STUDIES ON THE TAXONOMY AND KERATINASE ACTIVITY OF SOME KERATINOPHILIC FUNGI FROM POULTRY FARMS OF JAMMU DISTRICT

THESIS
SUBMITTED TO THE UNIVERSITY OF JAMMU
FOR AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN BOTANY
1995

SANJANA KAUL

DEPARTMENT OF BIOSCIENCES
UNIVERSITY OF JAMMU
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CERTIFICATE

Certified that :

1. the thesis entitled, "Studies on the taxonomy and keratinase activity of some keratinophilic fungi from poultry farms of Jammu district," embodies the work done by Ms. Sanjana Kaul under my supervision for the period required under statutes,

2. the candidate has put in the attendance in the department for the period required,

3. the thesis being submitted for the degree of Doctor of Philosophy by Ms. Sanjana Kaul has not been submitted for any other degree and is worthy of the consideration for the award of Ph.D degree of the University of Jammu, and

4. the conduct of the research scholar remained satisfactory during the period of research.

30/8

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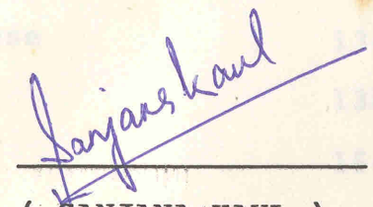

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CHAPTER I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Keratinophilic fungi are a group of highly specialized fungi which possess the ability to degrade hard keratin and utilize it as a source of protein. They are capable of invading the keratinized tissues (skins, hair, nails, claws, spines etc.) of man and animals, some growing more or less in the superficial layers and others invading more deeply and usually producing a host response. Such keratinophilic fungi which are capable of causing diseases (dermatophytosis) of skin and hair in man and animals are commonly called as dermatophytes. These fungi, by virtue of their ability to colonize epidermal appendages, may become a source of sanitary danger to human health and accordingly, from time to time they have drawn the attention of various medical and veterinary epidemiologists.

It has been observed that keratinophilic fungi are vigorous and self-sufficient saprophytes as long as environmental conditions are favourable. However, they may become parasitic by accident and after becoming pathogenic, they are able to survive and multiply at host's body temperature, causing further infection by invading fresh keratinized tissues. Infact, the potential

pathogenecity of keratinophilic fungi has been considered as a natural evolution from its presence in the soil (geophilic species) to invasion of cornified substrata in animals (zoophilic species) and man (anthropophilic species). Among the geophilic, zoophilic and anthropophilic species, the first group live in soil as saprophytes, whereas, the zoophilic dermatophytes represent an ecological group that has reached a higher degree of evolution during the process of adaptation to the condition of outer surroundings. They are mostly obligate parasites with a different range of animal hosts. The anthropophilic dermatophytes constitute an ecological group that has already lost connection with the original environment i.e., soil. In this group, specialization to the parasitic mode of life has reached the highest degree with a clear-cut specificity to a single host, man. This differentiation with respect to natural habitats and host preferences is believed to have played an important role in determining global distribution of these fungi.

Soil has been considered as an important natural reservoir and a link that relates different ecological groups together. Keratinous matter may be added to the soil from various sources in the form of feathers, hair, nail, horn, hoof, wool and related appendages, as wastes.

Hence, soil provides most conducive habitat for the growth and multiplication of these fungi. In recent years, unplanned exploitation of environment, greater population drifts, fast means of transport and general deterioration of hygiene have also contributed to the spread of these fungi. Attempts have been made to survey their viability in different habitats and soil has always furnished in various percentages the proof of the existence of these widespread, frequently cosmopolitan fungi (Alteras, 1971). In general, the ecological habitats mapped out and reported to be rich in keratinophilic fungi are the soils from densely populated areas, public gardens, play grounds, poultry farms, cattle farms and other places inhabited by animals and decomposed organic matter. However, it has been pointed out by Alteras (1971) that generally the sterile soil may harbour the presence of keratinophilic fungi for a longer period because of the lack of concurrence exerted by the common micro-flora of the soil.

Domestic animals and birds infected with dermatomycoses have also been recognized to act as a reservoir for zoophilic dermatophytes (Georg, 1960; Mantovani & Morganti 1977; Otcenasek, 1978; Alteras et al. 1986) and other potentially pathogenic fungi (Bagy, 1986). However, healthy domestic animals and birds can also act

as a reservoir for zoophilic dermatophytes and other potentially pathogenic fungi (Bagy, 1986; Bagy et al. 1985). These animals may thus act as a direct source of human and animal infections for subjects in contact with them or as an indirect source of infection by contaminating working areas and dwelling places (Rippon, 1982).

Keratin, an important natural material occurring in nature mainly in the form of hair, feather, wool, horn, hoofs, nails and cornified epidermis stimulates the development of keratinophilic fungi in soil and furthers their diffusion. All keratins are scleroproteins composed of long polypeptide chains bound laterally by disulfide linkages which maintain the three dimensional shape of the molecule, by giving it additional strength and rendering it more resistant to enzymatic digestion. A distinctive feature of keratin is its relatively high sulphur content due to the presence of the sulphur containing aminoacids-cystine, cysteine and methionine. Nickerson (1947) classified keratin into two distinct groups i.e., soft and hard keratins depending upon their cystine content. Soft keratin such as those found in skin, have a low cystine content i.e., approximately 2%, while hard keratin such as that in hair, has approximately 14% cystine. This difference in keratin molecule may be due to their

different process of formation by the keratinizing cells.

Ability of the fungi to colonize natural keratin is widespread and a considerable number of species have been reported belonging to aquatic fungi (Chytridiales and Saprolegniales), Zygomycetes and Deuteromycetes. It is now well established that the breakdown of keratin is carried out by the action of extracellular enzymes, keratinases. These keratinases of keratinophilic fungi have attracted much attention of dermatologists and mycologists as these enzymes have a powerful action on keratin which is almost always associated with the mycotic infection of man and animals. According to Vanbreuseghem (1953), two ecological groups of such fungi may be distinguished : 1) dermatophytes and other species which are able to attack native keratin by penetration and enzyme activity and 2) the saprophytic species which attack the keratin mainly by hyphal penetration and surface growth utilizing the more easily decomposable compounds of the keratin substrates. This process may differ in various soils but in general the common soil saprophytes with a high competitive ability constitute the early sequence in this colonization which is then followed by the keratinolytic species of the genera Chrysosporium, Keratinomyces, Microsporum and Trichophyton which cause a

complete decomposition of the complex substrate. English (1965), recorded that many fungi were found to grow on keratinized substrata in pure culture, whether or not these species normally colonize such materials in soil or on animal hosts. Some of these fungi appeared to degrade only cell membranes and cementing materials that form layers interspersed with keratin and only few appeared to utilize all components of the material. Therefore, it was concluded that many fungi are able to utilize at least some components of keratinized tissues, but the ability to degrade hard keratin is a specialized feature of keratinophilic fungi. Accordingly, English (1969) catagorised these fungi into following three groups :

(i) Keratinophilic - Fungus may or may not be able to digest keratinized substrate completely.

(ii) Keratinolytic - Fungus which can digest keratinized substrate completely.

(iii) Dermatophytes - Fungus belonging to one of the genera Keratinomyces, Microsporum, Trichophyton or Epidermophyton, whether pathogenic to man or animals or not. All the dermatophytes are keratinolytic in nature.

In addition to the availability of keratinous substrates, the occurrence and survival of keratinophilic

fungi may be influenced by various geonomic and climatic factors such as chemical composition of organic matter in soils, its quantity as source of nutrition and energy, soil humidity, pH and temperature, the depth of soil profile, soil texture and structure. Existence of keratinophilic fungi in soil is also influenced by the presence of other constituents of biocenotic complexes, namely the bacterial, actinomycete and fungal components which exert antagonistic effects on keratinophilic fungi (Srivastava et al. 1990). Some other factors which may influence their frequency of occurrence are moisture content and aeration of surface soil layers.

In Jammu and Kashmir state, keratinophilic fungi have been investigated from soils by very few workers (Garg, 1966; Kaul and Sumbali, 1992, 1994; Kaul et al. 1993). In view of this fact, the present research work was undertaken on the taxonomy and keratinase activity of some keratinophilic fungi from poultry farms of Jammu district. The objectives set forth for the present investigation were as follows :

A : Isolation, distribution and frequency occurrence of keratinophilic fungi from ten different poultry farm soils of Jammu district.

B : Isolation of keratinophilic fungi from feathers

of living poultry birds confined in the poultry farms under investigation.

C : Taxonomy of isolated keratinophilic fungal species.

D : Influence of soil factors on the occurrence of poultry soil inhabiting keratinophiles.

E : Evaluation of keratinolytic nature of individual isolates.

F : Preferential utilization of various keratin substrates by poultry soil and poultry feather isolates.

G : Invitro degradation of keratin by the isolated keratinophilic fungi and estimation of the released cysteine, total protein and keratinase.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

For many years research has been carried out in different parts of the world to assess relationships between man, his domestic animals and the keratinophilic fungi to verify the tendency of these fungi to follow human settlement. Infact, a qualitative and quantitative modification of some environmental factors which favour successively the establishment of these fungi, is consequent to the human presence. From the investigations completed upto now, it seems that the distribution of keratinophilic fungi in the soil largely depends on the existence of a relatively high quantity of keratinic material of either human or animal origin or both in soil.

The first report of a keratinophilic fungi growing saprophytically in nature was that of Szathmary (1936), who isolated Trichophyton primum (Trichophyton gypseum) from the mud of watercourses in the park of the University of Peco. Later, Emmons (1942), demonstrated that soil surveying is an excellent method for discovering the natural habitats of the fungi that are capable of causing human and animal diseases. In 1952, Vanbreuseghem introduced the hair-bait technique which has been of immense help in the selective isolation of keratinophilic

fungi from soil. Since then researchers from several countries have reported the occurrence and distribution of keratinophiles from different soils.

Isolation of keratinophilic fungi have been reported from **New Zealand** (Marples, 1964), **Sweden** (Gip and Paldrok, 1966; Palsson, 1968), **Canada** (Carmichael, 1962; Padhye et al. 1973; and Bakerspigel, 1974), **Czechoslovakia** (Otcenasek and Dvorak, 1964), **Romania** (Evolceanu and Alteras, 1966; Alteras and Evolceanu, 1967, 1968, 1969 a,b), **Israel** (Feuerman et al. 1975), **Iran** (Alilous and Asgar, 1973), **Jordan** (Ali-Shtayeh, 1988; Ali-Shatayeh and Asad- AlSheikh 1988), **Spain** (Pereiro, 1962; Aller et al. 1970; Guarro et al. 1981, 1987 a,b; Calvo et al. 1984; Punsola and Guarro, 1984; and Cano et al. 1987), **Argentina** (Varsavsky, 1964), **Denmark** (Sylvest, 1949), **United States** (Daniels,1954; Rippon and Medenica, 1964; Baxter, 1966,1969), **Pakistan** (Mohammed et al. 1971), **Kenya** (Muhammed and Lalji, 1978), **Japan** (Kominami, 1961), **Hungary** (Florian and Galgoczy, 1964), **Africa** (Ajello, 1956, 1962; Doupagne, 1959; Pattyn and Sassen, 1960, Vanbreuseghem and Lauwers, 1960; Al-Doory, 1968, 1969; Al-Doory and Kalter, 1967; Abou-Gabal and Abd-Elrahiem, 1973; Ahmed, 1975; Mostafa, 1977; Atia et al. 1981; Abdel-Fattah et al. 1982; Apinis and Clark, 1974), **South**

Dakota (Knudtson and Robertstad, 1970), Columbia (Cabrales, et al. 1966; Rogers, 1971), Australia (Frey 1959, 1965; Durie and Frey, 1955, 1957, 1962; Frey and Durie, 1956; Frey and Griffin, 1961; Dunne and Morahan, 1964a, b; McAleer, 1980), Italy (Ajello et al. 1965; Caretta and Piontelli, 1975; Mercantini, et al. 1978, 1980, 1983 , 1986, Caprilli et al. 1987) and Abaco Island (Volz, 1971) indicating their global distribution. Soil is thus recognized as the basic substrate for keratinophilic fungi and their universal occurrence in man's environment is considered to be of epidemiological significance.

In general, the ecological habitats mapped out and reported to be rich in keratinophilic fungi are the soils from densely populated areas rich in organic matter. However, Dabrowa et al. (1964), Randhawa and Sandhu (1965), Padhye & Thirumalachar (1967), and Al-Doory (1969) have reported the occurrence of keratinophilic fungi from marine and saline environments. Similarly survey of xeric low lying coastal areas of Galapagos Islands whose soil was essentially made up of disintegrated lava and, was low in organic matter revealed the presence of seven species of keratinophilic fungi (Ajello and Padhye, 1974). Interestingly, isolation of Microsporum, Trichophyton, and Chrysosporium species have also been made by Mercantini et al. (1989), from Antarctic soil during the scientific

expedition in 1986-1987, which indicates that very low temperatures and great scarcity of organic substrates do not prevent the occurrence of keratinophilic fungi.

A limited number of keratinophilic fungi have also been reported from air of Athens metropolitan area (Papavassiliou and Bartzokas 1975), air of a large hopital (Alteras and Lehrer, 1977), air filters (Acosta and Robertstad, 1979) and from air of Pavia, Italy (Franca and Caretta, 1984).

In India, studies on keratinophilic fungi commenced in the year 1955 when Dey and Kakoti isolated Microsporum gypseum from a soil sample collected from an animal house in Dibrugarh, Assam.

Since then comprehensive soil surveys from different regions of India have been made. Some important keratinophilic fungi isolated and published have been listed in table 1, overleaf.

Table 1 : Keratinophilic fungi isolated from Indian soils

Keratinophilic fungi 1	Source 2	Localities 3	Isolator 4
Acrodontium albaeo	various soils	Sagar	Kushwaha & Agrawal (1976b)
A. album	forest soils	-do-	Kushwaha & Agrawal (1977c)
Acremonium obclavatum	house dust	Kanpur	Nigam & Kushwaha (1989a)
Acremonium sp.	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
Allescherella sp.	-do-	-do-	-do-
Alternaria tenuis	forest litter, garden, crop field soils and bird's nests	Sagar	Singh & Agrawal(1983)
Aphanoascus reticulisporus	various soils	Orissa	Sur & Ghosh (1980a)
A. terreus	-do-	-do-	-do-
	cattle farm soils,	M.P. & U.P.	Jain (1983)
	hostel, park & play ground soils	Lucknow	Jain et al. (1985)
	forest soils	Kanpur	Nigam & Kushwaha (1990)

Contd.....

1	2	3	4
Aphanoascus sp.	forest soils and house dust	Kanpur	Nigam & Kushwaha(1989a,1990)
Arthroderma flavescens	soil & house dust	-do-	Nigam & Kushwaha (1987,1990)
A. gertleri	-do-	-do-	-do-
A. terreus	house dust	-do-	Nigam & Kushwaha (1989a)
A. tuberculatum	poultry farm soils	Poona	Padhye et al.(1966)
Aspergillus candidus	dairy farm soils	Jammu	Kaul & Sumbali (1994)
A. flavus	animal house, dairy farm, poultry farm soils	Jabalpur	Shukla & Rajak (1984)
	gelatin factory campus soils	-do-	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul and Sumbali (1994)
A. fumigatus	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
	animal house, dairy farm, poultry farm, forest litter and road side soils	Jabalpur	Shukla & Rajak (1984)
	rice field soils	Madras	Sundaram (1987)
	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)

(Contd.....

1	2	3	4
A. guircinus	various soils	Sagar	Singh & Agrawal (1983)
A. nidulans	various soils	-do-	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
A. niger	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
A. ochraceus	-do-	-do-	-do-
A. quadrilineata	dairy farm soils	Jammu	Kaul & Sumbali (1994)
A. quereinus	various soils	Sagar	Kushwaha & Agrawal (1976b)
	-do-	Kanpur	Kushwaha (1984)
A. sacchari	various soils	Sagar	Kushwaha & Agrawal (1976b)
A. sulfureus	-do-	-do-	-do-
A. sydowii	dairy farm soils	Jammu	Kaul & Sumbali (1994)
A. terreus	-do-	-do-	-do-
A. ustus	various soils	Sagar	Kushwaha & Agrawal (1976b)
	-do-	Kanpur	Kushwaha (1984)
Aspergillus sp.	road side soils	Jaipur	Iyer et al. (1989)

(Contd.....

1	2	3	4
Auxarthron brunneum	various soils	Mount Abu	Jain & Agrawal (1977)
Auxarthron conjugatum	forest soils	M.P. & U.P.	Jain (1983)
Bipolaris sp.	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
Botryotrichum keratinophilum	various soils	Sagar	Kushwaha & Agrawal (1976a,b)
	cattlefarm soils & bird's nests	-do-	Singh & Agrawal (1983)
B. piluliferum	various soils	-do-	Kushwaha & Agrawal (1976b)
Cephalosporium acremonium	-do-	-do-	-do-
	crop field soils	-do-	Singh & Agrawal (1983)
Chaetomium indicum	various soils	-do-	Kushwaha & Agrawal (1976b)
	poultry farm & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
C. globosum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	animal house floor sweepings & bird's nests	-do-	Singh & Agrawal (1983)
	soil	Kanpur	Kushwaha (1984)
Chrysosporium asperatum	hostel, park & play-ground soils	Lucknow	Jain et al. (1985)
C. carmichaeli	house dust soils	Kanpur	Nigam & Kushwaha (1987,1989,1990)
	public park & garden soils	Andaman Islands	Dixit & Kushwaha (1990)

(Contd.....

1	2	3	4
C. crassitunicatum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
C. evolceanuii	garden & crop field soils	Poona	Padhye et al. (1966)
	various soils	Orissa	Sur & Ghosh (1980a)
	garden, park & play-ground, river bank & hospital soils	Lucknow	Jain et al. (1985)
	dog habitat soils	Jaipur	Iyer et al. (1989)
	road side soils	Andaman Islands	Dixit & Kushwaha (1990)
	forest soil	Kanpur	Nigam & Kushwaha (1990)
C. farinicola	house dust	Kanpur	Nigam & Kushwaha (1989a,1990)
C. geophilum	hospital campus soils	-do-	Kushwaha & Srivastava (1989)
C. indicum	various soils	Poona	Padhye et al. (1966)
	marine soils	Bombay	Padhye et al. (1967)
	various soils	Orissa	Sur & Ghosh (1980a)
	pig habitat & horse stable soils	Patna	Verma et al. (1982)
	coastal soils	Goa	Deshmukh & Agrawal (1983)
	cattle farm, poultry farm, crop field & forest soils	M.P. & U.P.	Jain et al. (1985)
	various soils	Sagar	Singh & Agrawal (1983)

(Contd.....

1	2	3	4
C. indicum	various soils	Lucknow	Jain et al. (1985)
	house dust & grassland soils	Kanpur	Nigam & Kushwaha(1989a,1990)
	animal house, public park soils	Andaman Islands	Dixit & Kushwaha (1990)
	gelatin factory campus soils	Jabalpur	Hasija et al (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
C. keratinophilum	soils from banks of water courses	Srinagar	Garg (1965)
	cattle farm & poultry farm soils	M.P. &U.P.	Jain (1983)
	dairy farm, garden, kennel, river bank & poultry farm soils	Lucknow	Jain et al (1985)
	rice field soils	Madras	Sundaram (1987)
	house dust	Kanpur	Nigam & Kushwaha(1989a,1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
C. lucknowense	forest soils	Kanpur	Nigam & Kushwaha (1989a,1990)
	cattle farm soils	M.P. & U.P.	Jain (1983)
C. merdarium	cattle farm soils	Sagar	Deshmukh et al. (1985)
	house dust	Kanpur	Nigam & Kushwaha (1989a,1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)

(Contd.....

1	2	3	4
C. pannicola	forest soils	Kanpur	Nigam & Kushwaha (1989a,1990)
C. pannorum	dairy farm soils	Jammu	Kaul & Sumbali (1994)
C. queenslandicum	soil & house dust	Kanpur	Nigam & Kushwaha (1987, 1990)
	gelatin factory campus	Jabalpur	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali 1994
C. tropicum	rice field & sandy soils	Srinagar & Meerut	Garg (1965)
	various soils	Poona	Padhye et al. (1966)
	marine soils	Bombay	Padhye et al. (1967)
	various soils	Sagar	Kushwaha & Agrawal (1976b)
	-do-	Mount Abu	Jain & Agrawal (1977)
	various soils	Orissa	Sur & Ghosh (1980a)
	coastal soils	Goa	Deshmukh & Agrawal (1983)
	cattle farm, poultry farm, crop field & forest soils	M.P. & U.P.	Jain (1983)
	road side, forest litter & animal house soils	Jabalpur	Shukla & Rajak (1984)
	garden & poultry farm soils	Lucknow	Jain et al. (1985)
	forest soils	Kanpur	Kushwaha (1984)
	road side & animal habitat	Jaipur	Iyer et al. (1989)
	forest soils	Kanpur	Nigam & Kushwaha (1989a,1990)

(Contd.....

1	2	3	4
	animal house, public park & hospital soils	Andaman Islands	Dixit & Kushwaha (1990)
	gelatin factory campus soils	Jabalpur	Hasija *et al*. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
C. tuberculatum	forest soils	Kanpur	Nigam & Kushwaha (1989a)
C. sulfureum	Gelatin factory campus soils	Jabalpur	Hasija *et al*. (1990)
	Soil and house dust	Kanpur	Nigam & Kushwaha (1987,1990)
Chrysosporium anamorph of *Arthroderma curreyi*	road side and public park soils	Andaman Islands	Dixit & Kushwaha (1990)
Chrysosporium anamorph	poultry farm soils	Poona	Padhye *et al*. (1966)
of *Arthroderma tuberculatum*	various soils	Orissa	Sur & Ghosh (1980a)
Chrysosporium anamorph of *Ctenomyces serratus*	Soil	Srinagar & Ahmedabad	Garg (1965)
	poultry farm soils	Poona	Padhye *et al*. (1966)
		Goa	Deshmukh & Agrawal (1983)
	hostel, park & play-ground soils	Lucknow	Jain *et al*. (1985)
	animal house soils	Andaman Islands	Dixit & Kushwaha (1990)
Cladorrhinum sp.	various soils	Mount Abu	Jain & Agrawal (1977)

(Contd.....

1	2	3	4
Cladosporium chlorocephalum	various soils	Sagar	Kushwaha & Agrawal (1976b)
C. oxysporum	various soils	-do-	Singh & Agrawal (1983)
Ctenomyces serratus	marine soils	Bombay	Padhye et al. (1967)
	river bank, poultry farm & hostel soils	Lucknow	Jain et al. (1985)
Cunninghamella echinulata	compostpile & garden soils	Jaipur	Iyer et al. (1989)
C. elegans	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
	road side, poultry farm & animal house soils	Jabalpur	Shukla & Rajak (1984)
Curvularia geniculata	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
C. indica	various soils	-do-	Kushwaha & Agrawal (1976b)
	-do-	Kanpur	Kushwaha (1984)
C. lunata	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
	various soils	Sagar	Singh & Agrawal (1983)
C. subulata	various soils	Sagar	Kushwaha & Agrawal (1976b)
Cylindrocarpon lichenicoda	gelatin factory campus	Jabalpur	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)

(Contd.....

1	2	3	4
Eupenicillium sp.	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
Fusariella concinna	various soils	Sagar	Kushwaha & Agrawal (1976b)
	soil	Kanpur	Kushwaha (1984)
Fusarium brachygibbosum	various soils	Sagar	Kushwaha & Agrawal (1976b)
F. chlamydosporum	-do-	-do-	-do-
F. equiseti	-do-	Mount Abu	Jain & Agrawal (1977)
F. moniliforme	road side, forest litter & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
	gelatin factory campus soils	-do-	Hasija et al. (1990)
F. nivale	various soils	Sagar	Kushwaha & Agrawal (1976b)
	animal house floor sweepings	Jabalpur	Shukla & Rajak (1984)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
F. oxysporum	forest litter, poultry & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)

(Contd.....

1	2	3	4
F. poe	various soils	Sagar	Kushwaha & Agrawal (1976b)
F. roseum	-do-	-do-	-do-
	road side, forest litter & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
F. solani	gelatin factory campus soils	-do-	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
F. sporotrichoides	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	-do-	Singh & Agrawal (1983)
Geotrichum candidum	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
Gliocladium agrawalii	animal house floor sweepings	Kanpur	Kushwaha (1980a)
G. deliquescence	various soils	Sagar	Kushwaha & Agrawal (1976b)
G. penicilloides	various soils	Sagar	Kushwaha & Agrawal (1976b)
G. roseum	-do-	-do-	-do-
	soil	Kanpur	Kushwaha (1984)
Graphium cuneiferum	gelatin factory campus soil	Jabalpur	Hasija et al. (1990)
G. penicilloides	various soils	Sagar	Kushwaha & Agrawal (1976b)

(Contd.....

1	2	3	4
G. roseum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	soil	Kanpur	Kushwaha (1984)
Graphium cuneiferum	gelatin factory campus soil	Jabalpur	Hasija et al. (1990)
G. penicilloides	various soils	Sagar	Kushwaha & Agrawal (1976b)
	gelatin factory campus soil	Jabalpur	Hasija et al. (1990)
Gymnoascoideus petalosporus	various soils	Orissa	Sur & Ghosh (1980a)
Helminthosporium sativum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	animal house, dairy farm & poultry farm soils	Jabalpur	Shukla & Rajak (1984)
Humicola fuscoatra	various soils	Sagar	Kushwaha & Agrawal (1976b)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
H. grisea	gelatin factory campus soil	Jabalpur	Hasija et al. (1990)
Keratinomyces ajelloi	river bank soils	Patna	Verma et al. (1992)
Keratinophyton terreum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	Mount Abu	Jain & Agrawal (1977)
	soil	Kanpur	Kushwaha (1984)

(Contd.....

1	2	3	4
Malbranchea aurantiaca	cattle farm, poultry farm, crop field & forest soils	M.P. & U.P.	Jain (1983)
M. pulchella	poultry farm soils	Sagar	Kushwaha & Agrawal (1976b)
Malbranchea sp.	various soils		Jain & Agrawal (1979)
Microsporum canis	rice field soils	Madras	Sundaram (1987)
M. cookie	Dog habitat	Jaipur	Iyer et al. (1989)
M. fulvum	various soils	Sagar	Kushwaha & Agrawal (1976b)
	cattle farm, poultry farm,crop field & forest soils	M.P. & U.P.	Jain (1983)
	various soils	Kanpur	Kushwaha (1984)
	forest soils	Kanpur	Nigam & Kushwaha (1989a)
	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
M. gypseum	animal house soils	Assam	Dey & Kakoti (1955)
	various soils	Delhi	Randhawa et al. (1959)
	various soils	Poona	Padhye et al. (1966)
	marine soils	Bombay	Padhye et al. (1967)
	various soils	Sagar	Kushwaha & Agrawal (1976b)
	various soils	Orissa	Sur & Ghosh (1980a)

(Contd.....

1	2	3	4
	cattle farm, poultry farm, crop field & forest soils	M.P. & U.P.	Jain (1983)
	...	Kanpur	Kushwaha (1984)
	animal house, poultry farm, forest litter & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
	various soils	Lucknow	Jain et al. (1985)
	rice field soils	Madras	Sundaram (1987)
	cattle shed	Jaipur	Iyer et al. (1989)
	house dust	Kanpur	Nigam & Kushwaha (1989a)
	hospital, animal house, public park & road side soils	Andaman islands	Dixit & Kushwaha (1990)
	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
		Poona	Padhye et al. (1966)
M. nanum	kennel soils	Lucknow	Jain et al. (1985)
M. vanbreuseghemii	various soils		Sethi et al. (1957)
Microsporum sp.	forest soil	Kanpur	Nigam & Kushwaha (1990)
Mucor hiemalis	gelatin factory campus soils	Jabalpur	Hasija et al. (1990)

(contd.....

1	2	3	4
Mycelia sterilia	rice field soils	Madras	Sundaram (1987)
Myceliophthora anamorph of Arthroderma tuberculatum	house dust soils	Kanpur	Nigam & Kushwaha (1987,1989a)
Myceliophthora anamorph of Corynascus novoquineensis	-do-	-do-	-do-
Myceliophthora sp.	public park & garden soils	Andaman islands	Dixit & Kushwaha (1990)
Myrothecium atroviride	cattle farm soils	Sagar	Jain & Agrawal (1983)
Myxotrichum sp.	house dust	Kanpur	Nigam & Kushwaha (1989a)
Nannizzia sp.	various soils	Orissa	Sur & Ghosh (1980a)
	hospital, poultry farm, river bank, kennel & dairy farm soils	Lucknow	Jain et al. (1985)
Onygena sp.	house dust	Kanpur	Nigam & Kushwaha (1989a)
Orromyces spiralis	canal bed soils	Orissa	Ghosh & Sur (1985)
Paecilomyces fusisporus	various soils	Sagar	Kushwaha & Agrawal (1976b)
	animal house, dairy farm, & forest litter soils	Jabalpur	Shukla & Rajak (1984)
P. lilacinus	various soils	Mount Abu	Jain & Agrawal (1977)
	poultry farm & dairy farm soils	Jabalpur	Shukla & Rajak (1984)

(Contd.....

1	2	3	4
P. varioti	various soils	Sagar	Kushwaha & Agrawal (1976b)
Penicillium aurantiobruneum	-do-	-do-	-do-
P. duclauxi	-do-	-do-	-do-
P. liliacinum	-do-	-do-	-do-
	soil	Kanpur	Kushwaha (1984)
P. purpurogenum	gelatin factory campus soil	Jabalpur	Hasija *et al*. (1990)
P. rubrum	various soils	Sagar	Kushwaha & Agrawal (1976b)
Penicillium sp.	rice field soils	Madras	Sundaram (1987)
Periconia saraswatipurensis	poultry farm soils	Jabalpur	Shukla & Rajak (1984)
Pseudoarachniotus flavoluteus	various soils	Orissa	Sur & Ghosh (1980a)
P. orissi	canal bed soils	-do-	Ghosh & Sur (1985)
Pseudoarachniotus sp.	various soils	-do-	Sur & Ghosh (1980a)
Scopulariopsis brevicaulis	gelatin factory campus soils	Jabalpur	Hasija *et al*. (1990)
	dairy farm soils	Jammu	Kaul & Sumbali (1994)
Scopulariopsis sp.	gelatin factory campus soils	Jabalpur	Hasija *et al*. (1990)

(Contd.....

1	2	3	4
Syncephastrum racemosum	various soils	Sagar	Kushwaha & Agrawal (1976b)
Thermomyces sp.	gelatin factory campus soil	Jabalpur	Hasija et al. (1990)
Torula herbarum	forest litter	-do-	Shukla & Rajak (1984)
Trichoderma harzianum	variable soils	Sagar	Rao et al. (1975)
	various soils	-do-	Kushwaha & Agrawal (1976b)
T. hematum	-do-	-do-	-do-
T. viridae	-do-	-do-	-do-
	dairy farm & forest soils	Jabalpur	Shukla & Rajak (1984)
	gelatin factory campus soil	-do-	Hasija et al. (1990)
Trichoderma sp.	various soils	Mount Abu	Jain & Agrawal (1977)
Trichophyton ajelloi	animal house, forest litter & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
	rice field soils	Madras	Sudaram (1987)
T. equinum	cattle farm soils	M.P. & U.P.	Jain (1983)
T. evolceanui	variable soils	Meerut	Randhawa & Sandhu (1963)

(Contd.....

1	2	3	4
T. flavescens	house dust	Kanpur	Nigam & Kushwaha (1989a)
T. indicum	variable soils	Punjab	Randhawa & Sandhu (1963)
T. mentagrophytes	-do-	Calcutta & Dehradun	Puri (1961)
	variable soils	Poona	Padhye et al. (1966)
	various soils	Sagar	Kushwaha & Agrawal (1976b)
	cattle farm soils	M.P. & U.P.	Jain (1983)
	various soils	Kanpur	Kushwaha (1984)
T. rubrum	hospital floor & swimming pool sweepings	Poona	Padhye et al. (1966)
	various soils	Sagar	Kushwaha & Agrawal (1976b)
	-do-	M.P. & U.P.	Jain (1983)
	-do-	Kanpur	Kushwaha (1984)
	animal house & dairy farm soils	Jabalpur	Shukla & Rajak (1984)
T. simii	hospital, road side & garden soils	Jaipur	Iyer et al. (1989)
T. terrestre	various soils	Patna	Verma et al. (1982)
	rice field soils	Madras	Sundaram (1987)
	animal house soils	Andaman islands	Dixit & Kushwaha (1990)

(Contd.....

1	2	3	4
T. vanbreuseghemii	house dust	Kanpur	Nigam & Kushwaha (1989a)
Verticillium lecanii	various soils	Sagar	Kushwaha & Agrawal (1976b)
	-do-	Kanpur	Kushwaha (1984)
V. saksenii	forest soil	Sagar	Kushwaha (1980b)
Verticillium sp.	various soils	-do-	Kushwaha & Agrawal (1976b)

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The association, survival and dispersal of keratinophilic fungi with bird's feathers and nests has been recognized by various researchers (Ajello, 1953; Kuehn, 1960; Gierloff et al. 1961; Buhlmann and Reith, 1962; Hubschmann and Fragner, 1962; Pugh, 1964, 1965, 1966a,b; Rees, 1967a,b; Pugh and Evans, 1970a; and Sur and Ghosh, 1980a,b).

Pugh (1964), attributed the ragged appearance of black birds, Turdus merula in the early summer in England to the keratinophilic fungi. Later Pugh (1965) showed the presence of keratinophilic fungi on 36.6% of the experimental birds while Rees (1967a) recorded them on 40% birds in Australia. A close relationship was also observed to exist between the keratinophilic fungi and the birds of a specific order, e.g., Arthroderma curreyi and Turdus (Pugh, 1964), Ctenomyces serratus and members of Galliforme, especially patridges (Pugh, 1966a) and chickens (Rees, 1967b). Colonization of Chrysosporium species on birds feathers and their nests have been reported by several workers (Pugh, 1966a; Otcenasek et al. 1967; Rees, 1967a; Pugh and Evans, 1970a; Hubalek et al. 1973; and Sur and Ghosh, 1980b). Other species isolated from birds are Microsporum gypseum (Ajello, 1953; Pugh, 1966a; Otcenasek et al. 1967; Rees 1967a), M. cookei

(Otcenasek et al. 1967; Rees, 1967a; Pugh and Evans, 1970a), M. ripariae (Hubalek and Rush-Munro, 1973), Trichophyton gallinae (Gierloff et al. 1961, Kral, 1962), T. mentagrophytes (Buhlmann and Reith, 1962); T. terrestre (Pugh, 1965, 1966b; Otcenasek et al. 1967; Pugh and Evans 1970a; Hubalek, 1974), T. georgiae (Hubalek, 1970); T. violaceaum (Gierloff et al. 1961) and Arthroderma tuberculatum (Kuehn, 1960).

Pugh and Evans (1970a) found that the keratinophilic fungi isolated from birds were mesophilic. The high body temperature precludes these fungi from inner contour of feathers and makes them to confine to the outer surface. The feather fats constitute an important factor which conditions the growth of keratinophilic fungi. However, Sur and Ghosh (1980b) believed that the high fat content in the feather of Acridotheres tristis probably warded off the keratinophilic fungi.

A wide variety of fungi (6260) have also been isolated from the feathers of 92 species of free living birds in Czechoslovakia and Yugoslovia out of which 18.3 percent were noted to be keratinophilic (Hubalek, 1974). The isolation of Aphanoascus sp, A. terreus, Arthroderma ciferrii, A. flavescens, A. curreyi, A. multifidum, A. quadrifidum, A. tuberculatum, Chrysosporium tropicum, C.

evolceanui, C. serratus and Microsporum ripariae from the migratory birds gave an assumption that all these fungi could potentially be carried from one geographical region to another (Hubalek, 1974). Similarly, to ascertain information on the natural occurrence and distribution of certain keratinophilic fungi in Orissa, an investigation of feathers of wild birds and domestic fowls was undertaken by Sur and Ghosh (1980b) and six genera viz. Aphanoascus, Chrysosporium, Ctenomyces, Gymnoascoideus, Malbranchea and Rollandina were isolated. Recently Dixit and Kushwaha (1991) undertook a survey to find out the association of keratinophilic ascomycetes with six common birds of India viz., hen, pigeon, house sparrow, duck, parrot and crow. Sarangi and Ghosh (1991) also surveyed keratinophilic fungi inhabiting common house sparrow of coastal and intercoastal districts of Orissa with respect to three parameters - season, age, sex and found that Chrysosporium tropicum and Rollandina hyalinospora showed highest frequency of occurrence irrespective of the parameters.

High frequency of keratinophilic fungi have also been found in bird's nests and some commonly found species are Chrysosporium keratinophilum, Keratinomyces ajelloi and Trichophyton terrestre (Pugh, 1966b; Otcenasek et al.

1967). However, it has been pointed out by Pugh (1966b) and Pugh and Evans (1970a) that the spectrum of keratinophilic fungi in nests differs from that which is present on the birds.

Vanbreuseghem (1953) distinguished two ecological groups of keratinophilic fungi (i) dermatophytes and other species which are able to attack native keratin by penetration and (ii) Saprophytes which attack the keratin mainly by hyphal penetration and surface growth by utilizing the more easily decomposable compound of the keratin substrate. Griffin (1960), English (1963,1965), Kushwaha and Agrawal (1976b) and Nigam and Kushwaha (1989a) also supported this view while studying succession of fungi colonising human hair and other keratinous substrates. Kuester and Safwat (1978) reported keratin as a rich source of nitrogen that can only be decomposed by keratinophilic fungi.

In addition to the availability of keratinous substrates in soil, occurrence and survival of keratinophilic fungi has been reported to be influenced by various geonomic and climatic factors. Generally, they are considered to be mesophilic but some strains are thermotolerant and recent reports indicate that they can adapt themselves to adverse temperatures for their

survival (Pugh and Allsopp, 1982; Punsola and Guarro, 1984). Chmel et al (1972) found their presence to be directly proportional to the humus content of soil. Chmel and Vlacilikova (1975) studied the influence of depth and humus content of soil on the occurrence of keratinophilic fungi and found their highest frequency in the superficial layers.

The influence of pH on the distribution of soil keratinophilic fungi was first emphasized by Marples (1964). Later, Pugh (1966a) observed a distinct correlation between the pH of bird's nest and the presence of keratinophilic fungi. Ziegler (1966) and Bohme and Ziegler (1965, 1968) studied their distribution pattern in relation to the pH of soil and found their occurrence more frequent in weakly acidic to weakly alkaline soils. Hubalek (1974) carried out an extensive study on the occurrence of keratinophilic fungi at different substrate pH values and categorized them into three groups : (i) **acidophilic** such as Arthroderma uncinatum, A. curreyi and Chrysosporium tropicum in acidic soils; (ii) **neutrophilic** mainly Nannizia incurvata in near neutral soils and (iii) **alkalophilic** such as Arthroderma quadrifidum, Ctenomyces serratus and Chrysosporium keratinophilum in alkaline soils. Their grouping in these categories has further been confirmed by Chmel and Vlacilikova (1977).

Water constitutes a large proportion of the cell cytoplasm and is vital to life since most of the enzymatic reactions occur in aqueous solution. Pugh and Evans (1970b) observed a higher percentage of spore germination in case of Arthroderma uncinatum and Ctenomyces serratus at 90-100%. Chmel et al. (1972) found that higher humidity was associated with an increased number of isolations of Keratinophyton ajelloi but Trichophyton terrestre was found more frequently in soils having low water content. These findings show that K. ajelloi is hydrotolerant while T. terrestre is xerophilic in nature. Hubalek and Munro (1973) showed a greater incidence of keratinophilic fungi in bird's nests with 15-20% water content. Hubalek and Balat (1976) on further examination of the nests of tree sparrow (Passer montanus) found Chrysosporium keratinophilum and Arthroderma fulvescens as hygrophilic in nature.

The mechanism by which keratinophilic fungi attack and digest keratin has been a subject of much research. As early as 1926, Nannizzi suggested that ringworm fungi hydrolyse keratin but Tate (1929) could not detect any enzyme that could hydrolyse keratin. Nickerson (1947) suggested that the enzymes produced by the keratinophilic fungi may be able to act on a reduced form of keratin. Raubitschek (1961) and Weary et al. (1965)

speculated that keratinophiles could disrupt the keratinized tissues purely on mechanical basis without the involvement of chemical keratinolysis. English (1965) suggested that the breakdown of the disulphide bond of keratin molecule is brought about at high pH by the reducing agents. However, according to another view keratins are digested by certain chemical substances or enzymes produced by the keratinophilic fungi. Daniels (1953), determined qualitatively the release of amino acids into the culture medium, during the digestion of hair keratin by Microsporum canis. Weary & Canby (1967) determined keratinolytic activity of M. canis and M. gypseum by the release of soluble sulphydryl containing amino acids and polypeptides into the medium in quantities significantly greater than those released by controls. A fluorescent microscope examination of hair attacked by Trichophyton mentagrophytes showed that dermatophytes are able to penetrate the hair by enzymic digestion (Verma, 1966). Morphological studies with electron microscope also suggested, a possible induction of enzyme digestion of keratinized tissues leading to the breakage of disulphide bonds (Mercer and Verma, 1963; Miyazaki et al. 1966). It has also been discussed by Ruffin et al. (1976) that proteolytic activity and sulphytolysis are simultaneous and perhaps complementary phenomenon, exhibited by dermatophytes for transforming keratin into a nutritive

material. The proteolytic enzymes, particularly keratinases attracted the attention of dermatologists because of their powerful action on keratin. Production of these enzymes by keratinophilic/dermatophytic fungi has been demonstrated by other workers also (Noval and Nickerson, 1959; Evolceanu and Lazar, 1960; Chattaway et al. 1963; Nickerson and Durand, 1963; Kotrajaras, 1965; Weary et al. 1965; Verma, 1966; Shome, 1967; Day et al. 1968; Yu et al. 1968, 1969a,b, 1971; Collins et al. 1973; Eleuterio et al. 1973; Takiuchi, 1973; Takiuchi et al. 1973, 1982, 1984; Meevootisom and Niederpruem, 1979; Higuchi and Takiuchi, 1980; Jain and Agrawal, 1980; Singh, 1981; Higuchi et al. 1981; Das and Banerjee, 1982; Kushwaha, 1983; Biswas et al. 1988; Hasija et al 1990; Rajak et al. 1991a,b; Malviya et al. 1992a,b).

Keratinases have usually been found to be associated with the mycotic infections of man and animals also. Rippon and Garber (1969) related the activity of keratinase of dermatophytes with the severity of ringworm infection. Minocha et al. (1972) suggested that the Skin lesions are mediated by soluble keratinolytic enzymes produced by the dermatophytic fungi. Grappel and Blank (1972a,b) demonstrated the role of keratinase in

hypersensitive reactions. Recently, Das and Banerjee (1982) correlated, the failure of experimental infection in guinea pig by Trichophyton rubrum, with its inability to secrete the enzyme keratinase in vitro, showing, thereby, that the keratinophilic fungi, specially the dermatophytes possess specific enzymes that enable their parasitic growth on highly resistent keratinous substrates.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

ISOLATION OF KERATINOPHILIC FUNGI FROM POULTRY FARM SOILS

(a) Collection of soil samples :

Soil samples were collected from ten different poultry farms of Jammu district (Fig.1) as given below :

1. Machlian
2. Prem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Nagbani
9. Maralia
10. Ranbir Singh Pura

All the samples were collected by scraping a layer of soil not exceeding 5 cm in depth and were brought to the Laboratory in pre-sterilized polythene bags for investigations.

(b) Preparation of keratin baits :

Poultry feathers were used as keratinic substrate for the isolation of keratinophilic fungi from poultry farm soil samples. They were cut into small pieces, washed with distilled water, air dried and sterlized by autoclaving at 15 lbs/sq. inch steam pressure for 20 minutes.

FIGURE 1 : Location map of Surveyed poultry farms from Jammu district.

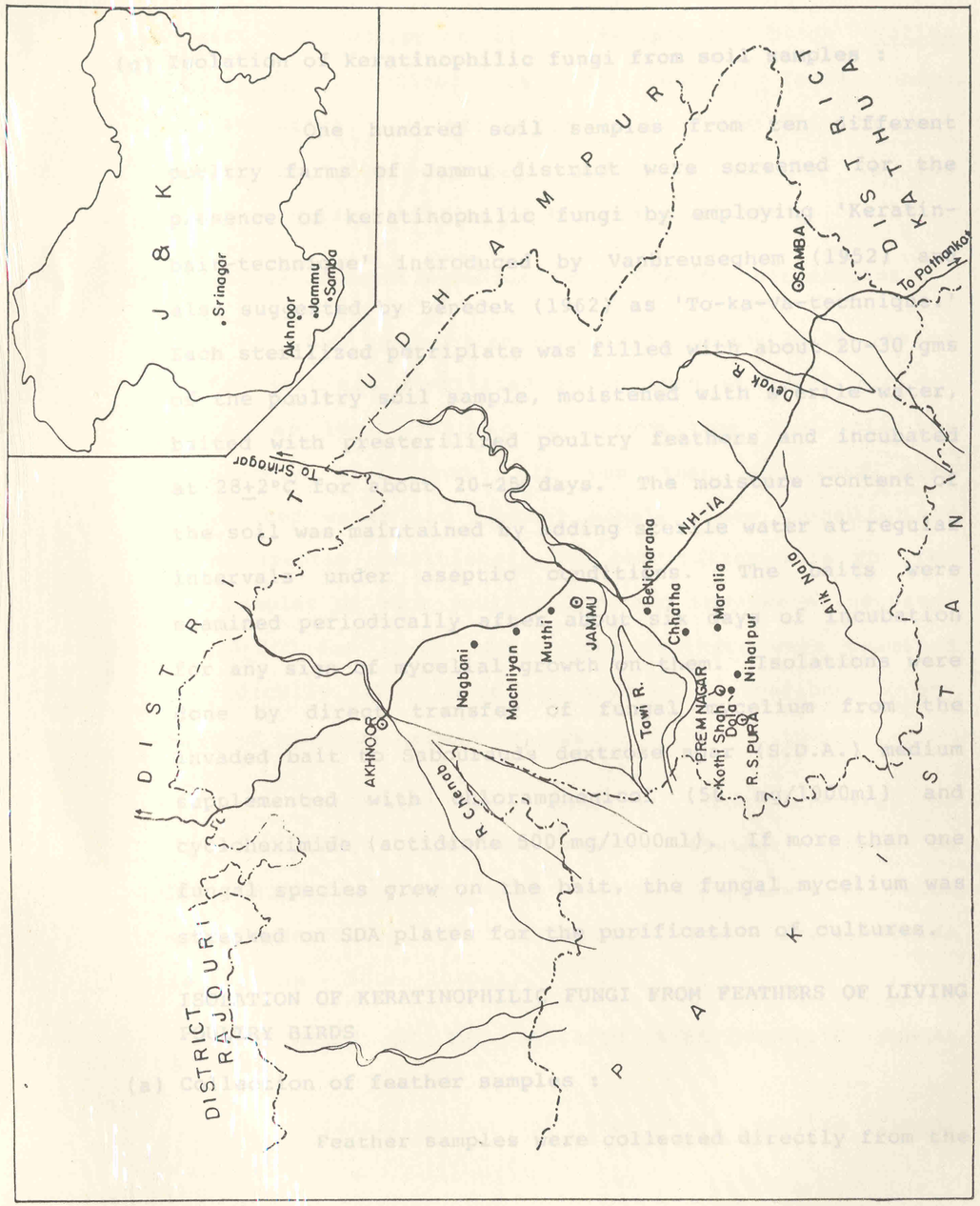


FIG. 1. LOCATION MAP OF SURVEYED POULTRY FARMS FROM JAMMU DISTRICT.

(c) Isolation of keratinophilic fungi from soil samples :

One hundred soil samples from ten different poultry farms of Jammu district were screened for the presence of keratinophilic fungi by employing 'Keratin-bait-technique' introduced by Vanbreuseghem (1952) and also suggested by Benedek (1962) as 'To-ka-Va-technique.' Each sterilized petriplate was filled with about 20-30 gms of the poultry soil sample, moistened with sterile water, baited with presterilized poultry feathers and incubated at 28±2°C for about 20-25 days. The moisture content of the soil was maintained by adding sterile water at regular intervals under aseptic conditions. The baits were examined periodically after about six days of incubation for any sign of mycelial growth on them. Isolations were done by direct transfer of fungal mycelium from the invaded bait to Sabouraud's dextrose agar (S.D.A.) medium supplemented with chloramphenicol (50 mg/1000ml) and cycloheximide (actidione 500 mg/1000ml). If more than one fungal species grew on the bait, the fungal mycelium was streaked on SDA plates for the purification of cultures.

ISOLATION OF KERATINOPHILIC FUNGI FROM FEATHERS OF LIVING POULTRY BIRDS

(a) Collection of feather samples :

Feather samples were collected directly from the

breast and underparts of living poultry birds (Gallus domesticus) confined in the poultry farms under investigation. Collection of samples was done in pre-sterilized polythene bags and were brought to the laboratory for isolation of keratinophilic fungi.

(b) **Isolation of keratinophilic fungi from feather samples :**

Each petriplate containing about 20-30 gms of garden soil was sterilized in an oven at 180°C for 4-5 hours, at least thrice, to ensure that the soil was completely sterilized. It was then moistened with sterilized water and feather samples were placed on it under aseptic conditions. Ten petriplates were run for the samples of each poultry farm and they were incubated at 28±2°C for 20-25 days. The feathers were examined periodically after about six days of incubation for any sign of mycelial growth on them.

Isolations were done by direct transfer of fungal mycelia from the invaded feather sample to Sabouraud's dextrose agar medium supplemented with chloramphenicol (50 mg/1000ml) and cycloheximide (actidione - 500mg/1000ml).

IDENTIFICATION OF THE ISOLATED KERATINOPHILIC FUNGAL SPECIES

Identification of the keratinophilic fungal

species isolated from poultry farm soils and feathers of living poultry birds was done by studying their macro and micro morphological characters. For inducing the isolated species to form perfect states, they were grown at 28°C for 3 months on oatmeal agar, phytone yeast extract medium and dextrose salt agar medium supplemented with sterilized human hair as the keratinous substrate.

Relevant literature and various keys used for the identification were those given by Hesseltine and Dorothy (1955), Barron et al. (1961), Barnett (1962), Carmichael (1962), Raper and Fennel (1965), Mukerji (1966), Gilman (1967), Booth (1971), Ellis (1971), Barron (1972), Ainsworth et al. (1973a, 1973b), Sigler and Carmichael (1976), Alexopoulos and Mims (1979), Van Oorschot (1980) and Chabasse (1988). Identity of the fungal isolates was also confirmed from International Mycological Institute, U.K., and from Indian Type Culture Collection, I.A.R.I., New Delhi.

PHOTOGRAPHY AND DRAWINGS :

For coloured photography of cultures Kodak Gold 35mm film and Ashai pentax camera were used. Olympus PM 6, 35mm Camera and Agfa Copex, PAN AHU "TRI 13" photographic film were used for microphotography. Sketches were made with Erma make, prism type camera

lucida using different lens combinations.

MAINTENANCE AND PRESERVATION OF CULTURES :

All the purified cultures were maintained on sterilized SDA slants at 10°C and subsequent subculturing was done after every six months. Each isolate was cultured and maintained in triplicate.

MEDIA USED :

During these investigations, a number of solid media were used for the isolation and sporulation of various keratinophilic fungi. Following is the list of media used alongwith their constituents.

(a) Sabouraud's dextrose agar medium :

Dextrose	-	40.0g
Peptone	-	10.0g
Agar-agar	-	20.0g
Distilled water	-	1000ml

(b) Potato sucrose agar medium :

Sucrose	-	20.0g
Potato broth	-	500ml
Agar-agar	-	20.0g
Distilled water	-	500ml

(c) Czapek's - Dox agar medium :

$NaNO_3$	-	2.0g
K_2HPO_4	-	1.0g
$MgSO_4\ 7H_2O$	-	0.5g
KCl	-	0.5g
$FeSO_4 \cdot 7H_2O$	-	0.01g
Sucrose	-	30.0g
Agar-agar	-	20.0g
Distilled water	-	1000ml

(d) Phytone yeast extract medium

Phytone	-	10g
Dextrose	-	40g
Yeast extract	-	5g
Agar-agar	-	20g
Distilled water	-	1000ml

(e) Dextrose - salts agar medium :

Dextrose	-	10mg
Sodium chloride	-	10mg
Ammonium sulphate	-	10mg
Dibasic potassium phosphate	-	10mg
Biotin	-	100ug
Agar-agar	-	20g
Distilled water	-	1000ml

(f) Oatmeal agar medium :

Oatmeal - 30g

Agar - agar - 20g

Distilled water - 1000ml

OCCURRENCE AND FREQUENCY OF KERATINOPHILIC FUNGI

The percentage of occurrence and frequency were calculated as follows :

$$\% \text{ occurrence} = \frac{\text{No. of positive samples}}{\text{Total No. of samples}} \times 100$$

$$\% \text{ frequency} = \frac{\text{No. of isolates of a fungus}}{\text{Total No. of isolates}} \times 100$$

EVALUATION OF KERATINOLYTIC NATURE OF INDIVIDUAL ISOLATES

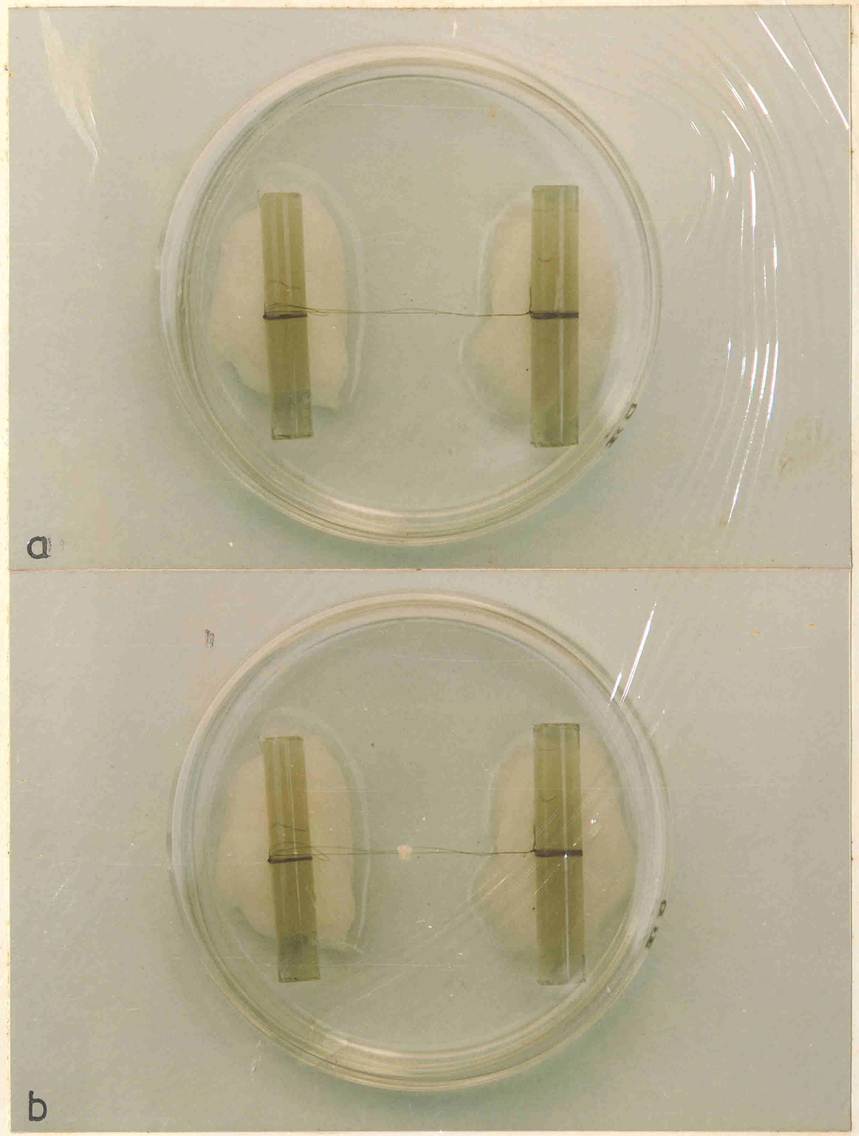
In order to confirm the keratinolytic nature, each fungal isolate was inoculated in the middle of a sterile human hair tied at each end to a piece of glass rod and kept in a paired sterile petriplate under aseptic conditions, as shown in Plate 1. To provide a humid atmosphere for the growing fungus, moistened cotton swabs were spread at the bottom of each glass rod. In addition to this, the cotton swabs also kept the glass rods in position. The whole assembly was kept in an incubator at 28°C for a period of 10-35 days. The fungus developed a colony on hair, continued to grow and after few days broke the hair at the point of inoculation indicating, thereby,

PLATE 1 : Method for evaluating keratinolytic nature of fungi.

a. Sterile human hair tied to glass rod pieces.

b. Test fungus developing a colony on the hair at inoculation point.

PLATE 1



digestion of hair keratin.

PREFERENTIAL UTILIZATION OF KERATIN BY POULTRY SOIL AND FEATHER ISOLATES

(a) Preparation of keratin baits :

In order to check the substrate affinity of the isolated keratinophilic fungi, different types of keratin baits were used viz. chicken feathers, peacock feathers, human hair, human nails and horse hair. The baits were thoroughly washed with water, air dried at room temperature and were then cut into small pieces. Finally, they were washed four times with chloroform : methanol (1:1 v/v), rinsed with distilled water, air dried and sterlized by tyndalization (80°C for 30 minutes on three successive days with intermediate incubation at 28±2°C).

(b) Preferential utilization of keratin :

Petridishes (8" diameter) were half filled with garden soil and sterilized in an oven at 180°C for 4-5 hours on three successive days. The soil was then moistened with sterile water and the inoculum of the test fungus was added. Different presterilized baits were kept on the inoculated soil and the petriplates were incubated at 28±2°C. Preferential utilization and the degree of colonization of different baits shown by various keratinophilic fungi was recorded after an incubation

period of 30 days.

INFLUENCE OF SOIL FACTORS ON THE OCCURRENCE OF POULTRY SOIL INHABITING KERATINOPHILES

Poultry farm soils were analysed for the presence of essential macro and micro elements required for the growth of keratinophilic fungi. The elements analysed were organic carbon, humus, total nitrogen, total phosphorus, potassium, magnesium, calcium and iron.

(a) Organic carbon

Organic carbon of the soil was determined by Walkley and Black's (1935) rapid titration method. In this method, soil samples were digested with chromic and sulphuric acids making use of the heat of dilution of H_2SO_4. The excess of chromic acid, not reduced by the carbon of the soil was determined by titration with standard ferrous sulphate or ferrous ammonium sulphate solution.

(b) Humus (Organic matter)

It was calculated by multiplying the Walkley Black value (i.e. % of carbon in the soil) by a factor of 1.724.

% of organic matter in soil = % carbon in soil x 1.724.

(c) Total nitrogen

Total nitrogen present in the soil sample was

determined by Kjeldahl's method (Vogel, 1964). The principle of the method is the conversion of the nitrogen of the nitrogenous substance into ammonia by boiling with excess of concentrated sulphuric acid. Ammmonia is fixed by the sulphuric acid as ammonium sulphate which is then determined by adding an excess of caustic alkali and distilling off the liberated ammonia into standard acid.

(d) Phosphorus

Determination of available phosphate in the soil samples was done colorimetrically (Chopra and Kanwar, 1976). This method is based on the extraction of available phosphorus from the soil by shaking with 0.5 N sodium bicarbonate solution adjusted to pH 8.5. The blue colour was compared colorimetrically at 640mm in a balanced cell colorimeter 102 (Systronics) and phosphorus was calculated from the standard curve.

(e) Magnesium

Magnesium was determined by using Titan Yellow (sodium salt of dihydrothio-p-toluidine sulphonic acid) as the dye (Vogel, 1964). In this method, magnesium hydroxide is precipitated with sodium hydroxide solution in the presence of Titan yellow resulting in the formation of a red lake whose colour intensity was measured at 535nm with a spectrophotometer (Perkin Elmer model).

(f) Potassium

Available potassium was extracted from the soil samples by shaking it with Morgan's reagent containing sodium acetate and acetic acid. The extract on treatment with sodium cobalt nitrite and alcohol produces a turbidity whose colour represents the concentration of potassium, which is then compared with standard potassium chloride solution and thus the amount of potassium present in the soil sample was calculated (Chopra and Kanwar, 1976).

(g) Calcium

Calcium was determined titrimetrically with EDTA (Versene method) (Chopra and Kanwar, 1976). The method is based on the fact that calcium forms stable complex with EDTA (ethylene diamine tetra acetic acid disodium salt).

A known volume of the sample is titrated with standard versenate 0.01N solution using murexide (ammonium purpurate) indicator in the presence of NaOH solution. The end point is a change of colour from orange red to purple at pH 12, when the whole of calcium forms a complex with EDTA.

(h) Iron

Iron was determined spectrophotometrically (Vogel, 1964) at 525nm by using Perkin Elmer model.

Salicylic acid and ferric ions form a deep coloured complex with a maximum absorption at about 525nm. This complex is used as the basis for the photometric titration of ferric ions with standard EDTA solution (0.10M).

(i) Soil pH

Took about 10g of the soil sample and added 25 ml of distilled water. Stirred it thoroughly and after a lapse of about 30 minutes, put the glass electrode in the soil suspension. Connected the electrode to the pH meter which had already been checked with a standard buffer of known pH. Read the pH of the soil from the scale.

INFLUENCE OF RELATIVE HUMIDITY, TEMPERATURE AND HYDROGEN ION CONCENTRATION ON THE GROWTH OF CHRYSOSPORIUM SPECIES

(a) Relative humidity :

To study the effect of relative humidity on the growth of Chrysosporium species viz. C. keratinophilum, C. tropicum (Isolate I&II), C. queenslandicum, C. pannorum and C. indicum, Sabouraud's dextrose agar plated petridishes were inoculated with a 5mm disc of the test fungus. After two hours of incubation at 28±2°C, the petridishes were inverted over other petridishes of equal diameter containing 15ml mixture of sulphuric acid and water (Stevens, 1916). Petridishes were sealed with cellophane

tape at the junction of the base and the lid and further incubated at 28±2°C. Radial growth in mm was recorded after 3,6,9 and 12 days of incubation by taking mean of the two diagonals of the colony from the lower surface of the petridishes as suggested by Brancato and Golding (1953). The experiment was run in triplicate for each species at each RH value.

Various levels of relative humidity within the range of 20-90 percent were maintained with stock solutions (equal volumes of concentrated sulphuric acid and distilled water) and distilled water according to the method of Buxton and Mellanby (1934).

Relative humidity	Stock solution in ml.	Distilled water in ml.
20	709	114
30	686	226
40	539	306
50	514	420
60	374	396
70	348	510.3
80	294	640
90	161	712

For 0 and 100% relative humidity, only concentrated sulphuric acid and distilled water respectively were used.

(b) Temperature

Chysosporium species were grown on SDA plates by inoculating the plates with a 5mm disc of the freshly grown seven days old culture of the test organism. Incubation was carried out in triplicate for each species at 20°C, 25°C, 30°C, 35°C, 40°C and 45°C. Radial growth of each test fungus was determined in mm after 3,6,9 and 12 days of incubation.

(c) Hydrogen ion concentration

Effect of different pH on the growth of Chrysosporium species was evaluated by inoculating a 5mm disc of the fungus on SDA plates (in triplicate) maintained at pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with the help of 0.5 M dipotassium hydrogen phosphate (K_2HPO_4) for alkaline and 0.5 M dihydrogen potassium phosphate (KH_2PO_4) for acidic. The petriplates were incubated at 28±2°C and radial growth of each test fungus was determined in mm after 3, 6, 9 and 12 days of incubation.

IN VITRO DEGRADATION OF KERATIN BY DIFFERENT KERATINOPHILIC ISOLATES.

(a) Preparation of keratin substrate :

Human hair was washed four times with chloroform methanol (1:1 V/V) and then with water and finally air dried at room-temperature. The hair was then cut into pieces of about 1 cm length, weighed into portions of 50mg

and sterlized by tyndallization (80°C for 30 minutes on three successive days with intermediate incubation at 28±2°C).

(b) **Preparation of media for inoculation :**

Two media (Medium A and Medium B) were prepared for each fungal species.

Medium A : K_2HPO_4-1.5g, $MgSO_4.7H_2O$-0.025g, $CaCl_2$-0.025g, $FeSO_4.7H_2O$-0.015g, $ZnSO_4.7H_2O$-0.005g distilled water 1000ml; pH 7.0 and 500 mg of presterlized human hair.

Medium B : Glucose - 0.9g, $MgSO_4.7H_2O$-0.6g, thiamine - 0.01g, Pyridoxine-0.01g, inositol-0.05g, 0.028 M phosphate buffer 1000ml, pH 7.8.

Two sets (one in medium 'A' and the other in medium 'B') were run for the isolated keratinophilic species. Each fungus was grown in 100ml capacity Erlenmeyer flasks containing 50ml of sterilized basal medium supplemented with 500 mg of presterilized human hair as a keratin source.

(c) **Preparation of fungal inoculum**

Flasks containing sterilized media were inoculated with inoculum discs (0.6cm) obtained from periphery of actively growing seven days old subcultures. Flasks containing medium with a disc of agar without the

fungus served as control. Four test flasks and one control set were maintained for each isolate and were incubated at 28±2°C for 40 days in static condition.

(d) Removal of sample

At the end of the growth period, fungal mat and hair were separated from culture medium by filtering through Whatman filter paper number 42. Culture filterate of four test flasks was pooled together, centrifuged at 5000 rpm for five minutes and the supernatant was collected and assayed for change in alkalinity, release of cysteine, protein and keratinase.

i) Change in alkalinity :

pH change was recorded potentiometrically by glass electrode.

ii) Estimation of cysteine :

It was done by the method of Ramakrishna et al. (1979).

Took 4 ml of the supernatant sample and added 15 ml of potassium biphthalate-hydrochloric acid buffer (pH 3.3), 2ml of 0.2% metol solution and 3ml of 0.01 M potassium dichromate solution successively. It was diluted to 25ml with distilled water in a calibrated volumetric flask and the absorbance was measured at 510nm after 160 minutes against a corresponding reagent blank prepared in the same manner. The cysteine content was

computed from an appropriate calibrationcurve, prepared in a similar manner using cysteine as standard and values were expressed in µg of cysteine per ml of the sample.

iii) **Estimation of Proteins :**

Protein estimation was done following the method of Lowry *et al.* (1951) by using bovine serum albumin as standard.

Preparation of reagents :

Reagent A : 2% Na_2CO_3 in 0.1N NaOH

Reagent B : 0.05% $CuSO_4.5H_2O$ in 1% Sodium Potassium tartarate.

Reagent C : Mix 50ml of reagent A and 1ml of reagent B. (Alkaline Copper Sulphate)

Folin - ciocalteu reagent : It was prepared by diluting it with equal volume of distilled water.

Took 0.2ml of the supernatant sample and put 0.8ml of distilled water to raise its final volume to 1ml. Added 5ml of alkaline copper reagent (Reagent C) and allowed the mixture to stand at room temperature for about 10 minutes. Then added 0.5ml of dilute Folin- ciocalteu reagent with immediate mixing and allowed it to stand again at room temperature for about 30 minutes. After this period, absorbance of the sample was read in a

spectrophotometer at 660 mm. 1ml of distilled water treated in a similar manner served as control. Net protein was calculated with the help of standard graph prepared similarly by using freshly prepared bovine serum albumin as standard and the values were expressed as ug of protein per ml of an enzyme sample.

(iv) Estimation of keratinase :

Human hair was treated with chloroform: methanol (1:1 v/v) four to five times, washed thoroughly with distilled water, air dried and finally sterilized by tyndallisation. Extracellular keratinase was measured as per the method of Yu et al. (1968) with slight modification. 50mg of presterilized human hair was incubated with 6ml of enzyme sample (from two media, Medium A and Medium B) diluted with 4ml of 28 mM phosphate buffer, pH 7.8. The reaction flasks containing reaction mixture (in triplicate) were then kept in a water bath at 37°C for 2 hours and then immediately immersed in ice water for 5 minutes, in order to stop the reaction. The above mixture was then filtered and the absorbance of the clear solution was measured spectrophoto-metrically at 280nm. An increase in 0.100 absorbance value at 280 nm was considered as an enzyme unit/ml of culture filtrate (1KU=0.100 corrected absorbance). Boiled enzyme sample served as the control.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

DISTRIBUTION AND FREQUENCY OF KERATINOPHILIC FUNGI IN POULTRY FARMS OF JAMMU DISTRICT.

During the period, January 1992 to December 1993, ten different poultry farms of Jammu district situated at Machlian, Prem Nagar, Nihalpur Simbal, Kothi Shahdaula, Akhnoor, Belicharana, Muthi, Nagbani, Maralia and Ranbir Singh Pura (Fig. 1) were screened for the presence of keratinophilic fungi. As depicted in table 2, a total of 35 keratinophilic isolates representing 27 species belonging to 12 genera (Chrysosporium, Malbranchea Scopulariopsis, Microascus, Gliocladium, Chaetomium, Circinella, Curvularia, Sepedonium, Fusarium, Aspergillus and Penicillium) were isolated by using keratin - bait - technique.

A Keratinophilic fungi from poultry farm soils

Data on the distribution of keratinophilic fungi (Table 3) indicates that all the poultry soil samples were positive (100%) for these fungi. This observation can be correlated with higher influx of poultry droppings and various other remains of the poultry birds like dropped off feathers, claws etc. in this soil which makes it

Table 2 : Keratinophilic fungi isolated during the survey

Keratinophilic fungi	Poultry Farms									
	1	2	3	4	5	6	7	8	9	10
Chrysosporium keratinophilum	▲●	▲●	▲●	△●	▲●	△○	△○	△●	▲●	▲○
C. queenslandicum	△○	△○	▲○	△●	△○	▲○	▲●	△○	△○	▲●
C. tropicum strain I	▲○	▲●	▲●	△○	▲●	▲●	▲○	▲●	▲●	△○
C. tropicum strain II	△○	▲○	▲○	▲○	△○	△○	△○	▲○	△○	△○
C. pannorum	△○	▲○	△○	△○	△○	△○	▲○	▲○	▲○	△○
C. indicum	△○	△○	△○	△●	△○	△●	△○	△○	△●	△●
Malbranchea flava	△○	△○	▲○	▲○	▲○	△○	△○	△○	▲○	△○
M. chrysospoiroidea	△○	△○	△●	△●	△○	△●	△●	△○	△○	△●
Scopulariopsis brevicaulis	△●	△●	▲●	▲○	▲○	△●	▲●	△○	▲●	▲○
Microascus manganii	▲○	△○	△●	△○	▲●	▲○	△○	△○	△○	△○
Gliocladium virens	▲○	△○	△○	▲○	△○	▲○	▲○	△○	▲○	▲○
Circinella muscae	▲○	△○	▲○	△○	▲○	△○	△○	▲○	△○	△○
Sepedonium maheshwarianum	△○	△○	△●	△●	△○	△○	△○	△●	△●	△●
Chaetomium globosum	△●	△●	△○	△●	△●	△○	△○	△●	△○	△○
Curvularia lunata	△●	△○	△●	△●	▲●	△●	△●	△○	△○	△●
Fusarium solani	△○	△○	△○	△○	▲○	△○	▲○	△○	△○	▲○
F. moniliforme	△●	△○	△○	▲●	▲○	▲●	△●	▲●	▲○	△●
Aspergillus candidus	▲○	▲○	△○	▲○	▲○	▲○	△○	△○	▲○	△○
A. deflectus	△○	△○	▲○	▲○	△○	△○	▲○	▲○	▲○	△○
A. flavus	△●	△●	△●	▲●	▲●	▲○	△●	△○	▲●	▲●
A. sydowii	△○	△○	△○	▲○	△○	△○	▲○	△○	▲○	△○
A. versicolor	▲○	▲○	▲○	▲○	▲○	▲○	▲○	△○	△○	△○
A. ochraceous	▲○	△○	△○	△○	△○	△○	△○	▲○	▲○	▲○
A. nidulans	△●	△●	△●	△●	△○	△○	△○	△○	△○	△○
Penicillium citrinum	▲○	▲○	▲○	▲○	▲○	△○	▲○	△○	▲○	▲○
P. griseofulvum	△○	▲○	△○	▲○	▲○	△○	△○	▲○	△○	△○
P. aurantiogriseum	△○	▲○	▲○	△○	△○	△○	△○	▲○	△○	△○
P. chrysogenum	△○	△○	△○	△○	△○	△●	△●	△○	△○	△○

Key to Symbols :

▲: Soil isolate present
△: Soil isolate absent
●: Feather isolate present
○: Feather isolate absent

Poultry farms :

1. Machlian
2. Prem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Nagbani
9. Maralia
10. Ranbir Singh Pura.

Table 3 : Prevalence of Keratinophilic fungi in different Poultry farm soils of Jammu

No. of soil samples examined 100 (10 samples from each poultry farms)

No. of soil samples positive 100

Occurrence (%) 100.0

Fungi Recorded	Number of Soil Samples Positive										Occurrence
	1	2	3	4	5	6	7	8	9	10	%
Chrysosporium keratinophilum	6	7	6	-	5	-	-	-	10	42	42
C. queenslandicum	-	-	9	-	-	8	5	-	-	7	29
C. tropicum strain I	7	9	5	-	7	10	6	5	5	-	54
C. tropicum strain II	-	3	7	5	-	-	-	4	-	-	19
C. pannorum	-	8	-	-	-	-	3	4	3	-	18
Malbranchea flava	-	-	6	10	7	-	-	-	10	-	33
Scopulariopsis brevicaulis	-	-	5	7	6	-	7	-	6	4	35
Microascus manganii	4	-	-	-	3	5	-	-	-	-	12
Gliocladium virens	6	-	-	5	-	7	8	-	8	4	38
Circinella muscae	5	-	2	-	4	-	-	4	-	-	15
Fusarium moniliforme	-	-	-	3	-	4	-	2	6	-	15
F. solani	-	-	-	-	3	-	5	-	-	3	11
Aspergillus candidus	6	6	-	5	7	4	-	-	5	-	33
A. deflectus	-	-	5	4	-	-	5	3	5	-	22
A. flavus	-	-	-	5	6	6	-	-	5	6	28
A. orchraceus	6	-	-	-	-	-	-	4	6	3	19
A. sydowii	-	-	-	5	-	-	5	-	4	-	14
A. versicolor	4	3	4	5	4	5	3	-	-	-	28
Penicillium aurantiogriseum	-	5	4	-	-	-	-	4	-	-	13
P. citrinum	5	5	7	3	3	-	5	-	4	6	38
P. griseofulvum	-	4	-	4	4	-	-	5	-	-	17
Fungal species recorded from each poultry farm	8	8	10	12	12	8	10	8	13	8	

Total No. of fungal speices 20

Poultry farms :

1. Machlian
2. Prem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Maralia
9. Maralia
10. Ranbir Singh Pura.

keratin rich. All these factors create conditions which are favourable to the development and diffusion of keratinophilic fungi. Previous investigations of geophilic keratinomycetes from poultry farms (Padhye et al. 1966; Jain, 1983; Jain and Agrawal, 1977) and other sites (Chmel et al. 1972; Sur and Ghosh, 1980a,b), have also shown that their distribution in soil largely depends on many ecological factors, especially on the existence of keratinous material of either human or animal origin or both. These fungi play a vital role in nature in the breakdown of keratinous substrates into simpler organic compounds, and some of them due to their ability to grow on keratin residues may also prove to be pathogens or potential pathogens for man and animals as many of the keratinophilic fungi previously thought to be nonpathogenic have been reported from various clinical conditions of skin of man and animals (Bhaskaran et al. 1977).

Perusal of data on the frequency of keratinophilic fungi (Table 4) reveals that two genera viz., Chrysosporium and Aspergillus were recovered from the soils of all the poultry farms and formed the bulk of the isolated keratinophiles. Maximum frequency of Chrysosporium was noted in the poultry farm soils of Prem Nagar and Nihalpur Simbal, whereas, Aspergillus occurred most

Table 4 : Percentage frequency of keratinophilic fungi in the soil samples of each Poultry farm.

Fungi recorded	Poultry Farms									
	1	2	3	4	5	6	7	8	9	10
Chrysosporium keratinophilum	12.2	14.0	10.3	-	8.5	-	-	-	13.0	19.5
C. queenslandicum	-	-	15.5	-	-	16.3	9.6	-	-	17.0
C. tropicum strain I	14.2	18.0	8	-	11.9	20.4	11.5	14.2	6.5	-
C. tropicum strain II	-	6.0	12.0	8.1	-	-	-	11.4	-	-
C. pannorum	-	16.0	-	-	-	-	5.7	11.4	3.9	-
Malbranchea flava	-	-	6.8	16.3	11.9	-	-	-	13.0	-
Scopulariopsis brevicaulis	-	-	8.6	11.4	10.2	-	13.4	-	7.8	9.7
Microascus manganii	8.1	-	-	-	5.0	10.2	-	-	-	-
Gliocladium virens	12.2	-	-	8.1	-	14.2	15.3	-	10.4	9.7
Circinella muscae	10.2	-	3.4	-	6.8	-	-	11.4	-	-
Fusarium moniliforme	-	-	-	4.9	-	8.1	-	5.7	7.8	-
F. solani	-	-	-	-	5.0	-	9.6	-	-	7.3
Aspergillus candidus	12.0	12.0	-	8.1	11.9	8.1	-	-	6.5	-
A. deflectus	-	-	8.6	6.5	-	-	9.6	8.5	6.5	-
A. flavus	-	-	-	8.1	10.2	12.2	-	-	6.5	14.6
A. ochraceus	12.2	-	-	-	-	-	-	11.4	5.2	7.3
A. sydowii	-	-	-	8.1	-	-	9.6	-	7.8	-
A. versicolor	8.1	6.0	6.8	8.1	6.8	10.2	5.7	-	-	-
Penicillium aurantiogriseum	-	10.0	6.8	-	-	-	-	11.4	-	-
P. citrinum	10.2	10.0	12.0	6.5	5.0	-	9.6	-	5.2	14.6
P. griseofulvum	-	8.0	-	4.9	6.8	-	-	14.2	-	-

Poultry farms:

1. Machlian
2. Prem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Nagbani
9. Maralia
10. Ranbir Singh Pura.

frequently in Kothi Shahdaula and Machlian poultry farms (Fig. 3).

Among the various keratinophilic genera isolated, Chrysosporium which is related to dermatophytes for being the anamorph of some species of Arthroderma and other members of gymnoascaceae was represented by C. tropicum strain I & II, C. keratinophilum, C. queenslandicum and C. pannorum (Table 3). Within this group, C. tropicum strain I appeared in soil samples of eight poultry farms and showed highest occurrence of 54% (Fig. 2). This result is in agreement with the previous findings of other workers who also observed C. tropicum to be predominant in many habitats (Pugh and Mathison, 1962 ; Ajello et al. 1965; Garg, 1965, 1966; Dunne and Morahan, 1964a,b; Padhye et al. 1966; Ajello and Alpert, 1972; Ajello and Padhye, 1974; Caretta and Piontelli, 1975; Kushwaha and Agrawal, 1976b; Jain and Agrawal, 1977; Guarro et al. 1981, Sur and Ghosh,1980a,b; Abdel - Fattah et al. 1982; Calvo et al. 1984; Ali-Shtayeh et al. 1989; Al-Musallam, 1990; Sundara Singh et al. 1990; Soon, 1991). This world wide distribution of C. tropicum suggests its successful competing ability with other keratinophilic fungi in colonizing keratinous substrates. Moreover, dominance of C. tropicum in most of the habitats of the world also

Fig-2

A - Chrysosporium keratinophilum

B - C. queenslandicum

C - C. tropicum strain I

D - C. tropicum strain II

E - C. pannorum

F - Malbranchea flava

G - Scopulariopsis brevicaulis

H - Microascus manganii

I - Gliocladium virens

J. Circinella muscae

K - Fusarium moniliforme

L - F. solani

M - Aspergillus candidus

N - A. deflectus

O - A. flavus

P - A. ochraceus

Q - A. sydowii

R - A. versicolor

S - Penicillium aurantiogriseum

T - P. citrinum

U - P. griseofulvum

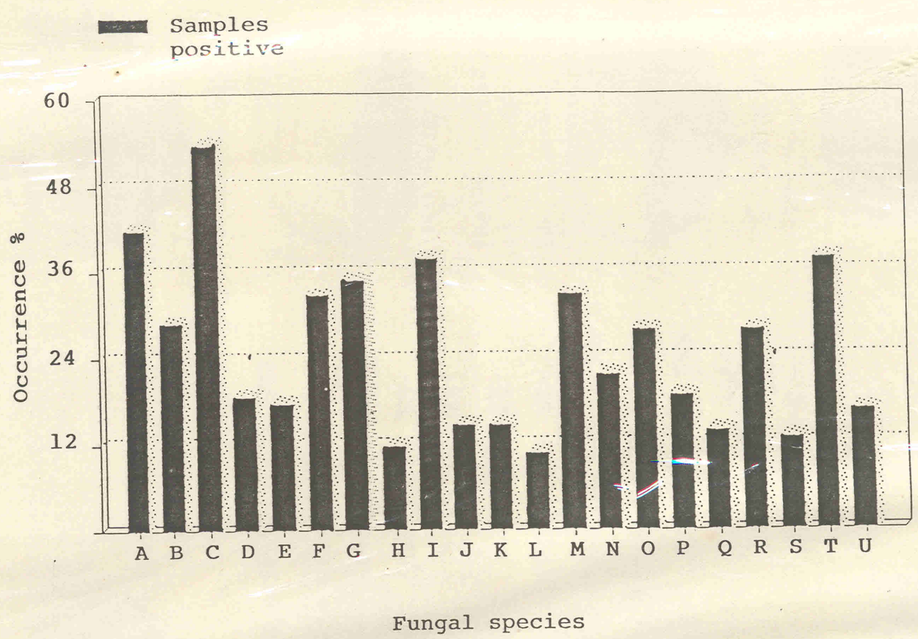


Fig. 2 : Occurrence of keratinophilic fungi in poultry farm soils.

Fig-3

Keratinophilic fungi

A - Chrysosporium sps.

B - Malbranchea sp.

C - Scopulariopsis sp.

D - Microascus sp.

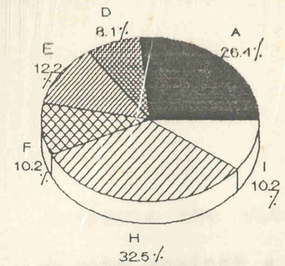
E - Gliocladium sp.

F - Circinella sp.

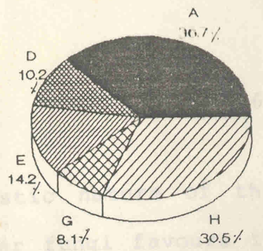
G - Fusarium sps.

H - Aspergillus sps.

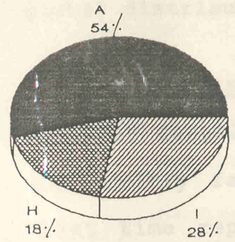
I - Penicillium. sps.



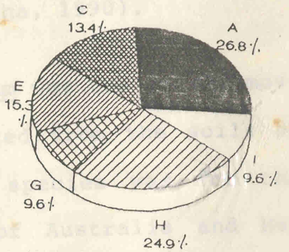
Machlian



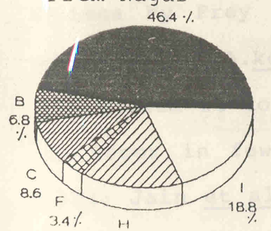
Belicharana



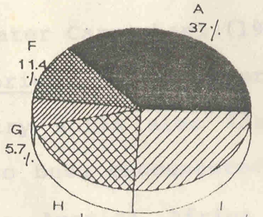
Prem Nagar



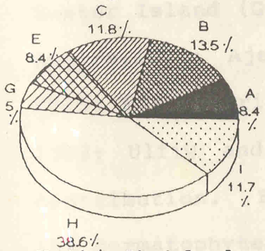
Muthi



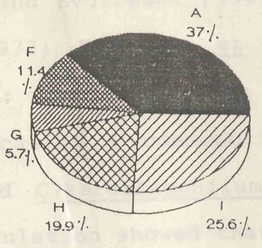
Nihalpur Simbal



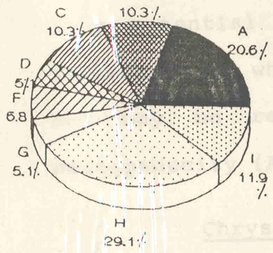
Nagbani



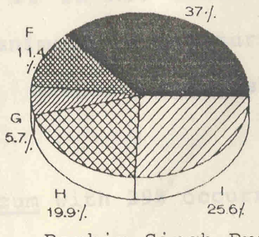
Kothi Shahdaula



Maralia



Akhnoor



Ranbir Singh Pura

Fig. 3 : Frequency of keratinophilic soil isolates.

supports the view that the antagonistic nature of this fungus against a large number of other fungi favours its global distribution (Nigam and Kushwaha, 1990).

Chrysosporium keratinophilum was the second most frequent fungal species (42%) isolated from the soils of six poultry farms (Fig. 2). This species was for the first time reported in the soils of Australia and New Guinea by Frey (1959), who classified it in the genus Aleurisma as A.keratinophilum, but later Carmichael (1962) transfered it to the genus Chrysosporium. It is reported to occur in few other Indian poultry soils also (Jain, 1983; Jain et al. 1985) and has also been encountered in various other soils from Europe, Asia, America, Africa and Easter Island (Garg, 1966; Alteras and Evolceanú ,1969 b; Rogers,1971; Ajello and Alpert, 1972; Feuerman et al. 1975; Michel-Brien and Monier, 1981; Abdel-Fattah et al. 1982; Ulfig and Korcz, 1983) indicating a cosmopolitan distribution. Frey (1959), related C.keratinophilum to the dermatophytes as guinea pig inoculation showed that it is a potential pathogen. However, it is not considered pathogenic as when inoculated into animals it may survive and may be reisolated also, but without manifesting pathogenecity (Ajello et al. 1964).

Chrysosporium queenslandicum with 29% occurrence

was recovered from only four poultry farms (Nihalpur Simbal, Muthi, Belicharana and Ranbir Singh Pura). It is a keratinophile which was first described by Apinis and Rees (1976) along with its teleomorph as Apinisia queenslandica on the feathers of domestic fowl from Southern Queensland. From India it has been reported from soil samples taken from houses, gelatin factory campus and dairy farms (Nigam and Kushwaha, 1987, 1990; Hasija et al. 1990; Kaul and Sumbali,1994).

Chrysosporium pannorum was also recovered from four poultry farms but it showed comparatively low percentage occurrence (18%). There is only a single report of its isolation from Indian soils (Kaul and Sumbali, 1994), although it has a wide distribution in the soils of other countries and has been even isolated from the skin and hair of man and animals (Carmichael, 1962; Chabasse,1988; Ali- Shtayeh and Sheikh, 1988).

Prevalence of Malbranchea flava was noted in only four poultry farms under investigation (Nihalpur Simbal, Kothi Shahdaula, Akhnoor and Maralia) and it showed an occurrence of 33%. Although, two other keratinophilic species of Malbranchea viz.M.aurantiaca and M.pulchella have been reported from few Indian soils

(Kushwaha and Agrawal, 1976b; Jain, 1983), isolation of M. flava has not been reported so far from India and hence is a new record and an addition to the Indian mycoflora. Records of keratinophilic strains of Malbranchea species from other countries are also very few (Orr and Kuehn, 1972; Ajello and Padhye, 1974). A scrutiny of available literature also revealed that keratinophilic strains of Gliocladium virens, Circinella muscae and Microascus manganii recovered in the present investigation with 38%, 15% and 12% occurrence respectively have not been reported earlier from India and are, therefore, new additions to this unique group. However, there are reports of Microascus manganii having been isolated from toe nails, tinea capitis and some lesions in human beings (De-Vries, 1983).

Scopulariopsis brevicaulis was recovered from six poultry farms with 35% occurrence. It has been reported earlier also by Hasija et al. (1990), Kaul and Sumbali (1994), while surveying the geophilic keratinophiles. This species has also been found in the droppings of birds and sea gulls of Antarctica (Dodge and Baker,1938) and is known to be associated with animal folds (Ali- Shtayeh et al.1988; Al-Musallam, 1990). There are even reports of S. brevicaulis as the causative agent

of onychomycoses in humans (Fragner and Belgan, 1974; Onsberg, 1980).

Fusarium moniliforme and F. solani were prevalent less frequently (15% & 11% respectively) in the investigated poultry farms. Distribution of these two species of Fusarium in soil is world wide and have been recorded as keratinophilic earlier also by Kushwaha and Agrawal (1976b), Shukla and Rajak (1984), Ali-Shtayeh and Sheikh (1988), Hasija et al. (1990) and Kaul and Sumbali (1994). F. solani is known to cause infections in hospitalized patients (Zimmermann, 1962; English, 1965); and has also been reported as an agent of mycotic keratitis (Emmons, 1963).

Six species of Aspergillus recovered during the survey were A. candidus (33%), A. flavus (28%), A. versicolor (28%), A. deflectus (22%), A. ochraceus (19%) and A. sydowii (14%). Among these A. candidus was found to be predominant (Table 3). The only report of keratinophilic nature of A. candidus and A. sydowii are from the dairy farm soils of Jammu (Kaul and Sumbali, 1994). A. flavus a potential pathogen has also been reported keratinophilic elsewhere (Kushwaha and Agrawal,

1976b; Shukla and Rajak, 1984; Ali- Shtayeh *et al*. 1988; Hasija *et al*. 1990; Kaul and Sumbali, 1994) and is known to be medically important (Rippon, 1982). *A. deflectus* and *A. versicolor* are not reported earlier as keratinophilic and are, thereby, new additions to the list of keratinophilic fungi.

Keratinophilic strains of *Penicillium citrinum*, *P. griseofulvum* and *P. aurantiogriseum* were also isolated during the present study. *P. citrinum* recovered from eight poultry farms had 38% occurrence, whereas, *P. griseofulvum* and *P. aurantiogriseum* were much less prevalent with 17% and 13% occurrence respectively. They have not been reported as keratinophilic from Indian soils. However, occurrence of keratinophilic *Penicillium* species have been reported elsewhere from nesting material and soils frequented by various birds (Pugh, 1965; Hubalek and Balat, 1976).

In the present investigation, none of the pathogenic dermatophytes were recorded. Failure in isolating the dermatophytes from poultry soils may be because these fungi have become so specialized in their growth requirements that they can survive and maintain themselves only on living animal hosts (Ajello, 1956). However, a few isolates have been reported earlier from

poultry farm soils of Poona, Sagar and Jabalpur (Padhye et al. 1966; Jain 1983; Singh and Agrawal, 1983; Shukla and Rajak, 1984), which seem to be chance isolations and do not afford proofof a constant infection source.

B. Keratinophilic fungi from feathers of living poultry birds

During the period under survey, feathers were collected directly from the living poultry birds (Gallus domesticus) and were assessed for the presence of keratinophilic fungi. A perusal of data (Table 5) shows that all the feather samples investigated were found to be positive (100%) for the presence of keratinophilic fungi and maximum number of species (9) were recovered from the feathers of poultry birds inhabiting poultry farms at Nihalpur Simbal and Kothi Shahdaula.

As depicted in table 5, positive samples yielded fourteen species of keratinophilic fungi classified in ten genera of perfect and imperfect forms. Among these, Aspergillus flavus occurred most frequently (42%) whereas, the only other Aspergillus species recovered was A. nidulans and it showed 17% occurrence. These findings are in accordance with those of Pugh (1966b), who also observed species of Aspergillus to be fairly abundant on Indian birds. These two species are also reported as potential

Table 5 : Prevalence of Keratinophilic fungi on Poultry Bird feathers

No. of petridishes sampled with feathers 100 (10 for each poultry farm)

No. of petridishes positive 100

Occurrence (%) 100.0

Fungi recorded	Number of Petridishes positive										Occurrence
	1	2	3	4	5	6	7	8	9	10	
Chrysosporium keratinophilum	6	6	2	6	3	-	-	6	5	5	22
C. queenslandicum	-	-	-	7	-	-	5	-	-	-	12
C. tropicum strain I	-	7	5	-	3	10	-	6	5	5	41
C. indicum	-	-	-	8	-	9	-	-	6	-	23
Malbranchea chrysosporoidea	-	-	6	5	-	6	5	-	-	5	27
Chaetomium globosum	6	5	-	4	5	-	-	6	-	6	23
Curvularia lunata	4	-	5	-	3	5	5	-	-	3	25
Sepedonium maheshwarianum	-	-	7	6	-	-	-	4	6	-	23
Microascus manganii	-	-	8	-	4	-	-	-	-	-	12
Fusarium moniliforme	5	-	-	2	-	6	4	5	-	-	22
Aspergillus flavus	7	8	6	4	4	-	5	-	4	4	42
A. nidulans	2	7	3	5	-	-	-	-	-	-	17
Scopulariopsis brevicaulis	3	6	5	-	-	6	4	5	-	-	22
Penicillium chrysogenum	-	-	-	-	-	4	3	-	-	4	11

Poultry farms:

1. Machlian
2. Prem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Nagbani
9. Maralia
10. Ranbir Singh Pura.

pathogens for humans and animals either alone or in association with other potential pathogens (De-Vries, 1983).

Next in order of occurrence was Chrysosporium tropicum strain I (41%), being isolated from feathers of birds inhabiting seven poultry farms. Other species of Chrysosporium isolated from feathers included C. indicum (23%), C. keratinophilum (22%) and C. queenslandicum (12%). Isolation of these four species of Chrysosporium from the feathers indicate it as a rich source of keratinous material on which they grow saprophytically. Similar observations have been recorded from dropped off feathers of chickens (Padhye et al. 1966), from feathers of domestic fowls and wild birds (Rees, 1967a,b; Apinis and Rees, 1976), from birds and their nests (Pugh and Evans, 1970a; Hubalek, 1974) and from soil rich with feathers of various birds (Otcenasek et al. 1978; Sur and Ghosh, 1980a,b).

Malbranchea chrysosporoidea was recovered from the feathers as the third most frequently occurring keratinophilic species (27%). It has not been reported from India and, thereby, constitutes an addition to the existing fungi of India. However, two other species of Malbranchea viz. M. fulva and M. pulchella are reported to

be associated with the feathers of Passer domesticus (Hubalek, 1974; Sarangi and Ghosh, 1991).

Occurrence in decreasing order of other keratinophilic fungi recovered from feathers of living poultry birds was Curvularia lunata (25%), Chaetomium globosum (23%), Sepedonium maheshwarianum (23%), Fusarium moniliforme (22%), Scopulariopsis brevicaulis (22%), Microascus manganii (12%) and Penicillium chrysogenum (11%). Among these, Chaetomium globosum and Curvularia lunata are reported earlier also to be fairly abundant on Indian birds (Pugh, 1966b) but Singh (1993) reported low incidence of Chaetomium from Ghana bird's sanctuary. Similarly, association of Scopulariopsis brevicaulis and Penicillium species with bird's nesting material have been reported (Pugh, 1965; Hubalek and Balat, 1974; Singh, 1993) but there is no previous report of isolating S. brevicaulis, Penicillium chrysogenum, Microascus manganii, Fusarium moniliforme and Sepedonium maheshwarianum from feathers of birds.

Data on the frequency of keratinophilic feather isolates (Table 6) shows that genus Chrysosporium was recovered from the feathers of birds belonging to all the investigated poultry farms. The second most frequent genus isolated was Aspergillus with maximum frequency in

Table 6 : Percentage frequency of keratinophilic fungi isolated from feathers of living Poultry birds

Fungi recorded	Poultry Farms									
	1	2	3	4	5	6	7	8	9	10
Chrysosporium keratinophilum	20.0	15.0	4.2	12.7	13.6	-	-	22.2	17.2	-
C. queenslandicum	-	-	-	14.8	-	-	15.1	-	-	15.6
C. tropicum strain I	-	17.5	10.6	-	13.6	21.7	-	22.2	17.2	-
C. indicum	-	-	-	17.0	-	19.5	-	-	20.6	15.6
Malbranchea chrysosporoidea	-	-	12.7	10.6	-	13.0	15.1	-	-	9.3
Chaetomium globosum	20.0	12.5	-	8.5	22.7	-	-	22.2	-	-
Curvularia lunata	10.3	-	10.6	-	13.6	10.8	15.1	-	-	18.7
Sepedonium maheshwarianum	-	-	14.8	12.7	-	-	-	14.8	20.6	12.5
Microascus manganii	-	-	17.0	-	18.1	-	-	-	-	-
Fusarium moniliforme	10.6	-	-	4.2	-	13.0	12.12	18.5	-	15.6
Aspergillus flavus	23.3	20.0	12.7	8.5	18.1	-	15.1	-	13.7	12.5
A. nidulans	6.6	17.5	6.3	10.6	-	-	-	-	-	-
Scopulariopsis brevicaulis	10.0	15.0	10.6	-	-	13.0	18.1	-	10.3	-
Penicillium chrysogenum	-	-	-	-	-	8.6	9.0	-	-	-

Poultry farms :

1. Machlian
2. Praem Nagar
3. Nihalpur Simbal
4. Kothi Shahdaula
5. Akhnoor
6. Belicharana
7. Muthi
8. Nagbani
9. Maralia
10. Ranbir Singh Pura.

feathers from Prem Nagar poultry farm (Fig. 5). An interesting correlation observed between the soil and feather isolates was that Chrysosporium and Aspergillus emerged as the most frequent genera in both the habitats. It is possible that since poultry birds spend most of the time on the ground, they may readilyacquire keratinophilic fungi from the soil. Therefore, to a certain extent, the colonization of birds feathers by keratinophilic fungi is a reflection of the qualitative and quantitative representation of microfungi occurring in the soil of the biotype inhabited by birds. Similar influence of active contact with soil upon the frequency of keratinophilic mycoflora in feathers was also proved by Sarangi and Ghosh (1991) in house sparrow (Passer domesticus).

Fig-4

a - Chrysosporium keratinophilum

b - C. queenslandicum

c - C. tropicum strain I

d - C. indicum

e - Malbranchea chrysosporoidea

f - Chaetomium globosum

g - Curvularia lunata

h - Sepedonium maheshwarianum

i - Microascus manganii

j - Fusarium moniliforme

k - Aspergillus flavus

l - A. nidulans

m - Scopulariopsis brevicaulis

n - Penicillium chrysogenum

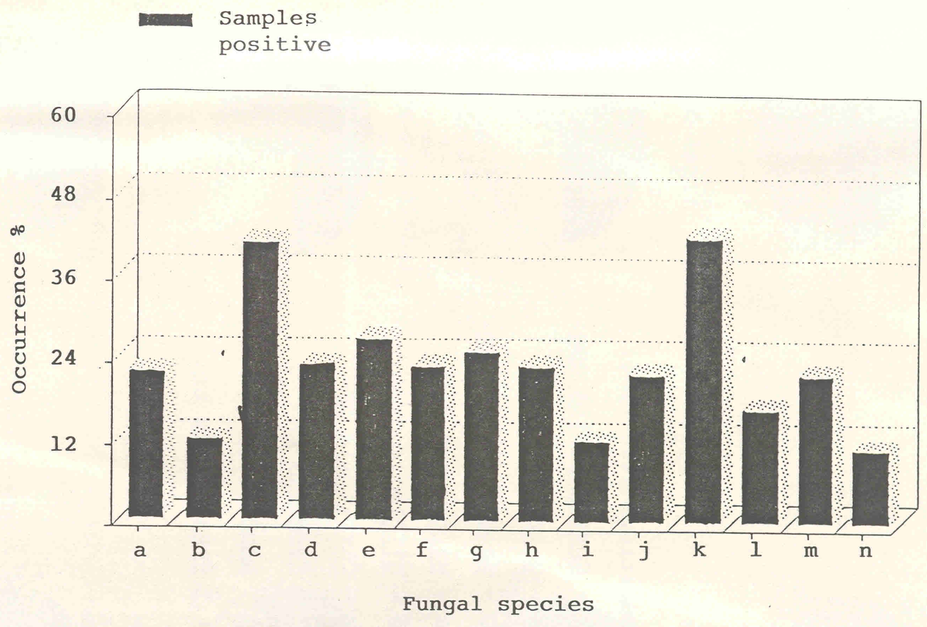


Fig. 4 : Occurrence of keratinophilic fungi on poultry bird feathers.

Fig-5

Keratinophilic fungi

A - Chrysosporium sps.

B - Malbranchea sp.

C - Scopulariopsis sp.

D - Microascus sp.

E - Sepedonium sp.

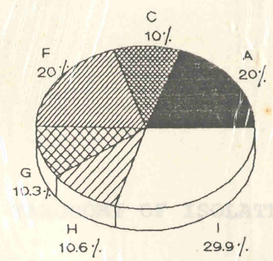
F - Chaetomium sp.

G - Curvularia sp

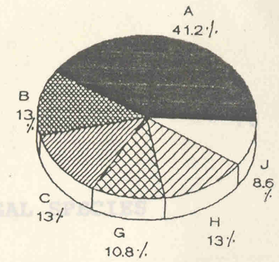
H - Fusarium sps.

I - Aspergillus sps.

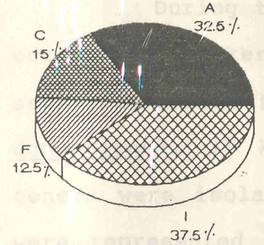
J - Penicillium sps.



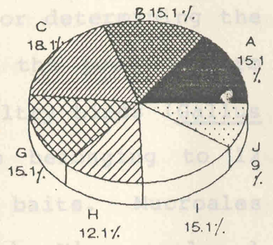
Machlian



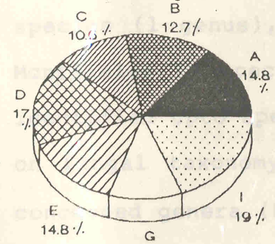
Belicharana



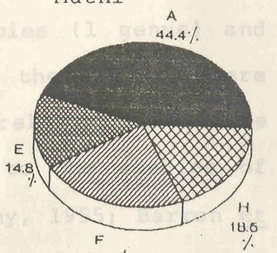
Prem Nagar



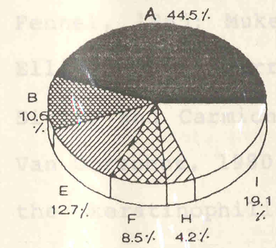
Muthi



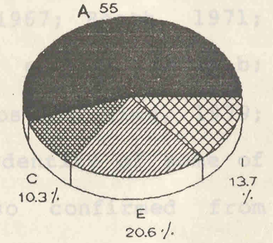
Nihalpur Simbal



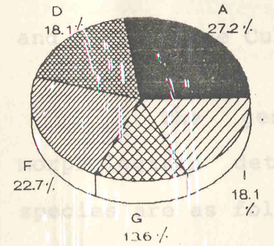
Nagbani



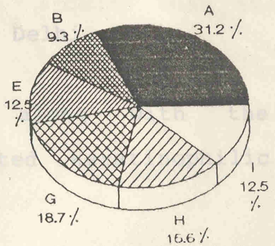
Kothi Shahdaula



Maralia



Akhnoor



Ranbir Singh Pura

Fig. 5 : Frequency of keratinophilic feather isolates.

TAXONOMY OF ISOLATED KERATINOPHILIC FUNGAL SPECIES

During the survey conducted for determining the occurrence of keratinophilic fungi in the poultry farm soils and on the feathers of living poultry birds (Gallus domesticus), 27 keratinophilic species belonging to 12 genera were isolated by using keratin baits. Mucroales were represented by 1 species (1 genus), Microascales 1 species (1 genus), Chaetomiales 1 species (1 genus) and Moniliales 24 species (9 genera). All the isolates were identified upto species level by using relevant literature on fungal taxonomy and specific keys to the species of concerned genera (Hesseltine and Dorothy, 1955; Barron et al. 1961; Barnett, 1962; Carmichael, 1962; Raper and Fennel, 1965; Mukerji, 1966; Gilman, 1967; Booth, 1971; Ellis, 1971; Barron, 1972; Ainsworth et al. 1973a,b; Sigler and Carmichael, 1976; Alexopoulos and Mims, 1979; Van Oorschot, 1980; Chabasse, 1988). Identity of some of the keratinophilic isolates was also confirmed from International Mycological Institute, Kew, England (U.K.) and Indian Type Culture Collection, New Delhi, India.

The generic descriptions along with the morphological details of the isolated keratinophilic species are as follows :

I. Chrysosporium Corda

The name Chrysosporium was introduced by Corda (in Sturm, 1833) for the single species, C. corii Corda. Later Carmichael (1962) reduced the genera Geomyces, Emmonsia, Myceliophthora and Blastomyces to synonymy with Chrysosporium making the genus morphologically heterogenous. Since then, several new species were described and have been incorporated in a new taxonomic revision of Chrysosporium and allied genera by Van Oorschot (1980).

Colonies of Chrysosporium are usually spreading, white to cream - coloured, pale brown or yellow, felty or powdery. Hyphae mostly hyaline, smooth-walled, branched. Fertile hyphae bearing terminal and lateral conidia (aleuriospores) either sessile or on short protrusions or side branches; conidia subhyaline or pale yellow, thin or thick walled, subglobose, clavate, pyriform or obovoid, 1 celled, rarely 2-celled, truncate. Intercalary conidia (arthrospores) sometimes present, solitary, occasionally catenate, sub-hyaline or pale yellow, broader than the supporting hyphae, normally 1 celled, truncate at both ends. Chlamydospores occasionally present.

In the present investigation, five species of

Chrysosporium were isolated and later identified by following the descriptions and illustrations proposed by Carmichael (1962), Van Oorschot (1980) and Chabasse (1988). The morphological details of these species are as follows :

Ia. Chrysosporium keratinophilum D. Frey ex Carmichael

This species was first reported from the soils of Australia and New Guinea by Frey (1959) as Aleurisma keratinophilum.

Colonies on Sabouraud's dextrose agar (SDA) white to cream, powdery, dense, flat, dry (Plate 2). Mycelium hyaline, thin-walled, smooth, septate, branched; aerial hyphae fertile 1-5.5 µm wide; racquet hyphae present. Aleuriospores terminal and lateral, sessile or on short protrusions, solitary, smooth, thick-walled, obovoid to clavate, 1-celled measuring 6.4-12.8 x 4-5.6µm, with wide basal scars. Arthrospores less abundant, solitary, subhyaline, smooth, thick walled, cylindrical to barrel shaped measuring 6.4-9.6 x 3.2-4 µm, of the same width as or slightly wider than the supporting hyphae (Fig.6).

The identity of this keratinophilic fungus was

PLATE 2 : Chrysosporium keratinophilum D. Frey ex Carmichael.

a. Colonies on SDA after 12 days of incubation

b. Terminal and lateral aleuriospores borne on the side branches x 1000

c. Racquet hyphae x 1000

d. Smooth walled aleuriospores x 1000

e. Arthrospores x 1000.

PLATE 2



FIGURE 6

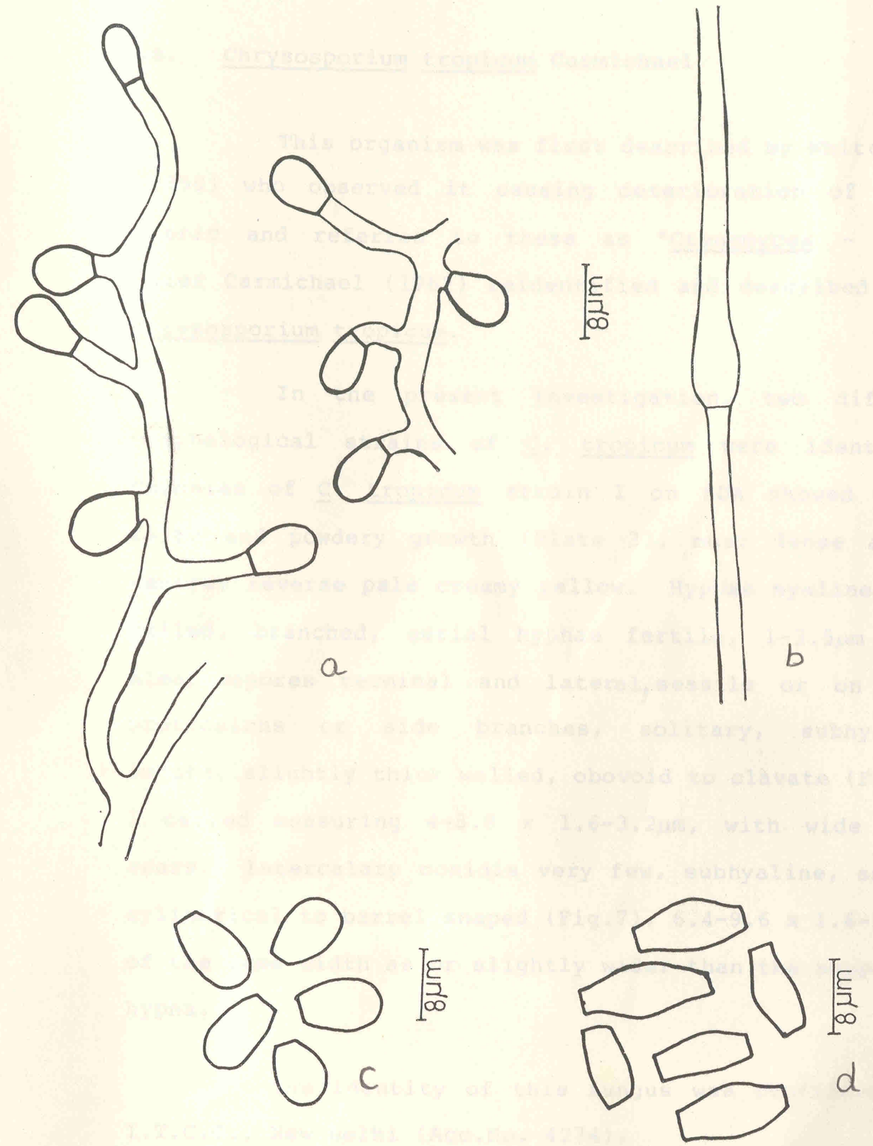


FIGURE 6 : Camera lucida drawings of **Chrysosporium keratinophilum** Frey ex. Carmichael.

a. Hyphae bearing aleuriospores.

b. Racquet hypha

c. Thick walled aleuriospores.

d. Barrel shaped intercalary spores.

confirmed from I.T.C.C. New Delhi (Ref. No. 382).

Ib. *Chrysosporium tropicum* Carmichael

This organism was first described by White *et al*. (1950) who observed it causing deterioration of woolen fabric and referred to these as "*Ctenomyces* - like." Later Carmichael (1962) reidentified and described it as *Chrysosporium tropicum*.

In the present investigation, two different morphological strains of *C. tropicum* were identified. Colonies of *C. tropicum* strain I on SDA showed white, felty and powdery growth (Plate 3), most dense at the centre; reverse pale creamy yellow. Hyphae hyaline, thin walled, branched, aerial hyphae fertile, 1-3.5µm wide. Aleuriospores terminal and lateral, sessile or on short protrusions or side branches, solitary, subhyaline, smooth, slightly thick walled, obovoid to clavate (Fig.7), 1 celled measuring 4-8.8 x 1.6-3.2µm, with wide basal scars. Intercalary conidia very few, subhyaline, smooth, cylindrical to barrel shaped (Fig.7), 6.4-9.6 x 1.6-3.2µm, of the same width as or slightly wider than the supporting hypha.

The identity of this fungus was confirmed from I.T.C.C., New Delhi (Acc.No. 4274).

PLATE 3 : Chrysosporium tropicum Carmichael Strain I.

a. Colonies on SDA after 12 days of incubation

b. Terminal and Lateral aleuriospores borne on the side branches x 1000

c. Racquet hyphae x 1000

d. Smooth aleuriospores x 1000

e. Arthrospore x 1000.

PLATE 3

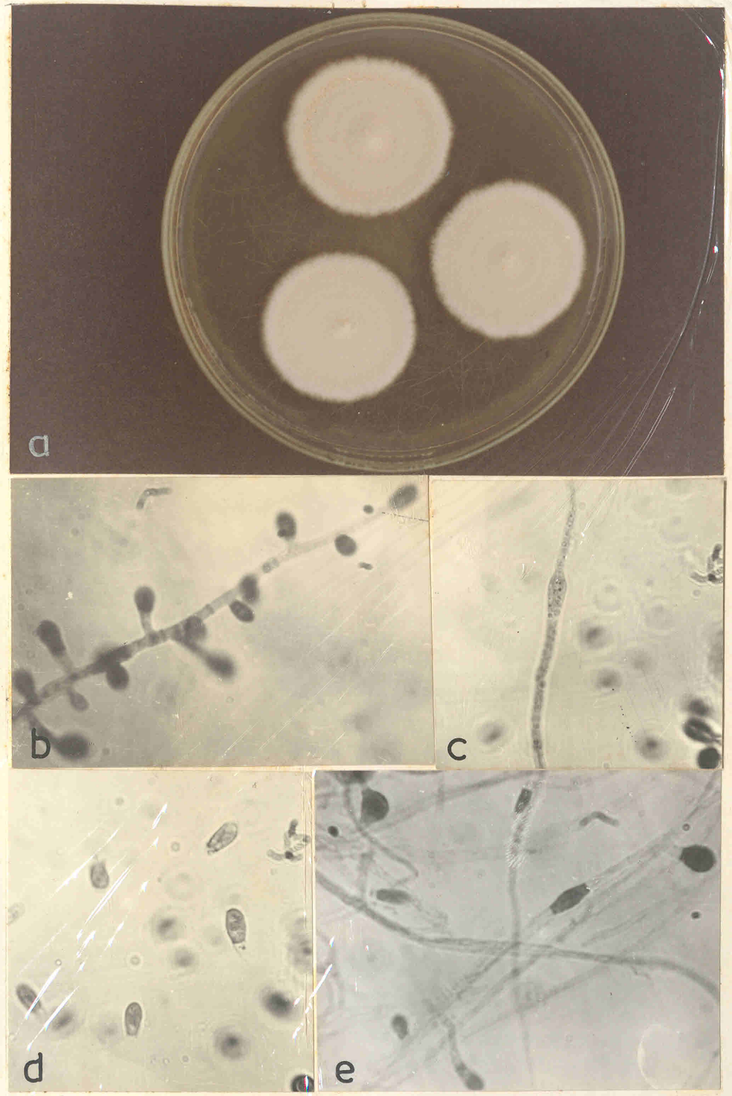


FIGURE 7 : Camera lucida drawings of **Chrysosporium tropicum** Carmichael strain I

a. Hyphae bearing aleuriospores

b. Racquet hypha

c. Mature aleuriospores

d. Arthrospores.

FIGURE -7

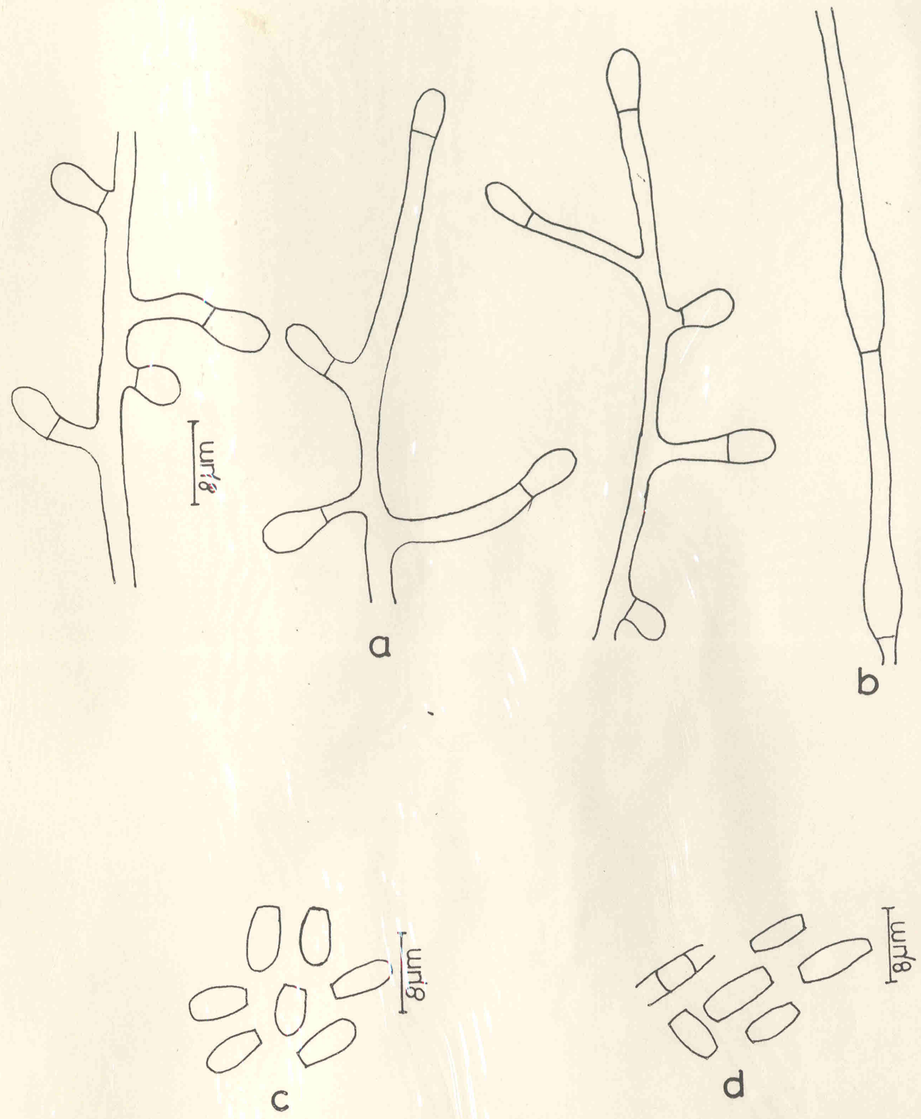


PLATE 4 : Chrysosporium tropicum Carmichael Strain II

a. Colonies on SDA after 12 days of incubation

b. Terminal and lateral aleuriospores borne on the side branches x 1000

c. Smooth walled aleuriospores x 1000.

PLATE 4



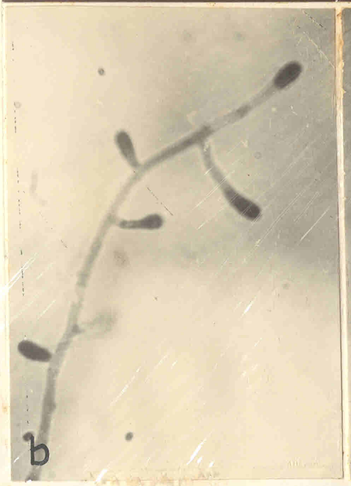




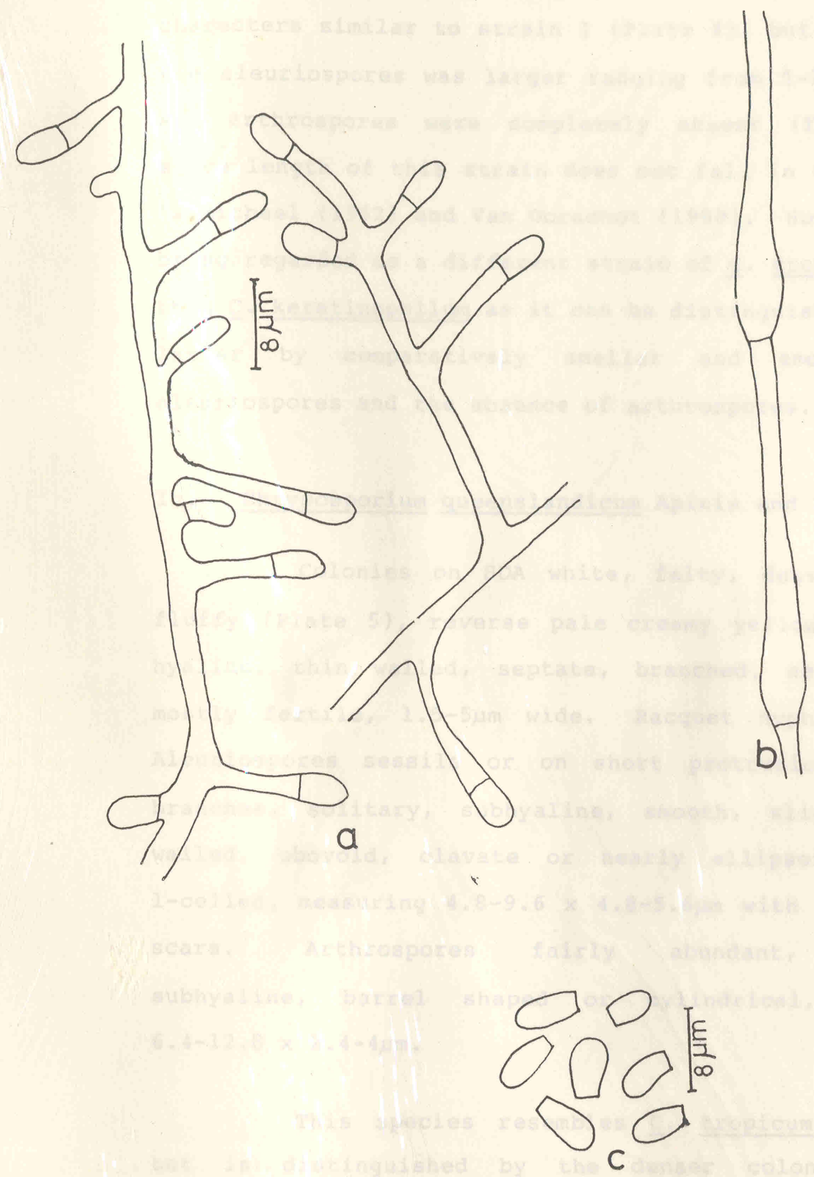
FIGURE 8 : Camera lucida drawings of <u>Chrysosporium tropicum</u> Carmichael Strain II

a. Hyphae bearing aleuriospores

b. Racquet hypha

c. Mature aleuriospores.

FIGURE - 8



Chrysosporium tropicum strain II showed cultural characters similar to strain I (Plate 4), but the size of the aleuriospores was larger ranging from 5-11 x 1.6-3µm and arthrospores were completely absent (Fig.8). The spore length of this strain does not fall in the range of Carmichael (1962) and Van Oorschot (1980). However, it is being regarded as a different strain of C. tropicum rather than C. keratinophilum as it can be distinguished from the latter by comparatively smaller and smooth walled aleuriospores and the absence of arthrospores.

Ic. Chrysosporium queenslandicum Apinis and Rees.

Colonies on SDA white, felty, dense, slightly fluffy (Plate 5), reverse pale creamy yellow. Mycelium hyaline, thin walled, septate, branched, aerial hyphae mostly fertile, 1.5-5µm wide. Racquet hyphae present. Aleuriospores sessile or on short protrusions or side branches, solitary, subhyaline, smooth, slightly thick walled, obovoid, clavate or nearly ellipsoid (Fig.9), 1-celled, measuring 4.8-9.6 x 4.8-5.6µm with broad basal scars. Arthrospores fairly abundant, solitary, subhyaline, barrel shaped or cylindrical, measuring 6.4-12.8 x 2.4-4µm.

This species resembles C. tropicum Carmichael but is distinguished by the denser colony and the

PLATE 5 : Chrysosporium queenslandicum Apinis and Rees

a. Colonies on SDA after 12 days of incubation

b. Terminal and lateral aleuriospores borne on the side branches x 1000

c. Racquet hyphae x 1000

d. Aleuriospores x 1000.

PLATE 5

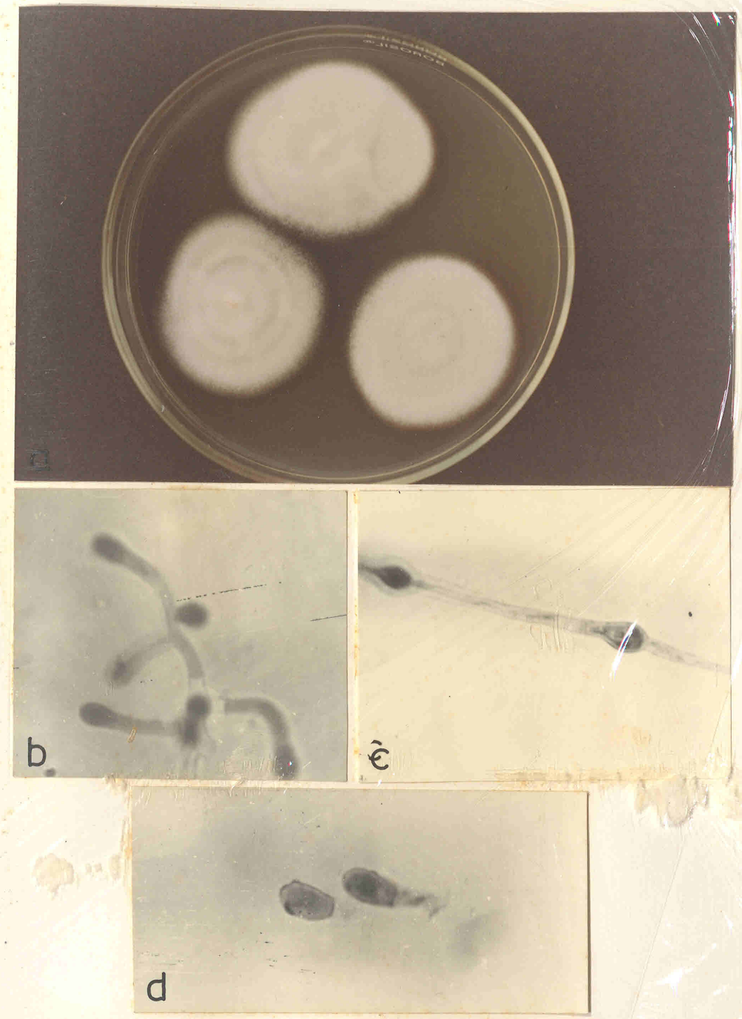


FIGURE 9 : Camera lucida drawings of <u>Chrysosporium queenslandicum</u> Apinis & Rees

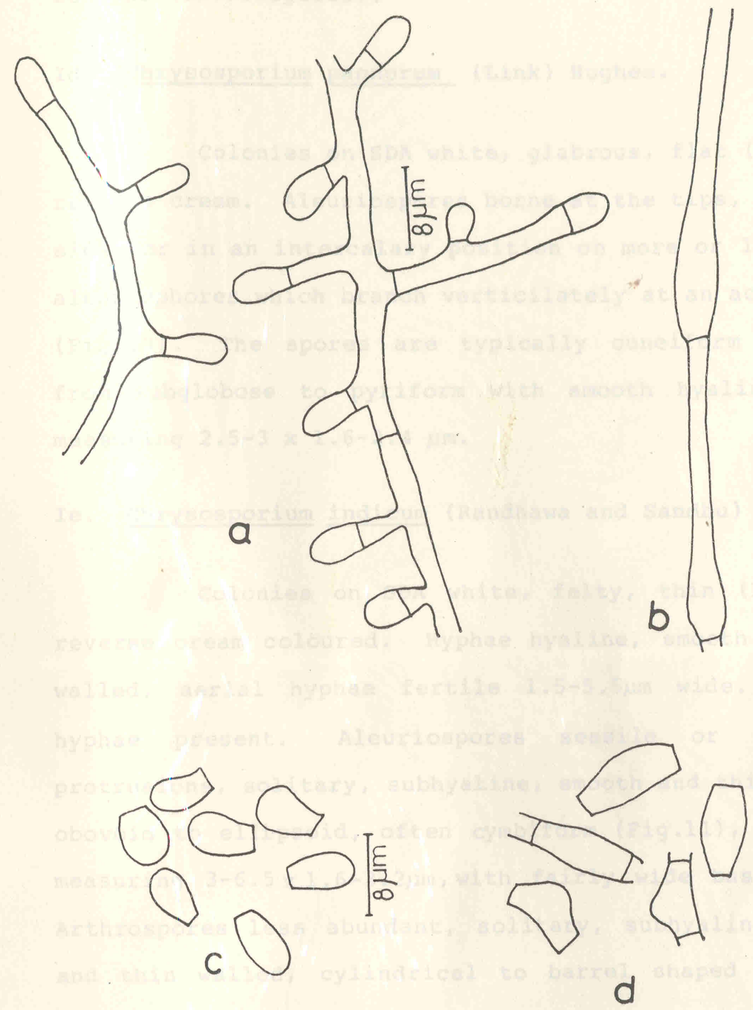
a. Hyphae bearing aleuriospores

b. Racquet hypha

c. Thick walled aleuriospores

d. Barrel shaped arthrospores.

FIGURE-9



abundance of cylindrical to barrel shaped intercalary conidia (arthrospores).

Id. Chrysosporium pannorum (Link) Hughes.

Colonies on SDA white, glabrous, flat (Plate 6), reverse cream. Aleuriospores borne at the tips, along the sides or in an intercalary position on more or less erect aleuriophores which branch verticilately at an acute angle (Fig.10). The spores are typically cuneiform but vary from subglobose to pyriform with smooth hyaline walls, measuring 2.5-3 x 1.6-2.4 µm.

Ie. Chrysosporium indicum (Randhawa and Sandhu) Garg

Colonies on SDA white, felty, thin (Plate 7), reverse cream coloured. Hyphae hyaline, smooth and thin walled, aerial hyphae fertile 1.5-5.5µm wide. Racquet hyphae present. Aleuriospores sessile or on short protrusions, solitary, subhyaline, smooth and thin walled, obovoid to ellipsoid, often cymbiform (Fig.11), 1-celled, measuring 3-6.5 x 1.6-3.2µm, with fairly wide basal scars. Arthrospores less abundant, solitary, subhyaline, smooth and thin walled, cylindrical to barrel shaped measuring 5.6-9 x 2-3.2 µm.

PLATE 6 : Chrysosporium pannorum (Link) Hughes

a. Colonies on SDA after 12 days of incubation

b. Terminal and lateral aleuriospores x 1000

c. Aleuriospores x 1000.

PLATE 6



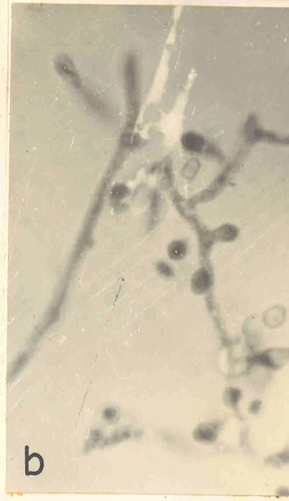




FIGURE 10 : Camera lucida drawings of Chrysosporium pannorum (Link) Hughes

a. Hyphae bearing aleuriospores

b. Racquet hyphae

c. Aleuriospores.

FIGURE-10

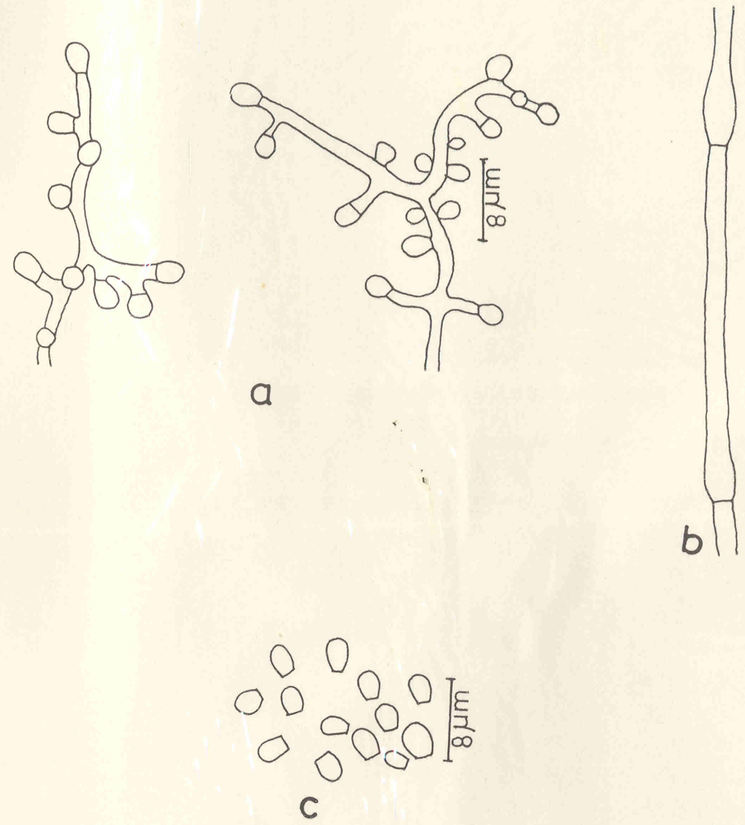


PLATE 7 : Chrysosporium indicum (Randhawa and Sandhu) Garg

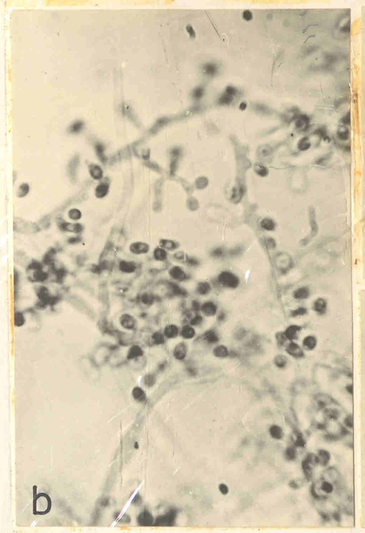
a. Colonies on SDA after 12 days of incubation

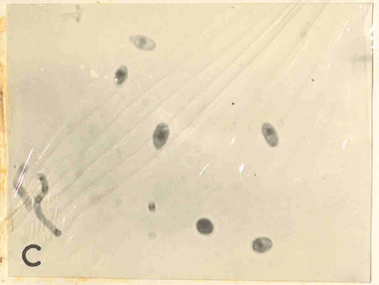
b. Terminal and lateral aleuriospores x 1000

c,d Aleuriospores x 1000.

PLATE 7







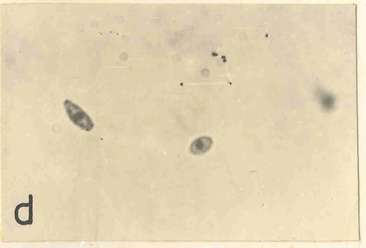


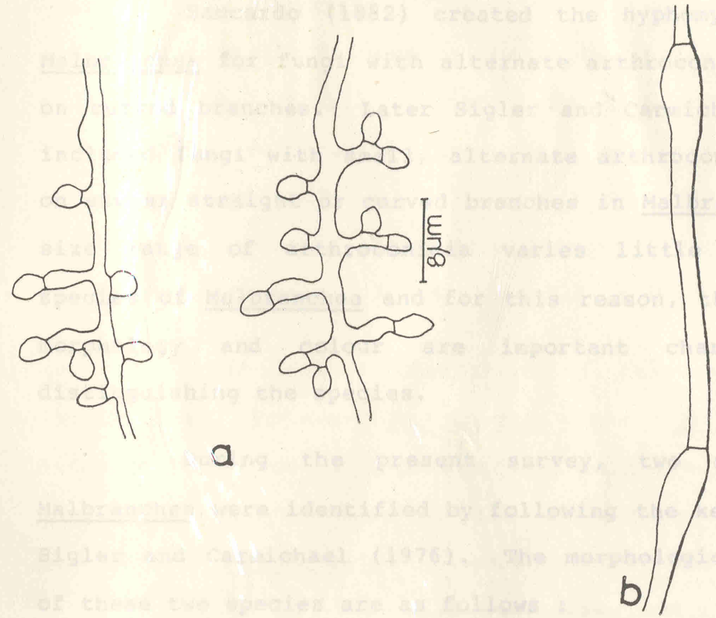
FIGURE 11 : Camera lucida drawings of Chrysosporium indicum (Randhawa & Sandhu) Garg

a. Hyphae bearing aleuriospores

b. Racquet hypha

c. Mature aleuriospores.

FIGURE-11



II. *Malbranchea* Saccardo

Saccardo (1882) created the hyphomycete genus *Malbranchea* for fungi with alternate arthroconidia formed on curved branches. Later Sigler and Carmichael (1976) included fungi with small, alternate arthroconidia borne on either straight or curved branches in *Malbranchea*. The size range of arthroconidia varies little among the species of *Malbranchea* and for this reason, the colonial morphology and colour are important characters in distinguishing the species.

During the present survey, two species of *Malbranchea* were identified by following the key given by Sigler and Carmichael (1976). The morphological details of these two species are as follows :

IIa. *Malbranchea chrysosporoidea* Sigler and Carmichael

Colonies on SDA dense, powdery, with floccose central umbo, zonate, charactistically bright tangerine orange, centre and periphery often white to pale yellow, reverse orange (Plate 8). Fertile hyphae bearing arthroconidia are of two types. Straight, slightly broader, branched primary hyphae, 2-4 µm wide, develop intercalary arthroconidia and later form multiple straight

or curved deflected lateral branches (Fig.12). The fertile branches bear intercalary arthroconidia formed terminally or laterally. The width of the arthroconidia and aleurioconidia forming on the hypha is identical with that of the hypha. Intercalary arthroconidia are cylindrical, truncate, hyaline, later pale orange, orange in mass, measuring 3.2-11.2 x 1.6-2.4 µm. Aleurioconidia are smooth, hyaline, later pale orange, orange in mass, cuneiform, measuring 3.2-6.4 x 2.4-3.2 µm (Fig.12).

Morphological characters of this species are intermediate between Malbranchea and Chrysosporium. Although formation of aleurioconidia is characteristic of Chrysosporium, yet some of the characters favour its placement in Malbranchea. These characters are :

1. The width of the aleurioconidia and intercalary arthroconidia does not exceed that of the supporting hypha.
2. Intercalary conidia predominate in the primary hyphae which is consistent with other species of Malbranchea.
3. The diameter of the fertile hyphae fits well within the rather narrow range for Malbranchea.

Another interesting character of M. chrysosporoidea is that its orange colonies resemble those of Malbranchea state of Auxarthron conjugatum and Malbranchea aurantiaca.

PLATE 8 : Malbranchea chrysosporoidea Sigler and Carmichael

a,b. Colonies on SDA after 12 days of incubation

a. Colony obverse b. Colony reverse

c. Curved deflected lateral branches bearing aleurioconidia x 1000

d. Fertile branch bearing arthrocondia and aleuriocondia x 1000

e. Aleurioconidia x 1000

PLATE 8

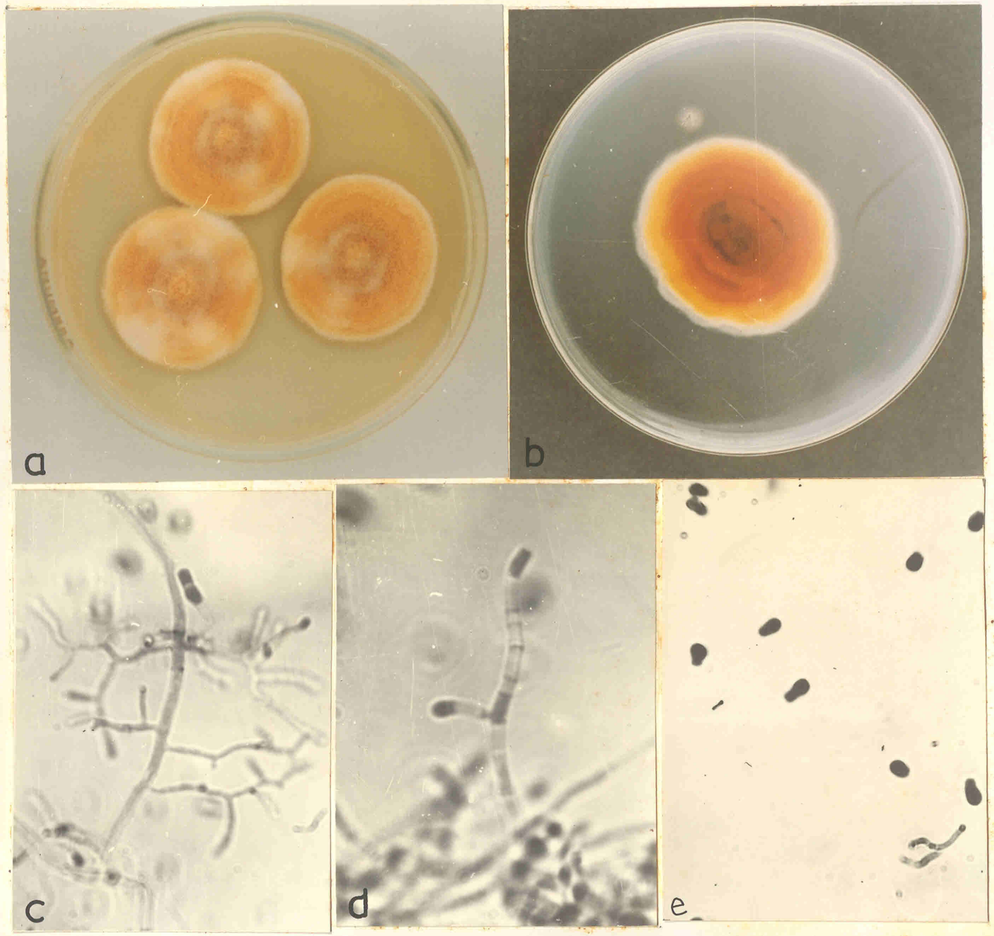


FIGURE 12 : Camera lucida drawings of **Malbranchea chrysosporoidea** Sigler & Carmichael

a. Hyphae bearing aleurioconidia and arthroconidia

b. Hyphae bearing arthroconidia

c. Racquet hypha

d. Aleurioconidia

e. Arthroconidia

FIGURE-12

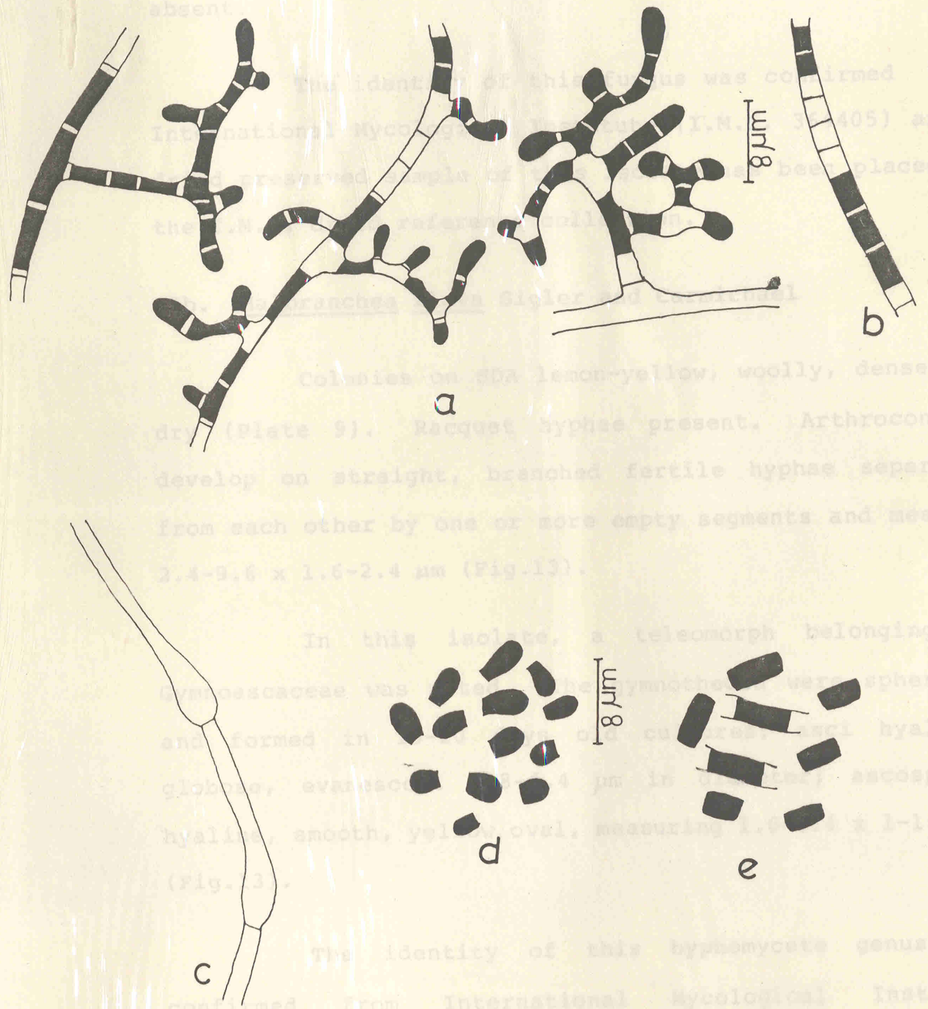


PLATE 9 : Malbranchea flava Sigler and Carmichael

a,b. Colonies on SDA after 12 days of incubation
a. Colonies obverse b. Colony reverse

c. Hyphae bearing arthrospores x 1000

d. Racquet hyphae x 1000

e. Coiled ascogonia x 1000

f. Asci bearing ascospores x 1000.

PLATE 9

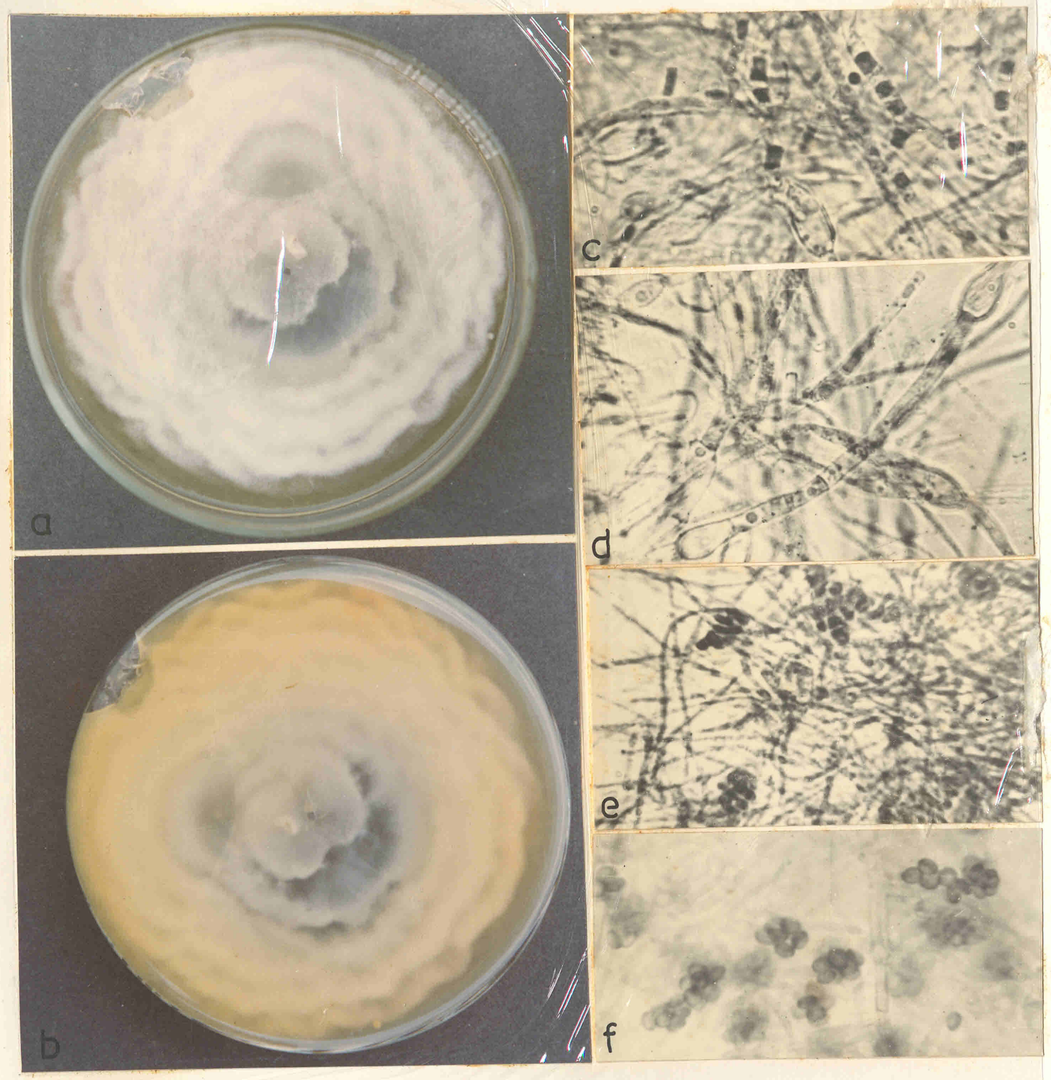


FIGURE 13 : Camera lucida drawings of Malbranchea flava Sigler & Carmichael.

a. Hyphae bearing arthroconidia

b. Arthroconidia

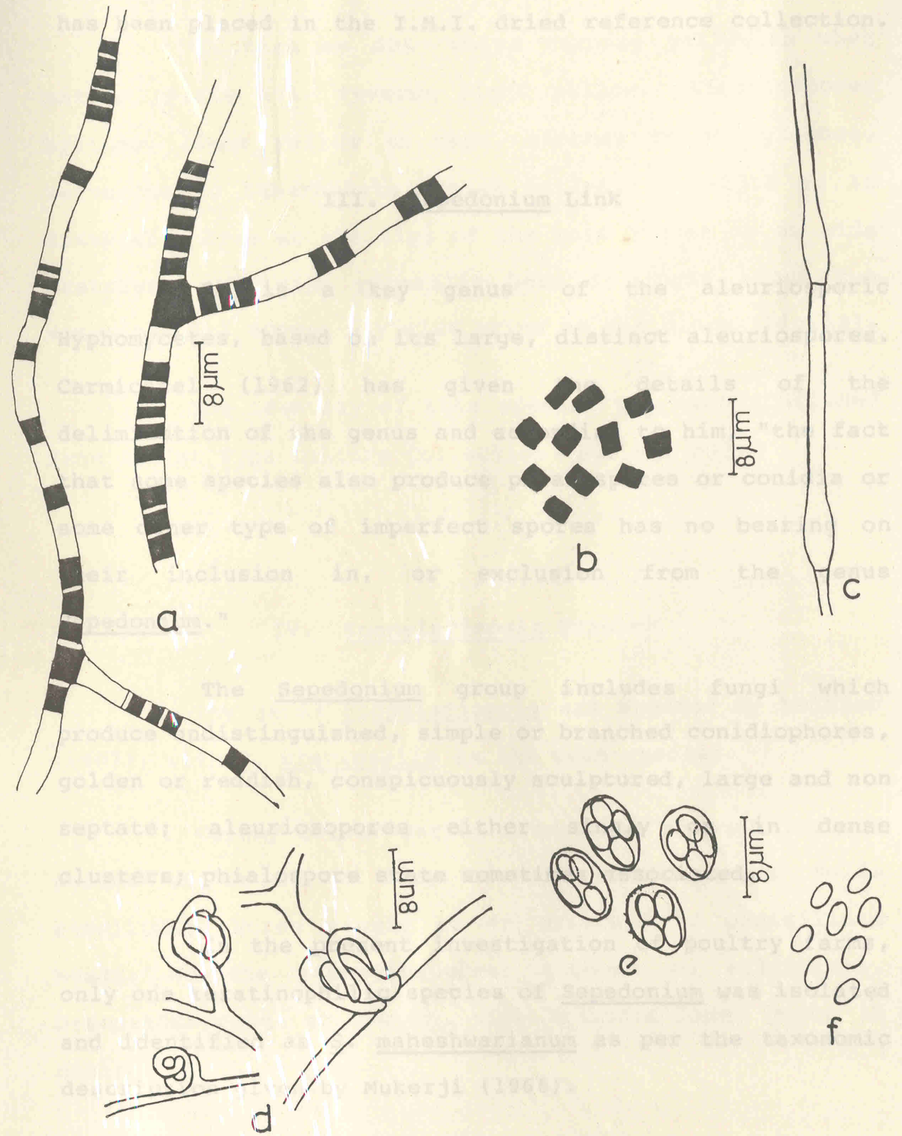
c. Racquet hypha

d. Coiled ascogonia

e. Asci bearing ascospores

f. Ascospores.

FIGURE-13



(I.M.I. 364404). A dried preserved sample of this isolate has been placed in the I.M.I. dried reference collection.

III. Sepedonium Link

It is a key genus of the aleuriosporic Hyphomycetes, based on its large, distinct aleuriospores. Carmichael (1962) has given the details of the delimitation of the genus and according to him, "the fact that some species also produce phialospores or conidia or some other type of imperfect spores has no bearing on their inclusion in, or exclusion from the genus Sepedonium."

The Sepedonium group includes fungi which produce undistinguished, simple or branched conidiophores, golden or reddish, conspicuously sculptured, large and non septate; aleuriosopores either singly or in dense clusters; phialospore state sometimes associated.

In the present investigation of poultry farms, only one keratinophilic species of Sepedonium was isolated and identified as S. maheshwarianum as per the taxonomic description given by Mukerji (1966).

IIIa. Sepedonium maheshwarianum Mukerji

Colonies on SDA white turning yellowish when mature (Plate 10), reverse light yellow. Aleuriospores hyaline, light yellow in mass, globose to sub-globose, verrucose to tuberculate, thick walled, 11.2-20.8 µm in diameter, borne at the tips of the main hyphae or on side branches. The tips generally bear a single spore but sometimes 2-3 spores may also arise at one place (Fig.14).

The identity of this species was also confirmed from Indian Type Culture Collection Ref.No. 833.

IV. Scopulariopsis Bainier

The genus Scopulariopsis was erected by Bainier (1907), with S. brevicaulis as the type species.

Colony colour varies from white through various shades of brown to deep brown. Conidiogenous cells annellides, borne singly or in branched or penicillate heads; conidia (annellospores) globose to elliptical, 0-septate, rough or smooth, with truncate base, borne in chains.

PLATE 10 : Sepedonium maheshwarianum Mukerji

a. Colony on SDA after 12 days of incubation

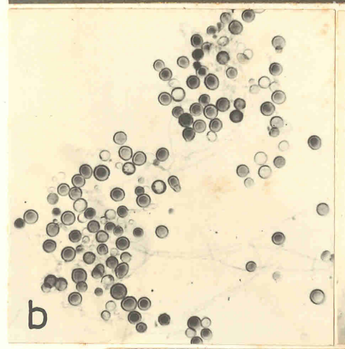
b. Globose, thick-walled aleuriospores x 1000

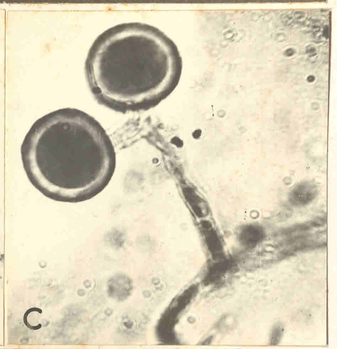
c. Aleuriospores borne on side branch x 1000

d. Aleuriospores with thick double wall x 1000.

PLATE 10







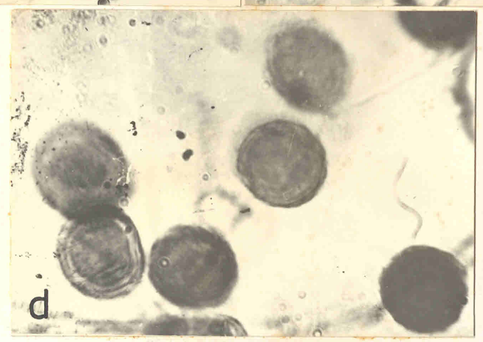


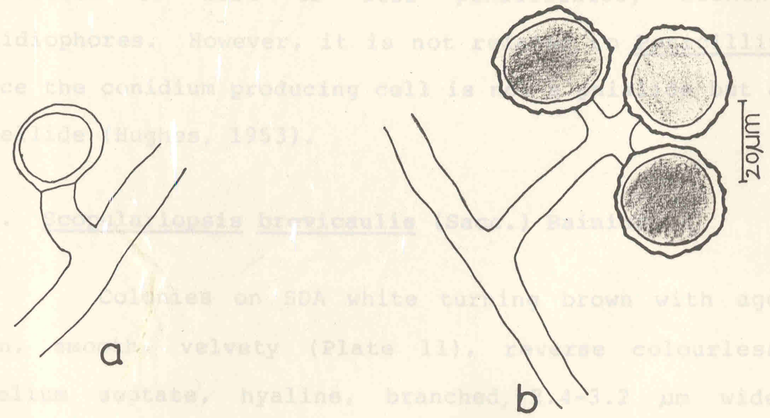
FIGURE 14 : Camera lucida drawings of Sepedonium maheshwarianum Mukerji

a. Hypha bearing young aleuriospore

b. Hypha bearing mature aleuriospores

c. Mature aleuriospores.

FIGURE-14



This genus resembles Penicillium in the production of more or less penicillately branched conidiophores. However, it is not related to Penicillium since the conidium producing cell is not a phialide but an annellide (Hughes, 1953).

IVa. Scopulariopsis brevicaulis (Sacc.) Bainier

Colonies on SDA white turning brown with age, thin, smooth, velvety (Plate 11), reverse colourless. Mycelium septate, hyaline, branched, 2.4-3.2 µm wide. Conidiophores short, sporogenous cells (annellophores) variable, short, swollen, flask shaped or cylindrical, produced singly or in groups or in complex penicillate arrangements. Spores globose to sub-globose, somewhat lemon shaped, produced in long chains, frequently truncate at the attachment point, smooth turning coarsely rough at maturity, one celled, hyaline, measuring 4.8-8.8 µm in long axis (Fig.15).

V. Microascus Zukal

It is the perfect state for several species of Scopulariopsis Bainier. The perithecia are black, almost

PLATE 11 : Scopulariopsis brevicaulis (Sacc.) Bainier

a. Colonies on SDA after 10 days of incubation

b,c. Annellophores bearing annellospores x 1000

d. Young, smooth walled annellospores in chains x 1000

e. Mature, rough walled annellospores in chains x 1000.

PLATE 11



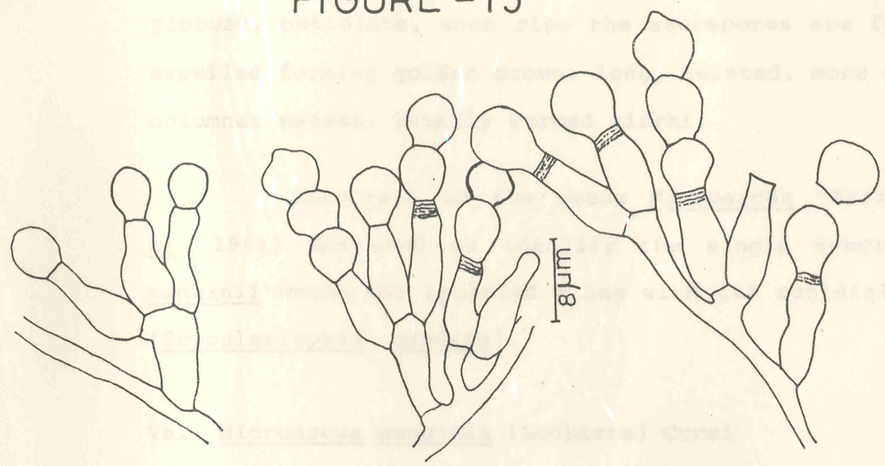
FIGURE 15 : Camera lucida drawings of Scopulariopsis brevicaulis (Sacc.) Bainier

a. Annellophores bearing annellospores

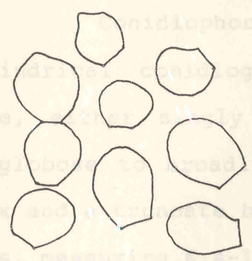
b. Young annellospores

c. Mature annellospores.

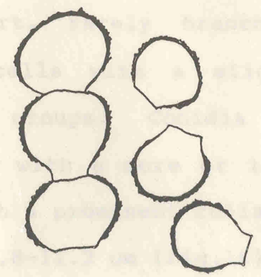
FIGURE -15



a



b



c

globose, ostiolate, when ripe the ascospores are forcibly expelled forming golden brown, long, twisted, more or less columnar masses, usually termed cirrhi.

Monograph on the genus Microascus (Barron, et al. 1961) was used to identify the single species, M. manganii which was isolated along with its conidial state (Scopulariopsis candida).

Va. Microascus manganii (Loubiere) Curzi

Colonies white (Plate 12); perithecia black, globose, ostiolate, measuring 135-180µm in diameter; asci globose; ascospores relatively large, heart-shaped, measuring 4.8-6.4 x 3.2 µm (Fig.16).

Conidiophores short, rarely branched bearing cylindrical conidiogenous cells with a slight swollen base, either singly or in groups. Conidia in chains, subglobose to broadly ovate with a more or less rounded apex and a truncate base with a prominent collar, white in mass, measuring 6.4-11.2 x 4.8-11.2 µm (Fig.16).

A living culture of this fungal isolate has been placed in the I.M.I. culture collection under reference number I.M.I. 364406.

PLATE 12 : Microascus manganii (Loubiere) Curzi

a. Colony on SDA after 12 days of incubation

b. Annellophore bearing annellospore x 1000

c,d. Annellospores x 1000

e. Perithecia x 100

f. Ruptured perithecium x 100

g,h. Ascospores x 1000

PLATE 12

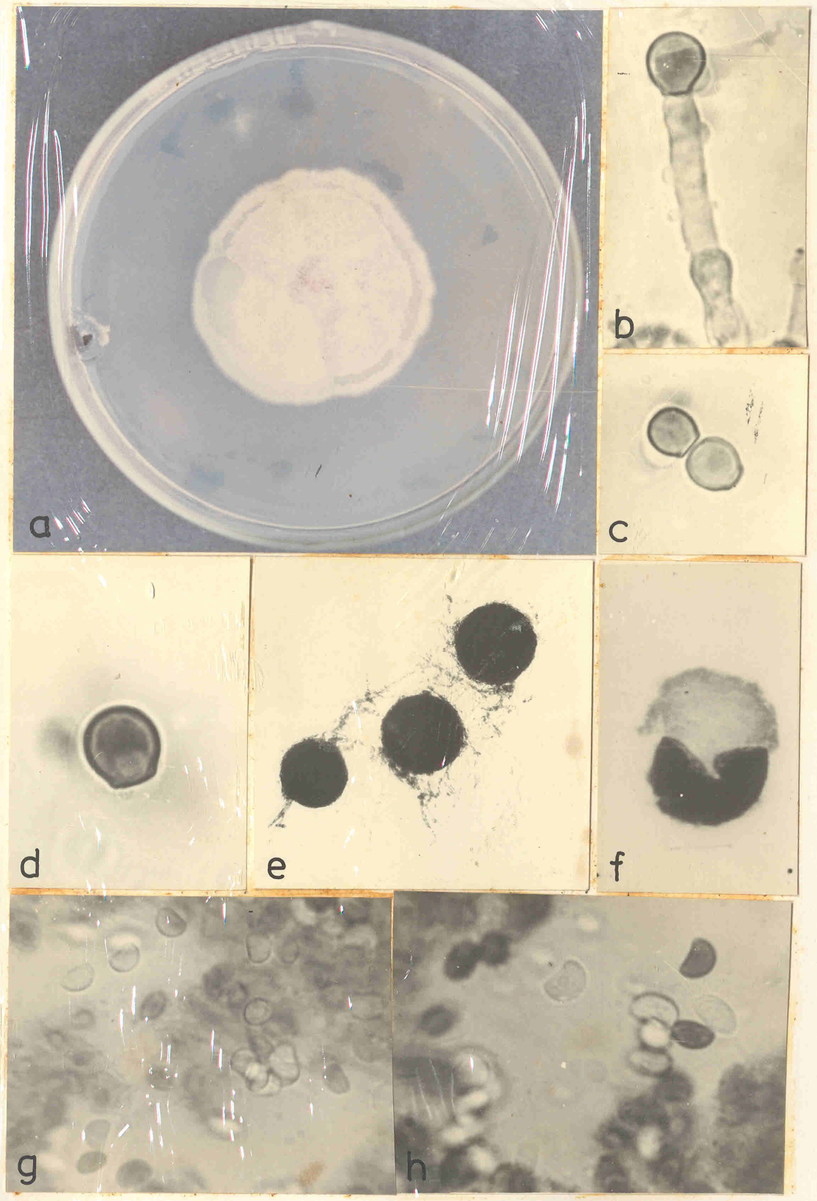


FIGURE 16 : Camera lucida drawings of Microascus manganii (Loubière) Curzi

a. Imperfect state (Scopulariopsis candida) of M. manganii showing annellophores bearing annellospores.

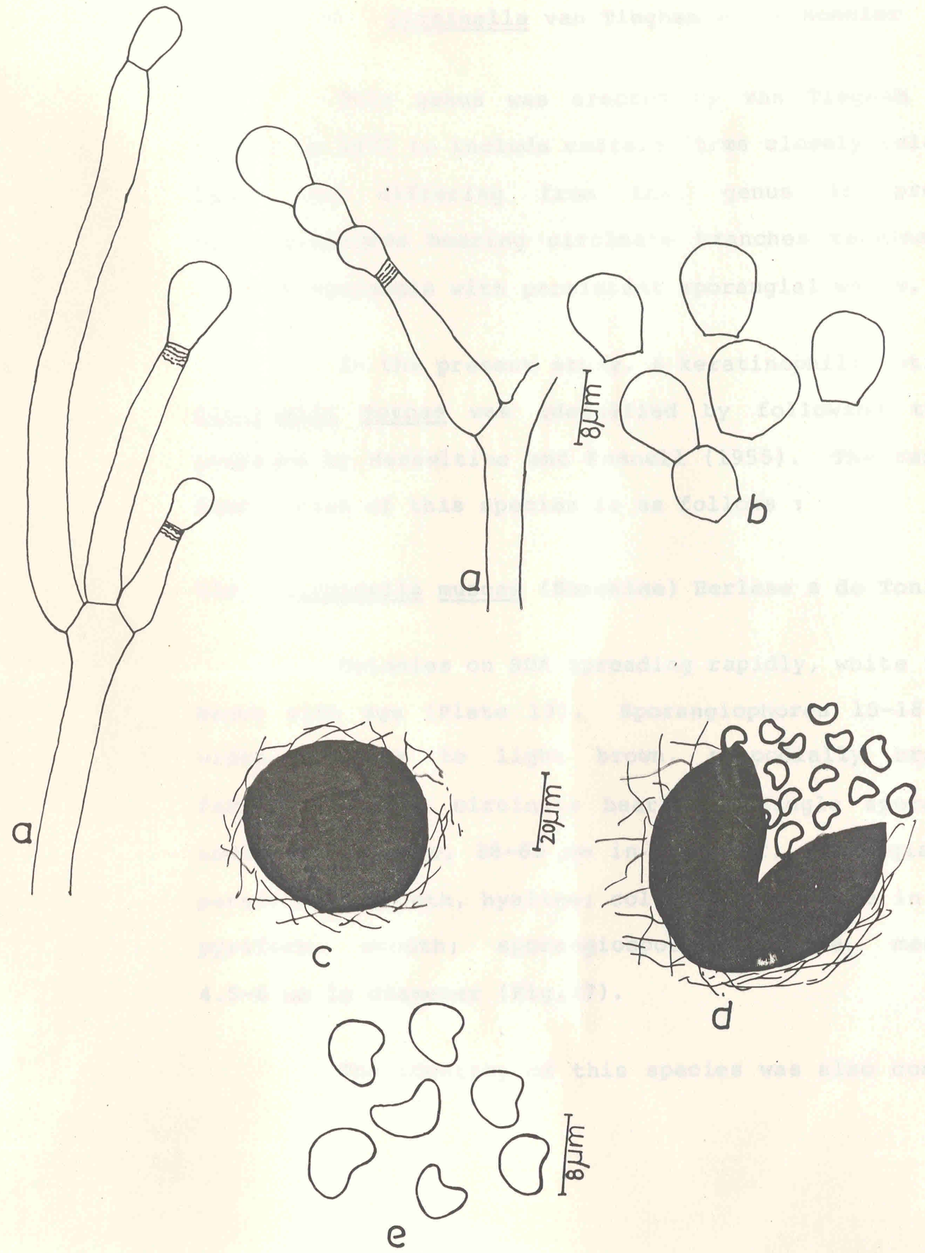
b. Mature annellospores

c. Perithecium

d. Ruptured perithecium

e. Ascospores.

FIGURE - 16



VI. Circinella van Tiegham et Le Monnier

This genus was erected by van Tiegham and Le Monier in 1873 to include certain forms closely related to Mucor but differing from that genus in producing sporangiophores bearing circinate branches terminated by globose sporangia with persistent sporangial walls.

In the present study, a keratinophilic strain of Circinella muscae was identified by following the key proposed by Hesseltine and Fennell (1955). The taxonomic description of this species is as follows :

VIa. Circinella muscae (Sorokine) Berlese & de Toni

Colonies on SDA spreading rapidly, white turning brown with age (Plate 13). Sporangiophores 15-18 µm in width, hyaline to light brown, sympodially branched, fertile branches circinate bearing a single sporangium; sporangia globose, 28-65 µm in diameter; sporangial wall persistent, smooth, hyaline; columellae variable in shape, pyriform, smooth; sporangiospores globose, measuring 4.5-6 µm in diameter (Fig.17).

The identity of this species was also confirmed

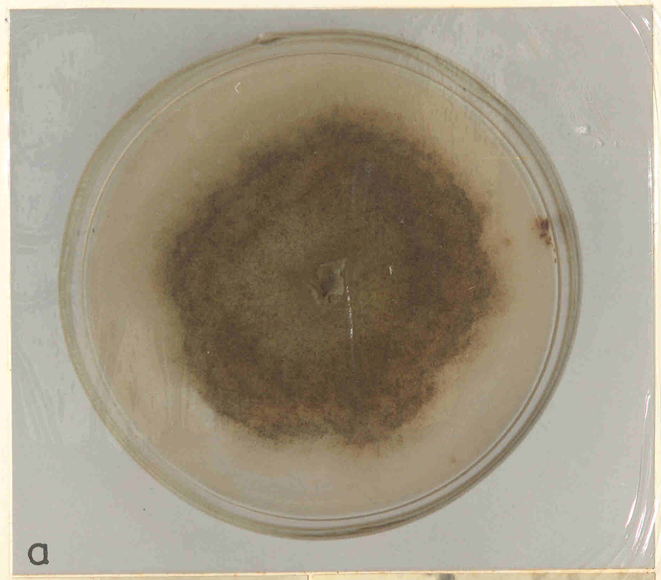
PLATE 13 : Circinella muscae (Sorokine) Berlese & de Toni

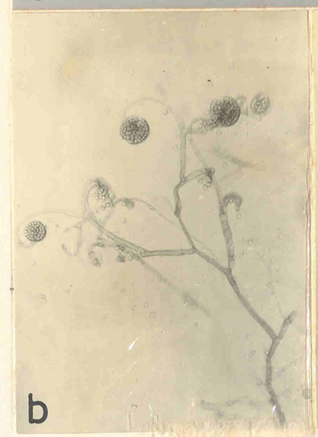
a. Colony on SDA after 12 days of incubation.

b. Circinate branches bearing sporangia x 100

c. Globose sporangiospores x 1000.

PLATE 13





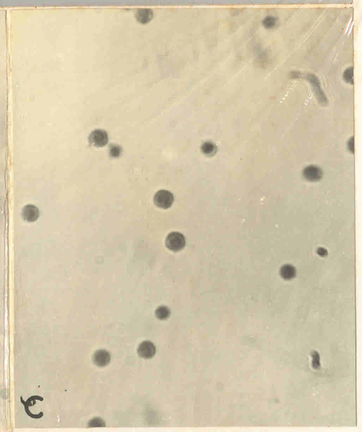


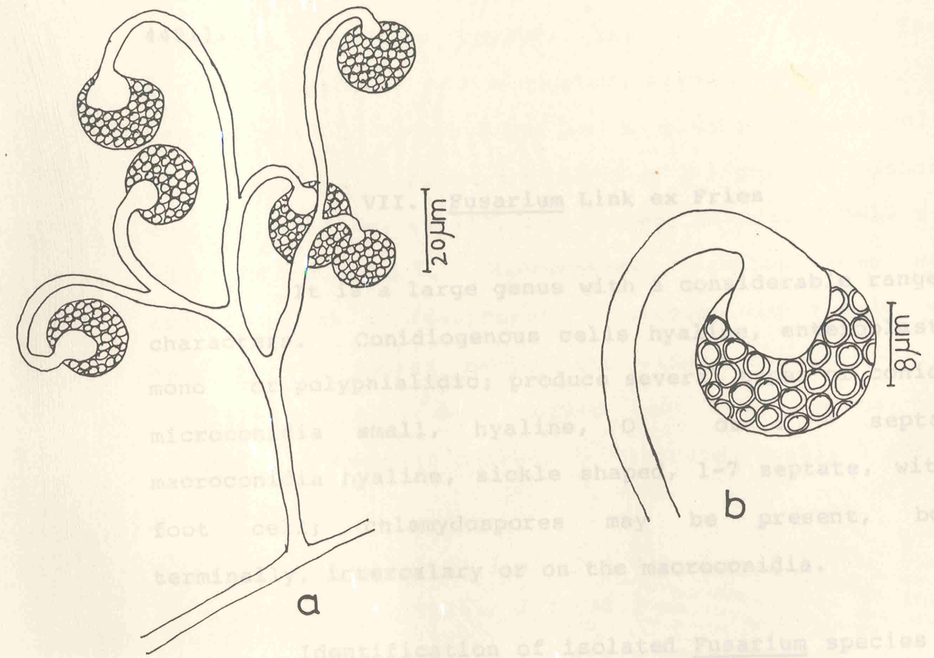
FIGURE 17 : Camera lucida drawings of Circinella muscae (Sorokine) Berlese & de Toni.

a. Sporangiophore bearing sporangia

b. Sporangium bearing sporangiospores

c. Mature sporangiospores.

FIGURE -17



from Indian Type Culture Collection, New Delhi (Acc. No. 4491).

VII. Fusarium Link ex Fries

It is a large genus with a considerable range of characters. Conidiogenous cells hyaline, enteroblastic, mono or polyphialidic; produce several types of conidia; microconidia small, hyaline, 0 or 1 - septate; macroconidia hyaline, sickle shaped, 1-7 septate, with a foot cell; chlamydospores may be present, borne terminally, intercalary or on the macroconidia.

Identification of isolated Fusarium species was done by following the monograph authored by Booth (1971) which stresses the importance of the conidiogenous cell in identification.

VIIa. Fusarium solani (Mart.) Sacc.

Colonies on potato sucrose agar (P.S.A.) dense, floccose, greying white (Plate 14); reverse brown. Microconidia develop abundantly in fresh cultures after 2-3 days. They are formed from lateral conidiophores,

which are initially merely elongated lateral phialides narrowing slightly towards the apex. Later formed microconidiophores are elongated, sparsely branched, each branch usually terminating in a single cylindrical to subulate phialide measuring 40-70 x 2-3 µm. Microconidia oval to fusiform, usually one celled, measuring 7-12 x 2.4 -3.2 µm (Fig.18). Macroconidia develop from short multibranched conidiophores. Macroconidia fusoid with many of the spores having the widest diameter in the penultimate cell, well marked foot cell, apical cell pointed and somewhat beaked, 1-5 septate, mostly 3 septate measuring :

1 Septate - 11.2-32.0 x 3.2-4.0 µm
2 Septate - 12.0-30.0 x 3.0-4.2 µm
3 Septate - 25.6-36.8 x 3.2-4.0 µm
4 Septate - 25.8-40.0 x 3.2-4.0 µm
5 Septate - 25.0-40.0 x 3.2-4.2 µm.

Chlamydospores develop in cultures after 10-14 days of incubation, globose to oval, smooth to rough, terminal and intercalalry, solitary or in chains, measuring 7-12 x 9-11µm (Fig.18).

The identity of this species was confirmed from Indian Type Culture Collection, New Delhi (Ref. No. 386).

PLATE 14 : Fusarium solani (Mart.) Sacc.

a. Colony on PSA after 12 days of incubation

b. Microconidia borne on monophialidic conidiophores x 100

d. Macroconidia borne on conidiophores x 400

c. Microconidia x 1000

e. Macroconidium x 1000

f. Chlamydospores x 1000.

PLATE 14

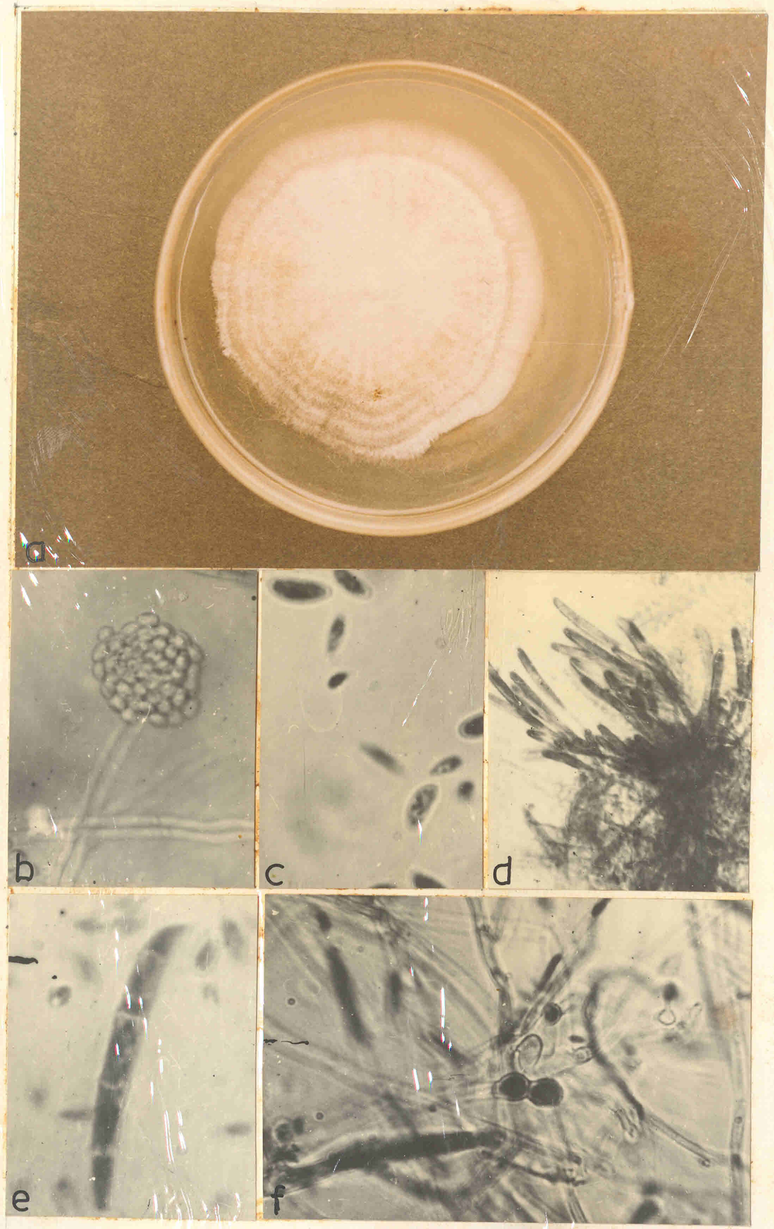


FIGURE 18 : Camera lucida drawings of Fusarium solani (Mart.) Sacc.

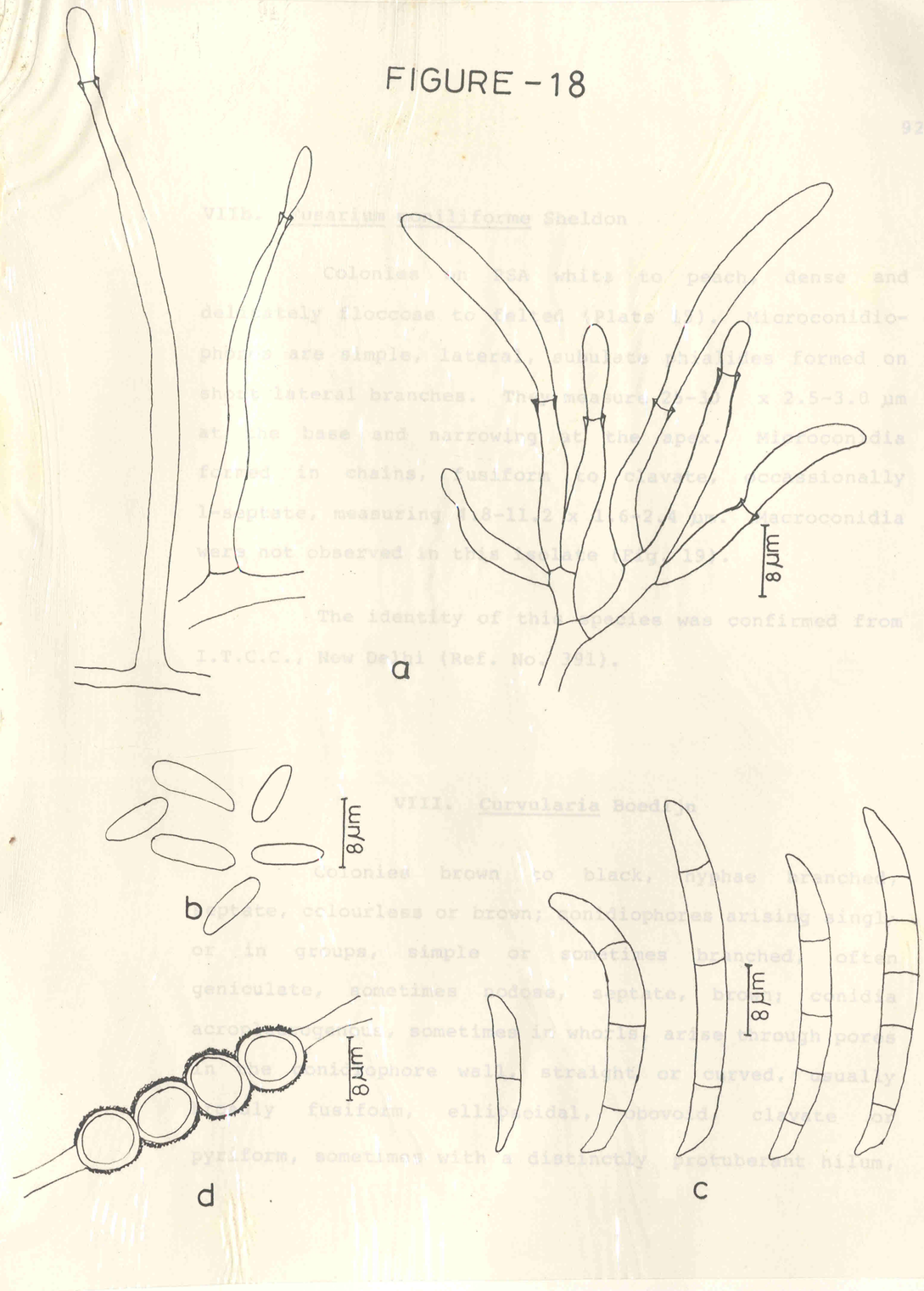
a. Conidiophores bearing conidia

b. Microconidia

c. Macroconidia

d. Chlamydospores.

FIGURE - 18



VIIb. Fusarium moniliforme Sheldon

Colonies on PSA white to peach, dense and delicately floccose to felted (Plate 15). Microconidiophores are simple, lateral, subulate phialides formed on short lateral branches. They measure 25-30 x 2.5-3.0 µm at the base and narrowing at the apex. Microconidia formed in chains, fusiform to clavate, occassionally 1-septate, measuring 4.8-11.2 x 1.6-2.4 µm. Macroconidia were not observed in this isolate (Fig. 19).

The identity of this species was confirmed from I.T.C.C., New Delhi (Ref. No. 391).

VIII. Curvularia Boedijn

Colonies brown to black, hyphae branched, septate, colourless or brown; conidiophores arising singly or in groups, simple or sometimes branched, often geniculate, sometimes nodose, septate, brown; conidia acropleurogenous, sometimes in whorls, arise through pores in the conidiophore wall, straight or curved, usually broadly fusiform, ellipsoidal, obovoid, clavate or pyriform, sometimes with a distinctly protuberant hilum,

PLATE 15 : Fusarium moniliforme Sheldon

a. Colony on PSA after 12 days of incubation

b. chains of microconidia on phialides x 1000

c,d. Phialides bearing microconidia x 1000

e. Microconidia x 1000.

PLATE 15

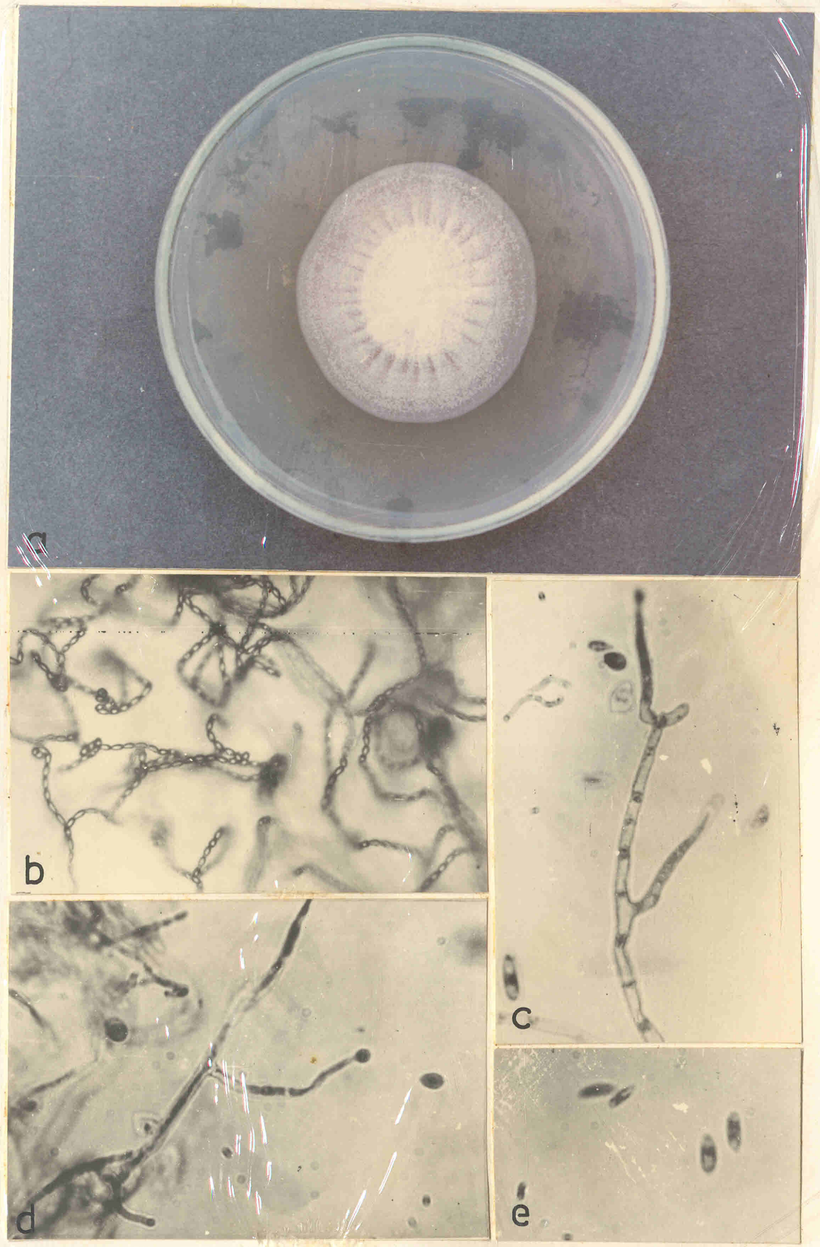


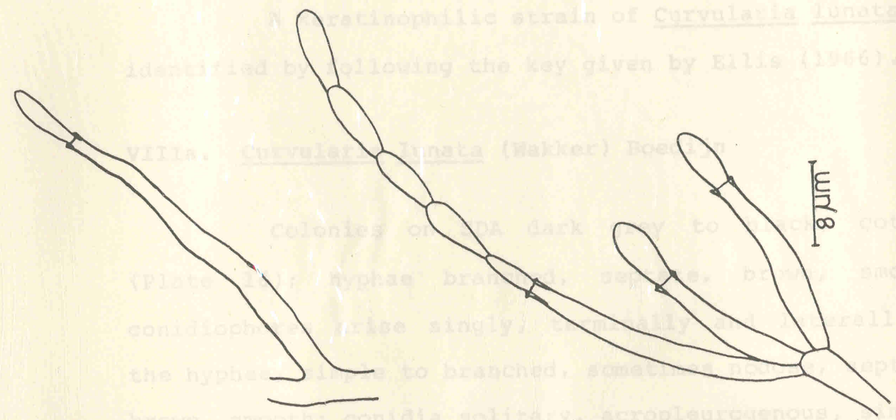
FIGURE 19 : Camera lucida drawings of Fusarium moniliforme Sheldon

a. Conidiophores bearing microconidia

b. Aseptate microconidia

c. Septate microconidia

FIGURE-19



transversely septate, often with one or more cells larger and darker than the others, smooth or verrucose.

A keratinophilic strain of Curvularia lunata was identified by following the key given by Ellis (1966).

VIIIa. Curvularia lunata (Wakker) Boedijn

Colonies on SDA dark grey to black, cottony (Plate 16); hyphae branched, septate, brown, smooth; conidiophores arise singly, terminally and laterally on the hyphae, simple to branched, sometimes nodose, septate, brown, smooth; conidia solitary, acropleurogenous, simple, smooth, pale, often curved, clavate, broadly fusiform with three transverse septa, third cell from the base larger and darker than the others, hilum protuberant, measuring 17.6-25.6 x 6.4-11.2 µm (Fig. 20).

IX. Gliocladium Corda

Conidiophores erect or suberect, septate, hyaline, branching at the apex in a penicillate or verticillate fashion, ultimate sporogenous cells phialides; phialides sometimes divergent, more often

PLATE 16 : Curvularia lunata (Wakker) Boedijn

a. Colony on CDA after 12 days of incubation

b. Conidiophores bearing conidia x 100

c. Conidiophore bearing a conidium x 1000

d,e. Conidia x 1000.

PLATE 16

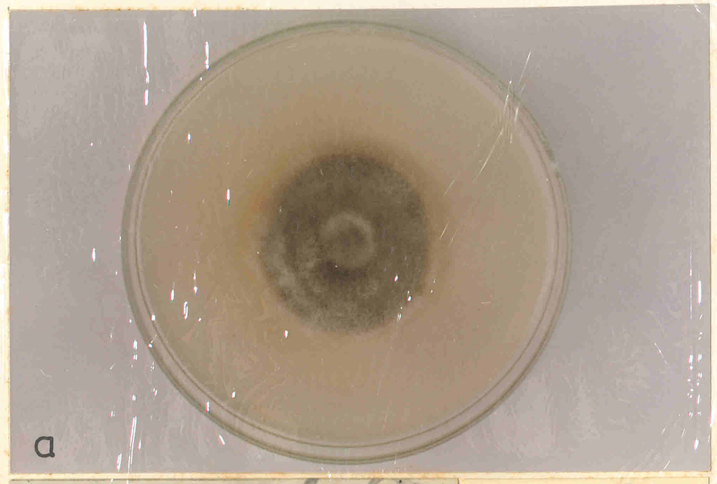
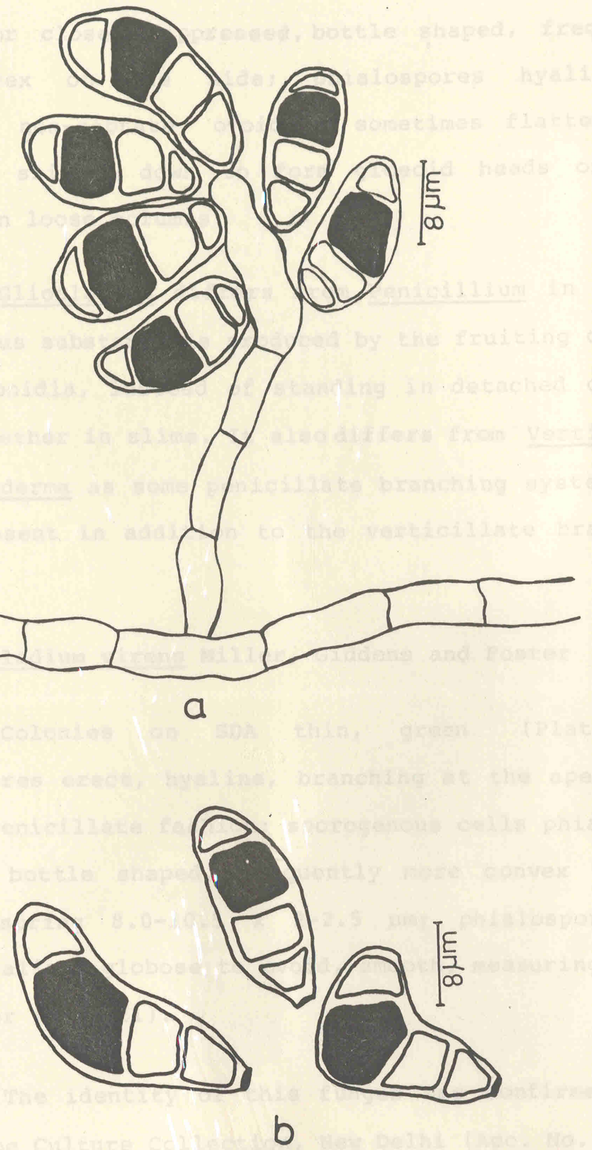


FIGURE 20 : Camera lucida drawings of Curvularia lunata (Wakker) Boedign

a. Conidiophore bearing conidia

b. Mature conidia

FIGURE-20



slightly or closely appressed, bottle shaped, frequently more convex on one side; phialospores hyaline or pigmented, non-septate, ovoid or sometimes flattened on one side, sliming down to form gloeoid heads or held together in loose columns.

Gliocladium differs from Penicillium in that a mucilaginous substance is produced by the fruiting organs, and the conidia, instead of standing in detached chains, adhere together in slime. It also differs from Verticillium and Trichoderma as some penicillate branching systems are always present in addition to the verticillate branching system.

IXa. Gliocladium virens Miller, Giddens and Foster

Colonies on SDA thin, green (Plate 17) conidiophores erect, hyaline, branching at the apex in a somewhat penicillate fashion; sporogenous cells phialides; phialides bottle shaped, frequently more convex on one side, measuring 8.0-10.5 x 2-2.5 µm; phialospores in chains, hyaline, globose to ovoid, smooth, measuring 3-4µm in diameter (Fig. 21).

The identity of this fungus was confirmed from Indian Type Culture Collection, New Delhi (Acc. No. 4278).

PLATE 17 : <u>Gliocladium virens</u> Miller, Giddens and Foster

a. Colony on SDA after 12 days of incubation.

b. Phialides bearing phialospores x 1000.

c. Globose phialospores x 1000.

PLATE 17

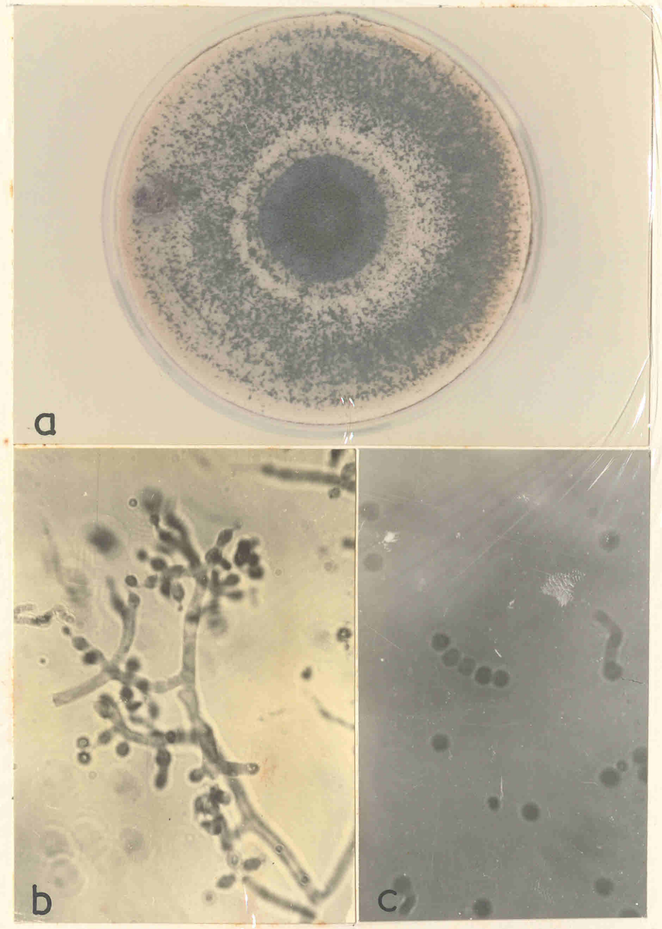
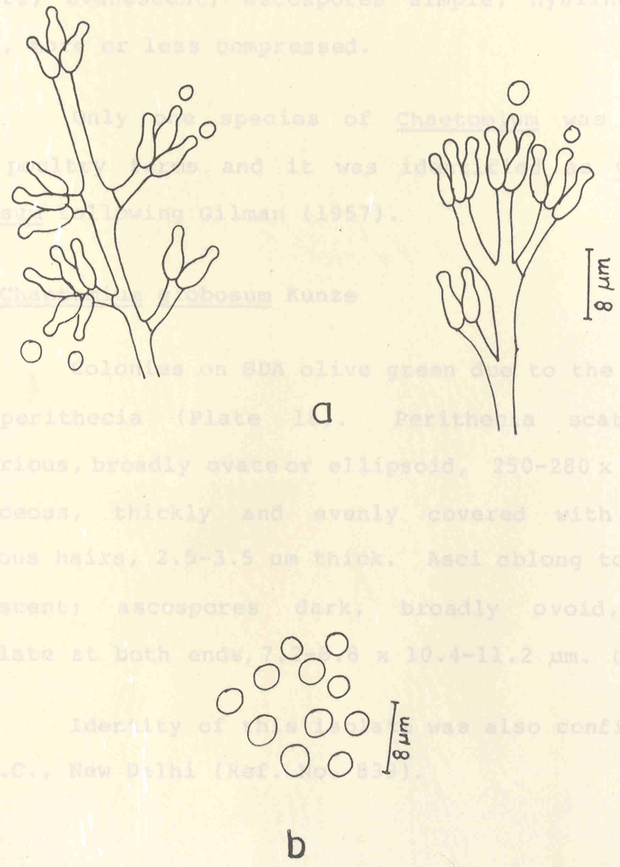


FIGURE 21 : Camera lucida drawings of Gliocladium virens Miller, Giddens and Foster

a. Conidiophores bearing conidia

b. Mature conidia.

FIGURE 21



X. Chaetomium Kunze and Schmidt.

Perithecia superficial, thin-membranous with an apical tuft of bristles and usually with an ostiole; asci clavate, evanescent; ascospores simple, hyaline to dark brown, more or less compressed.

Only one species of Chaetomium was recovered from poultry farms and it was identified as Chaetomium globosum following Gilman (1957).

Xa. Chaetomium globosum Kunze

Colonies on SDA olive green due to the colour of the perithecia (Plate 18). Perithecia scattered or gregarious, broadly ovate or ellipsoid, 250-280 x 200-245µm, olivaceous, thickly and evenly covered with slender, flexuous hairs, 2.5-3.5 µm thick. Asci oblong to clavate, evanescent; ascospores dark, broadly ovoid, faintly apiculate at both ends, 7.2-8.8 x 10.4-11.2 µm. (Fig. 22).

Identity of this isolate was also confirmed from I.T.C.C., New Delhi (Ref. No. 839).

XI. Aspergillus Micheli ex Fries

Mycelium hyaline, septate, partly submerged or

PLATE 18 : Chaetomium globosum kunze

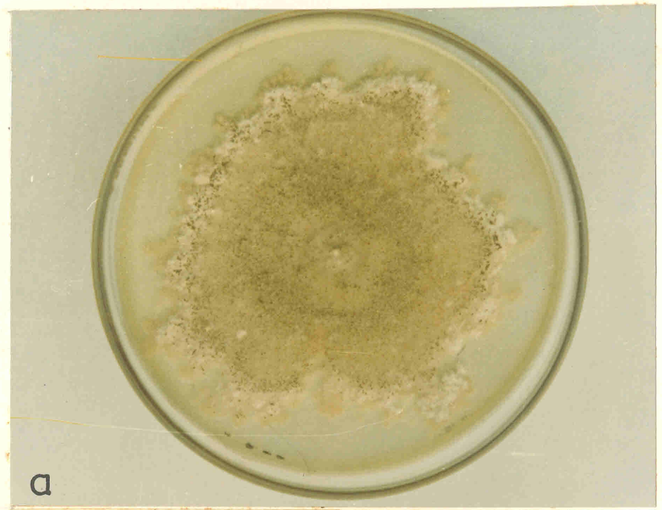
a. Colony on SDA after 15 days of incubation

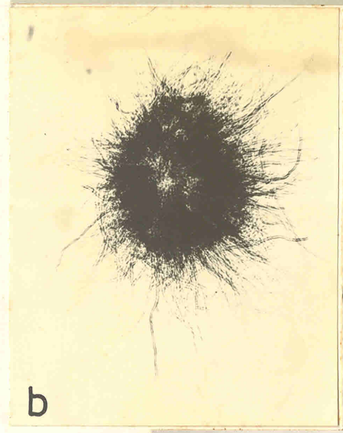
b. Perithecium x 100

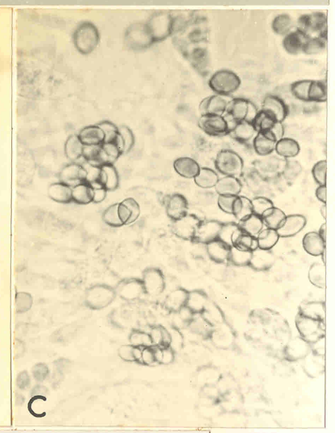
c. Apiculate ascospores x 400

d. Ascospores x 1000.

PLATE 18







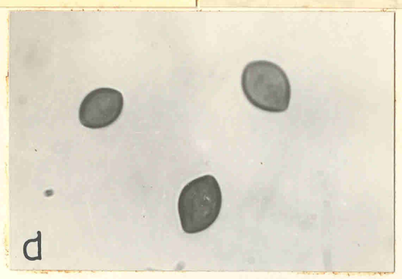


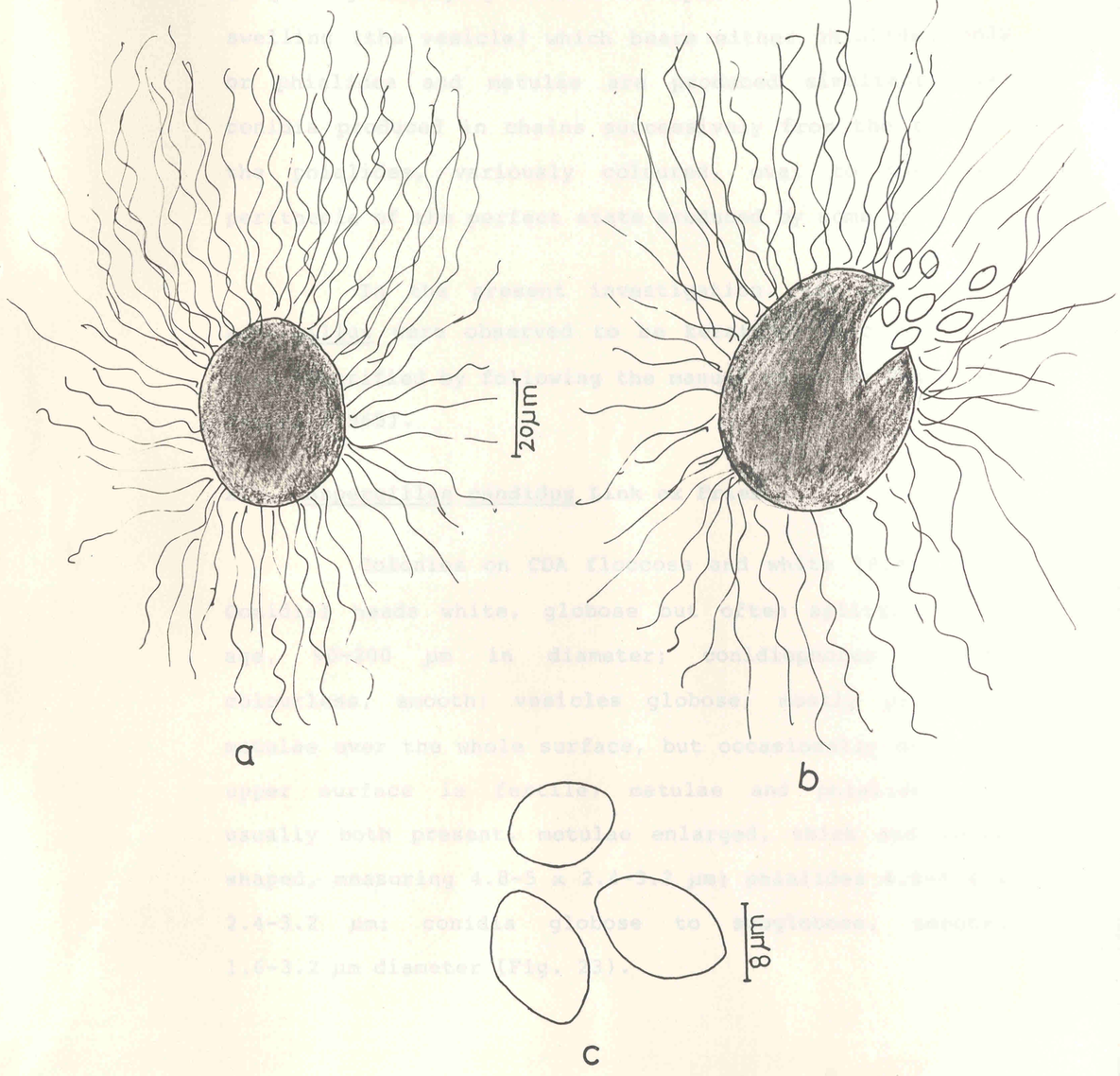
FIGURE 22 : Camera lucida drawings of Chaetomium globosum Kunze

a. Perithecium

b. Ruptured perithecium

c. Ascospores.

FIGURE-22



partly aerial; conidiophores smooth, rough or pitted, frequently enlarging towards the apex and terminating in a swelling (the vesicle) which bears either phialides only or phialides and metulae are produced simultaneously; conidia produced in chains successively from the tips of the phialides, variously coloured, oval to globular, perithecia of the perfect state produced by some species.

In the present investigation, six species of Aspergillus were observed to be keratinophilic and they were identified by following the manual given by Raper and Fennel (1965).

XIa. Aspergillus candidus Link ex Fries

Colonies on CDA floccose and white (Plate 19). Conidial heads white, globose but often splitting with age, 90-200 µm in diameter; conidiophores erect, colourless, smooth; vesicles globose, mostly producing metulae over the whole surface, but occasionally only the upper surface is fertile; metulae and phialides are usually both present, metulae enlarged, thick and wedge shaped, measuring 4.8-5 x 2.4-3.2 µm; phialides 4.8-6.4 x 2.4-3.2 µm; conidia globose to subglobose, smooth, 1.6-3.2 µm diameter (Fig. 23).

PLATE 19 : Aspergillus candidus Link ex Fries

a. Colonies on CDA after 12 days of incubation

b. Conidial head x 1000

c. Globose conidia x 1000.

PLATE 19

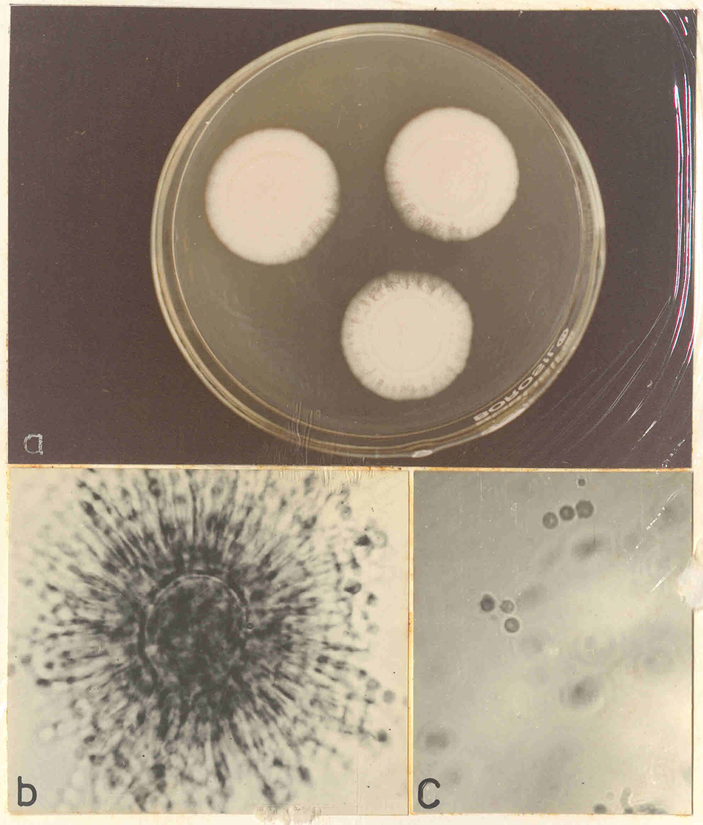


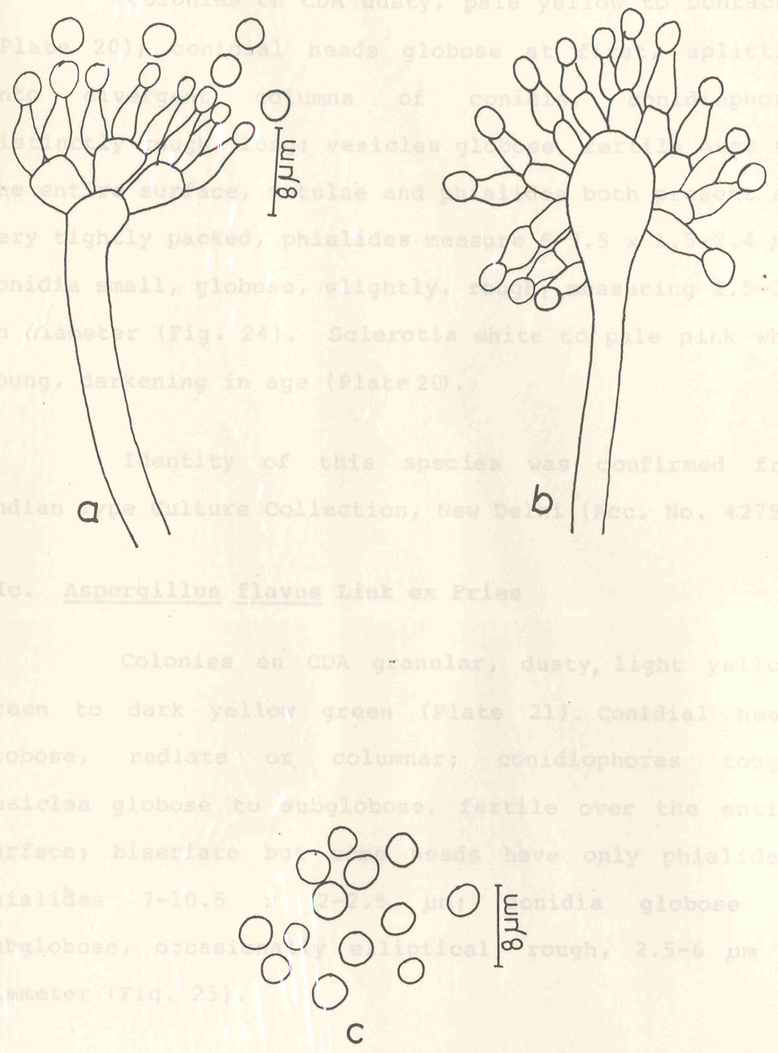
FIGURE 23 : Camera lucida drawings of Aspergillus candidus Link ex Fries

a. Reduced conidial head

b. Mature conidial head with large mature phialides

c. Mature conidia.

FIGURE -23



XIb. Aspergillus ochraceus Wilhelm

Colonies on CDA dusty, pale yellow to ochraceus (Plate 20); conidial heads globose at first, splitting into divergent columns of conidia, conidiophores distinctly rough, long; vesicles globose, fertile over the the entire surface, metulae and phialides both present and very tightly packed, phialides measure 6-9.5 x 1.5-2.4 µm; conidia small, globose, slightly, rough, measuring 2.5-3µm in diameter (Fig. 24). Sclerotia white to pale pink when young, darkening in age (Plate 20).

Identity of this species was confirmed from Indian Type Culture Collection, New Delhi (Acc. No. 4275).

XIc. Aspergillus flavus Link ex Fries

Colonies on CDA granular, dusty, light yellow, green to dark yellow green (Plate 21). Conidial heads globose, radiate or columnar; conidiophores rough; vesicles globose to subglobose, fertile over the entire surface; biseriate but some heads have only phialides; phialides 7-10.5 x 2-2.5 µm; conidia globose to subglobose, occasionally elliptical, rough, 2.5-6 µm in diameter (Fig. 25).

PLATE 20 : Aspergillus ochraceus Wilheln

a. Colony on CDA after 12 days of incubation

b. Colony showing the formation of Sclerotia

c. Typical head x 1000

d. Slightly rough conidia x 1000

PLATE 20

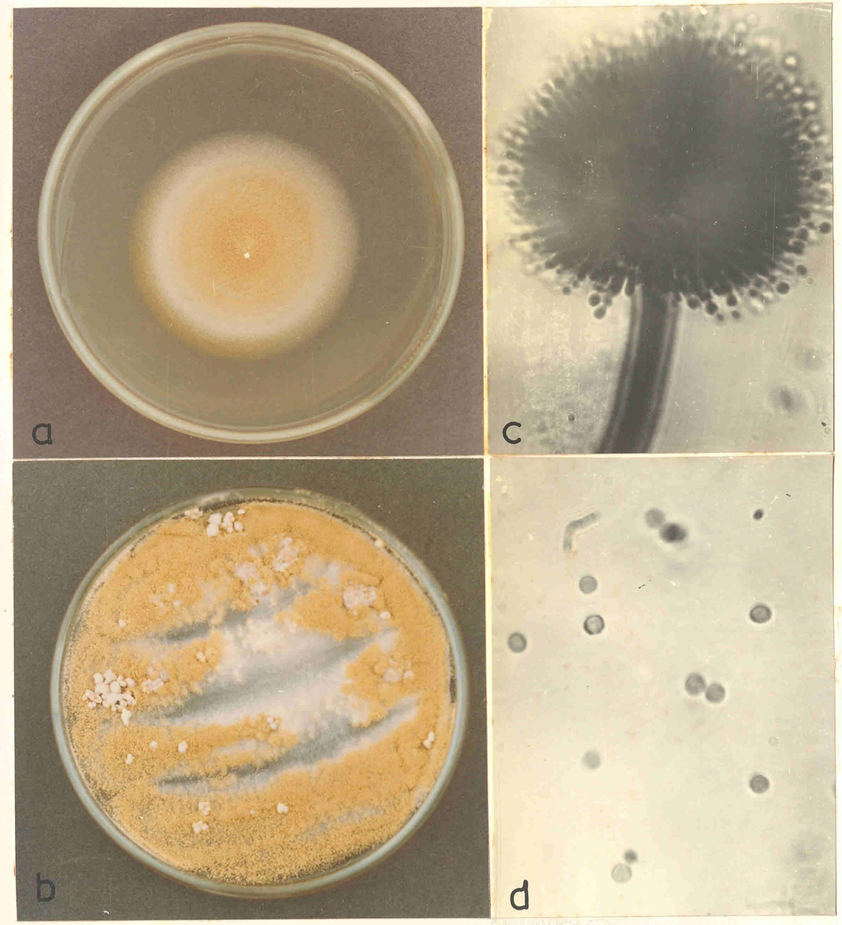
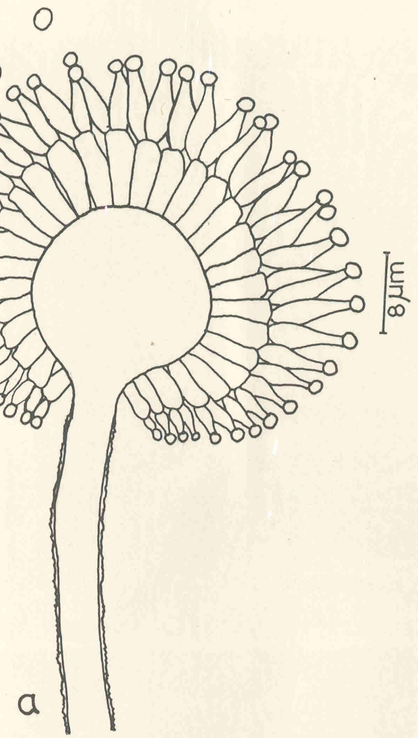


FIGURE 24 : Camera lucida drawings of Aspergillus ochraceus Wilhelm

a. Mature conidial head

b. Mature conidia.

FIGURE -24



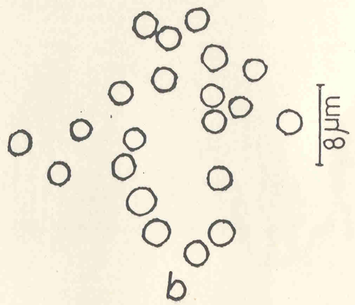


PLATE 21 : <u>Aspergillus flavus</u> Link ex Fries

a. Colony on CDA after 10 days of incubation

b. Conidial head with phialides borne directly on the vesicle x 1000

c. Globose conidia x 1000.

PLATE 21

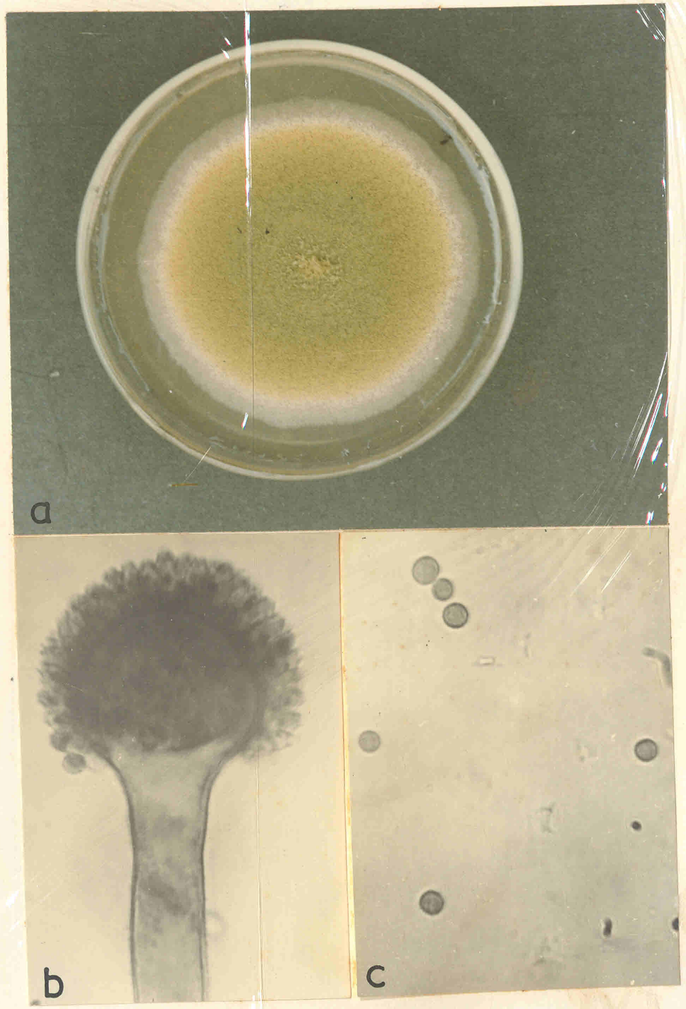


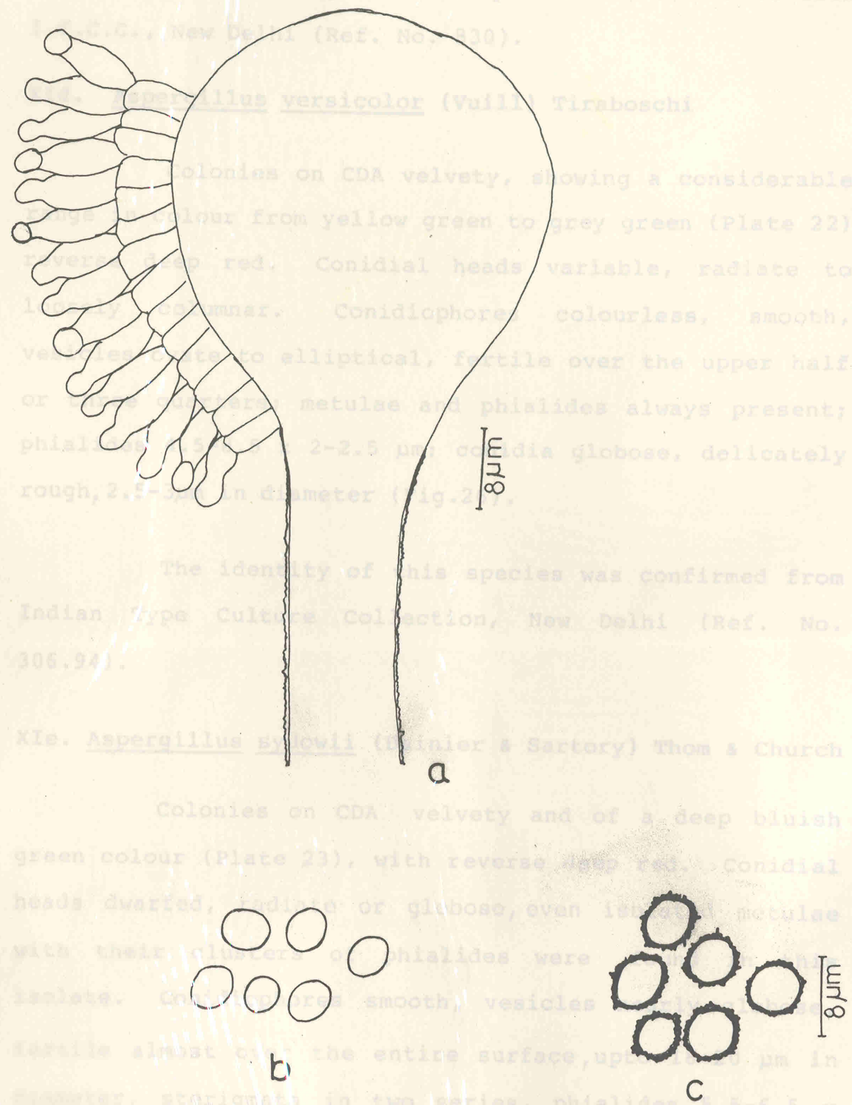
FIGURE 25 : Camera lucida drawings of Aspergillus flavus Link ex Fries

a. Mature conidial head

b. Young, smooth walled conidia

c. Mature, rough walled conidia.

FIGURE - 25



The identity of this species was confirmed from I.T.C.C., New Delhi (Ref. No. 830).

XId. Aspergillus versicolor (Vuill) Tiraboschi

Colonies on CDA velvety, showing a considerable range in colour from yellow green to grey green (Plate 22) reverse deep red. Conidial heads variable, radiate to loosely columnar. Conidiophores colourless, smooth, vesicles ovate to elliptical, fertile over the upper half or three quarters; metulae and phialides always present; phialides 4.5-8.5 x 2-2.5 µm; conidia globose, delicately rough, 2.5-3µm in diameter (Fig.26).

The identity of this species was confirmed from Indian Type Culture Collection, New Delhi (Ref. No. 306.94).

XIe. Aspergillus sydowii (Bainier & Sartory) Thom & Church

Colonies on CDA velvety and of a deep bluish green colour (Plate 23), with reverse deep red. Conidial heads dwarfed, radiate or globose, even isolated metulae with their clusters of phialides were found in this isolate. Conidiophores smooth, vesicles nearly globose, fertile almost over the entire surface, upto 18-20 µm in diameter, sterigmata in two series, phialides 5.5-6.5 x

PLATE 22 : <u>Aspergillus versicolor</u> (Vuill) Tiraboschi

a. Colonies on CDA after 10 days of incubation

b. Typical head x 1000

c. Conidia x 1000.

PLATE 22

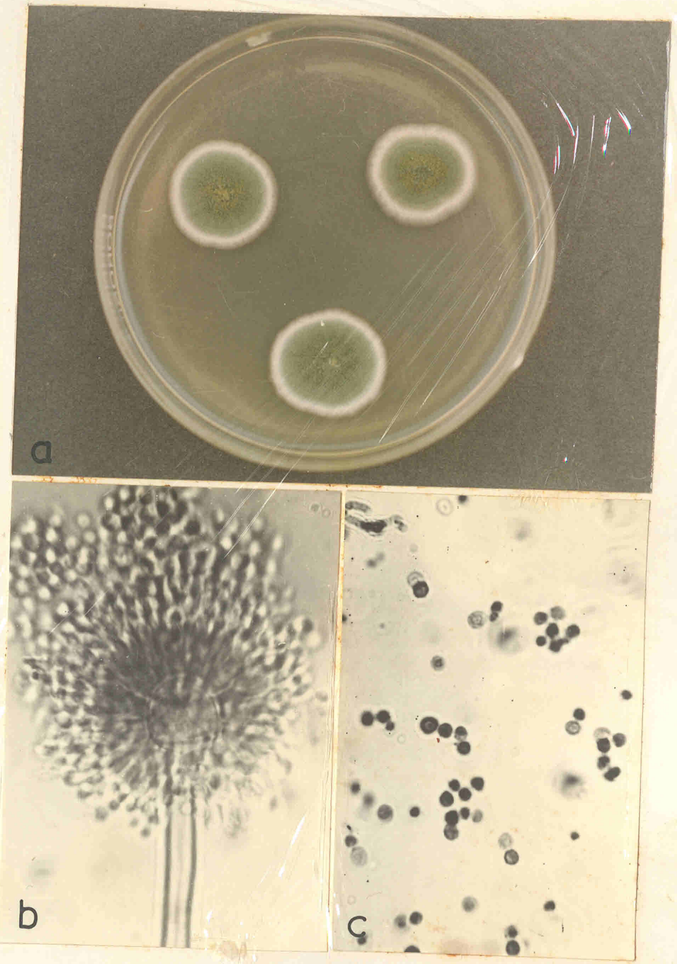


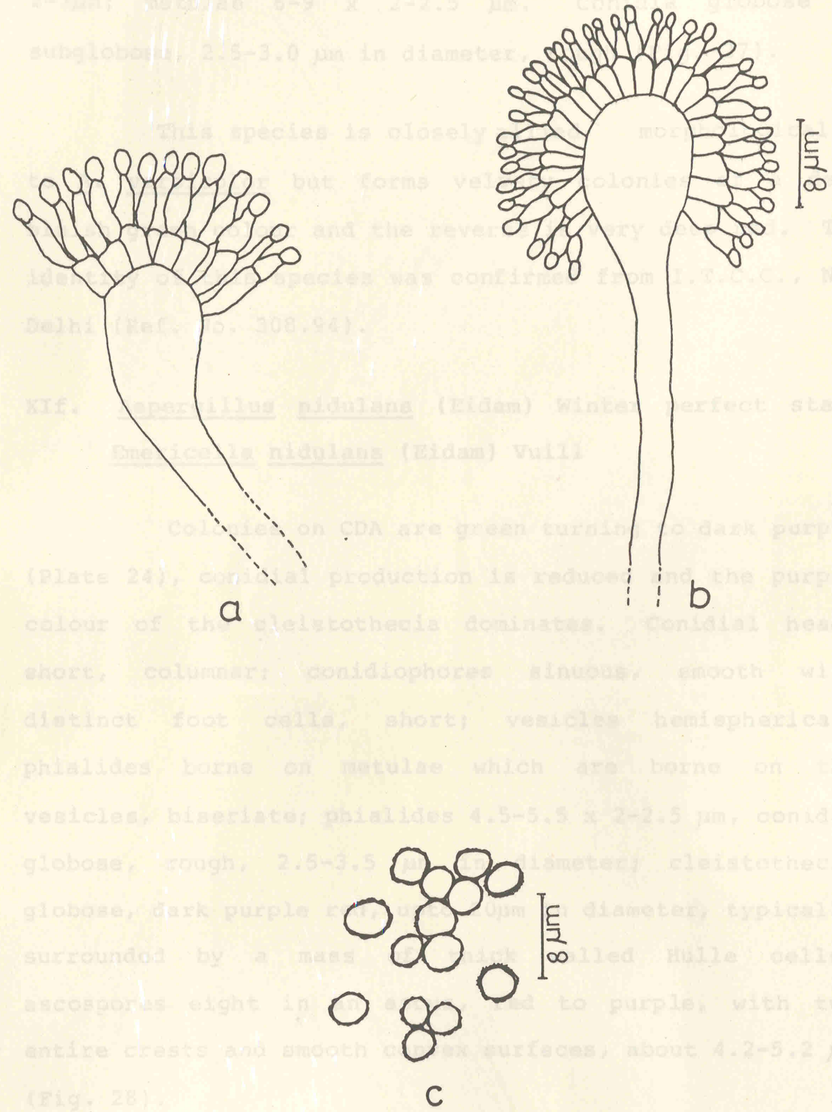
FIGURE 26 : Camera lucida drawings of <u>Aspergillus versicolor</u> (Vuill) Tiraboschi

a. Young conidial head

b. Mature conidial head

c. Mature, rough walled conidia

FIGURE-26



2-3μm; metulae 6-9 x 2-2.5 μm. Conidia globose to subglobose, 2.5-3.0 μm in diameter, rough (Fig. 27).

This species is closely allied morphologically to A. versicolor but forms velvety colonies of a deep bluish green colour and the reverse is very deep red. The identity of this species was confirmed from I.T.C.C., New Delhi (Ref. No. 308.94).

XIf. Aspergillus nidulans (Eidam) Winter perfect state Emericella nidulans (Eidam) Vuill

Colonies on CDA are green turning to dark purple (Plate 24), conidial production is reduced and the purple colour of the cleistothecia dominates. Conidial heads short, columnar; conidiophores sinuous, smooth with distinct foot cells, short; vesicles hemispherical; phialides borne on metulae which are borne on the vesicles, biseriate; phialides 4.5-5.5 x 2-2.5 μm, conidia globose, rough, 2.5-3.5 μm in diameter; cleistothecia globose, dark purple red, upto 20μm in diameter, typically surrounded by a mass of thick walled Hulle cells; ascospores eight in an ascus, red to purple, with two entire crests and smooth convex surfaces, about 4.2-5.2 μm (Fig. 28).

PLATE 23 : Aspergillus sydowii (Bainier & Sartory) Thom & Church

a. Colonies on CDA after 10 days of incubation

b,c. Conidial head x 1000

d. Conidia x 1000.

PLATE 23

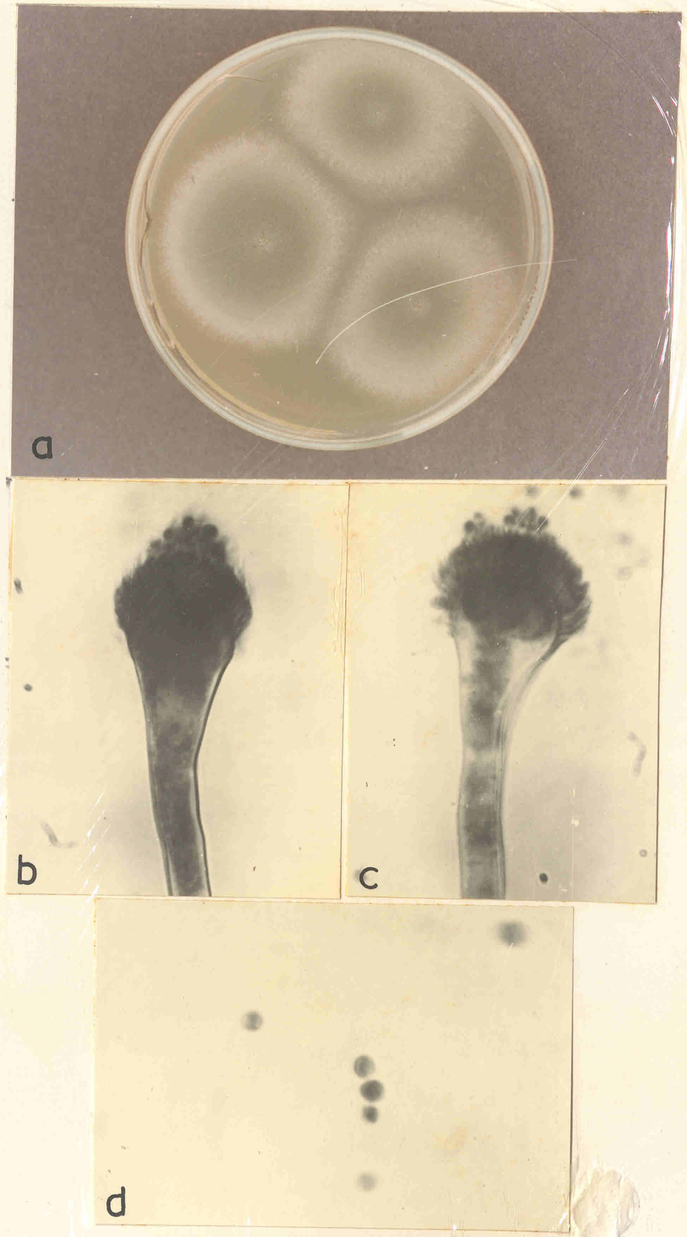


FIGURE 27 : Camera lucida drawings of <u>Aspergillus sydowii</u> (Bainier & Sartory) Thom & Church

a. Young conidial heads

b. Mature conidial head

b. Mature, rough walled conidia

FIGURE - 27

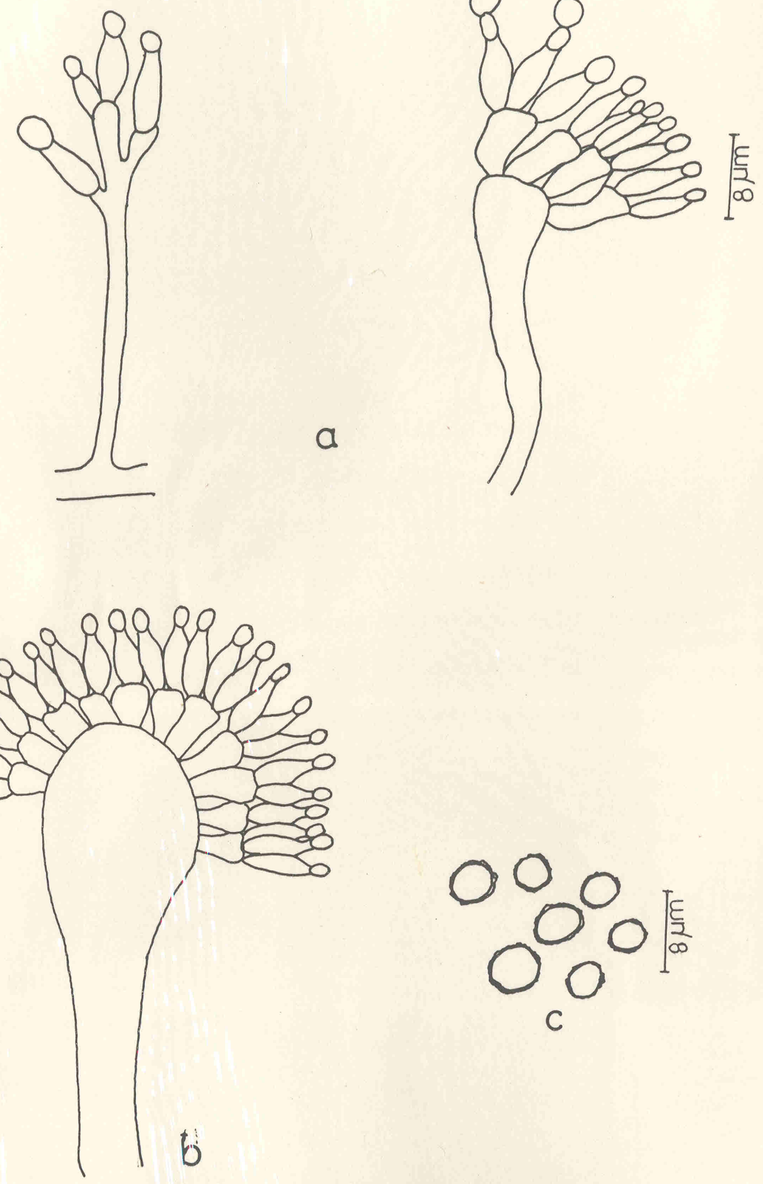


PLATE 24 : Aspergillus nidulans (Eidam) Winter
perfect slate Emericella nidulans (Eidam). Vuill

a. Colony on CDA after 15 days of incubation

b. Conidiophore bearing conidia x 1000

c. Conidia x 1000

d. Cleistothecium x 100

e. Hulle cells x 1000

f. Ascospores x 1000

PLATE 24

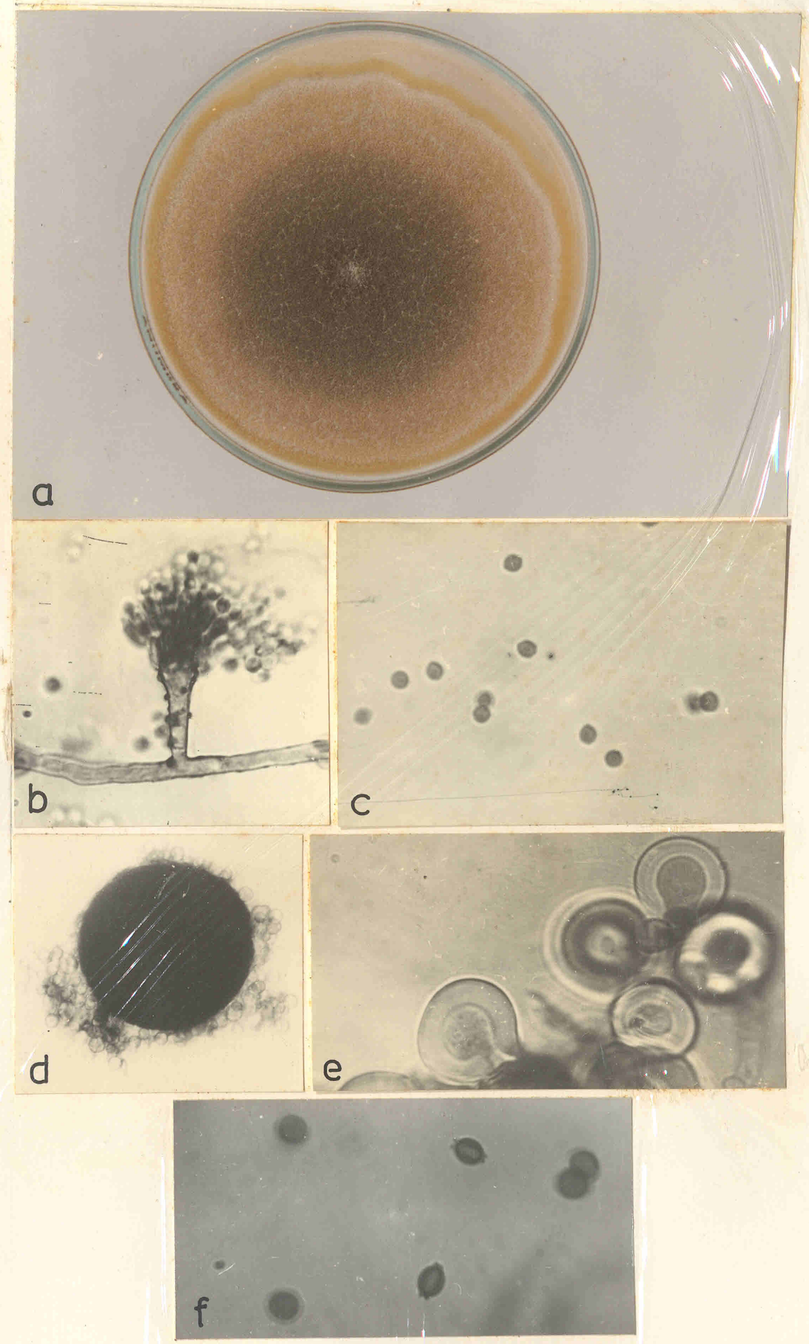


FIGURE 28 : Camera lucida drawings of Aspergillus nidulans (Eidam) Winter perfect state Emericella nidulans (Eidam) Vuill.

a. Conidial heads bearing metulae and phialides

b. Mature conidia

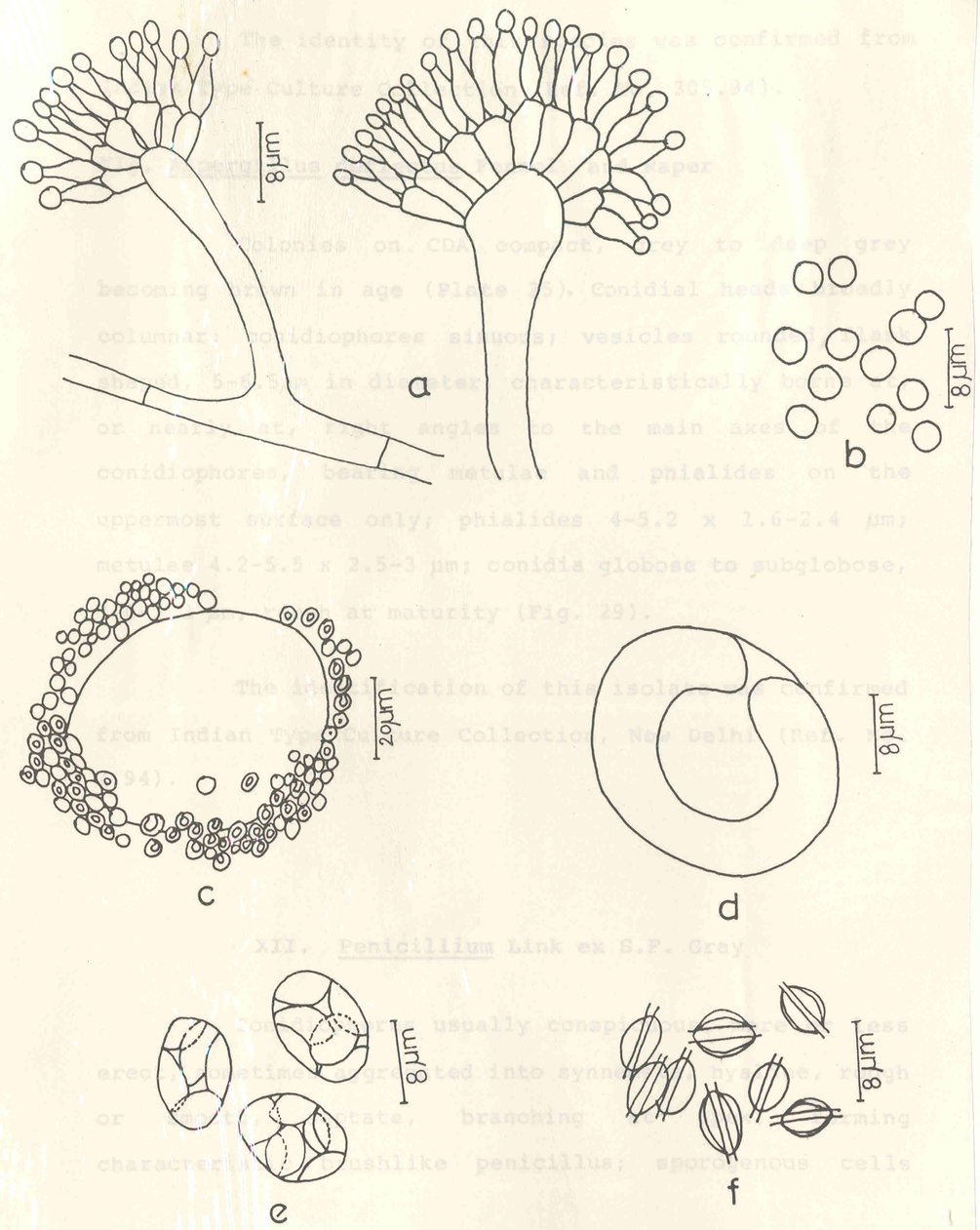
c. Cleistothecium surrounded by Hulle cells

d. Hulle cell

e. Asci bearing ascospores

f. Ascospores.

FIGURE -28



The identity of this species was confirmed from Indian Type Culture Collection (Ref. No. 305.94).

XIg. Aspergillus deflectus Fennel and Raper

Colonies on CDA compact, grey to deep grey becoming brown in age (Plate 25). Conidial heads broadly columnar; conidiophores sinuous; vesicles rounded, flask shaped, 5-6.5µm in diameter, characteristically borne at, or nearly at, right angles to the main axes of the conidiophores, bearing metulae and phialides on the uppermost surface only; phialides 4-5.2 x 1.6-2.4 µm; metulae 4.2-5.5 x 2.5-3 µm; conidia globose to subglobose, 3.0-3.2 µm, rough at maturity (Fig. 29).

The identification of this isolate was confirmed from Indian Type Culture Collection, New Delhi (Ref. No. 2.94).

XII. Penicillium Link ex S.F. Gray

Conidiophores usually conspicuous, more or less erect, sometimes aggregated into synnemata, hyaline, rough or smooth, septate, branching at apex; forming characteristic brushlike penicillus; sporogenous cells

PLATE 25 : Aspergillus deflectus Fennel and Raper

a. Colonies on CDA after 10 days of incubation

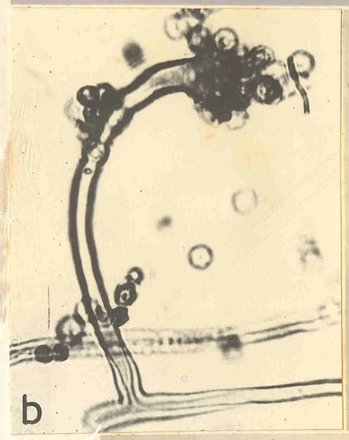
b. Young conidial head x 1000

c. Mature conidial head x 1000

d. Rough conidia x 1000

PLATE 25





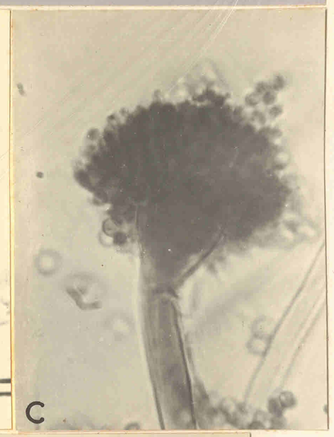




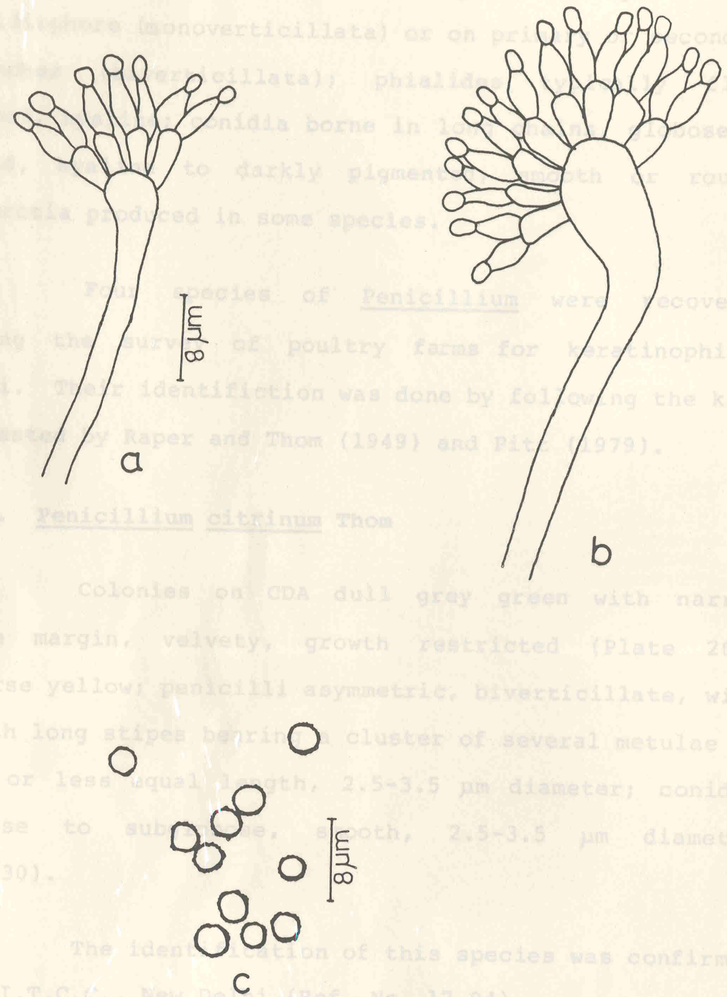
FIGURE 29 : Camera lucida drawings of Aspergillus deflectus Fenneland Raper

a. Young conidial head

b. Mature conidial head

c. Mature, rough walled conidia.

FIGURE – 29



phialides, borne in groups directly at the apex of the conidiophore (monoverticillata) or on primary or secondary branches (biverticillata); phialides typically flask shaped, hyaline; conidia borne in long chains, globose to ovoid, hyaline to darkly pigmented, smooth or rough; sclerotia produced in some species.

Four species of Penicillium were recovered during the survey of poultry farms for keratinophilic fungi. Their identifiction was done by following the keys suggested by Raper and Thom (1949) and Pitt (1979).

XIIa. Penicillium citrinum Thom

Colonies on CDA dull grey green with narrow white margin, velvety, growth restricted (Plate 26); reverse yellow; penicilli asymmetric, biverticillate, with smooth long stipes bearing a cluster of several metulae of more or less equal length, 2.5-3.5 µm diameter; conidia globose to subglobose, smooth, 2.5-3.5 µm diameter (Fig.30).

The identification of this species was confirmed from I.T.C.C., New Delhi (Ref. No. 17.94).

XIIb. Penicillium chrysogenum Thom.

Colonies on CDA blue green to bright green,

PLATE 26 : Penicillium citrinum Thom

a. Colonies on CDA after 10 days of incubation

b. Conidiophore bearing conidia x 1000

c. Conidia x 1000

PLATE 26

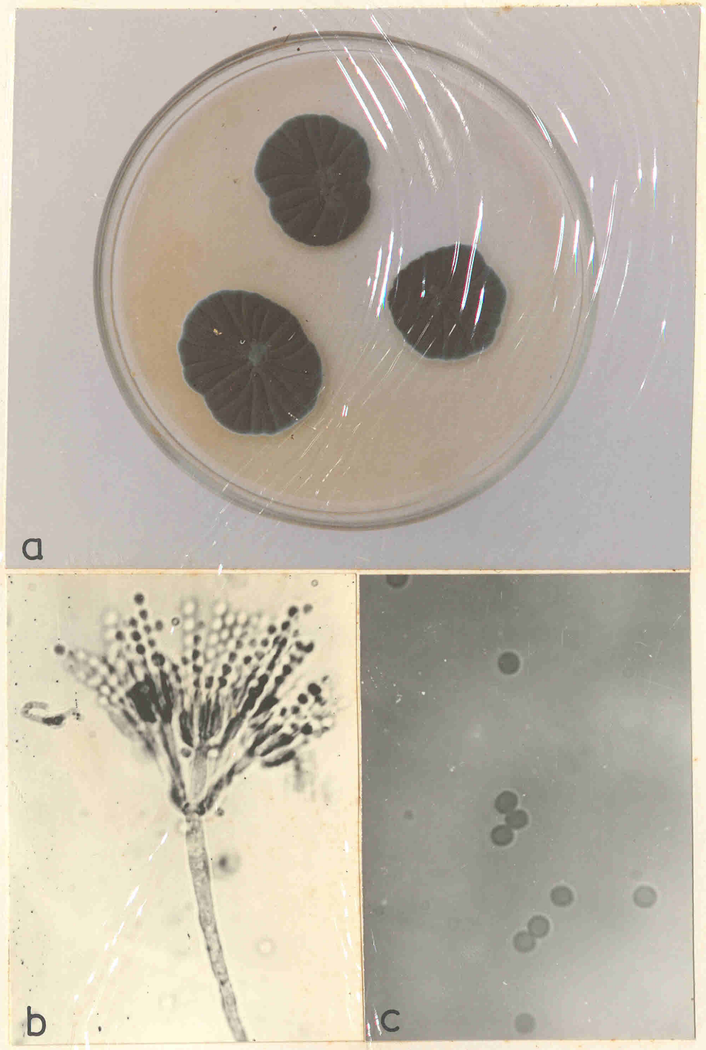
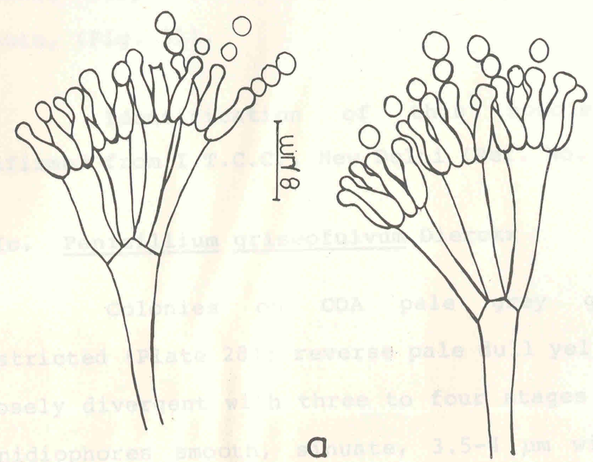


FIGURE 30 : Camera lucida drawings of Penicillium citrinum Thom

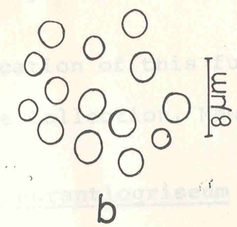
a. Conidiophores bearing metulae and phialides

b. Mature conidia.

FIGURE-30



a



b

broadly spreading with broad white margin during the growing period, smooth, velvety, becoming greyish in age (Plate 27), reverse yellow; penicilli fairly complex with divergent branches, smooth; stipes 2.5-4.2 µm in width; phialides ampuliform with a reduced neck, 6.5-8.5 x 2-2.8µm; conidia elliptical to subglobose, 2.5-3.5 µm in long axis, smooth, (Fig. 31).

Identification of this species was also confirmed from I.T.C.C., New Delhi (Ref. No. 842).

XIIc. Penicillium griseofulvum Dierckx

Colonies on CDA pale grey green, growth restricted (Plate 28); reverse pale dull yellow; penicilli loosely divergent with three to four stages of branching; conidiophores smooth, sinuate, 3.5-4 µm wide; phialides short, 4.5-6.5 µm long, crowded; conidia elliptical to subglobose, smooth, 2.5-3.2 µm in long axis, borne in divergent chains (Fig. 32).

Identification of this fungus was confirmed from Indian Type Culture Collection, New Delhi (Acc. No. 4276).

XIId. Penicillium aurantiogriseum Dierckx

Colonies on CDA dull blue green, with brighter

PLATE 27 : **Penicillium chrysogenum** Thom

a. Colonies on CDA after 10 days of incubation

b. conidiophore bearing conidia x 400

c. Conidiophore x 1000

d. Conidia x 1000.

PLATE 27



FIGURE 31 : Camera lucida drawings of <u>Penicillium chrysogenum</u> Thom

a. Mature conidiophores

b. Mature conidia.

FIGURE 31

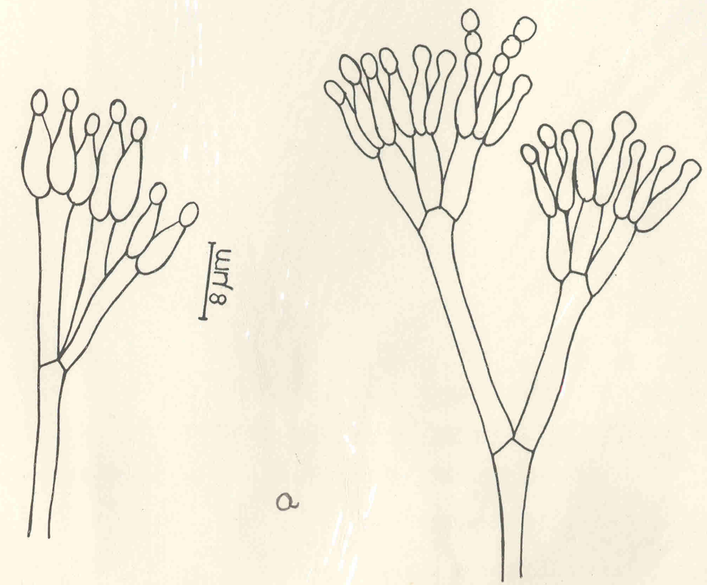


PLATE 28 : Penicillium griseofulvum Dierckx

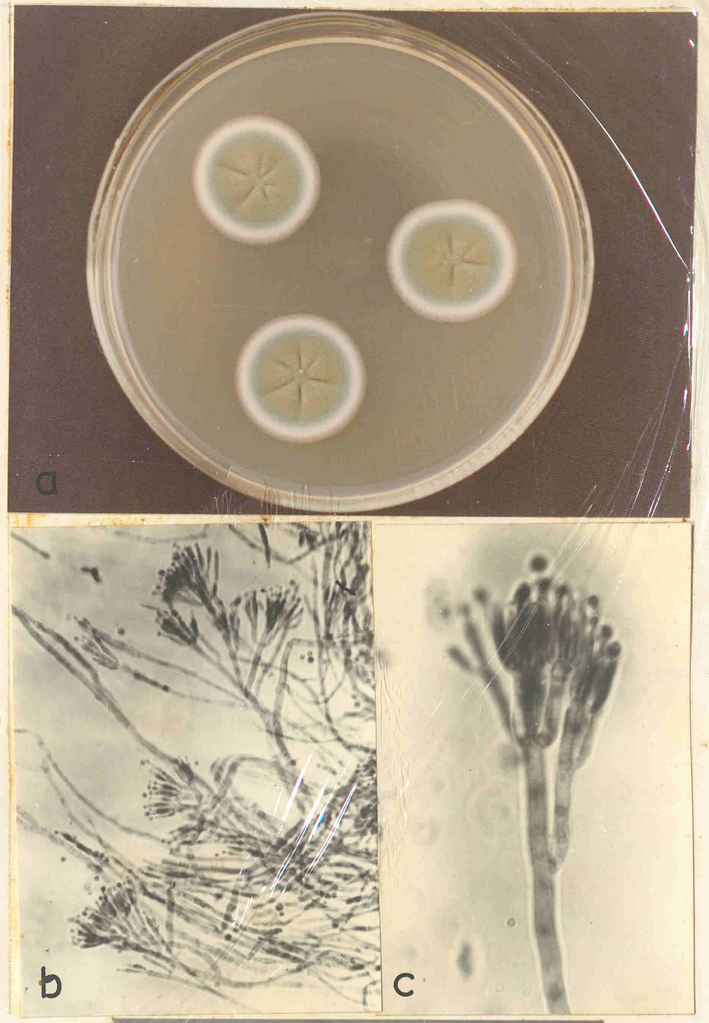
a. Colonies on CDA after 10 days of incubation

b. Conidiophores x 400

c. Conidiophores x 1000

d. Conidia x 1000

PLATE 28



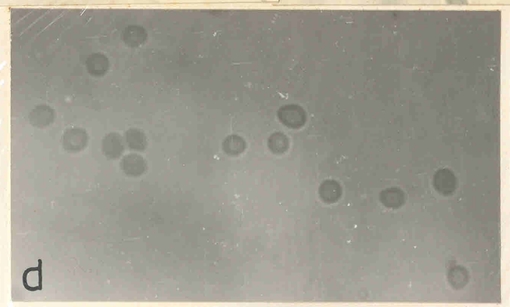
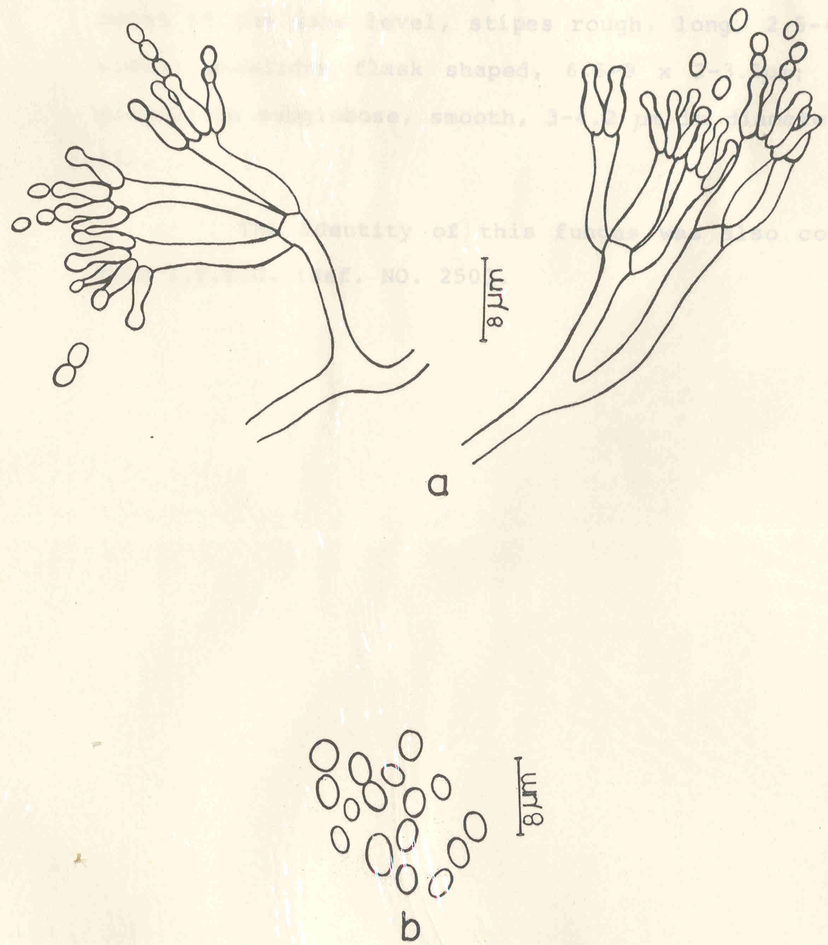


FIGURE 32 : Camera lucida drawings of Penicillium griseofulvum Dierckx

a. Mature conidiophores

b. Mature conidia.

FIGURE-32



zone inside the white margin, almost velvety, growth restricted (Plate 29), reverse pale; penicilli with two to three stages of branching, often with branches and metulae borne at the same level, stipes rough, long, 2.5-4 µm in width; phialides flask shaped, 6.5-9 x 2-3.5µm; conidia globose to subglobose, smooth, 3-4.2 µm in diameter (Fig. 33).

The identity of this fungus was also confirmed from I.T.C.C. (Ref. NO. 250).

FIGURE 33 : Camera lucida drawings of <u>Penicillium aurantiogriseum</u> Dierckx

a. Mature conidiophore

b. Mature conidia.

FIGURE -33

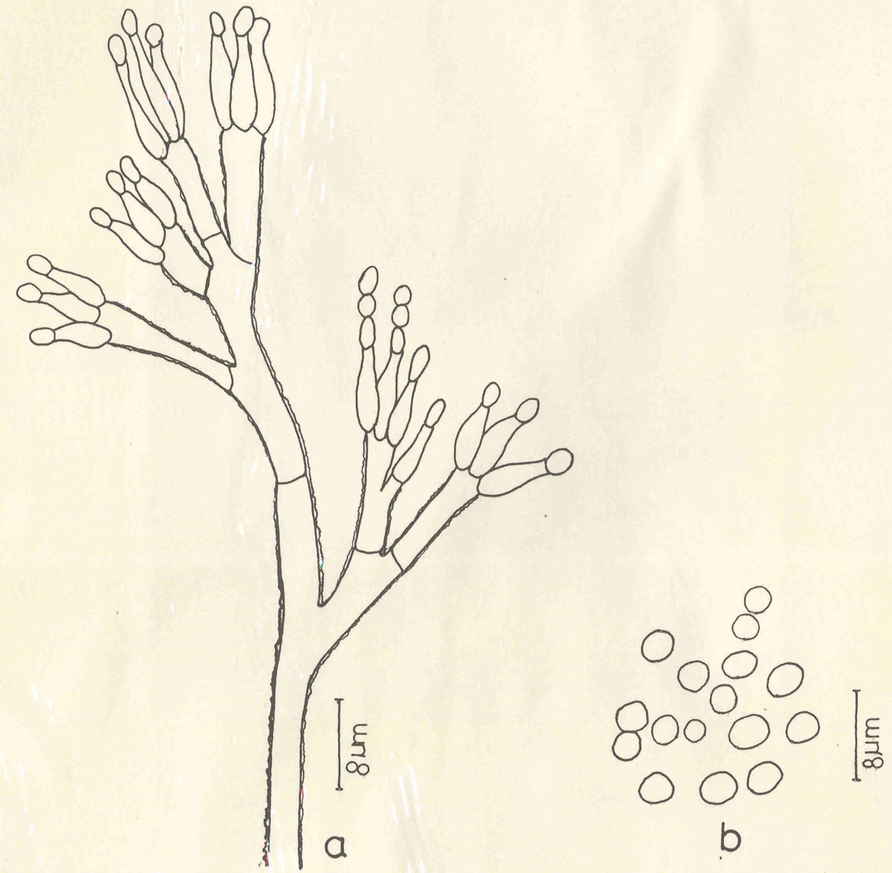


PLATE 29 : Penicillium aurantiogriseum Diercks

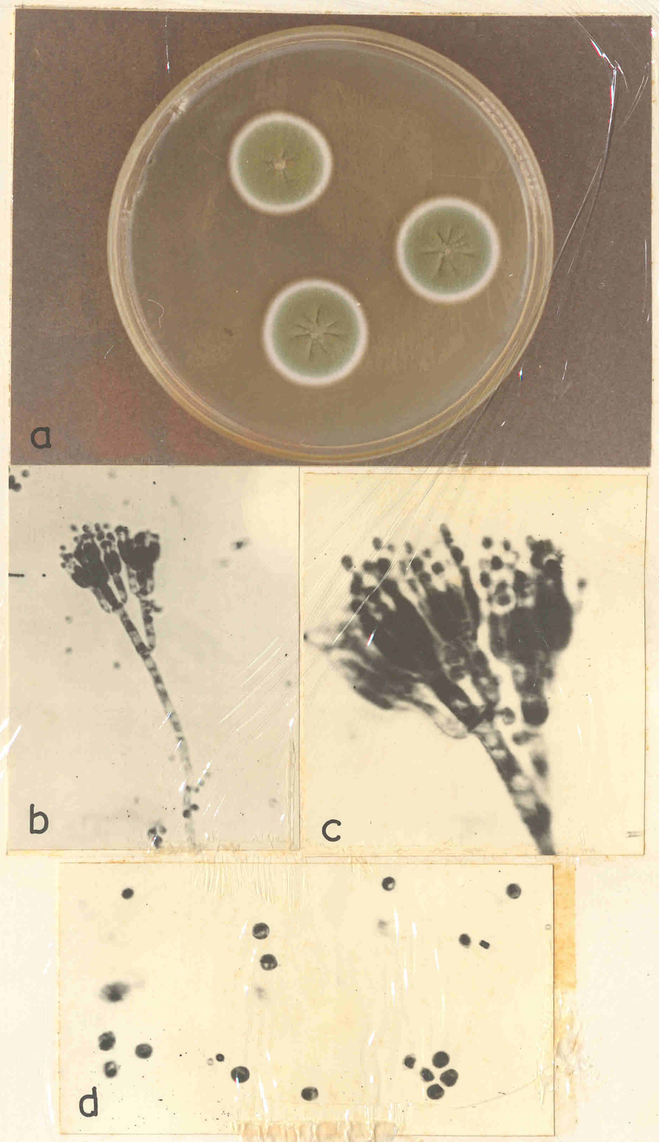
a. Colonies on CDA after 10 days of incubation

b. Conidiophore bearing conidia x 400

c. Conidiophore x 1000

d. Conidia x 1000

PLATE 29



INFLUENCE OF SOME ECOLOGICAL FACTORS ON THE OCCURRENCE OF POULTRY SOIL INHABITING KERATINOPHILES

Investigations were conducted to assess the ecologicalfactors governing the distribution and survival of keratinophilic fungi in the poultry farm soils and to discern correlation between them.

The soil characteristics of the investigated poultry farms depicted in Fig. 34 and listed in table 7 reveal that all the poultry farm soils were rich in humus (4.5-13.0%) and the occurrence of keratinophilic fungi was found to be generally directly proportional to the soil organic matter. This is proved by the fact that Maralia poultry farm soil which was richest in humus content (13%) elaborated maximum number of keratinophilic species (13), whereas, poultry farm soil of Ranbir singh Pura contained lowest humus content (4.5%) and proportionately only eight fungal species were recovered from it. Higher number of keratinophilic fungi in soils with high humus content has also been observed by other researchers (Chmel et al 1972; Ajello and Padhye, 1974; Chmel and Vlacilikova, 1975; Abdel-Fattah et al. 1982; Schonborn, 1983). Contradictory to these, distribution of few keratinophiles is reported to be irrespective of soil humus (Schonborn, 1968; Chmel et al. 1972; Ajello and Padhye, 1974; Muhammed

Table 7 : Characteristics of the poultry farm soils investigated

Poultry farms	pH/H_2O	Organic matter %	Organic carbon %	Nitrogen (Kjeldahl) %	C/N value	Phosphorus %	Potassium %	Magnesium %	Calcium %	Iron %	Total No.of fungi isolated	Fungal species isolated
Machlian	7.2	8.44	4.90	1.57	3.1	0.0026	0.024	1.00	2.11	1.68	8	Ck,Ct,Mm,Gv, Cm,Ac,Ao,Av,Pc
Prem Nagar	8.0	10.13	5.88	1.62	3.6	0.0019	0.024	0.50	1.62	1.82	8	Ck,Ct,Mm,Gv,Cm, Ac,Av,Pa,Pc,Pg.
Nihalpur Simbal	7.7	11.89	6.90	1.41	4.69	0.0022	0.023	1.08	3.80	1.47	10	Ck,Cq,Ct,Mf,Sb, Cm,Ad,Av,Pa,Pc.
Kothi Shahdaula	7.6	12.72	7.38	1.40	4.3	0.0025	0.023	0.53	1.76	1.96	12	Ct,Mf,Sb,Gv,Fm, Ac,Ad,Af,As,Av, Pc,Pg.
Akhnoor	7.2	11.99	6.96	1.56	6.3	0.0012	0.024	0.57	1.40	1.47	12	Ck,Ct,Mf,Sb,Mm, Cm,Fs,Ac,Af,Av, PC,Pg.
Belicharana	8.0	10.96	6.36	1.30	4.9	0.0016	0.024	0.21	0.51	1.61	8	Cq,Ct,Mm,Gv,Fm, Ac,Af,Av.
Muthi	7.6	11.06	6.42	1.81	3.5	0.0016	0.021	0.10	0.30	1.30	10	Cq,Ct,Cp,Sb,Gv, Fs,Ad,As,Av,Pc.
Nagbani	8.0	12.61	7.32	1.34	5.5	0.0031	0.023	0.50	1.02	1.40	8	Ct,Cp,Cm,Fm,Ad, Ao,Pa,Pg.
Maralia	7.5	13.03	7.56	1.70	5.4	0.0046	0.023	0.60	1.80	1.54	13	Ck,Ct,Cp,Mf,Sb, Gv,Fm,Ac,Ad,Af, Ao,As.Pc.
Ranbir Singh Pura	7.9	4.55	2.64	1.10	1.7	0.0033	0.023	0.41	0.87	2.10	8	Ck,Cq,Sb,Gv,Fs, Af,Ao,Pc.

Fungal species :
Ck - Chrysosporium keratinophilum
Cq - C. queenslandicum
Ct - C. tropicum
Cp - C. pannorum
Mf - Malbranchea flava
Gv - Gliocladium virens
Cm - Circinella muscae
Sb - Scopulariopsis brevicaulis
Mm - Microascus manganii
Fm - Fusarium moniliforme
Fs - F. solani
Pc - Penicillium citrinum
Pg - P. griseofulvum
Pa - P. aurantiogriseum
Af - Aspergillus flavus
Ad - A. deflectus
Ao - A. ochraceus
Av - A. versicolor
As - A. sydowii
Ac - A. candidus

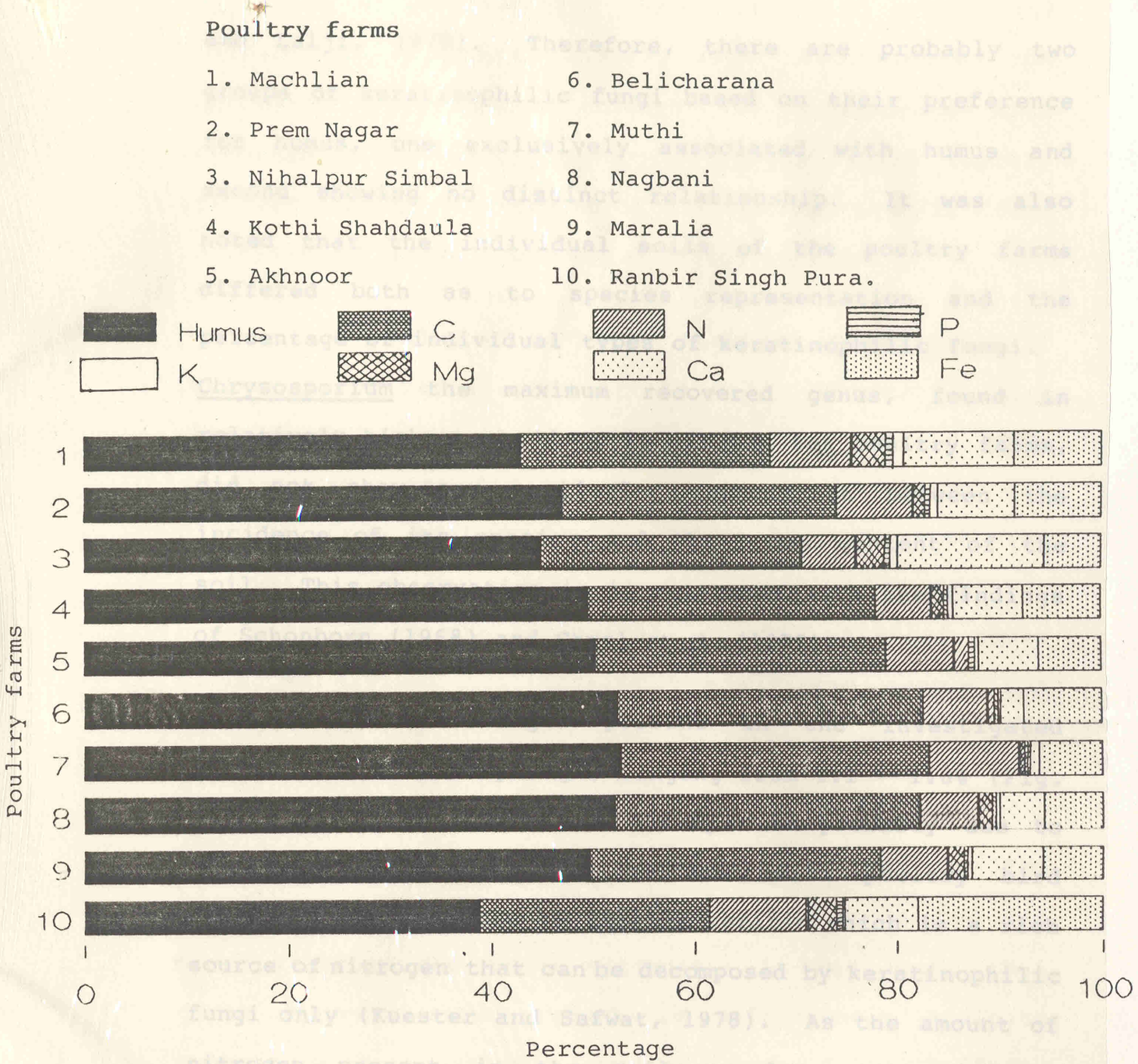


FIGURE 34 : Characteristics of poultry farm soils.

and Lalji, 1978). Therefore, there are probably two groups of keratinophilic fungi based on their preference for humus, one exclusively associated with humus and second showing no distinct relationship. It was also noted that the individual soils of the poultry farms differed both as to species representation and the percentage of individual types of keratinophilic fungi. Chrysosporium the maximum recovered genus, found in relatively high proportions in all the ten poultry farms, did not show any particular association between the incidence of its species and the humus content of the soil. This observation is in agreement with the findings of Schonborn (1968) and Chmel et al. (1972).

Total nitrogen present in the investigated samples was also very high, ranging from 1.1 - 1.8% (Fig. 34). This high content of nitrogen is probably due to continuous enrichment of the soil with poultry bird droppings and also due to feather keratin which is a rich source of nitrogen that can be decomposed by keratinophilic fungi only (Kuester and Safwat, 1978). As the amount of nitrogen present in the soil samples was not much variable, correlation between nitrogen content and distribution of individual genera could not be established. However, recovery of 8-13 keratinophilic species from each poultry farm, itself proves the

importance of nitrogen and carbon in their growth and distribution. Kunert (1982), also established the importance of nitrogen through his experiments with keratinophilic fungus Microsporum gypseum which rapidly utilized carbon and nitrogen of L - cystine, that is found in relatively large proportions in the keratin.

The ratio of carbon to nitrogen in the soils of poultry farms varied from 1.7 to 6.3. However, a close correlation between the occurrence of keratinophilic fungi and the C/N ratio did not emerge as the values of C/N differed from soil to soil (Table 7). As with the observation of Mercantini et al. (1980), no specific conclusion about the biological activity of these soils could be arrived at, because the nitrogen supply was partly from the decomposition of organic residue and partly from poultry bird excrements.

Poultry farm soils were also analysed for the presence of macro (phoshorus, potassium and magnesium) and micro (calcium and iron) elements. It was found that these elements were present in sufficiently adequate amounts with phosphorus ranging from 0.0012-0.0046%, potassium 0.021-0.024%, magnesium 0.10-1.08%, calcium 1.02-3.8% and iron 1.3-2.1%. These elements favoured distribution and luxuriant growth of the keratinophilic

fungi by playing a vital role in their structural and metabolic processes.

Data on the pH of these soils revealed that it varied from 7.2-8.0 (Table 7). These results show that the poultry soils were nearly neutral to weakly alkaline and favoured the prevalence of keratinophilic fungi as all the soil samples were positive for these fungi. A similar range of pH proved to be the most favourable for excretion of proteolytic enzymes (keratinase) by these fungi which is an indispensable condition for their growth on insoluble substrate like keratin. Therefore, it is probable that presence of keratinophilic fungi in alkaline soils may be due to activation of their enzyme activities under these conditions. On this basis, all the keratinophilic fungi recovered in the present investigation can be categorized as alkalophilic. These results are in agreement with the findings of Pugh and Mathison (1962), Bohme and Ziegler (1965) and Hubalek (1974) but in contradiction to Muhammad and Lalji (1978) who found that in Kenyan soils keratinophilic fungi were favoured by acidic conditions. Very acid soils are known to inhibit fungi even if organic matter is abundant, but weakly acid to weakly alkaline soils are optimal for keratinophilic fungi regardless of organic content

(Blaschke-Hellmessen, 1969; Bohme and Ziegler, 1968).

Therefore, it may be concluded from the present investigation that some ecological factors of soil like weakly alkaline pH and presence of high organic matter along with minerals in optimum concentrations influence the occurrence of increased number of soil keratinophiles. However, it is difficult to determine the order of importance of these factors as they are oftenly very closely related and show a collective effect.

As Chrysosporium species formed the bulk of keratinophiles isolated during the survey, experiments were also conducted to evaluate temperature, relative humidity and best pH for their optimum mycelial growth. It is evident from table 8 that all the species of Chrysosporium isolated during the survey could grow at relative humidity varying from 30-100% except for C. pannorum and C. indicum which could grow only at relative humidity varying from 30 to 80%. The radial growth of all the species increased gradually with an increase in the relative humidity, attaining maximum diameter at 60% R.H., beyond which it showed a gradual reduction (Fig. 35). Growth of all the tested Chrysosporium species at high relative humidity indicates their hygrophilic nature.

Table 8 : Effect of relative humidity on the mycelial growth of *Chrysosporium* species at different incubation periods

FUNGAL SPECIES	Incubation period in days	Colony diameter in mm at different RH									
		0%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Chrysosporium keratinophilum	3	*	*	*	*	5.0	5.0	5.0	6.0	*	*
	6	*	*	5.0	5.0	9.0	9.0	10.0	8.0	5.0	5.0
	9	*	*	6.0	7.0	15.0	19.0	15.0	12.0	8.0	7.0
	12	*	*	7.0	12.0	25.0	28.0	24.0	20.0	10.0	9.0
C.queenslandicum	3	*	*	*	*	7.0	8.0	9.0	6.0	*	*
	6	*	*	5.0	6.0	15.0	18.0	12.0	8.0	*	*
	9	*	*	7.0	10.0	25.0	24.0	19.0	10.0	7.0	7.0
	12	*	*	9.0	12.0	30.0	35.0	32.0	15.0	10.0	9.0
C. tropicum strain I	3	*	*	*	*	6.0	6.0	5.0	5.0	*	*
	6	*	*	5.0	6.0	15.0	17.0	10.0	8.0	*	*
	9	*	*	7.0	10.0	28.0	29.0	15.0	10.0	6.0	6.0
	12	*	*	10.0	15.0	35.0	38.0	19.0	15.0	10.0	9.0
C. tropicm strain II	3	*	*	*	*	6.0	6.0	5.0	5.0	*	*
	6	*	*	6.0	7.0	17.0	19.0	12.0	8.0	*	*
	9	*	*	7.0	12.0	29.0	31.0	15.0	11.0	6.0	6.0
	12	*	*	11.0	17.0	36.0	39.0	20.0	18.0	11.0	9.0
C. pannorum	3	*	*	*	5.0	6.0	7.0	7.0	5.0	*	*
	6	*	*	5.0	10.0	14.0	14.0	13.0	7.0	*	*
	9	*	*	7.0	11.0	17.0	18.0	15.0	9.0	*	*
	12	*	*	10.0	13.0	20.0	23.0	20.0	12.0	*	*
C. indicum	3	*	*	*	5.0	6.0	8.0	8.0	5.0	*	*
	6	*	*	5.0	11.0	14.0	15.0	12.0	7.0	*	*
	9	*	*	6.0	12.0	16.0	17.0	14.0	9.0	*	*
	12	*	*	9.0	13.0	18.0	20.0	17.0	11.0	*	*

* No growth.

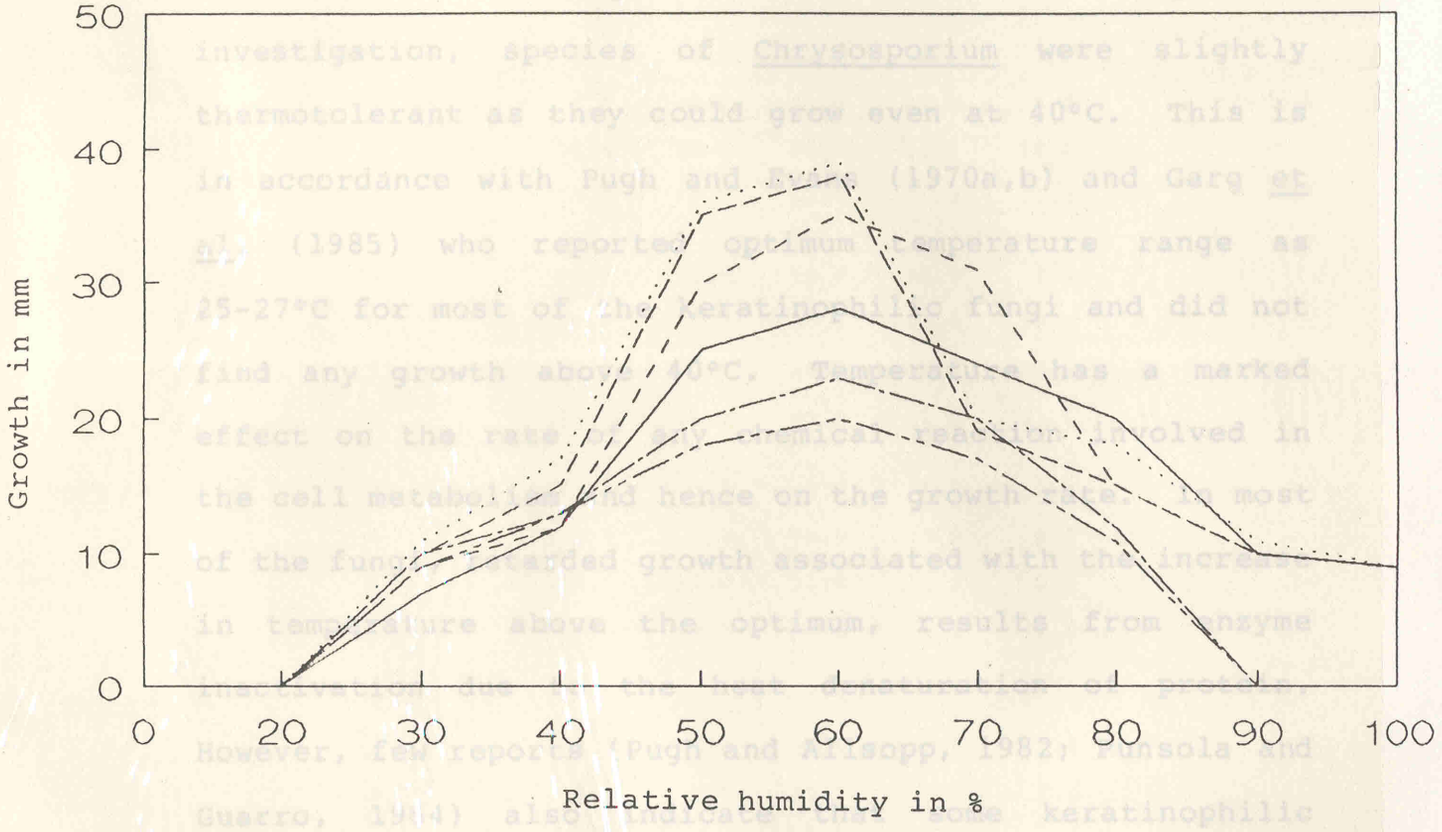


FIGURE 35 : Mycelial growth at different RH.

Hubalek and Balat (1976) also found C. keratinophilum to be hygrophilic.

Data on the temperature (Table 9), reveals that all the species of Chrysosporium could grow between temperatures 20-40°C, whereas no growth was observed at 45°C. Maximum radial growth of the Chrysosporium species occurred between 25 and 30°C, beyond which it reduced gradually (Fig. 36). Keratinophilic fungi are generally considered to be mesophilic in nature but in the present investigation, species of Chrysosporium were slightly thermotolerant as they could grow even at 40°C. This is in accordance with Pugh and Evans (1970a,b) and Garg et al. (1985) who reported optimum temperature range as 25-27°C for most of the keratinophilic fungi and did not find any growth above 40°C. Temperature has a marked effect on the rate of any chemical reaction involved in the cell metabolism and hence on the growth rate. In most of the fungi, retarded growth associated with the increase in temperature above the optimum, results from enzyme inactivation due to the heat denaturation of protein. However, few reports (Pugh and Allsopp, 1982; Punsola and Guarro, 1984) also indicate that some keratinophilic strains can adapt themselves to adverse temperatures for their survival.

Table 9 : Effect of temperature on the mycelial growth of *Chrysosporium* species at different incubation periods

FUNGAL SPECIES	Incubation period in days	Colony diameter in mm at different temperatures					
		20°c	25°c	30°c	35°c	40°c	45°c
Chrysosporium keratinophilum	3	*	*	11.0	10.0	6.0	*
	6	6.0	5.5	28.0	15.0	8.0	*
	9	8.0	8.0	35.0	25.5	12.0	*
	12	11.0	12.0	46.0	30.0	14.0	*
C. queenslandicum	3	5.5	6.0	13.5	12.0	8.0	*
	6	15.0	10.0	18.5	14.0	9.5	*
	9	18.0	15.0	30.0	21.0	12.0	*
	12	20.0	24.0	40.0	28.0	15.0	*
C. tropicum Strain I	3	*	6.0	10.0	5.5	7.0	*
	6	6.5	8.0	30.0	25.5	18.0	*
	9	10.0	11.0	35.0	28.0	28.0	*
	12	12.0	13.0	41.0	31.0	30.0	*
C. tropicum Strain II	3	*	5.5	13.0	10.0	7.5	*
	6	10.0	8.0	31.0	28.0	15.0	*
	9	12.0	10.0	38.0	30.0	25.0	*
	12	14.0	14.0	43.0	31.0	31.0	*
C. pannorum	3	*	5.5	8.0	7.0	5.5	*
	6	6.5	7.0	10.0	9.0	8.0	*
	9	10.0	11.0	14.0	12.0	9.5	*
	12	12.0	13.0	24.0	15.0	12.0	*
C. indicium	3	*	*	7.0	6.0	*	*
	6	5.5	6.0	10.0	8.0	5.5	*
	9	7.0	9.0	12.0	9.0	6.0	*
	12	11.0	12.0	20.0	11.0	10.0	*

* No Growth

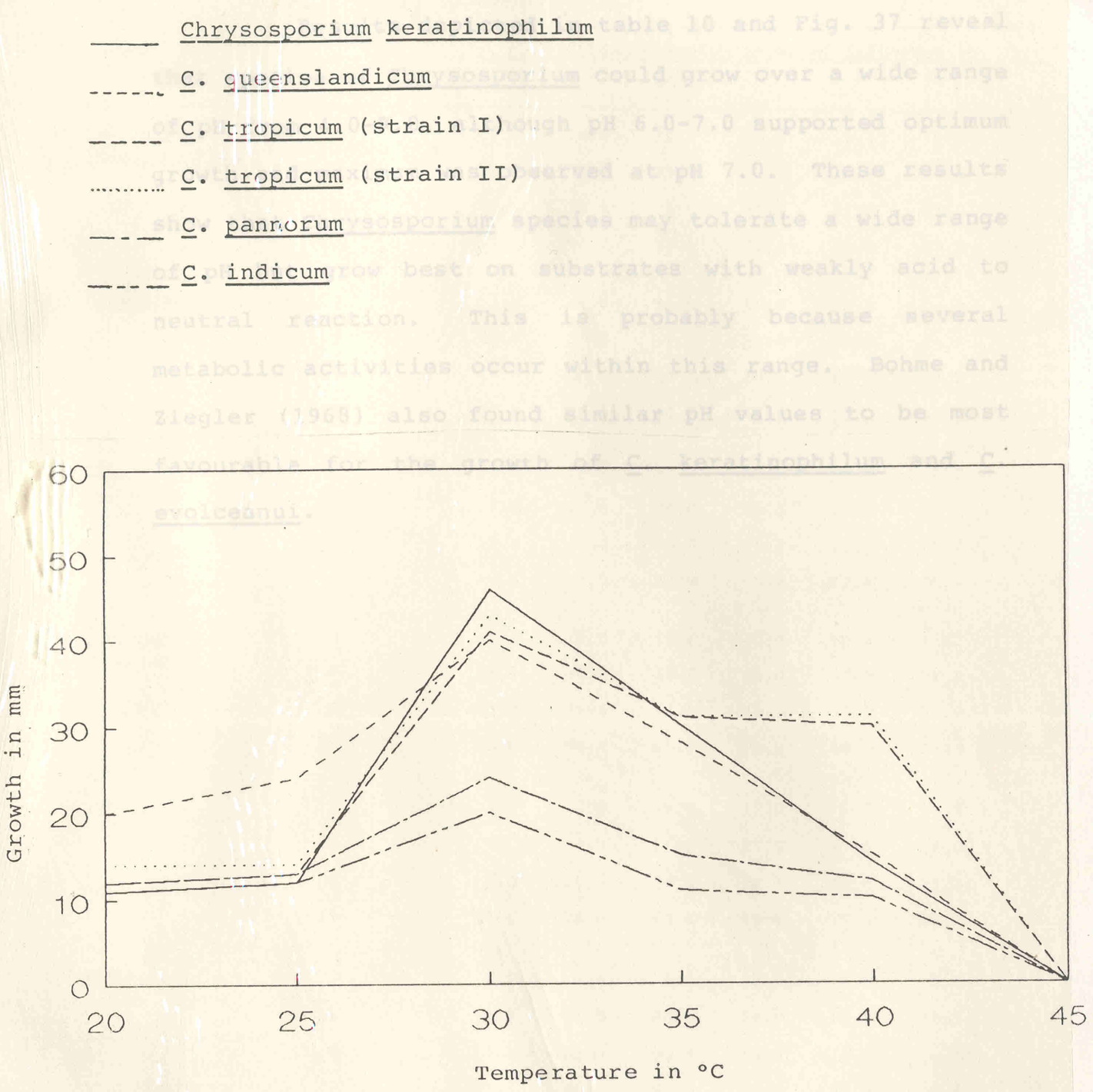


FIGURE 36 : Mycelial growth in relation to different temperatures.

Results depicted in table 10 and Fig. 37 reveal that species of Chrysosporium could grow over a wide range of pH from 4.0-9.0, although pH 6.0-7.0 supported optimum growth and maximum was observed at pH 7.0. These results show that Chrysosporium species may tolerate a wide range of pH but grow best on substrates with weakly acid to neutral reaction. This is probably because several metabolic activities occur within this range. Bohme and Ziegler (1968) also found similar pH values to be most favourable for the growth of C. keratinophilum and C. evolceanui.

Table 10 : Effect of pH on the mycelial growth of *Chrysosporium* species at different incubation periods

FUNGAL SPECIES	Incubation period in days	Colony diameter in mm at different pH					
		4.0	5.0	6.0	7.0	8.0	9.0
Chrysosporium keratinophilum	3	6.0	10.0	14.0	15.0	6.0	6.0
	6	20.0	31.0	36.0	39.0	20.0	20.0
	9	35.0	38.0	38.0	45.0	25.0	22.0
	12	55.0	64.0	68.0	70.0	38.0	32.0
C. queenslandicum	3	12.0	14.0	15.0	15.0	11.0	9.0
	6	30.0	35.0	37.0	37.0	28.0	24.0
	9	35.0	38.0	39.0	43.0	40.0	41.0
	12	50.0	55.0	60.0	65.0	60.0	53.0
C. tropicum Strain I	3	5.5	13.0	13.0	14.0	6.0	5.0
	6	17.0	27.0	28.0	30.0	20.0	15.0
	9	30.0	42.0	40.0	45.0	21.0	20.0
	12	50.0	70.0	75.0	75.0	43.0	40.0
C. tropicum Strain II	3	7.0	13.0	14.0	17.0	10.0	6.0
	6	23.0	30.0	32.0	34.0	27.0	21.0
	9	35.0	35.0	38.0	45.0	30.0	26.0
	12	53.0	62.0	68.0	70.0	60.0	48.0
C. pannorum	3	5.0	7.0	10.0	12.0	5.0	5.0
	6	17.0	24.0	26.0	27.0	21.0	18.0
	9	18.0	25.0	28.0	28.0	22.0	23.0
	12	20.0	26.0	40.0	40.0	25.0	24.0
C. indicum	3	5.0	5.0	8.0	8.0	6.5	6.0
	6	10.0	21.0	24.0	24.9	16.0	13.0
	9	12.0	25.0	35.0	38.0	20.0	18.0
	12	15.0	40.0	61.0	64.0	25.0	20.0

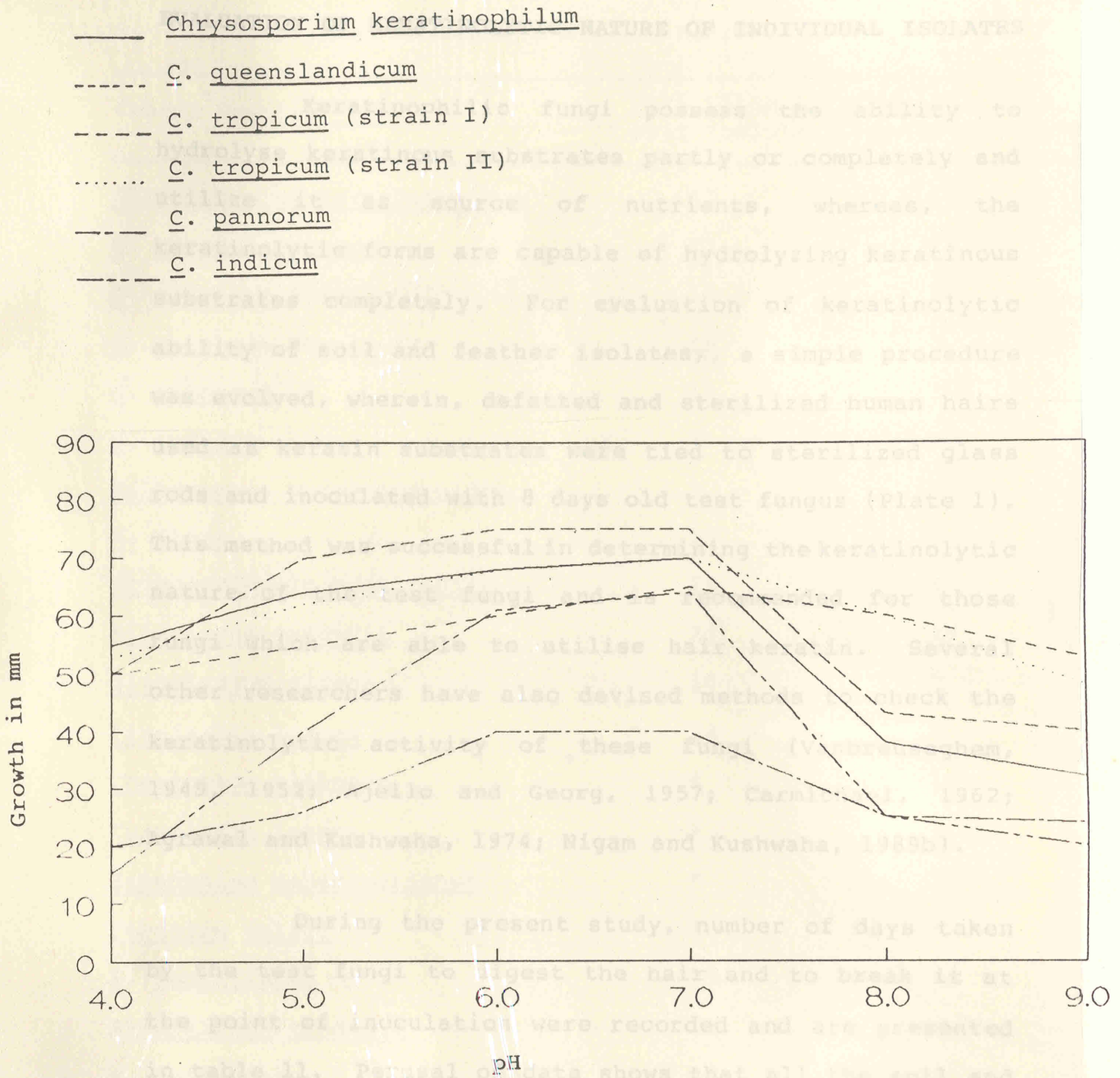


Figure 37 : Mycelial growth at different pH.

EVALUATION OF KERATINOLYTIC NATURE OF INDIVIDUAL ISOLATES

Keratinophilic fungi possess the ability to hydrolyse keratinous substrates partly or completely and utilize it as source of nutrients, whereas, the keratinolytic forms are capable of hydrolyzing keratinous substrates completely. For evaluation of keratinolytic ability of soil and feather isolates, a simple procedure was evolved, wherein, defatted and sterilized human hairs used as keratin substrates were tied to sterilized glass rods and inoculated with 8 days old test fungus (Plate 1). This method was successful in determining the keratinolytic nature of the test fungi and is recommended for those fungi which are able to utilise hair keratin. Several other researchers have also devised methods to check the keratinolytic activity of these fungi (Vanbreuseghem, 1949, 1952; Ajello and Georg, 1957; Carmichael, 1962; Agrawal and Kushwaha, 1974; Nigam and Kushwaha, 1989b).

During the present study, number of days taken by the test fungi to digest the hair and to break it at the point of inoculation were recorded and are presented in table 11. Perusal of data shows that all the soil and feather isolates were able to break the hair at the point of inoculation indicating, thereby, complete digestion of

Table 11 : Number of days taken by different keratinophilic fungi to break the human hair

Fungal Species	Days taken to break the hair	
	Soil isolate	Feather isolate
Chrysosporium keratinophilum	11	12
C. queenslandicum	17	19
C. tropicum Strain I	15	16
C. tropicum Strain II	18	*
C. pannorum	14	*
C. indicum	*	15
Malbranchea chrysosporoidea	*	18
M. flava	15	*
Scopulariopsis brevicaulis	20	18
Microascus manganii	22	24
Gliocladium virens	16	*
Circinella muscae	24	*
Chaetomium globosum	*	18
Curvularia lunata	*	20
Sepedonium maheshwarianum	*	30
Fusarium solani	28	*
F. moniliforme	24	26
Aspergillus candidus	20	*
A. deflectus	23	*
A. flavus	28	29
A. ochraceus	20	*
A. sydowii	25	*

Fungal Species	Days taken to break the hair	
	Soil isolate	Feather isolate
A. versicolor	23	*
A. nidulans	*	34
Penicillium aurantiogriseum	25	*
P. citrinum	28	*
P. chrysogenum	*	35
P. griseofulvum	27	*

* not present

hair keratin at the site. It was interesting to observe that the time taken for complete digestion of hair varied not only for different species of the same genus, but also for different isolates of the same species depending upon its degree of keratinolytic ability (Fig. 38). Comparatively early digestion of hair was brought about by Chrysosporium species, whereas, most of the Penicillium and Aspergillus species took longer time in completely hydrolyzing the hair keratin.

Among the various keratinophilic fungi tested, both the soil and feather isolates of Chrysosporium keratinophilum were found to be highly keratinolytic in comparison to others. The order of soil isolates with respect to the number of days taken to digest the hair keratin was :

Chrysosporium keratinophilum (11d) < C. pannorum (14d)<C. tropicum strain I and Malbranchea flava (15d)<Gliocladium virens (16d) < C. queenslandicum (17d) < C. tropicum Strain II (18d) < Scopulariopsis brevicaulis, Aspergillus ochraceus and A. candidus (20d) < Microascus manganii (22d) < A. deflectus and A. versicolor (23d) < Circinella muscae and Fusarium moniliforme (24d) < Aspergillus sydowii and Penicillium aurantiogriseum (25d) < P. griseofulvum (27d) < P. citrinum, Fusarium solani and Aspergillus flavus (28d).

Fig-38

Keratinophilic isolates

a. Chrysosporium keratinophilum
b. C. queenslandicum
c. C. tropicum (strain I)
d. Malbranchea chrysosporoidea
e. Malbranchea flava
f. Scopulariopsis brevicaulis
g. Microascus manganii
h. Gliocladium virens
i. Circinella muscae
j. Chaetomium globosum
k. Curvularia lunata
l. Chrysosporium tropicum (Strain II)
m. C. pannorum
n. C. indicum
o. Sepedonium maheshwarianum
p. Fusarium solani
q. F. moniliforme
r. Aspergillus candidus
s. A. deflectus
t. A. flavus
u. A. ochraceus
v. A. sydowii
w. A. versicolor
x. A. nidulans
y. Penicillium aurantiogriseum
z. P. citrinum
a'. P. chrysogenum
b' P. griseofulvum

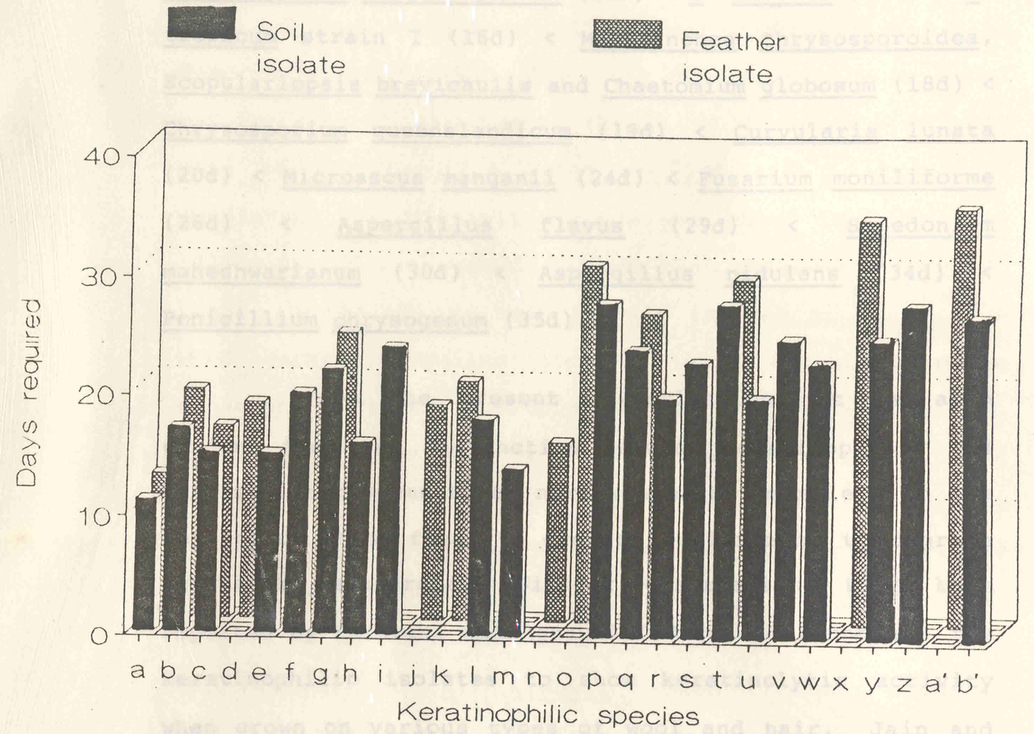


Figure 38 : No. of days taken by different keratinophilic isolates to break human hair.

In case of feather isolates, the order with respect to the number of days taken to completely hydrolyse the hair keratin was : Chrysosporium keratinophilum (12d) < C. indicum (15d) < C. tropicum strain I (16d) < Malbranchea chrysosporoidea, Scopulariopsis brevicaulis and Chaetomium globosum (18d) < Chrysosporium queenslandicum (19d) < Curvularia lunata (20d) < Microascus manganii (24d) < Fusarium moniliforme (26d) < Aspergillus flavus (29d) < Sepedonium maheshwarianum (30d) < Aspergillus nidulans (34d) < Penicillium chrysogenum (35d).

From the present investigation, it is also evident that the distinction between keratinophilic and keratinolytic seems to be narrow when based solely on the ability of these fungi to grow on, utilize or to degrade keratinous substrate. Similar observations have been reported by Kushwaha and Agrawal (1976b)who found all the keratinophilic isolates to show keratinolytic activity when grown on various types of wool and hair. Jain and Agrawal (1977) also found all the keratinophilic isolates excepting one to be keratinolytic in nature.

Microscopic examinations of hair samples attacked by keratinolytic moulds revealed extensive damage

including rupturing and blistering of hair cuticle (Plate 30). A characteristic effect of Scopulariopsis brevicaulis on human hair was the lifting of the scales of the cuticle to such an extent that the hair eventually gave a frayed appearance (Plate 30a,b). Fraying has also been noted by English (1965) in non-keratinolytic fungi and by Pinetti and Lostia (1966) in dermatophytes. In case of Chrysosporium keratinophilum and C. tropicum, undulation of the hair cuticle was noted in side view (Plate 30 c,d & e), which has not been observed with other fungi. Parts of the hair heavily invaded by the fungus were markedly swollen compared with untouched parts adjacent to them, possibly because the volume of the invading mycelium and spores was greater than that of the keratin it had replaced. Such swellings of hair at heavily invaded sites were also recorded by English (1976) while studying destruction of hair by C. keratinophilum. Cuticle of the hair was also found to be susceptible to the attack of A. sydowii which formed abundant conidiophores (Plate 30f). However, only few ruptures were noted in the cuticle and the damage of the cortex occurred very slowly. This is in accordance with the finding that Aspergillus species take longer time to digest and break the hair at inoculation point. The extent of structural damage of hair during the growth of

PLATE 30 : Degradation of human hair

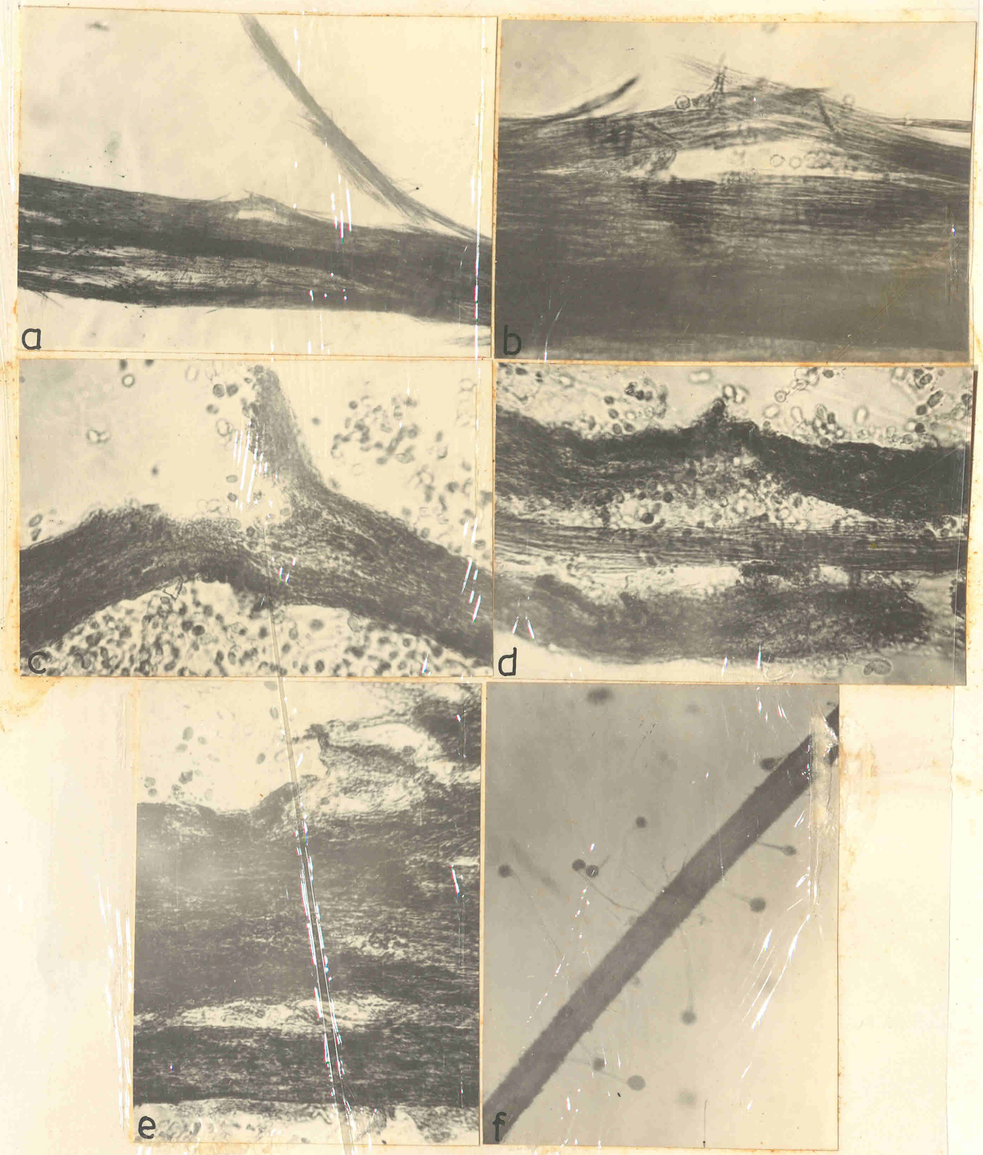
a,b. Showing lifting of cuticle by **Scopulariopsis brevicaulis** (a x 400; b x 1000)

c,d. Showing undulation of cuticle by **Chrysosporium keratinophilum** (c x 400; d x 1000)

e. Showing extensive disruption by **C. tropicum** and presence of its spores within the shaft (x 1000)

f. Showing conidiophores of **Aspergillus sydowii** arising from the slightly damaged shaft (x 100).

PLATE 30



these fungi is probably dependent on their ability to produce keratinase - a biochemical tool for keratin utilization.

PREFERENTIAL UTILIZATION OF KERATIN BY POULTRY SOIL AND FEATHER ISOLATES

Five different keratin substrates (human hair, horse hair, human nails, peacock feathers and poultry bird feathers) were used to assess preferential utilization and colonization of keratin by the isolated keratinophilic isolates. Visual observations were recorded after 20 days of incubation at 28±2°C and are presented in table 12.

A perusal of the data revealed differential colonising ability of the isolated keratinophiles on various keratinous substrates (Plates 31 & 32). It was noteworthy to observe that all the isolates grew on poultry feathers, peacock feathers and human hair, although with variable degree of colonization, whereas, only 17 and 14 species could colonize horse hair and human nails respectively (table 12). Similar observations have also been reported by Jain et al. (1985) who found nail as a poor bait in comparison to other keratinous substrates. These differences in the degree of colonization of different baits may be attributed to the soft and hard keratins that make up their composition. The ability of keratinophilic fungi to hydrolyse keratinous substances in nature makes them easy to invade the superficial tissues

Table 12 : Colonization of Keratinophilic fungi on different keratin substrates

Fungal Species	Baits used				
	Poultry feathers	Peacock feathers	Human hair	Horse hair	Human nails
Chrysosporium keratinophilum	+++	+++	+++	+++	+++
C. queenslandicum	+++	+++	+++	+++	+++
C. tropicum Strain I	+++	+++	+++	+++	+++
C. tropicum Strain II	+++	+++	+++	+++	+++
C. pannorum	++	++	+++	+++	+++
C. indicum	++	++	+++	+++	+++
Malbranchea flava	++	+++	+	+++	++
M. chrysosporoidea	+++	+++	+	++	+++
Scopulariopsis brevicaulis	+++	++	++	++	++
Microascus manganii	++	++	+	+	-
Gliocladium virens	++	+	+	++	++
Circinella muscae	++	+	+	-	-
Sepedonium maheshwarianum	+++	++	++	-	-
Chaetomium globosum	++	++	+	+	-
Curvularia lunata	+++	+++	++	+	-
Fusarium moniliforme	++	++	++	-	-
F. solani	++	++	+	-	-
Aspergillus candidus	++	+	+	+	+
A. flavus	++	++	+	+	++
A. ochraceus	++	+	+	+	++
A. sydowii	+	++	+	+	-

(Contd....

Fungal Species	Baits used				
	Poultry feathers	Peacock feathers	Human hair	Horse hair	Human nails
A. deflectus	++	+	+	-	-
A. versicolor	+	+	+	-	+
A. nidulans	+	+	++	++	-
Penicillium citrinum	+	++	+	-	+
P. chrysogenum	+	+	++	-	-
P. aurantiogriseum	+	+	+	-	-
P. griseofulvum	+	+	+	-	-
Total number of fungal species colonizing baits	27	27	27	17	14

Visual observation of growth :

+++ = excellent

++ = medium

+ = slow

- = no growth.

of man and animals. Although seldom involved in dermatophytoses they have the potential for it, as one can see natural evolution from keratin utilizing soil saprophytes to association and finally invasion of cornified substrate in living animals and man (Rippon, 1982).

In the present investigation, substrate specificity was found to be variable within the species of the same genus also. Among the isolated Chrysosporium species, C. keratinophilum, C. queenslandicum (Plate 31) and both the strains of C. tropicum showed luxuriant growth and maximum degradation of all the keratinous baits used, whereas, C. pannorum and C. indicum grew equally well on horse hair, human hair and human nails but showed moderate growth on feathers of peacock and poultry birds. The colonizing ability of Chrysosporium species on all the keratinous substrates also confirms the cosmopolitan nature of this genus. Since all the Chrysosporium species were observed to be growing either luxuriantly or moderately on various keratin residues, it is probable that they possess specific enzymatic system for degradation of keratin and it is, therefore, important to recognize their potential as pathogens. Chabasse et al. (1989) confirmed the pathogenic potential of C.

keratinophilum by testing it against guinea pig hair, which suggested its preference for keratin substrates of animal origin and its zoophilic nature. Earlier, Kushwaha (1983) also established maximum degradation of peacock feathers by C. tropicum and C. indicum. Moreover, these fungi by virtue of their ability to colonize various keratinous substrates and degrade them, also add to the carbon and nitrogen content of the soil.

Malbranchea chrysosporoidea and M. flava also showed excellent to moderate growth on all the keratin substrates except for human hair on which it grew slowly (Plate 32). Similarly, Scopulariopsis brevicaulis showed maximum preference for poultry feathers and moderate preference for the remaining keratin substrates; Sepedonium maheshwarianum grew luxuriantly on poultry feathers, moderately on peacock feathers and human hair but no growth was observed on horse hair and human nails; Curvularia lunata also showed preference for poultry and peacock feathers but no growth occurred on human nails. These results are in accordance with the findings of other workers (Pugh, 1971; Jain and Agrawal, 1980; Ali- Shtayeh, 1989), who also reported maximum degradation of birds feathers by some keratinophiles. The ability of all these fungi to hydrolyse feather keratin more

PLATE 31 : Differential colonising ability of

a. Chrysosporium keratinophilum

b. C. queenslandicum

c. Gliocladium virens.

PLATE 31

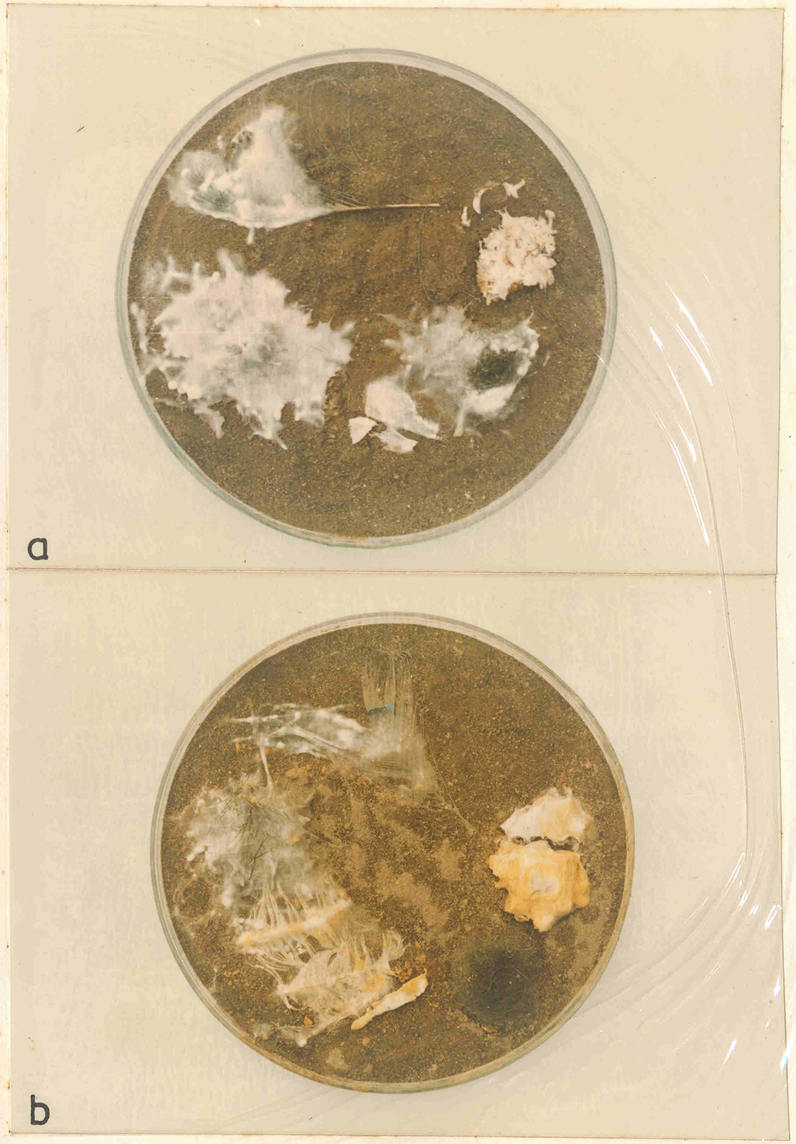


PLATE 32 : Differential colonising ability of

a. Malbranchea flava

b. M. chrysosporoidea.

PLATE 32



efficiently suggests their potential pathogenecity in birds although, so far, they have not been proved to be pathogenic.

Among the other keratinophilic fungi viz. Microascus manganii, Gliocladium virens, Circinella muscae, Fusarium spp, Penicillium spp. and Aspergillus spp, few were noted to be moderate colonizers, whereas, most of them grew very slowly on the tested keratin substrates. This may be due to low keratinase producing capabilities of individual fungi or due to the biochemical differences of the keratin present in various ectodermal appendages.

INVITRO DEGRADATION OF KERATIN BY DIFFERENT KERATINOPHILIC ISOLATES.

The results presented in tables 13 and 14 reveal that all the keratinophilic isolates tested could grow on human hair and degrade it, releasing sulphydryl containing compounds detected as cysteine, keratinase and total proteins. This finding closely corroborates to that of Weary et al. (1965) who reported that evidence of keratinolysis lies on the ability of fungi to release soluble sulphur containing aminoacids and polypeptides into the medium in quantities significantly greater than those released by controls. It was also noted that the release of cysteine, keratinase and proteins by soil isolates was slightly more than the releases by their corresponding feathers isolates.

Comparison of the data obtained from two different culture media showed that the glucose supplemented medium B supported maximum release of cysteine, protein and keratinase after the experimental period of 40 days incubation. Among the various carbon sources utilised, keratinophilic fungi are known to show preference for carbohydrates and exhibit stimulatory effect of glucose in inducing maximum enzyme production

(Jonsson, 1968; Ong and Gaucher, 1973; Singh, 1979; Singh, 1981; Singh and Agrawal, 1987a,b; Singh and Saksena, 1988; Singh, 1993).

Release of cysteine from the keratin :

The most distinctive character of keratin is its high cysteine content. The disulfide linkage between the amino acids give additional strength to keratin molecule, rendering it more resistant to enzymatic digestion.

In the present investigation, release of cysteine from human hair keratin was estimated for all the keratinophilic isolates obtained from poultry farm soils & poultry bird feathers. Among the soil isolates maximum cysteine was released in glucose supplemented medium by Chrysosporium tropicum strain I (28 µg/ml) followed by C. keratinophilum (24 µg/ml), C. pannorum, C. queenslandicum and Malbranchea flava (21 µg/ml), C. tropicum strain II (20 µg/ml), Scopulariopsis brevicaulis (18 µg/ml), Microascus manganii (15 µg/ml), Gliocladium virens, Circinella muscae and Aspergillus deflectus (12 µg/ml), Fusarium moniliforme, A. ochraceus and A. versicolor (11 µg/ml), F. solani, A. flavus, A. candidus, P. aurantiogriseum and P. citrinum (10 µg/ml), P.

griseofulvum (9 µg/ml) as shown in table 13.

Similar results were obtained for poultry feather isolates (table 14), where maximum cysteine was released in culture medium B by Chrysosporium tropicum strain I (27 µg/ml) followed by Malbranchea chrysosporoidea (26 µg/ml), C. indicum (25 µg/ml), C. keratinophilum (22 µg/ml), C. queenslandicum (21 µg/ml), Scopulariopsis brevicaulis and Curvularia lunata (18 µg/ml), Microascus manganii and Chaetomium globosum (15 µg/ml), Aspergillus nidulans (13 µg/ml), Penicillium chrysogenum (12 µg/ml), Fusarium moniliforme (11 µg/ml), Sepedonium maheswarianum and A. flavus (10 µg/ml). Since all the investigated isolates showed fairly good amount of cysteine (Fig 39) in the culture media, it proved their keratinolytic nature. Moreover, detection of cysteine in the culture media also supported the role of sulfitolysis during keratinolysis. Weary and Canby (1967) and Kunert (1973) also reported the release of cysteine by Trichophyton rubrum and Microsporum gypseum growing on wool and hair respectively. Similarly, Hasija, et al. (1990) tested in vitro 30 keratinophilic fungi for their ability to degrade human scalp hair and found that most of them could release cysteine. Keratin degradation is, therefore, a result of complex chain of reactions among which sulfitolysis takes place

Table 13 : Keratinolytic activity of fungi (soil isolates) on hair after 40 days of incubation.

Fungal Species	Change in pH		Cysteine µg/ml		Protein µg/ml		Keratinase KU	
	A*	B**	A	B	A	B	A	B
Chrysosporium keratinophilum	8.8	9.2	12.0	24.0	24.0	53.0	5.5	12.5
C. queenslandicum	8.1	9.0	15.0	21.0	14.0	45.0	6.2	9.5
C. tropicum strain I	8.6	8.9	14.0	28.0	20.0	55.0	6.0	11.8
C. tropicum strain II	8.0	8.6	13.0	20.0	18.0	40.0	7.0	10.0
C. pannorum	8.1	8.4	12.0	21.0	28.0	56.0	7.1	10.8
Malbranchea flava	8.6	9.0	14.0	21.0	29.0	40.0	5.8	12.0
Scopulariopsis brevicaulis	8.1	8.9	15.0	18.0	25.0	65.0	4.1	10.0
Microascus manganii	8.6	8.6	13.0	15.0	21.0	55.0	4.0	9.0
Gliocladium virens	7.9	8.1	15.0	12.0	17.0	40.0	6.5	7.0
Circinella muscae	7.2	7.9	8.0	12.0	12.0	30.0	2.5	5.0
Fusarium moniliforme	7.4	8.0	9.0	11.0	15.0	21.0	5.4	11.4
F. solani	7.2	8.3	8.0	10.0	12.0	48.0	6.1	10.8
Aspergillus ochraceus	7.8	8.5	10.0	11.0	21.0	45.0	2.0	8.9
A. flavus	7.9	8.5	9.0	10.0	20.0	55.0	3.0	7.9
A. sydowii	7.4	8.2	7.0	10.0	18.0	21.0	2.8	6.6
A. deflectus	7.2	8.4	8.0	12.0	17.0	19.0	3.8	5.5
A. versicolor	7.1	8.1	9.0	11.0	16.0	35.0	3.0	6.0
A. candidus	7.4	8.8	7.0	10.0	14.0	24.0	3.8	5.8
Penicillium griseofulvum	7.6	8.1	6.0	9.0	18.0	26.0	4.5	6.2
P. aurantiogriseum	7.4	8.4	9.0	10.0	12.0	31.0	4.0	6.0
P. citrinum	7.3	8.2	8.0	10.0	10.0	30.0	2.8	9.9

Initial pH of Medium A = 7.0; B = 7.8

*Culture medium A contained (g/l distilled Water) :

K_2HPO_4 ; 1.5g; $MgSO_4.7H_2O$: 0.025g; $CaCl_2$: 0.025g; $FeSO_4.7H_2O$: 0.015g; $ZnSO_4.7H_2O$: 0.005g

**Culture medium B contained (g/l 0.028M phosphate buffer) ;

Glucose : 0.9g; $MgSO_4.7H_2O$: 0.6g; thiamine : 0.01g; pyridoxine : 0.01g; inositol : 0.05g

Table 14 : Keratinolytic activity of fungi (feather isolates) on hair after 40 days of incubation.

Fungal species	Change in pH		Cysteine µg/ml		Protein µg/ml		Keratinase KU/ml	
	A*	B**	A	B	A	B	A	B
Chrysosporium keratinophilum	8.6	9.0	11.0	22.0	22.0	50.0	5.5	12.0
C. queenslandicum	8.0	9.2	15.0	21.0	14.0	44.0	6.0	9.5
C. tropicum strain I	8.6	8.8	13.0	27.0	20.0	54.0	6.0	11.8
C. indicum	8.5	8.8	10.0	25.0	25.0	45.0	6.8	11.0
Malbranchea chrysosporoidea	8.4	9.1	18.0	26.0	15.0	50.0	5.4	11.0
Scopulariopsis brevicaulis	8.0	8.8	14.0	18.0	24.0	60.0	4.0	10.5
Microascus manganii	8.0	8.4	13.0	15.0	20.0	51.0	4.0	9.0
Sepedonium maheshwarianum	7.6	7.9	13.0	10.0	33.0	52.0	6.1	7.9
Chaetomium globosum	7.9	8.5	13.0	15.0	14.0	24.0	2.5	5.5
Curvularia lunata	7.8	8.6	14.0	18.0	21.0	24.0	2.6	8.5
Aspergillus flavus	7.7	8.6	10.0	10.0	20.0	53.0	3.0	8.0
A. nidulans	7.5	8.3	10.0	13.0	24.0	35.0	3.0	5.8
Fusarium moniliforme	7.4	8.0	9.0	11.0	16.0	20.0	5.0	11.0
Penicillium chrysogenum	7.2	8.1	10.0	12.0	21.0	25.0	3.5	6.1

Initial pH of medium A = 7.0, B = 7.8

* Culture medium A contained (g/l distilled water) :

K_2HPO_4 : 1.5g; $MgSO_4 . 7H_2O$: 0.025g; $CaCl_2$: 0.025g; $FeSO_4 . 7H_2O$: 0.015g; $ZnSO_4$ 7HD-0.005g

**Culture medium B contained (g/l 0.028 M Phosphate buffer) :

Glucose : 0.9g; $MgSO_4 . 7H_2O$: 0.6g; thiamine : 0.01g; pyridoxine : 0.01g; inositol : 0.05g

significantly and involves the reduction of disulfide linkages of cysteine which are largely responsible for providing stability to keratin molecule.

Protein released from the keratinic substrate :

Degradation of hair keratin was also monitored by determining net protein release in the media. The results presented in tables 13 and 14 show that all the isolates of the tested keratinophilic fungi, released variable amount of total protein. However, protein released in culture medium A was quite lesser in quantity than that in the culture medium B (Fig. 40).

Among the soil isolates, maximum release of protein after the experimental period of 40 days of incubation was shown in culture medium B by Scopulariopsis brevicaulis (65 µg/ml), followed by Chrysosporium pannorum (56 µg/ml), C. tropicum Strain I, Microascus manganii and Aspergillus flavus (55 µg/ml), C. keratinophilum (53 µg/ml), Fusarium solani (48 µg/ml), C. queenslandicum and A. ochraceus (45 µg/ml), Gliocladium virens, Malbranchea flava and C. tropicum Strain II (40 µg/ml), A. versicolor (35 µg/ml), Penicillium aurantiogriseum (31 µg/ml), Circinella muscae and P. citrinum (30 µg/ml), P.

griseofulvum (26 µg/ml), A. candidus (24 µg/ml), A. sydowii and F. moniliforme (21 µg/ml), A. deflectus (19 µg/ml).

Maximum protein release among the feather isolates was again shown in culture medium B by Scopulariopsis brevicaulis (60 µg/ml), followed by C. tropicum strain I (54 µg/ml), A. flavus (53 µg/ml), Sepedonium maheshwarianum (52 µg/ml), Microascus manganii (51 µg/ml), C. keratinophilum and Malbranchea chrysosporoidea (50 µg/ml), C. indicum (45 µg/ml), C. queenslandicum (44 µg/ml), A. nidulans (35 µg/ml), P. chrysogenum (25 µg/ml), Chaetomium globosum and Curvularia lunata (24 µg/ml). Similar values of protein release after prolonged incubation have also been reported by Kunert (1973), Deshmukh and Agrawal (1982), Pandey et al. (1988) and Hasija et al. (1990).

Release of keratinase :

Net keratinolytic activity measured in keratinase units (KU) has been illustrated in Fig. 41. Perusal of the data shows that all the test isolates grown in the two media secreted significant amount of keratinase

which is known to be responsible for hydrolysis of keratin in nature. However, keratinase activity recorded after 40 days of incubation was found to be much higher in culture medium B which contained glucose than in culture medium A which was without glucose. Among the soil isolates, highest release of keratinase was elaborated in culture medium B by Chrysosporium keratinophilum (12.5 KU/ml), followed by Malbranchea flava (12 KU/ml), C. tropicum strain I (11.8 KU/ml), Fusarium moniliforme (11.4 KU/ml), C. pannorum (10.8 KU/ml), C. tropicum strain II, S. brevicaulis and F. solani (10 KU/ml), Penicillium citrinum (9.9 KU/ml), C. queenslandicum (9.5 KU/ml), Microascus manganii (9.0 KU/ml), Aspergillus ochraceus (8.9 KU/ml), A. flavus (7.9 KU/ml), Gliocladium virens (7 KU/ml), A. sydowii (6.5 KU/ml), Penicillium griseofulvum (6.2 KU/ml), P. aurantio griseum and A. versicolor (6.o KU/ml), A. candidus (5.8 KU/ml), A. deflectus (5.5 KU/ml) and Circinella muscae (5.0 KU/ml).

Feather isolates showed more or less similar keratinase activity with maximum release in culture medium B by C. keratinophilum (12.0 KU/ml), followed by C. tropicum Strain I (11.8 KU/ml), C. indicum, Malbranchea chrysosporoidea and F. moniliforme (11 KU/ml), S. brevicaulis (10.5 KU/ml), C. queenslandicum (9.5 KU/ml), Microasus

manganii (9 KU/ml), Curvularia lunata (8.5 KU/ml) A. flavus (8.0 KU/ml), Sepedonium maheshwarianum (7.9 KU/ml), P. chrysosgenum (6.1 KU/ml), A. nidulans (5.8 KU/ml), Chaetomium globosum (5.5 KU/ml). Production of extracellular keratinases during the growth of keratinofers have also been established by Yu et al.(1968, 1969a,b, 1971),Takiuchi et al.(1982), Rajak et al.(1991a).

It is also apparent from the data illustrated in Fig. 42, that there is a change in the pH of the medium towards alkalinity after the release of cysteine, protein and keratinase by the test fungi. Chesters and Mathison (1963) and Ziegler and Bohme (1969) proposed that the basis of keratinolysis is high deamination and as a result the medium is made alkaline. It was also noted that the fungi which showed strong keratinolytic ability rendered the culture medium more alkaline than the less keratinolytic ones. Similar changes in pH of the medium, supporting the keratinolytic isolates tested have also been reported by Pandey et al. (1988) and Hasija et al. (1990).

In the investigated soil isolates, maximum change in pH was induced by Chrysosporium keratinophilum (9.2) and minimum by Circinella muscae (7.9), whereas

among the feather isolates maximum increase in pH occurred with Chrysosporium queenslandicum (9.2) and minimum with Sepedonium maheshwarianum (7.9). This tendency of altering the pH of the medium by the keratinophiles also influences the keratin biodegradation (Jain and Agrawal, 1980; Rajamani and Hilda, 1988; Rajak et al. 1991b). Similarly, variable rates of degradation of keratin substrates under different pH conditions have been noted by Meevootisom and Niederpruem (1979). Few other researchers have also reported keratinolytic enzyme activity to be conspicuously higher at alkaline pH value than at pH below 7.0 (Dey et al. 1968; Yu et al. 1968, 1969a,b, 1971; Takiuchi and Higuchi, 1977; Higuchi and Takiuchi, 1980; Takiuchi et al. 1982; Sanyal et al. 1985). It is the production of these keratinases which makes the dermatophytes to invade the superficial tissues of man and animals (Chattaway et al. 1963; Meevootisom and Nieder pruem, 1979). Infact, keratinase activity of dermatophytes is considered to be one of the most important traits correlated with their capacity to infect and cause pathogenic syndromes in diverse kinds of living organisms (Minocha et al. 1972). Therefore, it can be concluded on the basis of these observations that invitro keratin digestion by the investigated fungi is predictive of their ability to cause infection in vivo and thus pose a formidable threat.

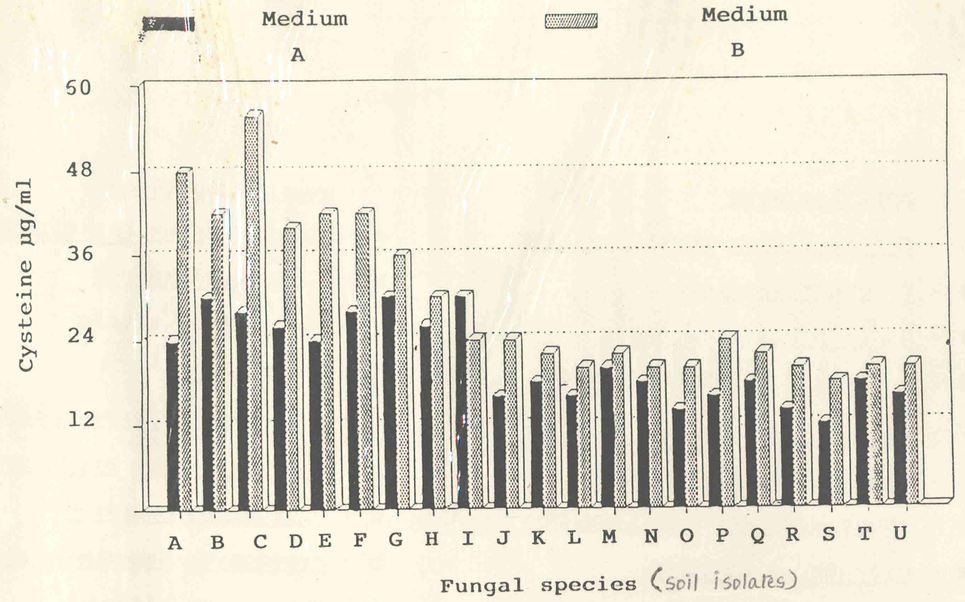
Fig. 39

Soil isolates

A - Chrysosporium keratinophilum
B - C. queenslandicum
C - C. tropicum strain I
D - C. tropicum strain II
E - C. pannorum
F - Malbranchea flava
G - Scopulariopsis brevicaulis
H - Microascus manganii
I - Gliocladium virens
J - Circinella muscae
K - Fusarium moniliforme
L - F. solani
M - Aspergillus ochraceus
N - A. flavus
O - A. sydowii
P - A. deflectus
Q - A. versicolor
R - A. candidus
S - Penicillium griseofulvum
T - P. aurantiogriseum
U - P. citrinum

Feather isolates

a - Chrysosporium keratinophilum
b - C. queenslandicum
c - C. tropicum strain I
d - C. indicum
e - Malbranchea chrysosporoidea
f - Scopulariopsis brevicaulis
g - Microascus manganii
h - Sepedonium maheshwarianum
i - Chaetomium globosum
j - Curvularia lunata
k - Aspergillus flavus
l - A. nidulans
m - Fusarium moniliforme
n - Penicillium chrysogenum



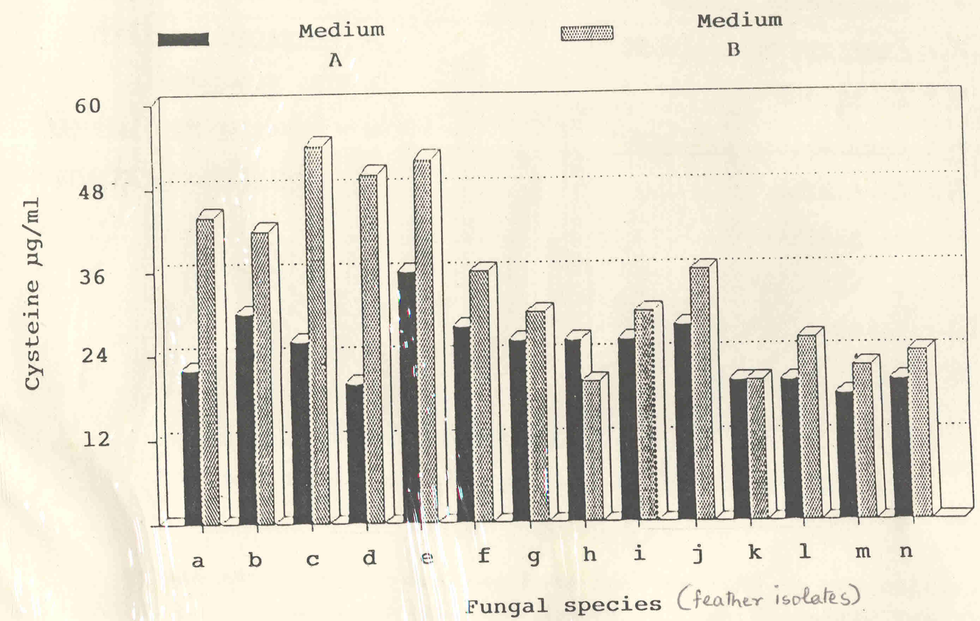


Figure 39 : Cysteine release from hair keratin in the culture media during the growth of keratinophilic fungi.

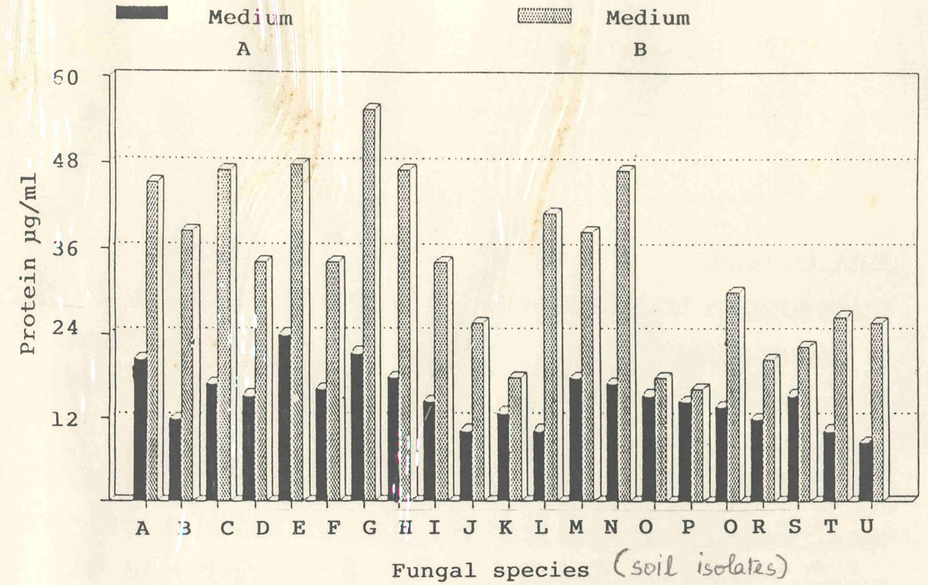
Fig.40

Soil isolates

A - Chrysosporium keratinophilum
B - C. queenslandicum
C - C. tropicum strain I
D - C. tropicum strain II
E - C. pannorum
F - Malbranchea flava
G - Scopulariopsis brevicaulis
H - Microascus manganii
I - Gliocladium virens
J - Circinella muscae
K - Fusarium moniliforme
L - F. solani
M - Aspergillus ochraceus
N - A. flavus
O - A. sydowii
P - A. deflectus
Q - A. versicolor
R - A. candidus
S - Penicillium griseofulvum
T - P. aurantiogriseum
U - P. citrinum

Feather isolates

a - Chrysosporium keratinophilum
b - C. queenslandicum
c - C. tropicum strain I
d - C. indicum
e - Malbranchea chrysosporoidea
f - Scopulariopsis brevicaulis
g - Microascus manganii
h - Sepedonium maheshwarianum
i - Chaetomium globosum
j - Curvularia lunata
k - Aspergillus flavus
l - A. nidulans
m - Fusarium moniliforme
n - Penicillium chrysogenum



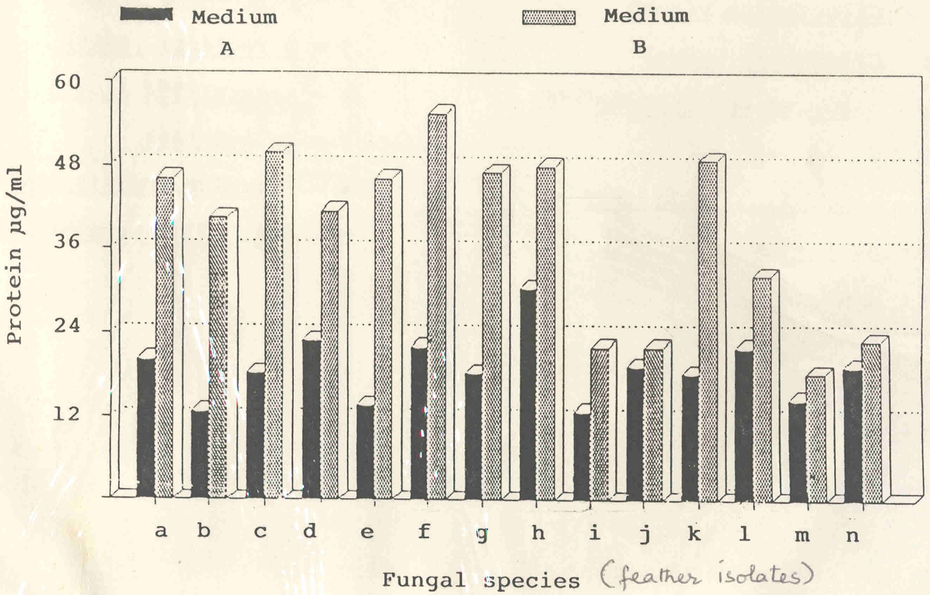


Figure 40 : Protein release from hair keratin in the culture media during the growth of keratinophilic fungi.

Fig.41

Soil isolates

A - Chrysosporium keratinophilum

B - C. queenslandicum

C - C. tropicum strain I

D - C. tropicum strain II

E - C. pannorum

F - Malbranchea flava

G - Scopulariopsis brevicaulis

H - Microascus manganii

I - Gliocladium virens

J - Circinella muscae

K - Fusarium moniliforme

L - F. solani

M - Aspergillus ochraceus

N - A. flavus

O - A. sydowii

P - A. deflectus

Q - A. versicolor

R - A. candidus

S - Penicillium griseofulvum

T - P. aurantiogriseum

U - P. citrinum

Feather isolates

a - Chrysosporium keratinophilum

b - C. queenslandicum

c - C. tropicum strain I

d - C. indicum

e - Malbranchea chrysosporoidea

f - Scopulariopsis brevicaulis

g - Microascus manganii

h - Sepedonium maheshwarianum

i - Chaetomium globosum

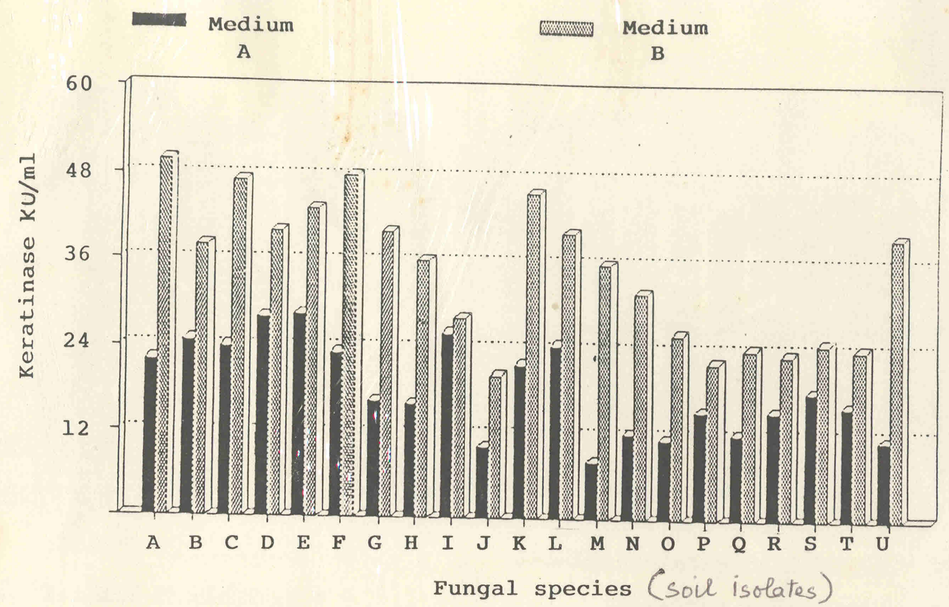
j - Curvularia lunata

k - Aspergillus flavus

l - A. nidulans

m - Fusarium moniliforme

n - Penicillium chrysogenum



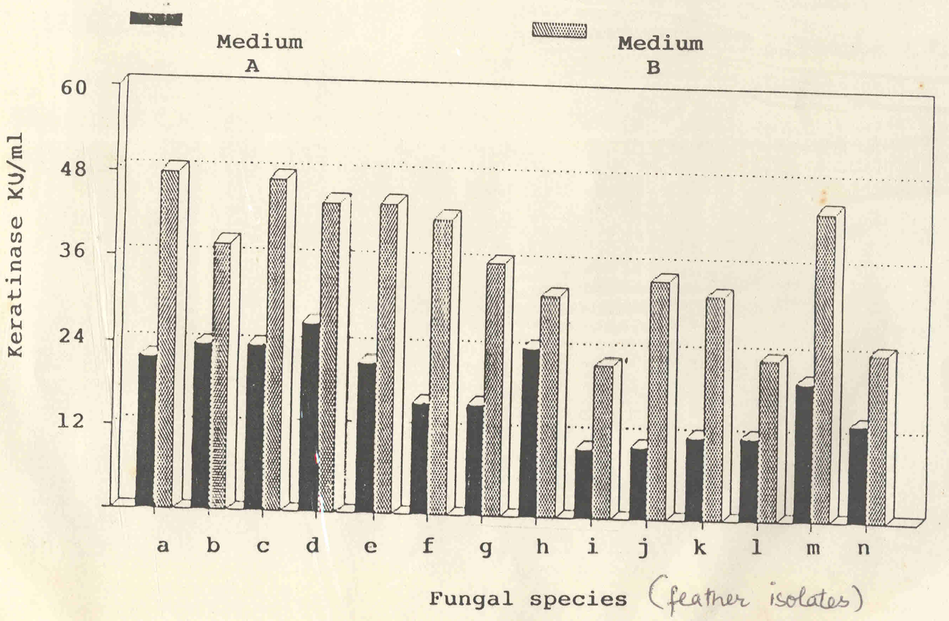


Figure 41 : Keratinolytic activity in keratinase units (KU/ml).

Fig.42

Soil isolates

A - Chrysosporium keratinophilum

B - C. queenslandicum

C - C. tropicum strain I

D - C. tropicum strain II

E - C. pannorum

F - Malbranchea flava

G - Scopulariopsis brevicaulis

H - Microascus manganii

I - Gliocladium virens

J - Circinella muscae

K - Fusarium moniliforme

L - F. solani

M - Aspergillus ochraceus

N - A. flavus

O - A. sydowii

P - A. deflectus

Q - A. versicolor

R - A. candidus

S - Penicillium griseofulvum

T - P. aurantiogriseum

U - P. citrinum

Feather isolates

a - Chrysosporium keratinophilum

b - C. queenslandicum

c - C. tropicum strain I

d - C. indicum

e - Malbranchea chrysosporoidea

f - Scopulariopsis brevicaulis

g - Microascus manganii

h - Sepedonium maheshwarianum

i - Chaetomium globosum

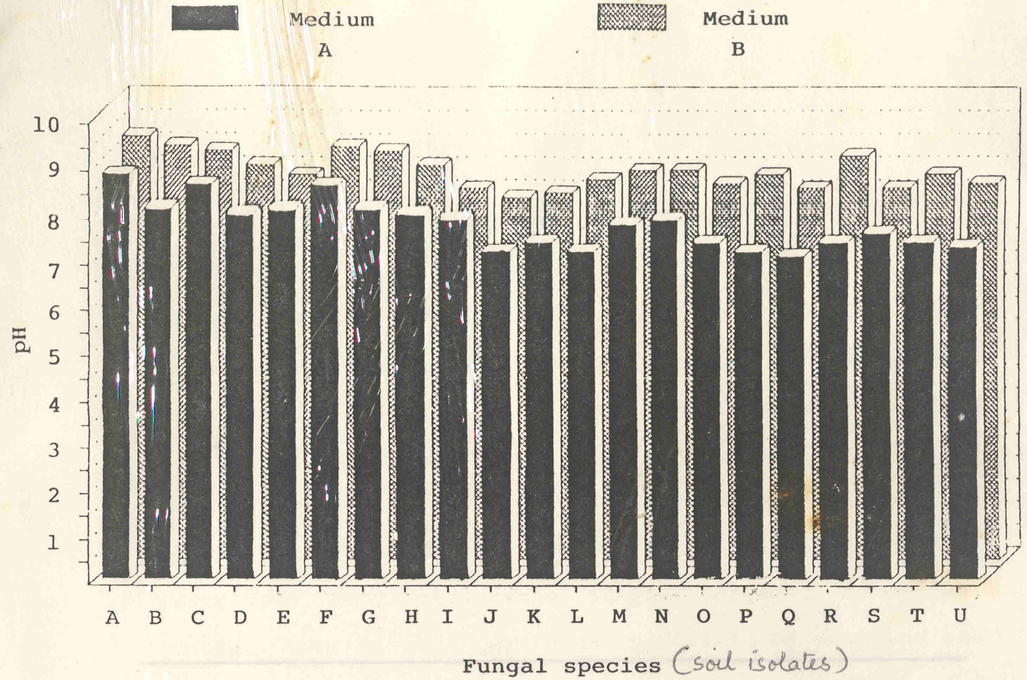
j - Curvularia lunata

k - Aspergillus flavus

l - A. nidulans

m - Fusarium moniliforme

n - Penicillium chrysogenum



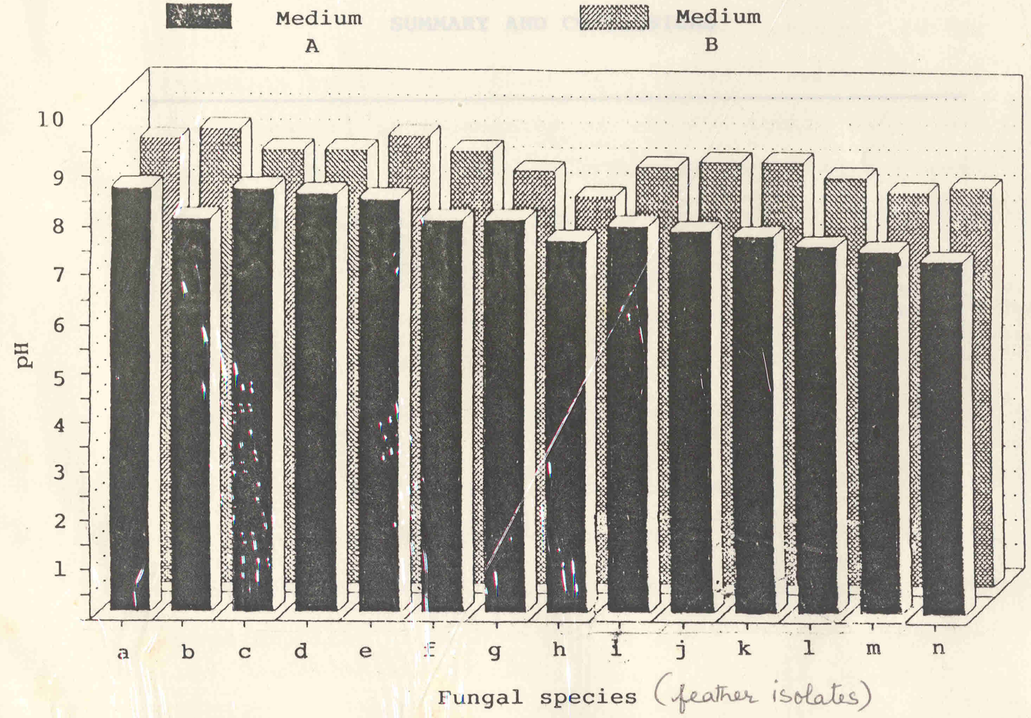


Figure 42 : Changes in pH during keratinolytic activity of fungi.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Keratinophilic fungi are a group of highly specialized fungi which possess the ability to degrade hard keratin (skin, hair, nails, claws, spines, feathers etc.) of man and animal and utilize it as a source of protein. They are vigorous and self-sufficient saprophytes which play a vital role in nature in the breakdown of keratinous substrates into simpler organic compounds. However, some of them may become parasitic by accident and after becoming pathogenic, they are able to survive and multiply at host's body temperature, causing futher infection by invading fresh keratinized tissues. Infact the potential pathogenecity of keratinophilic fungi has been considered as a natural evolution from its presence in the soil (geophilic species) to invasion of cornified substrata in animals (zoophilic species) and man (anthropophilic species). Therefore, soil is an important natural reservoir and link that relates these different ecological groups together.

Realizing the significance of this unique group of fungi, the present research work was undertaken to study the taxonomy and keratinase activity of some keratinophilic fungi from poultry farms of Jammu district.

During the period, January 1992 to December 1993, ten different poultry farms of Jammu district situated at Machlian, Prem Nagar, Nihalpur Simbal, Kothi, Shahdaula, Akhnoor, Belicharana, Muthi, Nagbani, Maralia and Ranbir Singh Pura were screened for the presence of keratinophilic fungi. A total of 35 keratinophilic isolates representing 27 species belonging to 12 genera were isolated by keratin-bait-technique. These keratinophilic species are as follows :

°* Chrysosporium keratinophilum D. Frey ex Carmichael

°* C. queenslandicum Apinis & Rees

°* C. tropicum Carmichael (Strain I)

* C. tropicum Carmichael (Strain II)

* C. pannorum (Link) Hughes

° C. indicum (Randhawa & Sandhu) Garg

* Malbranchea flava Sigler & Carmichael

° M. Chrysosporoidea Sigler & Carmichael

°* Scopulariopsis brevicaulis (Sacc.) Bainier

°* Microascus manganii (Loubiere) Curzi

* Circinella muscae (Sorokine) Berelse & de Toni

* Fusarium solani (Mart.) Sacc.

°* F. moniliforme Sheldon

° Curvularia lunata (Wakker) Boedign

* Gliocladium virens Miller, Giddens & Foster

* Aspergillus candidus Link ex Fries

°* A. flavus Link ex Fries

* A. versicolor (Vuill) Tira boschi

* A. deflectus Fennel & Raper

* A. ochraceus Wilhelm

° A. nidulans (Eidam) Winter

* A. sydowii (Bainier & Sartory) Thom & Church

* Penicillium citrinum Thom

* P. griseofulvum Dierckx

* P. aurantiogriseum Dierckx

° P. chrysogenum Thom

° Chaetomium globosum Kunze

° Sepedonium maheshwarianum Mukerji

°* Isolated from both poultryfarm soils and poultry bird feathers.

* Isolated from poultry farm soils only.

° Isolated from poultry bird feathers only.

During the course of investigation, all the poultry soil samples were found to be 100% positive for these fungi indicating, thereby, that poultry farm soils are favourable for the development of keratinophilic fungi. Two genera, viz. Chrysosporium and Aspergillus were most frequent, being recovered from the soils of all the poultry farms, and thus formed the bulk of the

isolated keratinophiles. Out of the 20 species of keratinophilic fungi isolated from poultry farm soils, Chrysosporium tropicum strain I showed highest occurrence (54%), followed by C. keratinophilum (42%), Gliocladium virens and Penicillicum citrinum (38%), Scopulariopsis brevicaulis (35%), Malbranchea flava and Aspergillus candidus (33%), Chrysosporium queenslandicum (29%), Aspergillus flavus and A. versicolor (28%), A. deflectus (22%), A. ochraceus and Chrysosporium tropicum strain II (19%), C. pannorum (18%), Penicillium griseofulvum (17%), Circinella muscae and Fusarium moniliforme (15%), Aspergillus sydowii (14%), Penicillium aurantiogriseum (13%), Microascus manganii (12%) and Fusarium solani (11%)

Keratinophilic fungi were also isolated from the feathers collected directly from the living poultry birds (Gallus domesticus). All the feather samples investigated were found to be 100% positive for the presence of keratinophilic fungi. Isolations yielded fourteen species of keratinophilic fungi classified in ten genera of perfect and imperfect forms. Aspergillus flavus showed highest occurrence (42%), followed by Chrysosporium tropicum strain I (41%), Malbranchea chrysosporoidea (27%) Curvularia lunata (25%), Chrysosporium indicum, Chaetomium globosum and Sepedonium maheshwarianum (23%),

Chrysosporium keratinophilum, Fusarium moniliforme and Scopulariopsis brevicaulis (22%), Aspergillus nidulans (17%), Chrysosporium queenslandicum and Microascus manganii (12%) and Penicillium chrysogenum (11%).

Frequency of keratinophilic feather isolates showed that Chrysosporium was recovered from the feathers of birds belonging to all the investigated poultry farms and the second most frequent genus was Aspergillus. As both these genera emerged most frequently in the poultry soils also, it is possible that the poultry birds acquire them from the soil. Therefore, to a certain extent, the colonization of birds feathers by keratinophilic fungi is a reflection of the qualitative and quantitative representation of microfungi occurring in the soil of the poultry farms.

In the present investigation, two different morphological strains of Chrysosporium tropicum were identified on the basis of spore character. In C. tropicum strain II, the arthrospores were completely absent and the size of the aleuriospores was larger (5-11 x 1.6 - 3.2 µm) than that of strain I (4-8.8 x 1.6-3.2 µm). Among the various keratinophiles isolated, Malbranchea flava and M. chrysosporoidea have not been reported so far from India and therefore, constitute new records as well as additions to the existing Indian mycoflora.

Morphological characters of M. chrysosporoidea were noted to be somewhat intermediate between Malbranchea and Chrysosporium, yet some significant characters favoured its placement in Malbranchea. Scrutiny of available literature also revealed that keratinophilic strains of Gliocladium virens, Circinella muscae and Microascus manganii recovered in the present investigation from poultry farm soils have not been reported earlier from India, and are, therefore, new additions to this unique group. Similarly, there is no previous record of Scopulariopsis brevicaulis, Penicillium chrysogenum, Microascus manganii, Fusarium moniliforme and Sepedonium maheshwarianum from feathers of birds.

Investigations were also conducted to assess the ecological factors governing the distribution and survival of keratinophilic fungi in the poultry farm soils. All the poultry farm soils were rich in humus and the occurrence of keratinophilic fungi was found to be generally directly proportional to the soil organic matter. Among the investigated poultry farms, Maralia poultry farm soil was richest in humus content (13.0%) and elaborated by maximum number of keratinophilic species (13), whereas, poultry farm soil of Ranbir Singh Pura contained lowest humus content (4.5%) and proportionally only eight fungal species were recovered from it. All the poultry

farm soils were found to be nearly neutral to weakly alkaline (pH 7.2 - 8.0) and organically rich with a high content of organic carbon (2.64-7.56%), nitrogen (1.1-1.81%), phosphorus (0.0012-0.0046%) potassium (0.021-0.024%), magnesium (0.10-1.08%), calcium (1.02-3.8%) and iron (1.3-2.1%). These soil factors influenced the occurrence of increased number of soil keratinophiles. However, order of importance of these factors could not be assessed as they are oftenly very closely related and show a collective effect.

Experiments were also conducted to evaluate temperature, relative humidity and best pH for the optimum mycelial growth of Chrysosporium species as they formed the bulk of the isolated keratinophiles. Most of the tested species of Chrysosporium could grow at R.H. varying 30-100%, but C. pannorum and C. indicum could grow only between 30-80% R.H. All the species attained maximum diameter at 60% RH indicating their hygrophilic nature. Similarly all the Chrysosporium species could grow between temperatures 20-40°C suggesting that they were slightly thermotolerant. However maximum radial growth occurred between 25-30°C. Chrysosporium species could also grow over a wide pH range (4.0-9.0), but pH6.0-7.0 supported maximum growth, probably because several metabolic

activities occur within this range.

Evaluation of keratinolytic ability of soil and feather isolates was done by a simple method, wherein, defatted and sterilized human hair used as keratin substrate was inoculated with the test fungus. The number of days taken by each isolate to break the hair was recorded. All the soil and feather isolates were able to break the hair at the point of inoculation indicating, thereby, complete digestion of hair keratin at the site. However, the time taken for complete digestion of hair varied not only for different species of the same genus, but also for different isolates of the same species depending upon their degree of keratinolytic ability. Early digestion of hair was brought about by Chrysosporium species particularly C. keratinophilum which took least time (11-12 days) to digest hair, whereas, most of the Penicillium and Aspergillus species took longer time in completely hydrolyzing the hair keratin.

Microscopic examinations of the hair samples attacked by keratinolytic moulds revealed extensive damage including rupturing and blistering of hair cuticle. Chrysosporium keratinophilum and C. tropicum resulted in undulation of the hair cuticle whereas, a characteristic

effect of Scopulalriopsis brevicaulis on human hair was the lifting of scales of the cuticle to such an extent that the hair eventually gave a frayed appearance. Cuticle of the hair was also found to be susceptible to the attack of Aspergillus sydowii which formed abundant conidiophores on the surface and only few ruptures in the cuticle. The extent of structural damage of hair is probably dependent on the ability of the test fungus to produce keratinase.

Preferential utilization and colonization of different keratin substrates (human hair, horse hair, human nails, peacock feathers and poultry bird feathers) by the isolated keratinophiles was also assessed. All the tested isolates grew on poultry feathers, peacock feathers, and human hair, although with variable degree of colonization, whereas, only 17 and 14 species could colonize horse hair and human nails respectively. Maximum colonization of all the keratinous substrates was shown by Chrysosporium species indicating that they possess specific enzymatic system for degradation of keratin and therefore, it is important to recognize their potential as pathogens. Substrate specificity was also found to be variable within the species of the same genus.

All the keratinophilic isolates were also tested

in vitro for their ability to degrade human hair keratin. It was observed that all the test isolates could degrade hair keratin by releasing sulphydryl containing compounds detected as cysteine, keratinase and total proteins. Release of these compounds by soil isolates was slightly more than the releases by their corresponding feather isolates. Moreover, out of the two culture media used, glucose supplemented medium B supported maximum release of cysteine, protein and keratinase after the experimental period of 40 days incubation.

The most distinctive character of keratin is its high cysteine content with disulphide linkage rendering it more resistant to enzymatic digestion. Among the soil isolates maximum cysteine was released by Chrysosporium tropicum strain I (28 µg/ml) and minimum by Penicillium griseofulvum (9 µg/ml), whereas among feather isolates maximum cysteine was released by Chrysosporium tropicum strain I (27 µg/ml) and minimum by Aspergillus flavus (10 µg/ml). Since all the investigated isolates showed fairly good amount of cysteine in the culture media, it proved their keratinolytic nature.

Degradation of hair keratin was also monitored by determining net protein release in the media. All the

isolates of the test fungi released variable amounts of total protein. Among the soil isolates, maximum release of protein was shown by Scopulariopsis brevicaulis (65 µg/ml) and minimum by Aspergillus deflectus (19 µg/ml), whereas, among the feather isolates maximum release was again shown by Scopulariopsis brevicaulis (60 µg/ml) and minimum by Curvularia lunata (24 µg/ml).

Net keratinolytic activity measured in keratinase units (KU) showed that all the test isolates secreted significant amount of keratinase which is known to be responsible for hydrolysis of keratin in nature. Among the soil isolates, highest release of keratinase was elaborated by Chrysosporium keratinophilum (12.5 KU/ml) and lowest by Circinella muscae (5.0 KU/ml). Feather isolates showed more or less similar keratinase activity with maximum release by Chrysosporium keratinophilum (12.0 KU/ml) and minimum by Chaetomium globosum (5.5 KU/ml).

A change in the pH of the medium towards alkalinity was also observed after the release of cysteine, protein and keratinase by the test fungi. In addition, it was noted that the fungi which showed strong keratinolytic ability rendered the culture medium more alakaline than the less keratinolytic ones. In fact the basis of keratinolysis is high deamination and as a result

the medium is made alkaline. All these observations made on the invitro keratin degradation by the test fungi is predictive of their ability to cause infection in vivo.

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