GENERAL SUMMARY
The term dermatophyte is used generally for filamentous fungi, bacteria and yeasts living on the surface of the skin is now restricted to parasitic fungi responsible for ringworm in man and animals. These fungi prefer to grow luxuriantly in the habitats rich in organic matter/keratin. The keratin material is added to the soil during animalization and occurs in the nature in the form of various animal appendages like hair, wool, feathers, nail, hooves, horns and also in outer keratinized layers of the skin. Most of these keratinophilic fungi are potential pathogenic to man and animals causing dermatophytic diseases.

During the survey of keratinophilic fungi in all 110 soil samples were collected from vicinity of Sagar. These samples were screened for the prevalence of keratinophilic and related dermatophytes. The samples yielded a total of 11 species of 5 different genera. The percentage distribution of each fungus is given in bracket against each species: Arthroderma benhamiae (2.72%), Chrysosporium crassitunicatum (2.72%), C. indicum (44.54%), C. tropicum (6.36%), Chrysosporium sp. I (0.90%), Chrysosporium sp. II (0.90%), Chrysosporium sp. III (0.90%), Malbranchea aurantiaca (3.63%), Microsporum gypseum (20.00%), M. fulvum (10.90%) and Trichophyton
sp. (1.81%). Among these C. indicum was isolated from all the habitats, showing its wide distribution in soils of Sagar. Fungi belonging to M. gypseum were found next in distribution. These findings strongly indicate that the well known dermatophytic species of Microsporum, Trichophyton and Arthroderma are of frequent occurrence in soils of Sagar. Out of these isolates only four fungal species i.e., Arthroderma benhamiae, Chrysosporium indicum, Malbranchea aurantiaca and Microsporum gypseum were selected for detailed physiological and biochemical studies.

The suitability of any culture medium depends upon the nature of an organism. In the present study seven different media were selected in which sabouraud's dextrose agar containing 1.0% peptone as nitrogen and 4.0% dextrose as carbon source has supported the best growth in most of the test organisms. All the test fungi exhibit maximum mycelial growth at 30°C temperature, M. aurantiaca and M. gypseum showed poor growth at 40°C while A. benhamiae and C. indicum fail to grow at this temperature. A temperature of 30°C was found to be the optimum for the spore germination of A. benhamiae, C. indicum, M. aurantiaca and M. gypseum with in 24 hours showing 51.0, 67.5, 70.0 and 72.0% germination respectively. A low temperature i.e., 20°C also supports the spore
germination of all the test fungi. Spore germination decreases by the increase in temperature up to 40°C. The study also revealed that germination of fungal spores is at a wide range of hydrogen-ion concentration (pH) i.e., at 4.0 to 9.0. The germination of all the fungal spores was found some what better in acidic medium than in the alkaline one. When these fungi were grown in the sabouraud's dextrose broth a gradual increase in the growth duration resulted in the increase of mycelial yield which was followed by a phase of no net growth or of autolysis. Maximum production of mycelium was recorded in those flasks harvested after 15 days of incubation. *M. gypseum*, *M. aurantiaca*, *A. benhamiae* and *C. indicum* showed 638, 500, 495 and 476 mg of mycelia, respectively. A gradual decrease in mycelial production was noted after 18 days of incubation.

In the present study four antibiotics i.e., griseofulvin, nystatin, tetracycline and chloramphenicol were taken to assess their antifungal properties against test fungi. Among test antibiotics griseofulvin was found to be most effective against all the test fungi. All the concentrations of this antibiotic showed cent percent inhibition in the growth of *C. indicum*. *M. gypseum* seems to be much susceptible showing no sporulation even at a mild dose of 50 ppm. *M. aurantiaca* was found
to be somewhat resistant in comparison to other test fungi showing 14.28, 22.85, 31.42, 32.57 and 34.85% growth inhibition at 50, 100, 150, 200 and 250 ppm concentration, respectively. Nystatin (Mycostatin) a polyene (tetraene) antifungal antibiotic showed its toxic effect against most of the test organisms. Higher dose of nystatin was found to be more effective than its mild dose. It showed fungitoxicity against A. benhamiae, M.aurantiaca and C.indicum, i.e., 60.00, 42.85 and 35.00% growth inhibition, respectively at a dose of 250 ppm. No sporulation was observed at 250 ppm concentration of nystatin in A. benhamiae, C.indicum and M.gypseum. Sporulation recorded was quite poor in M.aurantiaca in this treatment. Higher concentration of tetracycline (250 ppm) was found to be much effective against M.gypseum, C.indicum, M.aurantiaca and A. benhamiae showing 82.17, 72.5, 68.51 and 68% growth inhibition, respectively. No sporulation in M.gypseum was observed at its 250 ppm concentration. Chloramphenicol an antibacterial antibiotic found to be less effective against most of the test organisms.

In an experiment the sporostatic effect of antibiotic ointment was also tested. Mycostatin and griseafulvin which are known for their antifungal activity were tested against the keratinophilic fungi. The higher dose i.e., 6 mg of mycostatin showed 83.69%
inhibition in the spore germination of *A. benhamiae*. The same treatment was also found to be quite effective against *M. gypseum*, *C. indicum* and *M. aurantiaca* showing 78.94, 75 and 74.11% inhibition, respectively. In *C. indicum* 81.25% inhibition was recorded at a higher dose of griseofulvin. It was also found quite effective against other test fungi showing 78.26, 75.0, 78 and 70.58% inhibition in spore germination of *A. benhamiae*, *M. gypseum* and *M. aurantiaca*, respectively. Both the antibiotics showed their higher inhibitory capability with the increase in their doses.

The sensitivity of sulpha drugs, viz. sulphadiazine, sulphasemethoxazole, sulphamethoxazole, and sulphamethoxazole was assayed against keratinophilic fungi. Sulphadiazine and sulphamethoxazole were found to be most toxic for the mycelial growth in *M. aurantiaca* and *M. gypseum* showing cent percent inhibition at their higher concentration (500 ppm). With the gradual increase in a dose of sulpha drugs a reduction in the mycelial growth of keratinophilic fungi was noted. The effect of ointment containing sulphadiazine and sulphamethoxazole on the spore germination of test fungi was also evaluated. Sensitivity of sulpha ointment to fungal spores was found to be somewhat similar for all the test organisms at a higher dose (6 mg of sulpha drug mixed in 20 gm of white petroleum jelly) of sulphadiazine. It showed 84.21, 81.25, 80.48
and 76.59% inhibition in spore germination of *A. benhamiae*, *C. indicum*, *M. aurantiaca* and *M. gypseum*, respectively. *A. benhamiae* was found much sensitive at a higher dose (6 mg) of sulphamethoxazole showing 85.26% inhibition in spore germination. It was less effective for the spore germination of *M. aurantiaca* and *M. gypseum* at its mild dose.

Phenolic substances i.e., thymol, salicylic acid, B-nephthol and picric acid which are of use in medicines in the form of ointment or lotion against the skin diseases were assayed at different concentration (10, 20, 40, 50 and 100 ppm) against the keratinophilic fungi. It was noted that higher concentration (100 ppm) of thymol and B-nephthol caused cent percent inhibition in all the test fungi showing their fungicidal action. Salicylic acid and picric acid could not inhibited complete mycelial growth in any of the test fungi.

Sporostatic effect of phenolic compound i.e., thymol and salicylic acid which were showing strong fungitoxicity against test organisms were used in the present study. Four different doses of these phenolic compound were prepared in the form of ointment by mixing 1, 2, 4 and 6 mg phenolic compounds in 20 gm of petroleum jelly. A gradual increase in the inhibition of spore germination was noted by increasing the doses of phenolic
compound in the petroleum jelly based ointment. Sensitivity of spores of all the test organisms was found to be some what similar at a higher concentration i.e., 6 mg of salicylic acid. It showed 84.04, 83.69, 80.00 and 76.47% inhibition in spore germination of *A. benhamiae*, *M. gypseum*, *C. indicum* and *M. aurantiaca*, respectively. A mild dose 1 mg of salicylic acid showed 67.39, 42.22, 36.17 and 24.70% inhibition in spore germination of *M. gypseum*, *C. indicum*, *A. benhamiae* and *M. aurantiaca*, respectively. *A. benhamiae* was found to be sensitive at the higher doses of thymol i.e., 4 and 6 mg showing 78.72 and 84.04% inhibition, respectively. A drug dose of 6 mg caused 75.55, 72.82 and 70.58% inhibition in spore germination of *C. indicum*, *M. gypseum* and *M. aurantiaca*, respectively. Mild dose i.e., 1 mg of thymol was found to be less effective for spore germination of *M. gypseum* and *M. aurantiaca*.

Different hair dressing and vegetable oils i.e., groundnut, coconut, mustard and amla oil and seeds of mustard, ajwain, trigonella and garlic bulbs were mixed in the oil samples to determine their effects on the fungal spore germination. Some of these oils in combination with these seeds are used to cure skin and ear diseases caused by fungal pathogens. In the present study mustard oil was found to be most effective among the test oils. It showed cent percent inhibition.
in spore germination of *A. benhamiae* and *C. indicum*. This oil, when mixed with mustard, ajwain, trigonella seeds and garlic bulbs showed cent percent inhibition in spore germination of all the test fungi. In general, most of the oil samples showed more fungitoxic effect when boiled with mustard seeds.

In one of the experiments the vapours emanating from some volatile substances were tested against the growth and sporulation of keratinophilic fungi. For this purpose ten volatile substances i.e., ethyl alcohol, benzene, chloroform, isopropanol, formaldehyde, formic acid, pyridine, ethyl alcohol, acetaldehyde, n-butyric acid were selected. Chloroform, formaldehyde, formic acid, pyridine and n-butyric acid caused mycelial growth inhibition in all the test fungi. Maximum inhibition in growth i.e., 76.74% in *A. benhamiae* was recorded in the vapours emanated from chloroform. Maximum growth stimulation i.e., 6.97, 11.62, 16.27, 39.53 and 44.13% was noted when this organism was cultured in the presence of vapours emanating from benzene, ethyl alcohol, isopropanol, ethyl acetate and acetaldehyde, respectively. Ethyl acetate supported the best growth of *C. indicum*. Chloroform, ethyl alcohol, pyridine, isopropanol, formic acid, formaldehyde showed growth retarding property causing 44.14, 37.05, 10.08, 7.62, 3.44 and 3.26% growth inhibition, respectively against
M. aurantiaca. Maximum growth stimulation i.e., 5.48, 1.20 and 0.85% was noted when M. gypseum was cultured in the atmosphere saturated with the vapours of isopropanol, benzene and ethyl acetate respectively.

To evaluate the effect of fungistatic factors in soil, four different type of soils i.e., garden, forest, wheat field and roadside soil samples were collected from natural sites and used in the present study. Volatile emanations from garden soil caused 75% and 60% inhibition in M. aurantiaca and M. gypseum respectively. A. benhamiae and C. indicum showed only 30.58 and 50% inhibition respectively. The spores of these fungi seem to be not sensitive to the volatile emanations from the test soil samples.

The volatiles emanated from the forest soil showed cent percent inhibition against the spore germination of M. aurantiaca and M. gypseum, while only 85.88 and 85.71% inhibition in A. benhamiae and C. indicum, respectively was recorded. The volatiles emanated from wheat-field soil also showed cent percent inhibition in the spore germination of M. aurantiaca. Spores of the other test fungi were found to be quite susceptible to the volatile emanations of this soil. A total of 73.33, 42.85 and 40% inhibition in the spore germination of M. gypseum, C. indicum and A. benhamiae was recorded, respectively. The volatile emanations from the samples...
collected from road side showed 92.42, 84, 78.57 and 75% inhibition in A. benhamiae, M. gypseum, C. indicum and M. aurantiaca, respectively. These volatiles seem to be quite toxic showing maximum inhibition in most of the test keratinophilic fungi.

Antifungal activity of some essential oils of medicinal plants and their components have been also evaluated and discussed. The acetates of eugenol and geranyl were found to be most inhibitory against most of the keratinophilic fungi. The essential oils of Ocimum basilicum showed antifungal activity showing inhibition in the vegetative growth of all the test keratinophilic fungi. It is suggested that these oils can be employed as surface applicants as preventive measures against skin diseases caused by the test keratinophilic fungi.

Detailed experiments were performed to determined the changes in the free amino acid pool of A. benhamiae, C. indicum, M. aurantiaca and M. gypseum at different incubation periods. The data showed that keratinophilic fungi reach their optimum phase of the growth within 10 days and also found quite rich in amino acid content. The sporulation in most of the keratinophilic fungi was quite excellent after 5 days and upto 15 days of incubation. It is to be noted that during the phase of sporulation, vegetative mycelia required a large
number of amino acids to constitute the amino acid pool.

In vitro degradation of human hair was evaluated by the percentage weight loss. Keratinase activity was determined by Yu et al., (1969) and release of protein was evaluated by method described by Lowry et al., (1951). A total loss of 159 mg in the weight of hair in medium 'C' (K$_2$HPO$_4$; MgSO$_4$·7H$_2$O, NaCl; FeCl$_3$; Glucose) was noted after 30 days of incubation in the presence of A. benhamiae. In C. indicum a total loss of 85 and 120 mg was noted in medium 'A'. KH$_2$PO$_4$; MgSO$_4$·7H$_2$O; CaCl$_2$; FeSO$_4$·7H$_2$O; ZnSO$_4$) and in medium 'B' K$_2$HPO$_4$, MgSO$_4$·7H$_2$O; NaCl; FeCl$_3$) respectively after 45 days of incubation. The maximum keratinolytic activity was recorded in M. aurantiaca showing a total loss of 148 mg hair-keratin after 45 days of incubation. A gradual decrease in the alkalinity was noted by the release of protein in broth culture during the growth of the organisms on three different media was evaluated in terms of the activity and the nature an enzyme keratinase produced by these fungi. The net value was expressed as values of protein and keratinase activity i.e., the measured value in the test samples minus the sum of the values of keratin and fungus control. The keratinase activity was recorded near about equal and of similar nature in different incubation periods in all the
three culture conditions. Maximum keratinase activity, i.e., 14.32 KU/ml was exhibited by *A. benhamiae*. A rapid release of protein was observed after 45 days of incubation in *M. aurantiaca* (49.48 ug/ml) followed by *M. gypseum* (16.77 ug/ml), *C. indicum* (13.16 ug/ml) and *A. benhamiae* (12.11 ug/ml). Extensive damage to human hair was caused by *C. indicum* and *M. gypseum* while *M. aurantiaca* showed the rupturing and blistering in the hair. *A. benhamiae* could caused only minimal disruption of the substrate i.e., in hair pieces. Further detailed biochemical studies on keratinases of dermatophytes are needed for better understanding of the highly developed host parasite relationships of these fungi.

Data obtained from these studies will be of immense value for predicting the survival of these pathogenic fungi in natural habitats. These data will definitely be of much help in suggesting the proper and efficient therapy against the dermal diseases which are quite common in our country particularly in rural areas where persons remain in close contact with the soil and also come in close association with the diseased domesticated animals. The *in vitro* study of antibiotics, sulpha drugs and other related therapeutic agents can suggest their use against the mycotic infections caused by various species of dermatophytes and can help in solving the public health problem.