CHAPTER - I

GENERAL INTRODUCTION
Keratin are sclero-proteins composed of long polypeptide chains, are insoluble in dilute acid and alkali solutions and are resistant to the action of pepsin, trypsin and other non-substrate-specific proteases. The long axes of the peptide are oriented along the length of the keratin fibril in a spiral configuration (Mercer and Verma, 1963). A distinctive feature of keratin is its relatively high sulfur content due to the presence of the sulfur containing amino acids i.e., cystine, cysteine and methionien. The disulphide bonds are considered to be responsible for the stability of keratin and its resistance to enzymatic degradation. Nickerson (1947) classified keratin in to two distinct forms i.e., soft and hard keratins depending on 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. A number of workers admirably reviewed the histology, biochemistry and physiology of keratinization (Rothman, 1953; Birbeck and Mercer, 1957a; Mercer, 1961), and found that the origin of the keratin take place in living epithelial cells which are transformed by the process of keratinization, this
involves the transformation of cytoplasmic proteins into keratin fibres and a complete disintegration of the keratinized cells in the last. The cytoplasmic proteins which are transformed into keratin may bring a difference in different keratinic appendages of man and animals.

A number of saprophytic fungi and other microorganisms have been found thriving on variety of substrates in nature depending on their intrinsic potential to make use of the substrate concerned by hydrolysing it with the help of extracellular enzymes. In general the fungi which can produce enzymes and can hydrolyse keratinic substances are generally termed as 'keratinophilic'. However, English (1969) categorised these fungi in 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 the genera *Epidermophyton*, *Keratinomyces* or *Trichophyton* whether pathogenic to man and animal or not. All the dermatophytes are keratinolytic in nature.

The term 'keratinofers' has been advocated to name all such fungi that comes in any of the
above three categories of keratin loving fungi (Jain, 1977). The dermatophytic fungi are of great significance because of their pathogenic potential to cause diseases in man and animals. This group is an assemblage of many fungi that have variation in their ecology and pathology. On the basis of natural habitat and epidemiological standpoint, Ajello (1960) classified dermatophytes into three groups i.e., geophilic, anthropophilic, and zoophilic. The anthropophilic dermatophytes occur primarily on man and transmitted to animals causing dermatophytosis; Zoophilic dermatophytes are those which mainly parasitic to lower animals and transmitted to man through contact, while geophilic dermatophytes include species which are predominantly saprobic and occur mainly in the soil these may or may not be associated with pathogenic attack.

Although, dermatophytes undoubtedly existed in prehistoric times and have plagued lower animals and man for millions of years but the infections were long endured before their true nature was realized. The first recorded reference to a dermatophytic infection is attributed to Aulus Cornelius Celsus, the Roman encyclopedia, who in his 'De Re Medicina' written around 30 A.D., described a suppurative infection of the scalp that came to be known as the 'kerion of celsus' (Rosenthal, 1961). Cassius Felix (400 A.D.) was the
first who coined the term 'tinea' to mean ringworm
latter the term 'favus' was applied to honey coloured
lesions. In the first decade of 19th century Agostino
Bassi (1807) proved that an entamogenous fungus can
also cause diseases among animals (Rathore, 1987).
Hence, he is recognized as the discoverer of microbial
etiology of infections. In 1837 Robert Remak, noted
hyphae in the crust of the disease known as favus (Kisch,
1954). This was an epochal discovery since for the
first time a microorganism was incriminated as being
the cause of a human disease. Remak's discovery was
independently confirmed by David Gruby (1841a) who
carefully and compactly described several types of
dermatophytic infections; tinea favosa, ecotothrix and
endothrix, trichophytosis and microsporosis (Gruby,
1841b, 1842, 1843, 1844). Ottenasek (1979) gave an
ecological evidence for the existence of dermatophytes
in soil. It was emphasized that dermatophytes can
be divided into the primitive representatives are those
which are primarily geophilic and the advanced are
those termed as anthropophilic. The geophilic dermatophytes
are adopted to live and reproduce outside of man and
animals under the conditions corresponding to the
requirements of their metabolism, mainly in places
where they can utilize various forms of keratin.

The introduction of the term 'mycoses' by Raymond
Jacques Adrien sabouraud, a French giant, with the
publication of his classical work 'Les Teignes' in 1910 for fungal diseases has truly revolutionized the concept of dermatophytes and the development of medical mycology. This work is a classic in medical literature and includes observations over centuries. Gruby in 1843 discovered the genus Microsporum and described M. audouinii from clinical materials. Out of the three genera of fungi Imperfecti, in which dermatophytes are classified today, the Microsporum is the first. The second genus Trichophyton was described by per Hendrik Malmsteen in 1945 and the third, Epidermatophyton was established by Raymond Sabouraud in 1910. A fourth genus keratinomyces described by vanbreuseghem (1952a) is considered unnecessary and is redescribed since its characteristics are well covered by the genus Trichophyton. Discovery of sexual reproduction among the dermatophytes and related species serves to confirm the validity of the imperfect state in the classification of Microsporum and Trichophyton, with significant consistency the perfect states of Microsporum and Trichophyton species have been found to be classificable in the genera Nannizzia and Arthroderma, respectively (Ajello, 1968).

Several reviews have been published which described the dermatophytes (Ajello, 1960; 1974; Das Gupta et al., 1960; Taplin, 1976). Their descriptions showed that
some species of *Microsporum* and *Trichophyton* are not known to infect man or lower animals. However, since these species possess the morphological characteristics of the dermatophytes, they must perforce be classified therein on purely mycotaxonomic grounds until recently the various species of *Microsporum* and *Trichophyton* were defined, identified and distinguished from each other on the basis of the morphological and physiological properties of their imperfect states. Identification thus based on colonial expression, spore morphology and the physiological properties, i.e., vitamin deficiencies, ability to perforate hair, temperature requirements etc. Besides dermatophytes, certain other fungi are also known to cause infections in man and animals. To differentiate such infections from those caused by dermatophytes, the fungal infection have been categorised into two groups:

(i) Superficial mycoses or dermatomycoses and (ii) Systemic mycoses, that includes disease of man and animals caused by other than dermatophytes. A comprehensive account of all known fungal diseases has been compiled by many workers (Stockdala, 1953; George, 1959; Rebell and Taplin, 1970; Rippon, 1974; Sheklakov and Milich, 1974).

The available literature on fungal infections to man and animals indicate that there are several aspect particularly on the mode of survival and their
distribution in different habitats which are still to be worked out to have a clear understanding about fungal diseases.

The occurrence of keratinophilic fungi in soil was first reported by vanbreuseghem (1952b). Since then a number of other reports have been made by many workers who evaluated the distribution of keratinophilic fungi in soils of various countries (Ajello, 1953; 1956; Gorden, 1953; Deniels, 1954; Frey and Durie, 1956; Dawson and Gentles, 1959; Griffin, 1960; Pugh and Mathison, 1962; AlDoory, 1969; Khudtson and Robertstad, 1970; Masih Mohammad et al., 1971; DeBracalenti et al., 1975; Pugh and Hughes, 1975; Punsola and Guarro, 1984; Gueho et al., 1985). In India the first report of the occurrence of a keratinophilic fungus was made by Day and Kakoti in 1955, who isolated Microsporum gypseum from a soil sample collected from Dibrugarh, Assam. Later on several attempts have been made to survey of the keratinophilic fungi from different types of soils of India (Puri, 1961; Sarkar, 1962a,b; Mahapatra and Gugnani, 1964; Randhawa and Sandhu, 1965; Padhye, 1966; Jain, 1977; Singh, 1981; Deshmukh and Agrawal, 1983a,b; Kushwaha et al., 1985; Jain et al., 1993). Besides these, marine soils and coastal habitats have also been reported as good habitats for the occurrence of these fungi (Pawar et al., 1963; Padhye et al.,
1967; Jain et al., 1988). Similar reports have also been made from the Indian hills (Garg, 1966; Jain and Agrawal, 1977). While, other have found the occurrence of these fungi in bird's nest and feathers (Pugh and Evans, 1970; Pugh, 1966). Above reports indicate that the keratinophilic fungi are widely distributed in the environment which are generally inhabited by man or animals but still we do not known how many strains of these fungi are hidden and are playing role in the development of diseases in man and animals. Hence, it seems essential to continue such studies to develop better understanding about the habitats of pathogenic fungi and their role in development of diseases in man and animals.

Fungi inhabiting in various havitates are surviving there on the cost of available nutrients in them. It is well known that the soils of various nature differ greatly in their nutrient status thus fungi inhabiting in various types of soils may differ in their physiological and nutritional behaviour. The sporulation in fungi may also be influenced by various nutrients (Cochrane, 1958). Various factors that can govern the growth and sporulation in fungi may be the temperature and pH. A large number of workers have proved that the dermatophytes and keratinophilic fungi have a versatile range for their nutrition (Mosher et al., 1936; Hazen,
1951; George, 1952; Benham, 1953; Mathison, 1962; Chattaway et al., 1962; Pugh and Evans, 1970; Kushwaha and Agrawal, 1977; Jain, 1983). A majority of dermatophytes show very poor growth response to inorganic nitrogen sources (Robbins and Ma, 1945; George, 1951; Das Gupta and Shome, 1963). While, others have found better growth of most of the keratinophilic fungi and dermatophytes in complex organic nitrogen sources for their normal growth and reproduction (Kuehn and Orr, 1962; Kashyap et al., 1972). A few reports have also appeared on the selective requirements of amino acid as nutrients for some pathogenic fungi (Silva and Benham, 1952, Klein, 1953). Available literature indicate that the temperature and pH requirements of keratinophilic fungi have not been studied much though these fungi occur in the habits of varied temperature and pH range, such as, coastal habitats, sweepings of cattle farm and poultry farms, humus etc. (Pawar et al., 1963; Jain et al., 1968; Chmel, et al., 1972; Jain, 1977).

The in vitro study of antibiotics and sulpha drugs and other therapeutic agents against the dermatophytes can suggest their use to cure diseases caused by these pathogens in man and animals. Phenols are known to be most frequently involved in the disease resistance mechanisms of plants especially against fungal pathogens (Greathouse and Rigler, 1940; Walker and Stahmann,
1956; Friend, 1981, 1982). The orthodihydroxy phenols have been reported more fungitoxic to microorganisms than others (Le Toureau et al., 1957), and hence it may be useful for combating skin infections. The use of thymol in alcohol as an antifungal agent had been also described by some workers (Gill, 1932; Mac Bunery and Seourcey, 1936; Reeh, 1942; Johnston, 1944).

Mycotic infections caused by various species of dermatophytes are widely distributed and constitute a public health problem of great magnitude in some areas. Hajni et al. (1970) demonstrated that the incorporation of vegetative oil and fatty acids into sabouraud's dextrose agar medium inhibit the growth of dermatophytes. The activity of leaf extract of some plants such as Nerium indicum and Eucalyptus globulus were found highly inhibitory for the growth of six strains of Nannizzia fulva, N.gypsea and N.incurvata (Jain and Agrawal, 1976). The sensitivity of some keratinophilic fungi against some oils bailed with seeds/bulbs of some plants such as garlic, onion have also been evaluated for their antifungal properties (Jain, 1991). The essential oils and related compounds which are mainly the products from above ground parts of the seeds plants are insufficiently explored for their antifungal properties, probably this may be due to the narrow concept that microbes can produce more
potential antifungal substances like antibiotics. Few reports are there in literature showing the inhibitory effect of essential oils against keratinophilic fungi (Kushwaha, 1976; Jain and Agrawal, 1978a; Singh and Agrawal, 1978; Jain et al., 1980; Deshmukh and Jain, 1981). The results of these investigations were quite encouraging and suggest further investigations on different plant products for their use as antifungal agents.

The advent of the antibiotic era has raised the hope that the antibiotics would provide us with the much awaited chemotherapeutants effective in plant as well as in man and animal disease control. As far as fungal disease are concerned, the expectations has not been fulfilled to a significant level. The antifungal antibiotics are very few these includes griseofulvin, mycostatin, aureafungin and hamycine. Besides these some sulphonamides have also known to possess antifungal properties (Thompson, 1942; Wolf, 1946; Jain, 1977).

During microbial degradation of organic waste a number of volatiles have been produced. These volatiles are either produced by the microbes or by decaying organic matter and play an important role in fungistasis (Cholodny, 1948; Hora and Baker, 1970; Dennis and Webster, 1971; Hutchinson, 1973; Lockwood, 1977). It is now well established that fungi, practically in all habitats,
live in an atmosphere containing various volatile substances, whether these volatile factors are inhibitory or stimulatory for growth of keratinophilic fungi in nature is a subject of further enquiry.

Keratinase by most of the dermatophytes has been reported by Evolceanui and Maria (1960) and they found amino acids as the final products after the enzymatic breakdown. The keratinase of keratinofers and specially of dermatophytes has attracted the interest of dermatologists because this enzyme has a powerful action on keratin which are almost always associated with the mycotic infection of man and animals.

A review of literature indicates that several investigations particularly on the evaluation of antimycotic drugs including sulpha drugs and antibiotics are now well established as medical drugs. As the antibiotics are systemic in their action they can be used as protective and therapeutic. Of course results vary with the nature of the antibiotic and the host tested. The concentration of a particular drug or antibiotic, method of application, temperature and humidity play and important role in the outcome of this action. In the present investigation effects are being mode to evaluate the sensitivity of some selected drugs including antibiotic, phenols, sulphadurugs etc. on the growth and sporulation of four keratinophilic fungi.