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STUDIES ON THE OCCURRENCE OF FUNGI IN A WHEAT–FIELD. I. MESOPHILIC, THERMOPHILIC AND KERATINOPHILIC FUNGI IN SOIL

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ABSTRACT

From October 1982 to July 1983 a total of 1772 fungal colonies were recorded from a wheat-field soil in Lombardy. Meso- thermo- and keratinophilic fungi were found with the following breakdowns: mesophilic (59 species), ***Fusarium oxysporum, Lipomyces starkeyi, Penicillium janthinellum dominant; Cryptococcus albidus, Gibberella acuminata, Gliocladium roseum, Rhizopus stolonifer*** *frequent;* ***Bahusakala olivaceonigra, Pseudallescheria boydii, Truncatella angustata, Verticillium lecanii, Pyrenochaeta ilicis, Myrothecium roridum, Drechslera dematioidea*** *and others rare; thermophilic (15 species),* ***Aspergillus fumigatus*** *and* ***Rhizomucor pusillus*** *dominant;* ***Scytalidium thermophilum*** *and* ***Thermomyces lanuginosus*** *frequent; keratinophilic 11 species),* ***Myceliophthora*** *anamorph of* ***Ctenomyces serratus, Mycrosporum gypseum, fulvum complex*** *and* ***Chrysisporium merdarium*** *dominant;* ***Chrysosporium keratinophilum*** *and* ***Ctenomyces serratus*** *frequent. Incidence in all groups increased markedly during February and April.*

RESUMEN

[*Estudio sobre la presencia de hongos en campo de trigo. I Hongos mesofílicos, termofílicos y queratinofílicos en los suelos.*]

Desde Octubre 1982 a Julio 1983 un total de 1772 colonias fúngicas se aislaron desde suelos de trigales en Lombardía. Se estudiaron hongos mesófilos, termófilos y queratinófilos. Entre los mesófilos (59 especies) se detectan como dominantes: ***Fusarium oxysporum, Lipomyces starkeyi, Penicillium janthinellum;*** *como frecuentes,* ***Cryptococcus albidus, Gibberella acuminata, Gliocladium roseum, Rhizopus stolonifer;*** *y otros raros* ***Bahusakala olivaceonigra, Pseudallescheria boydii, Truncatella angustata, Verticillium lecanii, Pyrenochaeta ilicis, Myrothecium roridum, Drechslera dematioidea.*** *Entre los termófilos (15 especies) como dominantes,* ***Aspergillus fumigatus y Rhizomucor pusillus;*** *frecuentes,* ***Scytalidium thermophilum y Thermomyces lanuginosus.*** *Entre los queratinofílicos (11 especies), como dominantes* ***Myceliophthora*** *anamorfo de* ***Ctenomyces serratus, Microsporum gypseum - fulvum*** *complex y* ***Chrysosporium merdarium*** *como frecuentes,* ***Chrysosporium keratinophilum y Ctenomyces serratus.*** *Durante Febrero y Abril, la incidencia de todos los grupos aumenta notoriamente.*

INTRODUCTION

As part of a research proyect on soil and phylloplane fungi of wheat, maize and rice, we report the occurrence of mesophilic, thermophilic and keratinophilic fungi from Octobor 1982 to July 1983 in a wheat-field soil at Voghera in the province of Pavia (Lombardy).

MATERIALS AND METHODS

The soil

The wheat-field is at Voghera, Lombardy, a region with a continental climate of hot summers and hard winters. Seasonal rains occur in March-April and September-October.

Soil analysis made in 1982 showed the following composition and characteristics; sand 39,45 o/o; silt 36.95 o/o; clay 23.60 o/o; pH 7.85; total Nitrogen (Jodlbauer) 0.190 o/o; P2O5 (Ferrari's method)

115 ppm; K2O (Seay-Attoe-Truog) 720 ppm; organic Carbon bicromate oxidation method 1.273 o/o; humus 2.193 o/o; carbon-nitrogen ratio 6.700.

The field was sown with tender winter wheat, cultivar "San Pastore" having been a meadow in 1981.

It was ploughed in October 1982 and fertilized with P, K and N at 240 Kg/ha. Sowing was done in late October and harvesting the following July. Herbicides and fungicides were not used. Soil fungal analyses were made at monthly intervals in a plot of 28 m2: the October samples were collected before sowing.

Collection of soil samples and isolation of fungi

All the soil samples were always collected int the same sides of the field during the research. The soil samples were collected in October (before sowing), in November (after sowing), from January to June (during the cereal growth) and in July (after harvest).

The monthly soil samples were: three for the meso- thermo- and keratinophilic fungi isolation and other six for thermo- and keratinophilic fungi. The different number of soil samples for the different fungal groups was suggested because the thermophilic and keratinophilic population was scarcely representative in only three soil samples. The aim of this research was to evaluate the qualitative fungal population of soil during the life cycle of wheat. Each soil sample was collected by inserting sterile plastic specimen tubes (length 15 cm and internal diameter 30 mm) into the soil. Each soil sample was mixed and air dried.

Fungal analyses were made on the day at soil collection by three different methods.

MESOPHILIC FUNGI: fungal analysis of three soil samples monthly collected was by the dilution method (1/5000): 10 g of mixed soil from each sample were added to 500 ml of sterile water and shaken for 30 min. to obtain maximum dispersion. Five ml of this dilution were added to 495 ml of sterile water. Two ml of this dilution were poured per 16 cm diameter plate onto potato dextrose agar (PDA) to which rose Bengal (0.035 g/l), aureomycin (50 μg/ml) and streptomycin (0.1 mg/ml) had been added, and acidified to pH 4.5. Three plates from each sample were prepared for a total of nine plates. The plates were incubated at 22° C and examined at intervals over a period of 2-4 weeks for the recording and isolation of fungi. All fungal colonies recorded were counted.

THERMOPHILIC FUNGI: two g of each soil sample were plated, in duplicate, directly on Petri dishes containing malt extract agar (MEA) with the addition of rose Bengal (0.035 g/l), aureomycin (50 μg/ml) and streptomycin (0.1 mg/ml) and acidified to pH 4.5. Half of these dishes were incubated at 45° C and the others at 50° C for three days.

The termophilic or thermotolerant nature of the species isolated was determined by measuring their growth at 18° C and 50° C. For every soil sample, each fungal species was counted only once even if numerous isolates of this species were present in the plates of the same sample, because we wanted to record only the presence of every species.

KERATINOPHILIC FUNGI: sixty g of each sample were transferred aseptically into sterile Petri dishes of 16 cm diameter. These were moistened with distilled water containing cycloheximide at 2 o/oo and baited with autoclaved fragments of human and horse hair and fowl feathers.

The Petri dishes were incubated at 25° C and examined at intervals for up to 12 weeks being remoistened when necessary.

Keratinophilic fungi which developed were identified microscopically and cultured on YpSs (Bacto-yeast extract, 4 g; soluble starch, 15 g; dipotassium sulphate, 1 g; magnesium sulphate, 0,5 g; Bacto-agar, 20 g; water, 1 litre) and Sabouraud agar.

As this technique does not permit the counting of individual colonies, each fungal species was counted only once on a given Petri dish, even if it was present on all three kinds of baits.

RESULTS

MESOPHILIC FUNGI

Over the period a total of 1479 colonies represented by 59 species belonging to 36 genera were isolated from a total of 24 soil samples (Table I). The number of species varied monthly. eg. from 26 in February to 8 in November; these differences were not directly correlated with the number of fungal colonies.

Acremonium, Aspegillus, Cladosporium, Cryptococcus, Fusarium, Gliocladium, Lipomyces and **Penicillium** were the most common genera; among these **Aspergillus, Fusarium** and **Penicillium** were present all months and were the genera represented by the most species.

Among the 59 species recorded, 42 were present only after sowing, 14 both before and after sowing and 3 only before sowing. **Aspergillus,** the genus with the highest number of species (9 sp.), was represented before sowing with only one species (**A. alutaceus**).

Penicillium janthinellum and **Fusarium oxysporum** were the only two species occurring all months. **Lipomyces starkeyi** always occurred, except in November, with a great number of colonies in July. **Cryptococcus albidus** was regularly present from February to July. The genera **Arthrinium,**

Aureobasidium and **Stachybotrys**, althought of sporadic occurrence, were represented by more than one species; some genera were represented by only one species and several fungi were isolated only once.

THERMOPHILIC FUNGI

Thermophilic fungi isolated are summarized in Table 2.

Out of 81 soil samples examined, 77 (95%) contained thermophilic and thermotolerant fungi. Many soil samples yielded more than one thermophilic fungus.

Fifteen species of thermophilic and thermotolerant fungi were isolated: eight were truly thermophilic. The number of isolates and species was low in October before sowing and in July 1983, but it rose gradually during the cold months, with a maximum in February and April.

The thermotolerant **A. fumigatus** and the thermophilic **Rhizomucor pusillus** were the most frequent, with the former most abundant and always present. In addition to these two species, **Scytalidium thermophilum** and **Thermomyces lanuginosus** were recorded frequently.

KERATINOPHILIC FUNGI

Keratinophilic fungi recorded are listed in Table 3.

Among the 81 soil samples examined, 72 (88.9%) contained keratinophilic fungi. Many dishes yielded more than one fungus.

The highest number of species and isolates of these fungi was recorded in April.

The most common species was **Myceliophthora** anamorph of **Ctenomyces serratus** Eidam which represented 32.2% of the total isolates. This species occurred throughout the year and was most common in October, November, April and July. Its teleomorph **Ctenomyces serratus** Eidam was also often recorded. **Microsporum gypseum - fulvum** complex was the next most common species at 16.8% and was recorded more abundantly in spring. **Chrysosporium** represented the highest percentage of the total isolates, with **Ch. merdarium** and **Ch. keratinophilum** dominant and five isolates identified as **Ch. queenslandicum** Apihis & Rees (6). **Trichophyton terreste** was recorded only from two soil samples, in April; one produced fertile ascomata characteristic of **Arthroderma quadrifidum**. Only one isolate each of **T. gloriae** and **A. cuniculi** were obtained.

All species of **Chrysosporium** were more often isolated on feathers and horse hair, **Ct. serratus** and his anamorph on feathers, **M. gypseum - fulvum** complex on horse hair and human hair. Other fungi colonizing keratinized substrata included: **Alternaria alternata** (2 isolates), **Acremonium strictum** (1 is.), **Verticillium chlamydosporium** (2 is.), **Fusarium oxysporum** (1 is.), **Paecilomyces lilacinus** (3 iso.), **Penicillium citrinum** (4 is.) and **Scopulariopsis brevicaulis** (1 is.).

DISCUSSION

The following general conclusions may be drawn from this study. Our results confirm those of earlier researches making similar studies (5, 12, 27) showing that wheat-field soils are inhabited by a rich population of mesophilic, thermophilic and keratinophilic fungi.

Among mesophilic species, **Bahusakala olivaceonigra** was especially significant because it was recorded in Italy for the first time. This species is very similar to **Scytalidium flavo-brunneum** and **Bahusakala** state of **Aulographina pinorum** (26). The microscopic and colonial morphology of our strain conforms to that of **B. olivaceonigra**; though our strain was associated with **Chaetomium dolichotrichum** in culture, we could not say if it was the teleomorph or a contaminant.

We always isolated **F. oxysporum** and neither found true pathogens nor wheat diseases (in spite of no chemicals was applied): this could be a further confirmation that **F. oxysporum** plays a key role in the suppressive properties of certain soils (3).

The thermophilic fungi were abundant from November to April and Ellis (14) also recorded the thermophilic species **A. fumigatus, Chaetomium gracile, Myceliophthora thermophila** and **Thermomyces lanuginosus** from antarctic and subantarctic soils, in spite of the fact that this group of fungi occurs generally in temperate and tropical areas. It is also of interest that the thermotolerant strain of **A. fumigatus** was constantly present, while the mesophilic strain was very infrequent. Our data confirm earlier studies showing **A. fumigatus, Rhizomucor pusillus, Scytalidium thermophilum** and **Thermomyces lanuginosus** to be generally abundant in soil (4, 13), even if informations concerning thermophilic fungi from wheat fields are few (5, 23). Correlation between occurrence of these organisms and soil factors or vegetation has not geenerally been shown (15, 16). These fungi are of considerable importance not only as mediators of biological transformations, but also as cause of diseases in man or in animals. For instance **Rhizomucor pusillus** and **A. fumigatus** are potential pathogens to mammals (1, 10, 24, 25).

The keratinophilic species **Myceliphthora** anamorph of **Ctenomyces serratus** and **M. gypseum - fulvum** complex (among the most common species

isolated in this research) are found in soil all over the world (11, 21, 22). Instead, **Trichophyton gloriae** has a limited geographic distribution (2, 17). It is also worth noting the apparent absence of **Keratinomyces ajelloi**, a species which we recorded in maize and rice field soils (8, 9).

The strains identified as **Ch. queenslandicum** showed cultural characteristic and microscopic morphology very similar to those of **Ch. crassitunicatum** Kushwaha & Agraval (18) reduced to synonymy with **Ch. queenslandicum** by van Oorschot (20). Our strains had smaller conidia, with two distinctly visible layers. The keratinophilic fungi recorded, in spite of their world-wide distribution, are insignificant for human and animal pathology. Only **M. gypseum - fulvum** complex is considered a potential human pathogen causing ring worm of the scalp and glabrous skin.

Although the biological activity and ability of some species to compete with other fungal or microbial groups in the soil have been studied (7, 19, 28), an evaluation of the combined occurrence and biological activity of mesophilic, thermophilic and keratinophilic fungi has not yet been done. The incidence of these three fungal groups, and above all of certain dominant species, may have a biological significance in relation to the wheat plant: the fungi recorded in this wheat field soil although largely common cosmopolitan species are very different from those we found in rice and maize field soils using the same methods (8, 9). For a more complete understanding of these three fungal components of wheat field soils, it is important to investigate the interactions or potential activity among mesophilic, thermophilic and keratinophilic fungi and between these fungi and the wheat plants. Experiments in this area of research are in progress.

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TABLE 1

Number of colonies of mesophilic fungi from wheat-field soil during October 1982 to July 1983

	O	N	F	M	A	M	J	J	Total
Acremonium furcatum F. & V. Moreau ex Gams					1				1
A. murorum (Corda) W. Gams	2					2			2
A. strictum W. Gams			2		3		11	7	23
Alternaria alternata (Fr.) Keissler								4	4
Arthrinium anamorph of **Apiospora montagnei** Sacc.						3	1		4
A. phaeospermum (Corda) Ellis	2								2
Aspergillus alutaceus Berk. & Curt.	14	327				1			342
A. clavatus Desm.					1				1
A. flavipes (Bain. & Sart.) Thom. & Church			2	1	1	1	1		6
A. fumigatus Fres.							3		3
A. nidulans (Eidam) Winter					1				1
A. niger van Thieghem		32							32
A. ustus (Bain) Thom & Church		18			1				19
A. versicolor (Vuill.) Tiraboschi						1			1
A. wentii Wehmer			3	5	5	3			16
Aspergillus spp.						1		4	5
Aureobasidium pullulans (de Bary) Arn. v. **pullulans**			1					1	2

	O	N	F	M	A	M	J	J	Total
G. roseum Bain	3		1	9	3			5	21
Gliocladium sp.	1								1
Lipomyces starkeyi Lodd. & Kreg. v. Rij	1		7	16	7	20	6	160	217
Mucor racemosus Fresen	1		1					9	11
Myrothecium roridum Tode ex Steudel				1					1
Nectria inventa Pethybr.			2						2
Paecilomyces lilacinus (Thom) Samson	8					1			9
Penicillium brevicompactum Dierckx		3	3	1	1				8
P. chrysogenum Thom			1	1					2
P. expansum Link ex Gray			214	108					322
P. frequentans Westling								11	11
P. janthinellum Biourge	8	1	5	2	6	3	12	1	38
P. purpurogenum Stoll	1								1
P. restrictum Gilman & Abbott	1				1			38	40
P. rubrum Stoll					3	1			4
Penicillium spp.	1			3	1			2	7
Phoma herbarum Westend.			4		6		17		27
Phomopsis archeri Sutton					6				6
Pseudallescheria boydii (Negroni) McGinnis, Padhye & Ajello					1				1
Pyrenochaeta ilicis Wilson							17		17

	O	N	F	M	A	M	J	J	Total
Pythium sp.							1		1
Rhizopus stolonifer (Ehrenb. ex Link) Lind	4	6			1		1	2	14
Rhodotorula rubra (Demme) Lodder			1						1
Scopulariopsis chartarum (G. Sm.) Morton & G. Sm.							1		1
Stachybotrys chartarum (Ehrenb. ex Link) Hughes					1		3		4
Stachybotrys anamorph of **Melanopsamma pomiformis** (Pers. Fr.) Sacc.						1			1
Trichoderma harzianum Rifai	1		1	1	3	1			7
Truncatella angustata (Pers. ex Link) Hughes			2		1				3
Verticillium lecanii (Zimm) Viégas			1						1
Sterile cultures	7	4	3	1	4	4	37	2	62
Total monthly colonies	62	397	282	170	65	85	137	281	1479
Total monthly genera	15	5	19	11	15	11	16	16	
Total monthly species	17	8	26	15	24	17	18	18	

TABLE 2

N° of isolations of thermophilic fungi in wheat-field soil expressed as the number of Petri dishes on which each fungal species was recorded

		Oct	Nov	Jan	Feb	Mar	April	May	June	July	Total
TT	**Absidia corimbifera** (Cohn) Sacc. & Trotter						1				1
TT	**Aspergillus fumigatus** Fres.	2	4	6	9	8	8	9	8	9	63
TT	**A. nidulans** (Eidam) Wint.				1		1				2
TT	**A. niger** van Tieghem			1	1	2	1				5
TP	**Chaetomium thermophile** La Touche			1	1			1			3
TP	**Humicola grisea** Traaen var. **thermoidea** Cooney & Emerson		2	1	1						4
TP	**H. insolens** Cooney & Emerson			1	1						2
TP	**Malbranchea pulchella** var. **sulfurea** (Miehe) Cooney & Emerson		1		1		2				4
TP	**Mycelia sterilia**		2	6	5	3	2	1			19
TT	**Myceliophthora thermophila** (Apinis) v. Oorschot		1				1		1		3
TT	**Paecilomyces variotii** Bain				1	1	2	1			5
TT	**Penicillium** sp.		1								1
TP	**Rhizomucor pusillus** (Lindt) Schipper	1		2	1	2	3		8		17
TT	**Scopulariopsis carbonaria** Morton & Smith						1				1
TP	**Scytalidium thermophilum** (Cooney & Emerson) Austwick		4		1	1	1				7
TP	**Thermoascus aurantiacus** Miehe						1				1
TP	**Thermomyces lanuginosus** Tsiklinsky		2		1	1	2				6
	Number of isolation	3	16	19	24	18	26	12	17	9	144
	Number of genera	2	6	5	8	5	10	3	3	1	
	Number of species	2	6	6	11	6	12	3	3	1	

TT = thermotolerant TP = thermophilic

TABLE 3

N° of isolations of keratinophilic fungi in wheat field soil expressed as the number of Petri dishes on which each fungal species was recorded.

	Oct	Nov	Jan	Feb	Mar	Apr	May	June	July	Total
Arthroderma cuniculi Dawson	1									1
A. quadrifidum Dawson & Gentles						1				1
Chrysosporium indicum (Randawa & Sandhu) Garg	3					4				7
Ch. keratinophilum (Frey) Carmichael		3	1	4	1	3	4			16
Ch. merdarium (Link ex Grey.) Carmichael	3			4	2	3	3	4	3	22
Ch. queenslandicum Apinis & Rees	1				1				3	5
Ch. tropicum Carmichael				1	7	3				11
Ctenomyces serratus Eidam	1	1				1		6	1	10
Microsporum gypseum (Bodin) Guiart & Grigorakis		5				7	2	9	2	25
Myceliophthora anamorph of **Ctenomyces serratus** Eidam	9	9	1	2	2	8	2	7	8	48
Trichophyton gloriae Ajello								1		1
T. terrestre Durie & Frey						2				2
Number of isolations	18	18	2	11	13	32	11	27	17	149
Number of samples positives	9	9	1	9	8	9	9	9	9	
Average N° of fungi per sample	2	2	0.22	1.22	1.62	3.55	1.22	3	1.88	
Number of species	6	4	2	4	5	9	4	5	5	

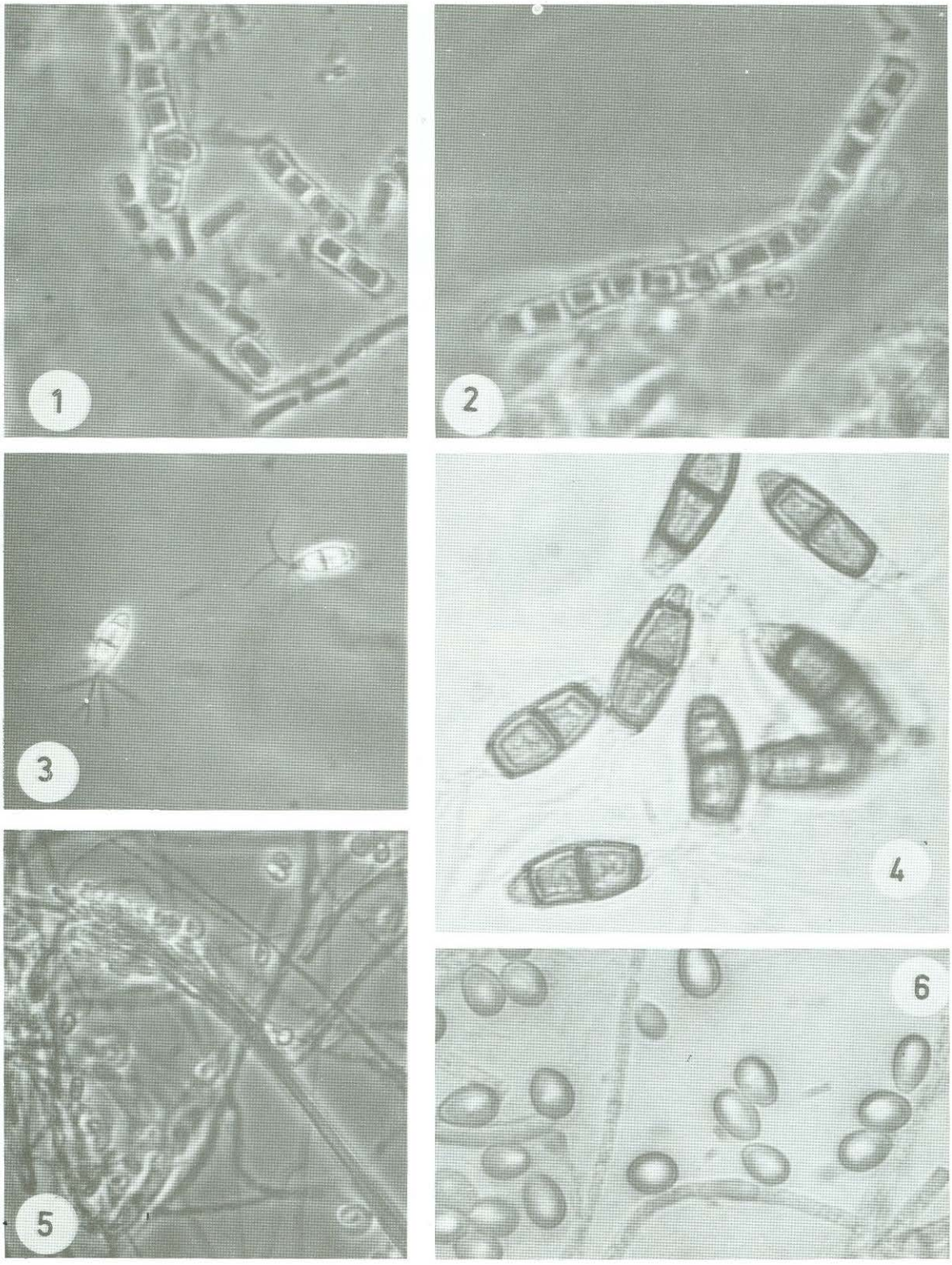
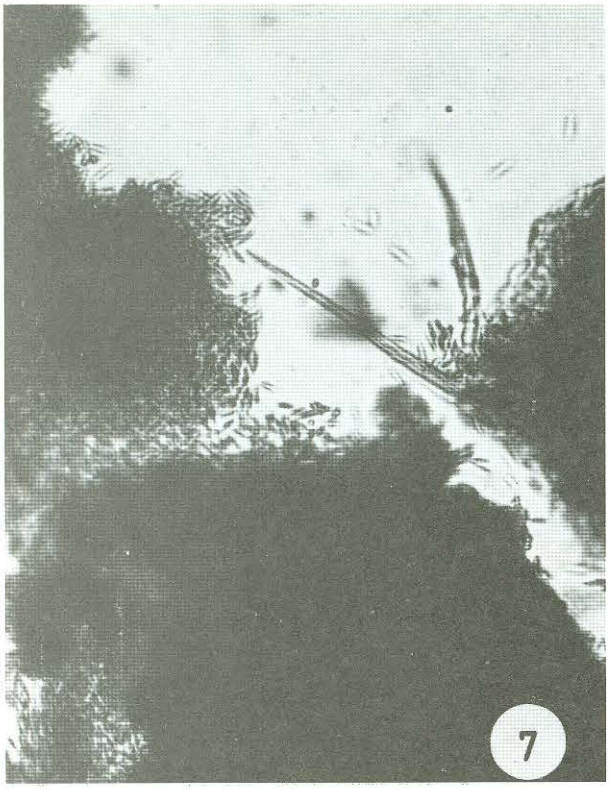
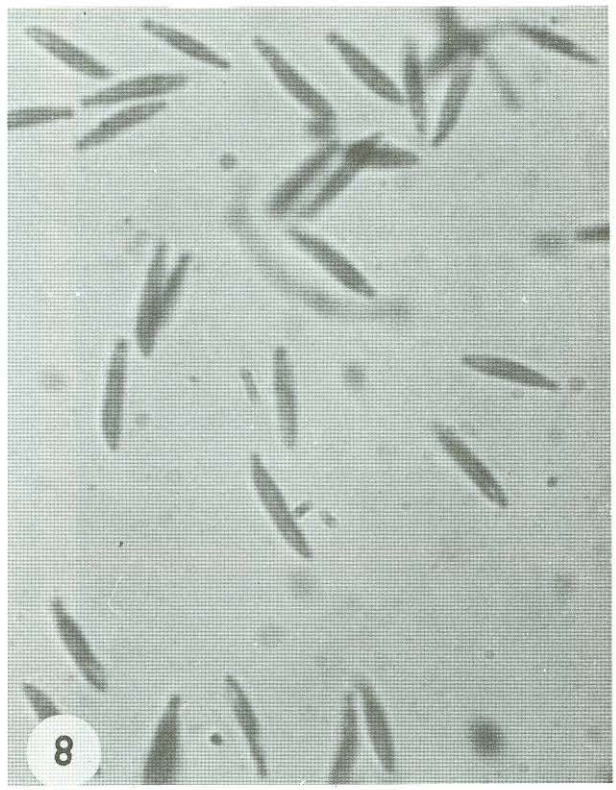
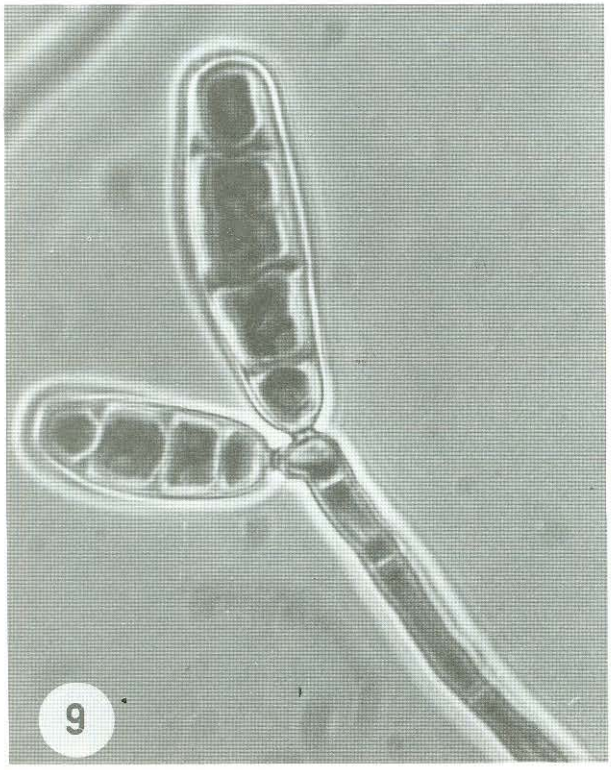


Fig. 1-2: **Bahusakala olivaceonigra;** mature fission arthroconidia x 1000. Fig. 3-4: **Truncatella angustata;** conidia with apical appendages branched irregularly x 400 and x 1000. Fig. 5-6: **Pseudallescheria boydii;** synnematous sporulation x 250 and conidia pyriform x 1000.







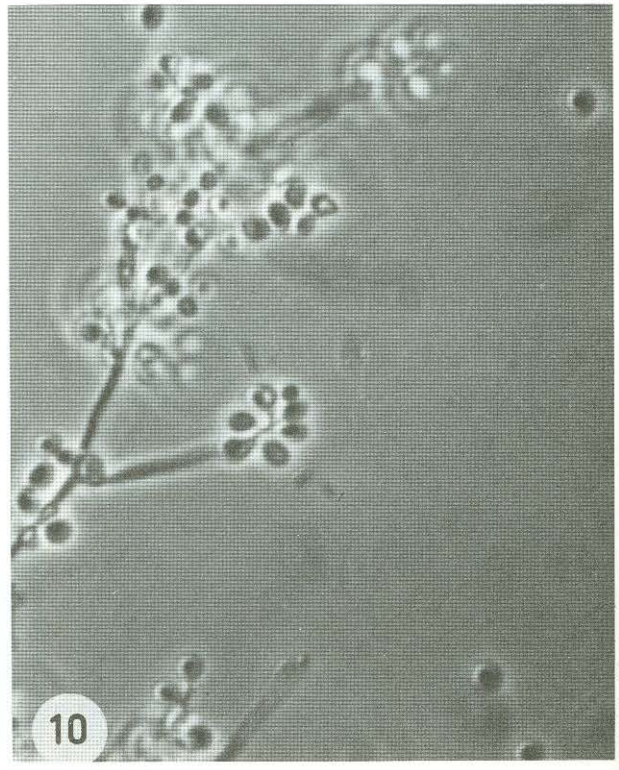
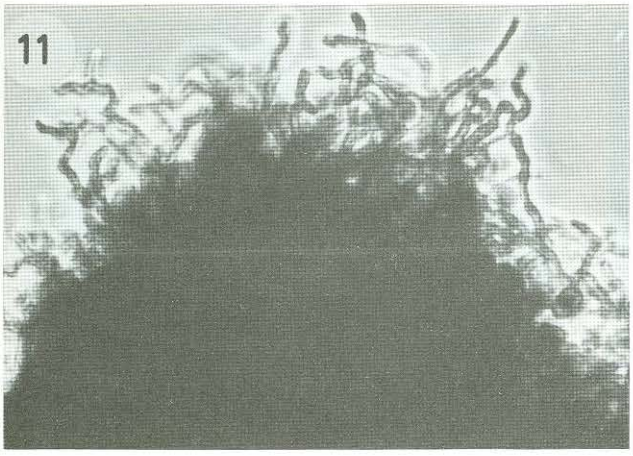
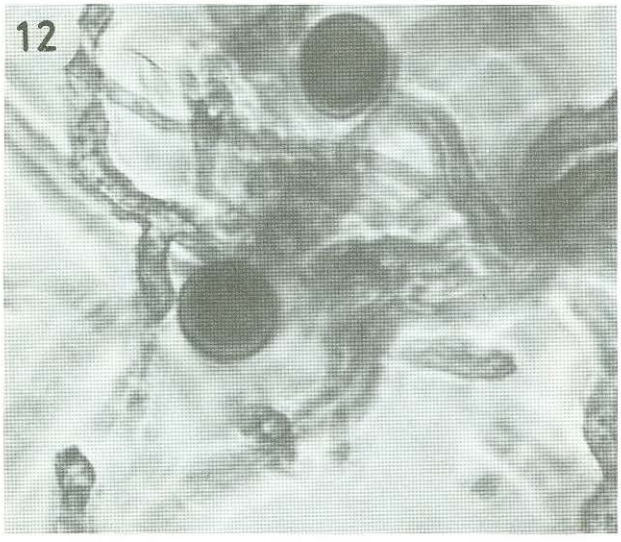
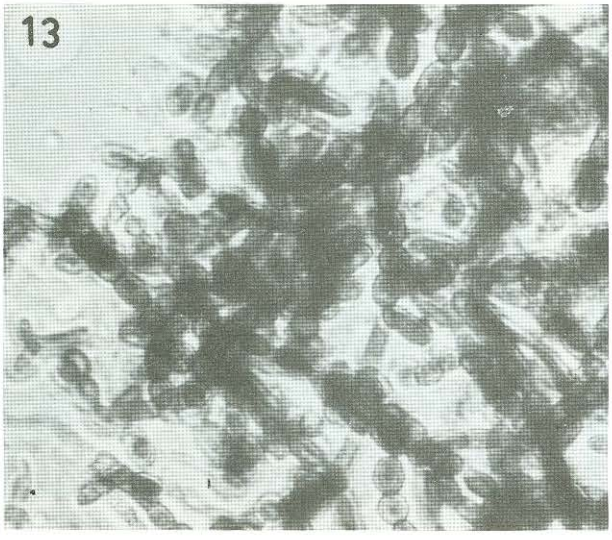
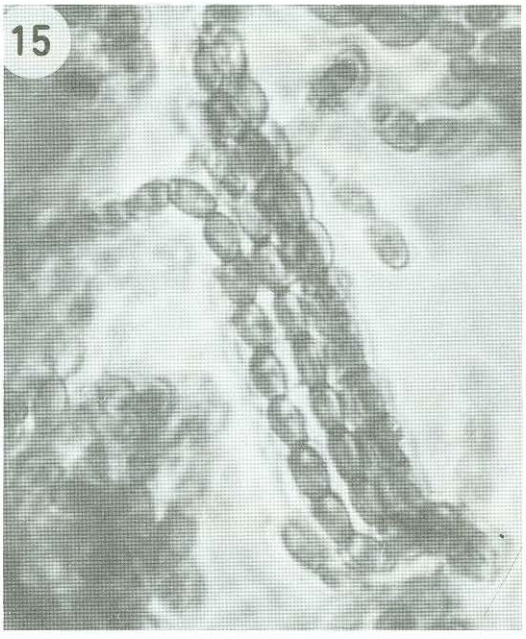


Fig. 7-8: **Pyrenochaeta ilicis**; pycnidium and setae x 250 and conidia x 1000. Fig. 9: **Drechslera dematioidea**; x 1000. Fig. 10: **Beauveria bassiana**; conidiophores and conidia x 1000.









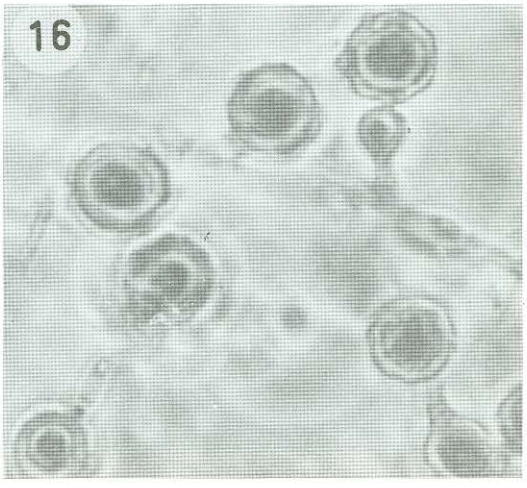
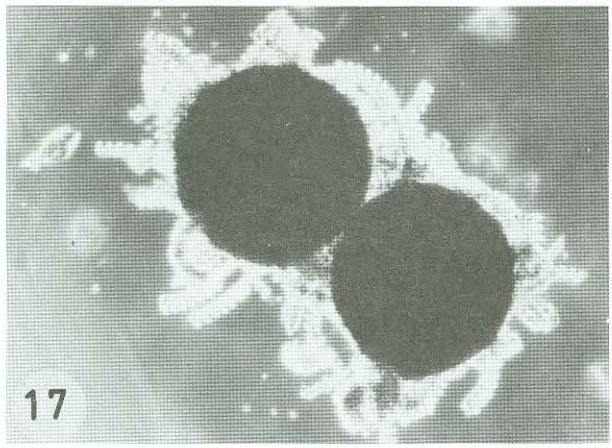
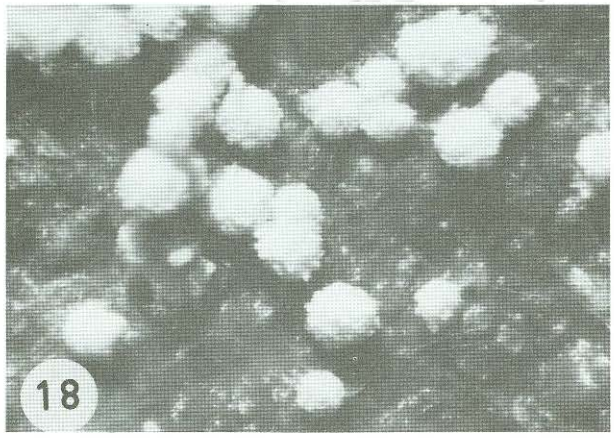
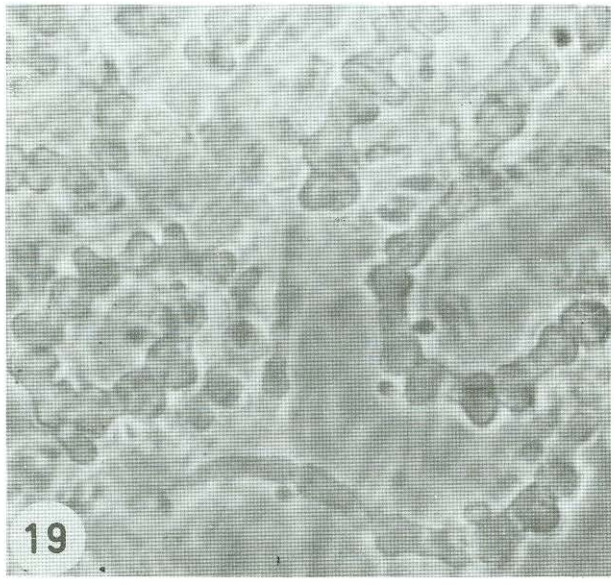
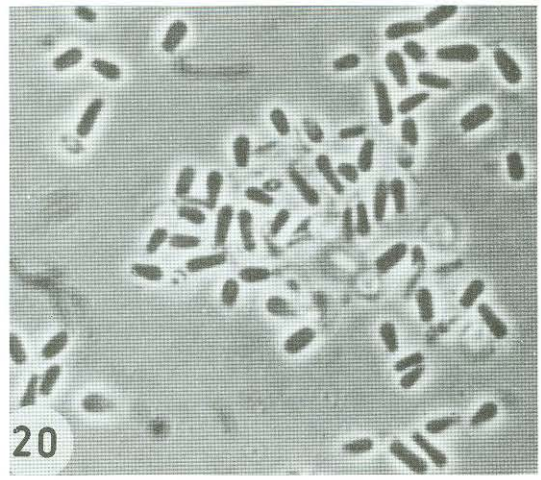


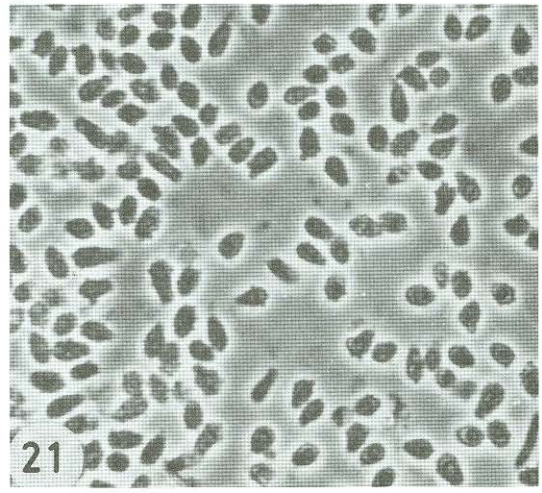
Fig. 11-12: **Chaetomium thermophile,** perithecium x 250 and detail of hairs and ascospores x 1000. Fig. 13: **Scytalidium thermophilum,** x 250. Fig. 14-15: **Scopulariopsis carbonaria,** conidiophore with conidial chains x 250. Fig. 16: **Thermomyces lanuginosus,** aleurioconidia x 1000.











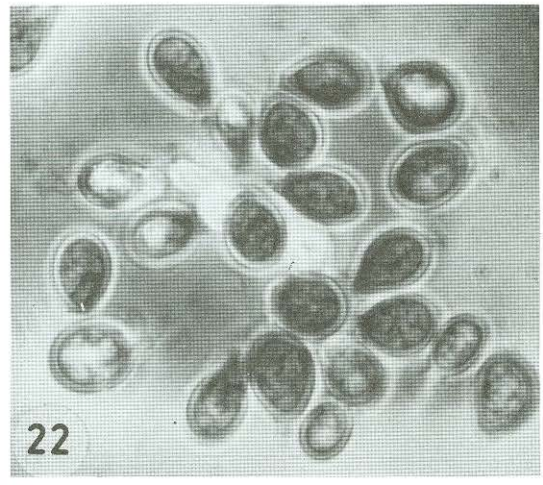


Fig. 17: **Ctenomyces serratus**, ascomata and appendages x 150. Figs. 18-19-20: **Arthroderma cuniculi**, ascomata x 15, peridial appendages x 1000 and microconidia x 400. Fig. 21: **Chrysosporium indicum**, microconidia x 400. Fig. 22: **Chrysosporium keratinophilum**, microconidia x 1000.

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