link		+				
* *Aspergillus foetidus* var. *pallidus* Naka., Simo and Watanabe	+		+	+	+	+
Aspergillus fumigatus Fres.	+			+		
**,* *Aspergillus oryzae* var. *microsporus* Yamamoto	+	+	+	+	+	+
Aspergillus sydowi (Bain. & Sart.) Thom & Church			+	+	+	
Aspergillus tamarii Kita					+	
Aspergillus terrus Thom	+		+	+	+	+
Aspergillus versicolor (Vuill.) Tiraboschi	+	+	+	+	+	+
Cladosporium cladosporioides (Fresen.) de Vries		+				
**,* *Cladosporium oxysporum* Berk. & Curt.					+	
Cladosporium sphaerospermum Penz.	+				+	
Curvularia senegalensis (Speg.) Subram			+		+	
**,* *Cylindrocarpon hederae* Booth	+			+		+
Eurotium chevalieri Mangin	+				+	
Fusarium sp.	+					
Monilia sitophila (Mont.) Sacc.	+		+	+		+
Mucor sp.	+		+	+	+	+
* *Paecilomyces varioti* Bainier				+		
Penicillum corylophilum Dierckx	+	+				
**,* *Penicillium crustosum* Thom				+		+
Penicillium frequentans Westling	+	+		+	+	+
**,* *Penicillium godlewskii* Zaleski					+	
**,* *Penicillium implicatum* Biourge	+	+				
Penicillium islandicum Sopp.	+			+		
* *Penicillium italicum* Wehmer	+					
**,* *Penicillium martensii* Biourge	+	+	+	+	+	
* *Phoma pomorum* Thüm				+		
Rhizopus sp.	+	+	+	+	+	+
Scopulariopsis brevicaulis (Sacc.) Bain	+	+		+		
Syncephalustrum racemosum Cohn ex Schroter		+				
* *Trichoderma harzianum* Rifai	+					

+: presence of fungus
*: new records to stored cereals in Taiwan
**: new records to fungal flora of Taiwan

prevalent in stored seeds. He preferred to use Malt-Salt agar to isolate a large number of fungi significant in storage deterioration, especially in *Aspergillus glaucus* and *Aspergillus candidus* group, than any other media tested. Ichinoe *et al.* (1973) indicated that in general, Potato Dextrose agar yielded more wide-species of molds including plant pathogen, while Malt-Salt agar exhibited rather restricted growth of colonies, especially *Penicillium* and *Aspergillus*, and this seemed to be convenient for a transfer of each colony in sub-culture. Since this paper was a preliminary investigation on stored feeds, we used the Czapek's solution agar just for its convenience on the identification of Aspergilli and Penicilli groups. However, we isolated 14 species of new records on the stored cereals to Taiwan, and several of them were believed to be new records to the fungal flora of Taiwan. Some of these new records have been isolated from different sources, such as *Penicillium implicatum* from "Panmix" (Sakabe & Kurata, 1973), *Penicillum implicatum*, *Paecilomyces varioti* from barley grains and wheat grains (Ichinoe *et al.*, 1973), *Aspergillus foetidus* var. *pallidus* from herbal drug (Horie *et al.*, 1979), *Absidia corymbifera* from dried barley grain (Flannigan, 1969). The possible toxin producing fungal species (Ciegler *et al.*, 1971) isolated are *Aspergillus clavatus* (Ascladiol), *Penicillium implicatum* and *Aspergillus terrus* (Citrinin), *Aspergillus flavus* (Aflatoxin), *Aspergillus fumigatus* (Fumigatin), *Penicillium islandicum* (Chlorine-containing peptide, Letroskyrin), *Aspergillus oryzae* var. *microsporus* (Maltoryzine), *Aspergillus clavatus* (Patulin), *Aspergillus versicolor* and *Aspergillus flavus* (Sterigmatocystin), *Eurotium chevalieri* (Xanthocillin X), *Fusarium* spp. (Butenolide & Scirpenes). A thorough survey of the distribution and prevalence in stored feeds in this island is still essential from both academic and economical points of view and should be traced carefully and continuously in coming future.

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Plate I

Fig. 1. *Penicillium corylophilum* (F-0101). PDA. 3 weeks.
Fig. 2. *Penicillium crustosum* (F-0102). PDA. 3 weeks.
Fig. 3. *Penicillium frequentans* (F-0103). PDA. 3 weeks.
Fig. 4. *Penicillium godlewskii* (F-0104). PDA. 3 weeks.
Fig. 5. *Penicillium implicatum* (F-0105). PDA. 3 weeks.
Fig. 6. *Penicillium islandicum* (F-0106). PDA. 3 weeks.
Fig. 7. *Penicillium italicum* (F-0107). PDA. 3 weeks.
Fig. 8. *Penicillium martensii* (F-0108). PDA. 3 weeks.
Fig. 9. *Paecilomyces varioti* (F-0301). PDA. 3 weeks.

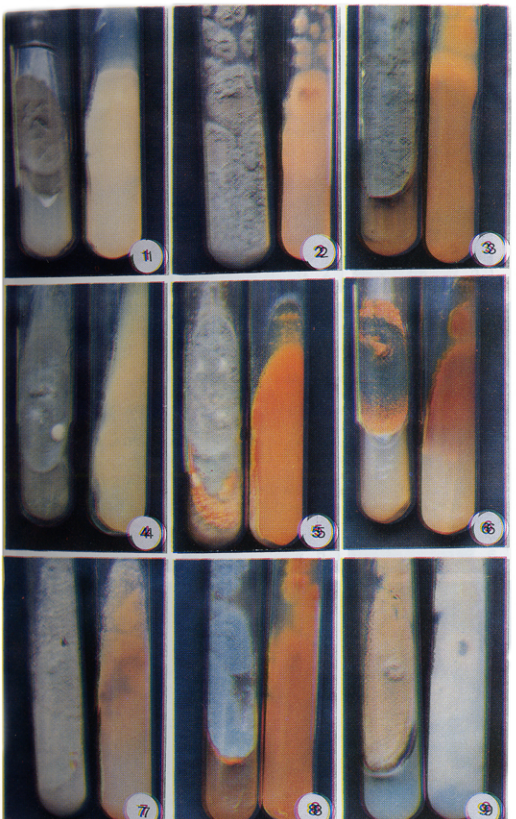
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Plate II

Fig. 1. *Aspergillus aculeatus* (F-0201). PDA. 3 weeks.
Fig. 2. *Aspergillus candidus* (F-0202). PDA. 3 weeks.
Fig. 3. *Aspergillus foetidus* var. *pallidus* (F-0205). PDA. 3 weeks.
Fig. 4. *Aspergillus tamarii* (F-0209). PDA. 3 weeks.
Fig. 5. *Aspergillus oryzae* var. *microsporus* (F-0207). PDA. 3 weeks.
Fig. 6. *Aspergillus sydowi* (F-0208). PDA. 3 weeks.
Fig. 7. *Aspergillus terrus* (F-0210). PDA. 3 weeks.
Fig. 8. *Aspergillus versicolor* (F-0211). PDA. 3 weeks.
Fig. 9. *Eurotium chevalieri* (F-0212). PDA. 3 weeks.


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Plate III

Figs. 1-2. *Aspergillus clavatus.* 1. Conidial heads showing characteristic clavate form of vesicle, ×154; 2. Portion of a conidial head further enlarged, showing closely packed single series of sterigmata, ×536. (F-0203).

Figs. 3-5. *Aspergillus flavus.* 3. Typical large to radiate head showing sterigmata in two series, ×880; 4. Some small heads showing single series of series of sterigmata, ×965; 5. Radiate head borne from roughened condiophore, ×670 (F-0204).

Fig. 6. *Aspergillus oryzae* var. *microsporus.* Conidial head, ×804. (F-0207).


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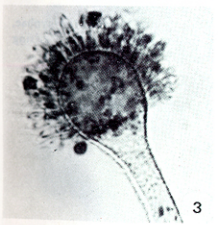
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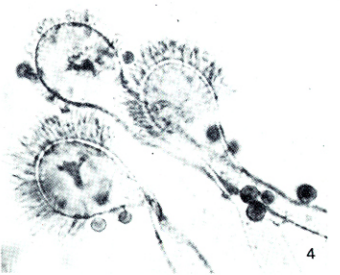
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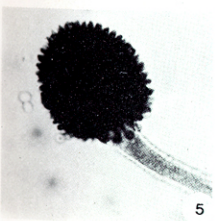
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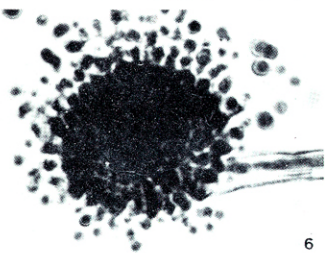
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Plate IV

Figs. 1-2. *Aspergillus terrus.* 1. Conidial heads showing compactly columnar form, ×201; 2. Portion of conidial head further enlarged, showing closely crowed biseriate sterigmata, ×804. (F-0210).

Figs. 3-4. *Aspergillus tamarii.* 3. Conspicuously roughened condia, ×804; 4. Conidial head, ×804. (F-0209).

Figs. 5-6. *Aspergillus sydowi.* 5. Small head, borne as a lateral branch on an aerial hyphae, ×804; 6. Typical large conidial head borne upon an erect conidiophore arising from the substratum, ×804. (F-0208).

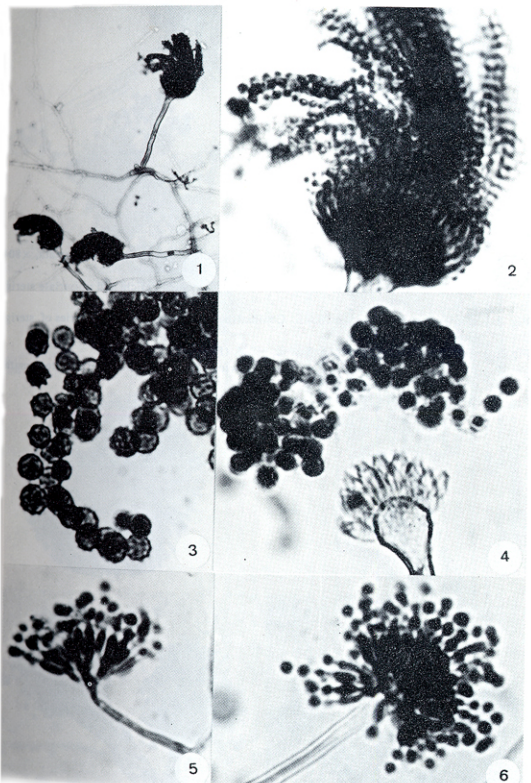
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Plate V

Fig. 1. *Aspergillus versicolor.* Conidial heads showing the double series of sterigmata, ×804. (F-0211).

Fig. 2. *Aspergillus aculeatus.* Conidial head showing pigmented vesicle and uniseriate sterigmata, ×670. (F-0201).

Fig. 3. *Aspergillus foetidus* var. *pallidus.* Conidial head showing the double series of sterigmata, ×643. (F-0205).

Fig. 4. *Aspergillus candidus.* Conidial head, ×804. (F-0202).

Fig. 5. *Aspergillus fumigatus.* Conidial heads showing the crowded uniseriate sterigmata, ×884. (F-0204).

Fig. 6. *Eurotium chevalieri.* Conidial heads, ×201. (F-0212).

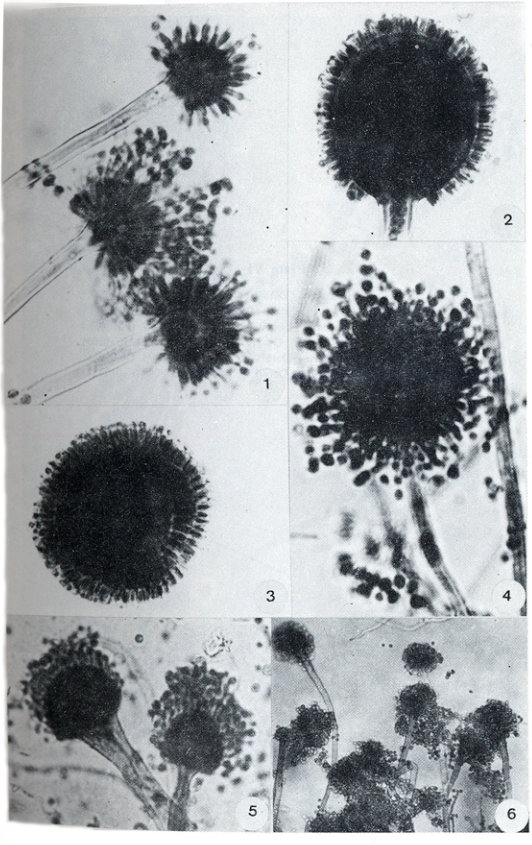
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Plate VI

Fig. 1. *Penicillum implicatum.* Detail of penicilli, ×804. (F-0105).
Fig. 2. *Penicillium italicum.* Detail of penicillus, ×804. (F-0107).
Fig. 3. *Penicillium crustosum.* Detail of penicillus, ×804. (F-0102).
Fig. 4. *Penicillium frequentans.* Detail of penicillus, ×804. (F-0103).
Fig. 5. *Penicllium islandicum.* Detail of penicillus, ×804. (F-0106).
Fig. 6. *Penicillium godlewskii.* Detail of penicilli, ×804. (F-0104).

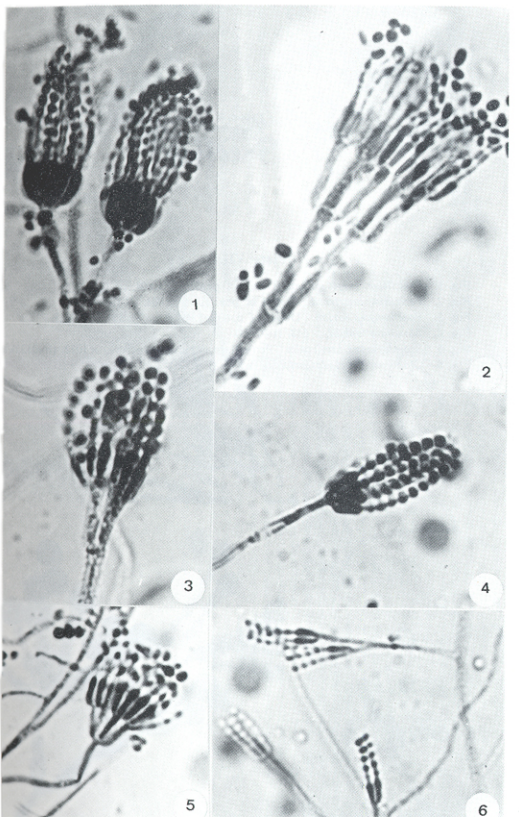
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Plate VII

Fig. 1. *Penicillium corylophilum.* Detail of penicilli, ×804. (F-0101).
Fig. 2. *Penicillium martensii.* Detail of penicillus, ×804. (F-0108).
Fig. 3. *Paecilomyces varioti.* Detail of conidial structure. ×804. (F-0301).
Fig. 4. *Monilia sitophila,* ×648. (F-0303).
Fig. 5. *Scopulariopsis brevicaulis.* Penicillate conidial structure, ×1072. (F-0302).
Fig. 6. *Cladosporium cladosporioides,* ×720. (F-0304).

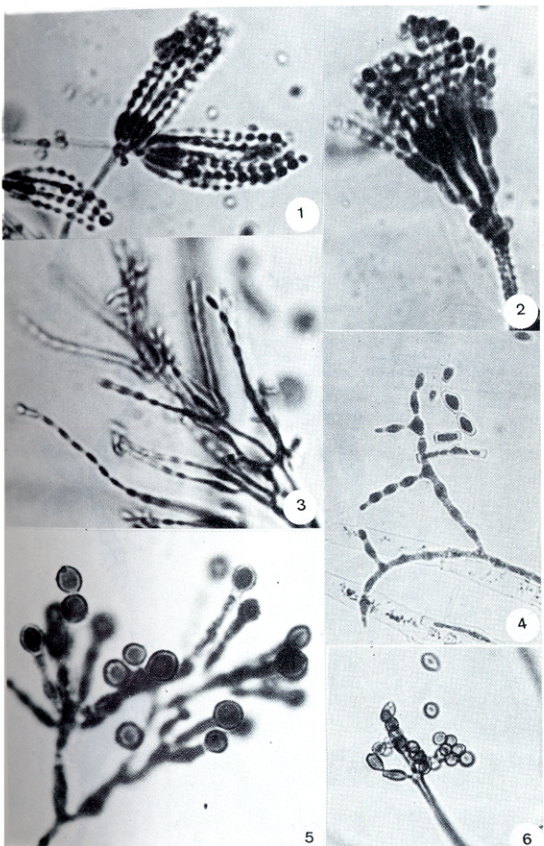
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Plate VIII

Fig. 1. *Curvularia senegalensis*, ×804. (F-0307).
Fig. 2. *Absidia corymbifera*, ×360. (F-0401).
Fig. 3. *Alternaria alternata*, ×480. (F-0308).
Figs. 4-5. *Rhizopus* sp. 4. Sporangia, ×360; 5. Rhizoids, ×324. (F-0403).
Fig. 6. *Cladosporium sphaerospermum*, ×804. (F-0306).
Fig. 7. *Trichoderm harzianum*, ×750. (F-0309).

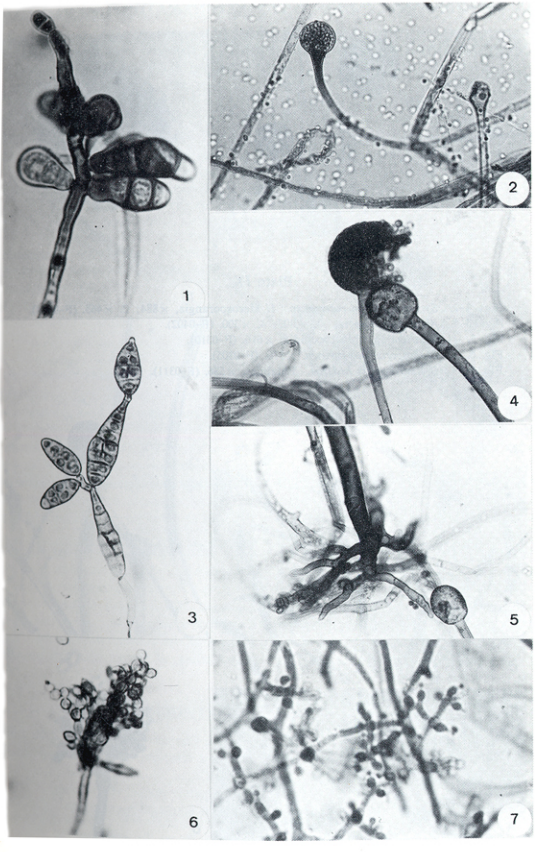
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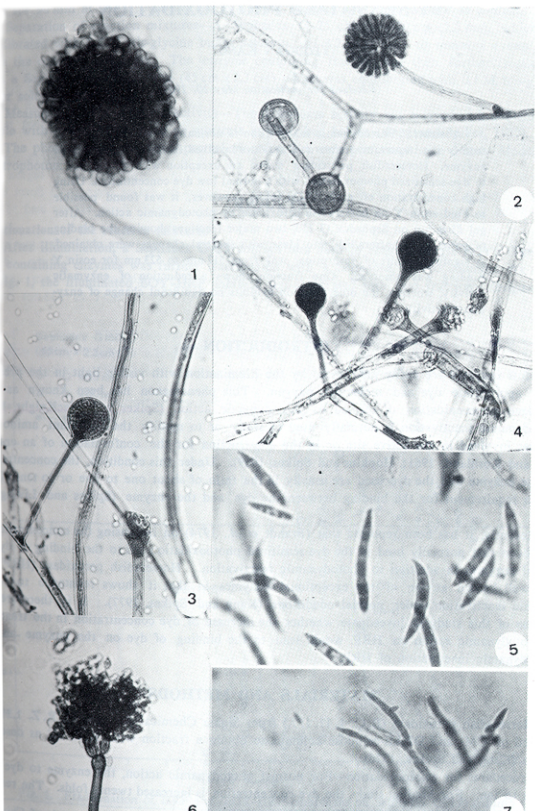
Plate IX

Figs. 1-2. *Syncephalustrum racemosum.* 1. Merosporangia, ×884; 2. ×468. (F-0404).
Figs. 3-4. *Absidia hyalospora.* 3. ×300; 4. ×300. (F-0402).
Fig. 5. *Fusarium* sp. Macroconidia. ×616. (F-0310).
Fig. 6. *Cladosporium oxysporum.* ×576. (F-0305).
Fig. 7. *Cylindrocarpon hederae.* Phialides. ×536. (F-0311).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