GENERAL SUMMARY
Otomycosis is one of the commonest findings in India during rainy season. A large number of people suffer from this disease in the central parts of India. In India majority of its population lives in villages. Illiteracy, poverty, lack of hygiene, health, education and medical care, all contribute to fungal infection of ear. Otomycosis goes unnoticed and unattended until it causes severe pain. A fungal infection is common in external ear canal (Post-operative mastoid cavities). Fungal flora of the air is now of great concern to man as possible sources of allergies and otomycosis in our country. Since fungus spores abound in the atmosphere, and might well enter the meatus only to lie dormant. Their detection by the culture of a swab taken from the meatus gives a clear picture of their role in the infection of the ear. However, the growth of moulds of the genus *Aspergillus*, *Penicillium*, *Rhizopus*, *Absidia* and also yeast-like fungi *Candida* on the walls of the external acoustic meatus. The promoting factors are general and local allergy, metabolic and neurohormonal disorders, and dysfunction of the ceruminous glands. Fungi grow to form a dense network of mycelium which causes inflammation of the skin.

Fungal infection of the external ear usually begins unnoticed by the patient. The main symptoms are
constant and severe itching (pruritis), increased
sensitivity of the acoustic meatus and the pinna, stuffiness and noise in the ears; in the absence of
exacerbation, pain in the ear is mild. Some patients
complain of headache on the involved side.

Mould-caused otomycosis is characterized by
pathological exudation in the external acoustic meatus,
which looks like wet blotting paper. The colour of the
discharge varies and depends on the colour of mycelium. It
can be dark-brown if the ear is affected by *Aspergillus
niger*, yellow or greenish in the infection with *A. flavus*
and greyish-black if infected with *A. fumigatus*. Otomycosis caused by the fungi of the genus *Candida* is
characterized by liquid serous discharge in the external
acoustic meatus with soft yellowish-white easily detachable
crusts throughout its entire length. The clinical picture
of otomycosis caused by yeast-like fungi resembles that
of weeping eczema of the external ear. Fungi, therefore,
could be mentioned as a normal mycoflora in the external
auditory meatus playing an important role in otomycosis
when conditioned by various factors paving the way for such
saprophytic organisms to gain a foothold in meatus.

In the present investigation, an effort has
been made to determine the prevalence of pathogenic fungi in external ear canal in some clinical specimens. Clinical specimens were obtained from the cases of infected ear canal and normal ear canal from the clinics of the district E.N.T. hospital.

For the present study 480 samples in all were collected that included 350 normal ears and 130 from infected ears, respectively. Mycological examination of these samples indicated fungal occurrence in 10.57 percent samples of normal ears, while a 43.84 percent samples belong to infected ears were found positive for fungal occurrence. A total of 16 fungal species belonging to 14 genera were isolated from all the samples examined during the present study. Amongst these 15 fungal species belonging to 13 genera were isolated from samples of the normal ear, while only 9 fungal species belonging to 7 genera were collected from samples of infected ear canal. (Their percentage is mentioned in the brackets). These are:

- **Aspergillus niger** (13.846 percent)
- **Aspergillus flavus** (6.923 percent)
- **Aspergillus fumigatus** (4.615 percent)
- **Penicillium nigricans** (4.615 percent)
- Absidia corymbefera  (6.153 percent)
- Candida albicans  (2.307 percent)
- Rhizopus sp.  (2.307 percent)
- Mucor sp.  (1.536 percent).

The most interesting finding of the present investigation was the high percentage of mixed fungal infections, as well as high percentage of Aspergillus sp. A large variety of symptoms and mycological appearance of otomycosis were encountered and studied in various individuals and in relation to the different types of fungi isolated.

A study of the external ear canal fungal flora isolated from clinical specimen has been made as ground work for the experimental section. For detailed physiological and biochemical studies five otomycotic fungi i.e., Aspergillus niger, A. flavus, Absidia corymbefera, Penicillium nigricans and Candida albicans were selected. Mycelial growth, sporulation and spore germination of these organisms were determined at different temperatures. These fungi grew well at 30°C. Candida albicans showed maximum mycelial growth, sporulation and spore germination at 35°C. It was found that growth rate decreases by the increase of
temperature up to 45°C. Spore germination of these fungi i.e., A. niger and A. flavus exhibited maximum germination at 6 hours light and 18 hours dark incubation in 24 hours. A. corymbifera, P. nigricans and C. albicans could grow even at 12 hours light and 12 hours dark incubation in 24 hours. The study also revealed that germination of fungal spores is at a wide range of hydrogen-ion concentration (pH). i.e., 4 to 9 pH. The germination of all the fungal spores was found somewhat better in alkaline than the acidic condition.

It is well known that some higher plants possess a number of antimicrobial ingredients in them. The fungitoxic effect of different plant extracts, i.e., bulb of onion (Allium cepa Linn.), bulb of garlic (Allium sativum Linn.), rhizome of ginger (Zingiber officinalis Rose), leaves of marry gold (Tagetes patula Linn.) and leaves of sukhdarshan (Hedychium coronarium Koeing.) against the test fungi was determined. Percentage inhibition of spore germination was also evaluated. The data revealed that the inhibition of spore germination in most of the test fungi, was prevented at higher concentration. Inhibitory activity was found to be more with the increase in the concentrations of plant extracts. Garlic, onion and ginger extracts inhibited the spore
germination in all the test fungi. Garlic bulb extract was found to be much more effective at higher concentration against *A. flavus*, *C. albicans*, *A. niger* and *P. nigricans*.

The cent percent inhibition in spore germination of *A. corymbefera* was recorded at a higher dose of garlic bulb and onion bulb extract caused 93.34 percent inhibition in the spore germination of *A. flavus*. Spores of other test fungi also showed more than 76 percent inhibition in spore germination in extract of onion bulb. Similar by the marry gold leaves, extract of sukhdarshan leaves were also found to be toxic to the spores of these fungi. In all the cases inhibition of spore germination was found directly proportional to the concentration of an extract in the medium.

Effect of some edible vegetable oils i.e., Mustard, Groundnut, Soyabean, Coconut and Amla hair oil was tested without and with heat treatment on the spore germination of test fungi. Amongst the oils tested mustard oil caused cent percent inhibition of the spore germination in most of the test organisms i.e., in *A. flavus*, *A. corymbefera*, *P. nigricans* and *C. albicans*. The oil as such, caused 86.37 percent inhibition in spore germination of *A. niger*. Coconut oil was also found cent percent
effective against spore germination in most of the test fungi. The spores of all the test fungi seem to be more resistant for two test oils i.e., groundnut and amla in comparison to other oils. In general, the test oils when heated for two minutes or fifteen minutes before testing, the increased inhibitory effect was noted against spore germination of almost all the test fungi.

Effect of some oil extracts of herbal seeds i.e., seeds of ajwain, mustard, trigonella and bulb of garlic were mixed in the above oil samples except amla hair oil to determine their effect on the spore germination. Some of the above oil in combination with these seeds are used to cure various skin and ear diseases caused by fungal pathogens. In the present study mustard oil with all the herbal seeds was found to show cent percent inhibition in spore germination. Mustard seed extract in coconut oil also caused cent percent inhibition in P. nigricans. In general all the oil samples showed their more fungitoxic effect when boiled with mustard seeds. The constituents of trigonella seem to be next to mustard seeds as far as their antifungal activity is concerned.

In the present investigation nine different varieties of perfums i.e., shamama, khas, musk, phulwari,
hina, jasmine, chandan, nagchampa and bela were tested against spore germination of all the test fungi. Most of the test perfumes emanating volatile vapours are toxic for germination of spores of the test pathogens. Volatile vapours emanating from some perfume i.e., musk, phulwari, jasmine, nagchampa and bela caused near about cent percent inhibition in spore germination of all the test fungi. Volatile emanating from chandan, khas and hina were not found much inhibitory for the test pathogens of otomycosis. The sporostatic effect of test fungi was determined in different types of fresh milk i.e., lactating woman, buffalo, cow and goat. Maximum sporostatic activity was recorded in cow milk against spore germination of A. niger, C. albicans, A. flavus and P. nigricans which accounts 82.31, 78.95, 76.00, and 65.46 percent inhibition of spores, respectively. In general A. niger and A. flavus were found to be sensitive to all the milk samples under test. spores of C. albicans were found to be somewhat resistant to most of the milk samples.

In the present study nine antibiotics i.e., mycostatin, griseofulvin, erythromycin, neomycin -sulphate, cephalexin, tetracycline, amoxycilline, ampicilline and chloramphenical were taken to assess their antifungal properties against test pathogens. Amongst test
antibiotics mycostatin was found to be most effective against all the test fungi its 300 ppm dose caused 64 to 94.60 percent inhibition in mycelial growth in all the test pathogens. Except C. albicans all the test fungi are found to be susceptible to the lower doses of this antibiotic. Griseofulvin also showed its toxic effect against the growth of A. niger, showing 90.97 percent inhibition in the mycelial growth at a concentration of 300ppm. Amongst the test organism A. niger was found quite susceptible to the higher concentration (i.e., 300ppm) of almost all the test antibiotics an inhibition of 92.05, 06.44, 07.00, 75.09 and 72.56 percent was recorded by cephalexine, chloramphenicol, amoxicilline, neomycin -sulphate, erythromycin, ampicilline and tetracycline, respectively. A. flavus was also found quite susceptible for the higher doses of these antibiotics. A reduction in sporulation in all the test organisms was also observed due to the presence of these antibiotic in the medium. In an experiment the sporostatic effect of antifungal antibiotic ointment was also tested. Mycostatin and griseofulvin which are known for their antifungal activity were tested against the otomycotic pathogens. When the higher dose i.e., 0.040 mg of griseofulvin was mixed in 20 gm of white petroleum jelly, 92.31 percent inhibition was observed in
A. niger while the other test fungi i.e., A. corymbefera, A. flavus, C. albicans and P. nigricans showed 91.42, 88.67, 78.73, and 47.28 percent inhibition, respectively. At a higher dose of mycostatin the inhibition of spore germination recorded in P. nigricans was cent percent. In other test fungi i.e., C. albicans, A. flavus, A. niger and A. corymbefera 97.88, 96.00, 95.39, and 95.30 percent inhibition in spore germination was recorded. Mild dose (0.005 percent w/w) of this antibiotic was found to cause 65.46, 63.83, 57.34 and 54.12 percent inhibition in P. nigricans, C. albicans, A. flavus and A. corymbefera, respectively. The spores of almost all the test fungi showed more than 50 percent inhibition of spore germination in the ointment having 0.005 percent antibiotic.

Fungitoxic effect of five phenolic compounds i.e., resorcinol, thymol, pyrocatachol, salicylic acid and hydroquinone was also tested against five otomycotic pathogens isolated from infected external ear canals. For this each phenolic compound was tested in its five different concentrations i.e., 25, 50, 75, 100 and 125 ppm. were incorporated in Sabouraud’s dextrose agar medium. Resorcinol was found with maximum fungitoxic effect at a dose of 125 ppm against C. albicans showing 78.67 percent inhibition in mycelial growth. In most of the test
pathogens 20 to 56.43 percent growth inhibition was caused by this phenol when used at a dose of 125 ppm in growth medium. Lower doses of resorcinol were also found to be toxic but to a lesser extent. Thymol and hydroquinone were found to be effective against most of the test pathogens. Thymol was found to be very effective against *P. nigricans* (91.23%), *C. albicans* (87.00%), *A. flavus* (73.86%) and *A. corymbifera* (68.02%). However, growth inhibition recorded in *A. niger* was only 24.54 percent at 125 ppm concentration. A gradual inhibition in the mycelial growth and sporulation in all the test organisms was noted by increasing the doses of all the phenolic compounds in the culture medium. Like pyrocatechol salicylic acid also showed its varied effects on the growth and sporulation of the test pathogens.

Sporostatic effect of the phenolic compounds i.e., Thymol and hydroquinone showed strong fungitoxic effect against test organisms used in the present experiment. Four different concentrations of these phenolic compounds were prepared in the form of ointment by mixing 0.005, 0.010, 0.020 and 0.040 percent of phenolic compound in 20 gm of white petroleum jelly. A gradual increase in the inhibition of spore germination was noted by increasing the percentage of phenolic compound in the petroleum jelly
based ointment. Higher dose of thymol i.e., 0.040 percent w/w was found to cause near about similar effect on spore germination of almost all the test fungi. At this dose 81.54 to 90.00 percent inhibition in spore germination of all the test fungi was observed. The lower dose of thymol in the ointment (0.005% w/w) was found to cause 31.92 to 56.00 percent inhibition in the spore germination in most of the test fungi. Hydroquinone at a dose of 0.040% w/w caused maximum inhibition in the spore germination of C. albicans (62.11%). Its lower dose i.e., 0.005 percent w/w was found to cause 41.16 percent inhibition in the spore germination of A. corymbifera in comparison to other test fungi.

In vitro efficacy of four sulfadrugs i.e., sulfadiazine, sulfamethoxazole, sulfaguanidine and sulfamoxol were taken in different doses (500, 1000, 1500, 2000 and 2500 ppm) to test their effectiveness against five pathogenic organisms for the control of mycelial growth and sporulation. Amongst the drugs tested sulfamethoxazole seems to be most effective against most of the test fungi showing 96.78, 90.53, 86.62, 54.16 and 43.91 percent inhibition in mycelial growth of P. nigriceps, A. flavus, A. corymbifera, A. niger and C. albicans, respectively at its 2500 ppm dose in the basal medium. Gradual reduction i
sporulation in all the test organisms almost in all the sulfadrugs have caused inhibition in sporulation, in comparison to their respective controls. Maximum inhibitory effect of sulfadiazine was recorded in A. niger showing 77.26 percent inhibition in the mycelial growth at a dose of 2500 ppm. Sulfaguanidine at its higher dose i.e., 2500 ppm proved to be most toxic against A. corymbifera causing 56.39 percent inhibition in the mycelial growth, while the same dose could not be found much effective against other test fungi. Sulfamoxol was found to be quite effective against most of the test fungi causing 47.16 to 85.86 percent inhibition in the mycelial growth. A gradual inhibition in the vegetative growth and sporulation in all the test fungi was noted by increasing the concentrations of the test sulfadrugs.

Effect of two sulfadrugs i.e., sulfadiazine and sulfamethoxazole on spore germination of the five test fungi was tested in the present study. For this ointment having different concentrations of the drugs (by mixing 1, 2, 4, and 6 mg of the drug in 20 gm base), were prepared in petroleum jelly and were then tested for their effect on spore germination. A gradual increase in the inhibition of spore germination was noted by increasing the concentration of test sulfadrugs, sulfadiazine at a higher dose (6 mg/20
gm jelly) caused 96.93, 94.67, 91.71, 88.19 and 78.95% inhibition in spore germination of A. niger, A. flavus, A. corymbifera, P. nigricans and C. albicans, respectively. Maximum effect of sulfamethoxazole was noted in A. niger causing 86.16 percent inhibition in spore germination. Lower concentration of this drug was not found much effective against C. albicans.

A remarkable antifungal activity was achieved by screening the sensitivity of otomycotic pathogens in different combinations of antibiotics and sulfadrgus. Combination of sulfadiazine with sulfamethoxazole was found to be highly toxic at a dose of 2500 ppm against A. flavus (76.13 %), A. corymbifera (73.83 %), and P. nigricans (73.69 %). A combination of mycostatin and griseofulvin was found to be most toxic for P. nigricans and A. niger when used at a concentration of 300 ppm, showing 85.97 and 85.55 percent inhibition in mycelial growth, respectively. A combination of mycostation with neomycin-sulphate was also found to cause maximum inhibition in A. niger showing 88.30 percent growth inhibition at 300 ppm concentration.

To evaluate the effect of fungistatic factors in soil, four types of soils i.e., forest soil, garden
soil, wheat-field soil, and road-side soil samples were collected from natural sites and used for the present investigation. Volatile emanations from forest soil caused 76 percent inhibition of spore germination in A. corymbifera, C. albicans and P. nigricans while only 87.15 and 84.62 percent inhibition in A. niger and A. flavus, respectively was recorded. The volatiles of wheat-field soil showed 60 percent inhibition in A. corymbifera only. Emanations from garden soil could cause only 60 to 89 percent inhibition in the spore germination of the test fungi. The volatile emanations of road-side soil caused 46.00 and 60.68 percent inhibition in spore germination of A. corymbifera and C. albicans, respectively.

The effect of vapours emanating from twelve different organic volatiles compounds i.e., formaldehyde, n-butyric acid, acetic acid, ethyl-acetate, carbon-disulphide, nepthalene, chloroform, petroleum-benzene, methanol, hydrogen-peroxide, carbon-dioxide and ammonia was evaluated against five test fungi. Maximum inhibition in vegetative growth was recorded when the fungi were grown in an atmosphere of ammonia showing no growth in all the test organisms. Carbon-disulphide was also found to be toxic showing maximum inhibition in A. corymbifera. Petroleum benzene was found to be toxic for A. corymbifera and A.
flavus. In most of the test organisms less than 50 percent inhibition was recorded. Toxicity of carbon-dioxide was found to be maximum in case of P. nigricans, A. flavus and A. niger. The gas was not found to be much effective for C. albicans and A. corymbefera. Inhibition of mycelial growth in methanol and acetic acid was maximum in most of the Ascomycetous fungi including the species of Aspergillus, Penicillium. A zygomycetous fungus i.e., A. corymbefera was found to be somewhat susceptible for these volatile substances. In most of the test fungi no sporulation was recorded in the presence of ammonia, methanol, hydrogen-peroxide, and carbon-dioxide. Except P. nigricans and C. albicans all the other test fungi showed no sporulation in an atmosphere saturated with carbon-disulphide.

For antagonistic activity twelve fungi including five test fungi causing otomycosis were selected. They were found to be of common occurrence in the external ear canal. Activity of all the twelve fungi was tested against five otomycotic pathogens. Amongst the test antagonists C. albicans was found to antagonise A. fumigatus. P. nigricans inhibit the growth of A. flavus. A. flavus and A. niger were found to inhibit the growth of A. fumigatus. However, most of the fungal species were not
found antagonistic to test fungi.

Data obtained from these studies are of immense value for predicting the survival of these pathogenic fungi in natural habitats. The present experiments and the data obtained are mere outline of the complex problems of the physiology and etiology of the fungal pathogens causing otomycosis. The data will definitely help in suggesting the proper and efficient therapy against this ear diseases which are quite common in our country particularly in rural areas where the children remain in close contact with the soil and where there is less modern medical facilities available to common people.