SPOROTRICHUM SCHENCKII ECOLOGY MAJOR LIPID CONSTITUENTS

ABSTRACT

The study was carried out to find significant lipid constituents of S. schenckii, purified lipid extract was assessed, and the ecology of S. schenckii was also studied.

Phospholipids, triglycerides, and cholesterol were the three major lipid constituents of S. schenckii (77.9%). Other lipids (22.1%) were too few to be quantitated.

16 different types of fungi were isolated out of 120 samples processed; the maximum isolations were of *Aspergillus* spp., the most typical contaminant fungus. There was no particular correlation between the fungus isolate and the type of sample or its collection site. The pattern of various fungal isolates was almost identical irrespective of the sample and place. None of the samples processed was positive for S. schenckii

S. schenckii, though said to be a saprophyte, was not grown in the 120 samples studied. The endemicity of the disease, however, points towards the existence of the fungus in the area.

INTRODUCTION

Sporotrichum schenckii (S. schenckii), a fungus with worldwide distribution, is sporotrichosis's causative agent. It is present in a mold form when growing in soil or cultures incubated at 25°C, but in leathery filamentous yeast when seen in tissues or culture at 37°C. (Schenck, 1898). It was first cultured and described by Schenck. It is responsible for a clinical entity broadly known as sporotrichosis.

S. schenckii is widely distributed in nature as a saprophyte. It can be recovered from soil, wood, dead plants, and decaying organic matter. High humidity and a temperature range between 79° - 84°F are favorable conditions for the saprophytic growth of the fungus (Dey et al., 1973). Sporotrichosis occurs around the world in temperate and tropical zones.

Sporotrichosis is a subacute or chronic granulomatous mycosis that may remain localized for months, may spread locally along the lymphatics, and may get disseminated involving bones and systems. Joints, lungs, and the central nervous The infection follows the entry of fungus into the body by accidental inoculation or inhalation of the contaminated material. There is no evidence of direct transfer of infection from man to man. Not only humans but the mule, dog,

horse, cattle, fowl, rats, mice, and chimpanzees may also be affected (Ormsby & Montgomery , 1955). Animals, however, are not an important source of infection for humans though several cases after handling infected cats have been described recently.

The Lymphocutaneous type is the most common form of sporotrichosis. This is a rare infection but has been reported on all continents. It is the most standard type of deep mycoses found in Mexico and other Latin American countries (Dominguez-Soto & Hojyo-Tomoka , 1983). Epidemics have been reported in the literature from time to time, the biggest epidemic being in South Africa in gold mines (Quintal , 2000). The workers in the mine became infected following injuries to the skin while working in the mines. The fungus grew as a saprophyte on the timbers used in the mines.

Sporotrichosis has also been reported in India. Most of the cases have been from North Eastern parts (Baruah et al., 1975), probably because climatic conditions are more favorable for the saprophytic existence of this fungus.

The existence of sporotrichosis has also been documented in Himachal Pradesh (Singh et al., 1968). Sporotrichosis is not as rare of a disease as it was previously thought to be, and the frequency of infection indicates its clinical importance. Infections have been reported in both sexes between the age group of 16 months to 81 years.

Clinically. a patient of sporotrichosis presents with the most common lymphocutaneous type of infection or disseminated sporotrichosis which may involve viscera, skeletal system, or mucosal surfaces. Commonly, the patient suffers from a chronic, non-healing ulcer that tends to spread along lymphatics and is usually present on the extremities.

The clinical diagnosis of lymphocutaneous type is straightforward, provided the condition is kept in mind. Other sporotrichosis types are difficult to diagnose, and laboratory help is needed. For the laboratory diagnosis of sporotrichosis, direct microscopy of the clinical material is of little value as S. schenckii is too less in quantity in the infected tissues to be demonstrated directly. Moreover, a biopsy is not indicated for fear of dissemination. Material from the lesion cultured on Sabouraud's Dextrose Agar (SDA) is the most dependable mode of diagnosis. Histopathological examination is not a very suitable method of diagnosis as the fungus is rarely demonstrated in tissues.

S. schenckii is a saprophyte present in the environment of man, such as plants, leaves, dead and decaying organic matter, wood, sphagnum moss, potting soil (Kenyon et al., 1984), rat droppings, and other environmental substrates.

Keeping in mind the occurrence of sporotrichosis in Himachal Pradesh, it is worth finding its saprophytic existence in and around Shimla.

MATERIAL AND METHODS

Culture of S. schenckii

A strain of S. schenckii (S- 22/87) isolated from a case of lympho cutaneous type of sporotrichosis was used in the study. This strain was being maintained in the Department of Microbiology on slants of Sabouraud's dextrose agar. S. schenckii was inoculated on SDA with chloramphenicol (.05 mg/ml).

Preparation of SDA with Chloramphenicol

The Peptone: 10 gm.

Agar: 20 gm.

Dextrose: 40 gm.

Distilled water: 1000 ml.

Chloramphenicol: 50 mg.

The ingredients except chloramphenicol were dissolved in distilled water, PH adjusted to 5.6, and autoclaved at 120°C for 10 minutes. The medium was allowed to cool to about 48°C, and 50 mg. of chloramphenicol suspended in 10 ml. of 95% alcohol was added. The medium was distributed in 9 ml. aliquots in sterilized 15x150 mm. test tubes. The tubes were kept in such a way that the medium solidified with a slant on the surface.

The isolated strain of S. schenckii was inoculated on SDA slants and incubated at 25°c. It grew as a moist mycelial colony with a membranous surface. On the 8th day of inoculation, the colony was carefully scrapped with a spatula, and the growth from all the tubes was pooled and weighed.

Preparation of Homogenate

The pestle and mortar were thoroughly cleaned and rinsed with chloroform-methanol 2: 1 (v/v). The scrappings were shifted to mortar, and the homogenate was prepared by mixing with chloroform-methanol solution till the whole of the growth was grinded to a smooth paste. The homogenized mixture was further processed for the extraction of lipids.

Extraction and Purification of Lipids

In the present study, the method of Folch et al. (1957) was followed for extracting the lipid of S. schenckii as given below:

1. The homogenized mixture (vide supra) was filtered in a graduated cylinder through whatman No.1 filter paper (previously washed with chloroform and methanol). The residue on the filter paper was washed three times with chloroform-methanol (21), and a known volume was made (20 ml for 1 gm).

2. To the filtrate, .7% Potassium chloride (20% of the total extract volume) was added and mixed vigorously. The mixture was allowed to stand to separate the aqueous and chloroform layers.

3. The upper aqueous layer was removed by aspiration with a pasteur pipette, and the lower layer was washed with 5 ml. of chloroform-methanol potassium chloride (.7%), 3:48:47 (v/v). The procedure was repeated 3 times.

4. The washed lower layer was transferred to a round bottom flask and evaporated to dryness in a flashy evaporator at a temperature below 45°c.

5. To the residue, 5 ml. of chloroform-methanol, 2:1 (V/V) containing 4% water (chloroform - methanol-water, 64:32:4, (V/V) was added and evaporated to dryness.

6. The residue was carefully transferred in 2 ml aliquots to a glass stoppered tube three times quantitatively. The solvent was evaporated, and the volume was made to 4 ml. with chloroform. The lipid extract was preserved at 0°c.

Total Lipid Estimation

For estimating total lipid, a 5 ml. beaker was used, and its initial weight was taken. 1 ml. of lipid extract was added with the help of a blowing pipette. The beaker was placed in a desiccator and allowed to remain there overnight for the lipid extract to dry. The beaker was weighed the next day, and the procedure was repeated till a constant weight of the beaker was obtained. Total lipid was calculated by subtracting the initial weight of the beaker from the final weight.

Total phospholipid estimation

Phosphorous content was estimated from a known volume of lipid extract and multiplied by 25 (taking an average molecular weight of 875 for a Phospholipid) to get the total phospholipid content.

Estimation of Phosphorous

In the present investigation, modified Bartlett's method was used as described below (Bartlett, 1959).

Principle

When an acid hydrolysate of any substance containing phosphorous was treated with molybdate, it formed phosphomolybdic acid with inorganic phosphate present. Phosphomolybdic acid was reduced by adding 1 - amino, 2 - naphthol, and 4 - sulfonic acid reagent to produce a blue color, the intensity of which was proportional to the amount of phosphorus present.

Reagents

1. Standard Phosphorous: Stock standard (1 mg Phosphorous/ml). 439 mg of potassium dihydrogen phosphate was dissolved in 100 ml of water.

Working standard (10mg/ml): 1 ml of the stock standard was diluted to 100 ml with distilled water.

2. 1 amino, 2 naphthol, 4 sulfonic acid (ANSA) reagent.

This was prepared by dissolving the following substances in distilled water and making the volume 100 ml. in a volumetric flask.

1 amino, 2 naphthol, 4 sulfonic acid - 200 mg.

Sodium metabisulfite - 12.00 gm.

Sodium sulfite (hydrated) - 2.4 gm.

or

Sodium sulfite (anhydrous) - 1.2 gm.

The solution was filtered and stored in the dark at 4°c.
3. Ammonium molybdate (2.5%): 2.5 gm of ammonium molybdate was dissolved in 100 ml of distilled water using heat.

4. Perchloric acid 70%.

Method

1. 1, 2, 3, 4, and 5 ugs of Phosphorous were taken in different test tubes.

2. .9 ml of perchloric acid was added to each tube. The tubes were kept in a heated sand bath and digested for about 10 minutes till the solution became colorless. Glass beads were added to avoid bumping. Minimum amounts of perchloric acid fumes were allowed to escape from the tubes.

3. The tubes were allowed to cool, and 7.0 ml of distilled water was added to each tube.

4. 0.5 ml of ammonium molybdate solution was added to each tube and mixed well.

5. 0.2 ml of ANSA reagent was added and mixed well.

6. The mouths of the tubes were covered with aluminum foil to ensure constant volume, and the tubes were kept in a boiling water bath for 7 minutes. The tubes were cooled, and the optical density of the color produced was measured at a wavelength of 830 mu with a Bausch and Lomb Colorimeter. After standardizing the method, the phosphorous content of lipid extract was estimated in a similar way.

Separation of phospholipids and neutral lipids

For the separation of phospholipid and neutral lipid fractions of lipid extract, thin layer chromatography (TLC) was used based on differential migration.

Preparation of plates

Glass plates 20 x 20 cm. in size were cleaned, rinsed with distilled water, and allowed to dry. Again the plates were washed with chloroform. Silica Gel G slurry was made by quickly mixing 30 gm of silica gel G and 60ml of distilled water. The plates were coated with the slurry to 0.5 mm thickness. The plates were allowed to dry and were put for pre-run in the solvent mixture to be used for development. It will remove any impurities left on the plate. When the pre-run was complete, the plates were kept in an oven at 110°C for one hour for activation.

Separation of Individual Phospholipids

Spotting

The plate, when still warm, was placed on the table, and spotting started from the left end of the plate. The different spots were about 2 cm apart in a straight line leaving about a 2 cm margin from the bottom. 7-10 ug of lipid phosphorous was spotted.

Plate Developing

A strip of filter paper soaked in a solvent system was kept on the inner wall of a rectangular jar containing a solvent system of chloroform-methanol ammonia, 6512514 (V/V), to provide proper saturation with solvent vapors. Then the plate was run in the jar. In this solvent, the neutral lipids moved along with the solvent front separating phosphatides in the order of Cardiolipin (C), Phosphatidyl ethanolamine (PE), Phosphatidylcholine (PC), Sphingomyelin (SPH), Phosphatidylinositol (PI), Phosphatidylserine (PS) and Lysophosphatidylcholine (LPC). Different Phospholipid spots were identified using authentic markers.

Detection of Spots on TLC plate

The plate, after developing, was exposed to Iodine vapors. The phospholipid fractions that appeared as yellowish brown spots were identified and marked with a sharp needle.

Estimation of Individual Phospholipids

The spots of different phospholipid fractions were directly scrapped off into the test tubes. Their phosphorous content was estimated after digestion by the method of Martin and Bartlett (1957). Silica gel did not interfere in the development of the color. In the end, the tubes were centrifuged at 2000 rpm for 10 minutes, and readings were taken using a red filter in a photoelectric Colorimeter. Phospholipid content was obtained by multiplying phosphorous content by 25. The percentage of phospholipids in a particular spot was calculated by considering the total amount of phospholipids spotted on the plate.

Separation of Neutral Lipids

Spotting of lipid extract was done on a silica gel G thin layer plate using .05 ml for each spot to separate phospholipids; standards run simultaneously.

The plate was developed in a solvent system of petroleum ether - diethyl ether acetic acid, 90:10:1 (V/V). The plate was exposed to iodine vapors. The Yellowish brown spots of triglyceride, cholesterol, and cholesterol ester were identified and marked with a sharp needle. Silica gel from the spots was directly scrapped off with the help of a sharp blade into the different labeled tubes. Free cholesterol, triglyceride, and cholesterol ester were eluted from the silica gel with a 5 ml aliquot of chloroform thrice. The extracts were under vacuum and processed for estimation of cholesterol and triglycerides.

Estimation of Cholesterol

Cholesterol was estimated according to the method of Zlatkis et al. (1953).

Reagents

1. Stock ferric chloride reagent: 5 gm. of anhydrous ferric chloride was dissolved in 50 ml of glacial acetic acid.

2. Working ferric chloride solution 1 ml of stock ferric chloride was diluted to 100 ml with concentrated sulphuric acid of analytical grade. For this purpose, 30 ml of concentrated sulphuric acid was taken in a graduated cylinder, and 1 ml of stock ferric chloride was added with stirring to prevent the formation of ferrous sulfate precipitate. The volume was made to 100 ml with concentrated sulphuric acid. Working ferric chloride solution was prepared freshly just before use.

3. Standard cholesterol 100 mg of cholesterol was dissolved in 100 ml of chloroform and stored in a cool place. The concentration of standard was 1 mg/ml.

Method

1. 20, 40, 50, 60, and 120 ugs. of cholesterol standards were taken in different test tubes and dried in an oven at 45°C.

2. 3 ml of glacial acetic acid was added to each tube.

3. 2 ml of working ferric chloride reagent was added and mixed well.

Tubes were kept in the dark for 30 minutes. After that, the optical density was measured at a wavelength of 540 mu in the Bausch and Lomb colorimeter. Blank was run simultaneously, which contained glacial acetic acid and working ferric chloride. After standardizing the method, cholesterol and cholesterol ester were estimated in the test sample.

Estimation of Triglyceride

Triglyceride estimation was done according to the method of Van Handel and Zilversmit (1957).

Principle

Triglyceride was hydrolyzed to glycerol and free fatty acids by refluxing with an alcoholic KoH solution. Glycerol formed a violet-colored complex with chromotropic acid in the presence of sodium sulfite and sodium periodate, which had maximum absorption at 570 mu.

Reagents

1. A standard triglyceride solution: 500 mg of trioleate was dissolved in 100 ml chloroform (500 mg / 100 ml).

2. Working standard: One ml of stock standard solution was diluted to 100 ml with chloroform (50 ug/ml).

3. 0.1 N alcoholic Potassium hydroxide (KOH): 280 mg of KOH was dissolved in 50 ml of absolute alcohol.

4. Sodium periodate (.05 m): 107 mg of Sodium periodate was dissolved in 10 ml of water.

5. Sodium sulfite (20%): 2 gm of sodium sulfite was dissolved in 10 ml water. Reagents No. 4 and 5 were prepared freshly just before estimation.

6. 0.4 N Sulphuric acid (H_2, SO_4).

7. 66% H_2 SO_4: 300 ml of conc. H_2 SO_4 was added slowly to 150 ml of distilled water.

8. Chromotropic acid: 500 mg of Chromotropic acid was dissolved in 10 ml of water, and then 250 ml of 66% H2SO4 was added to it and mixed well. This was filtered through glass wool and preserved in a brown glass bottle at 4°c. The glass wool was treated with 66% H_2 SO_4 before being used for filtration.

Method

1. 25, 50, 75, 100, and 125 ugs of triglyceride standards were taken in different test tubes.

2. The chloroform of the standard solution in different test tubes was evaporated.

3. 0.5 ml of alcoholic KOH solution was added to each tube.

4. Tubes were kept at 70°C for 20 minutes and subsequently kept in a boiling water bath till the smell of alcohol disappeared.

5. 0.2 ml of 0.4 N H_2 80 was added to each tube.

6. 0.1 ml of Sodium periodate solution was added and kept for 10 minutes.

7. 0.2 ml of Sodium sulfite solution was added to each tube.

8. 8 ml of chromotropic acid was added to each tube, and the tubes were kept in a boiling water bath for 30 minutes.

9. The tubes were cooled under tap water, and the optical density was read at a wavelength of 570 mu in a Bausch and Lomb colorimeter. A blank consisting of reagents without triglyceride standard was run simultaneously.

A similar procedure was adopted for estimating the tubes containing eluted dried triglyceride fractions.

Isolation of S. schenckii from the environment

For the isolation of S. schenckii, a total of 120 samples were collected from different sources in and around Shimla town. Samples were collected as follows -

A. Central Potato Research Institute, Shimla - 171 002.

30 samples:
- 7 decaying leaves samples
- 5 potting soil samples
- 5 decaying wood samples
- 5 straw samples
- 5 moss samples
- 3 bark samples.

B. Indira Gandhi Medical College, Shimla - 171 001.

30 Samples:
- 8 decaying wood samples
- 7 sphagnum moss samples
- 5 decaying leaves samples
- 5 tree bark samples
- 3 straw samples
- 2 rat dropping samples.

C. Institute of Advanced Studies, Shimla - 171 005.

25 Samples:
- 5 potting soil samples

5 decaying leaves samples
5 decaying wood samples
5 tree bark samples
5 rose thorns samples.

D. Central Fruit Research Centre, Mashobra, Shimla - 171 007.

20 Samples: 5 potting soil samples
5 decaying leaves samples
5 decaying wood samples
5 straw samples.

E. Wild Flower Hall, Charabra, Shimla - 171 007.

15 Samples: 5 decaying leaves samples
4 sphagnum moss samples
2 potting soil samples
2 tree bark samples
2 thorn samples.

Method of collection

All 120 samples were collected from different places during June 2020 to Sept. 2020. These collections were made at randomly selected sites, and all the samples were scooped into labeled plastic bags; the mouths of the bags were closed and taken to the laboratory. These were kept at room temperature for some days before being processed to decrease bacterial contamination.

The samples were processed by the method of Howard and Orr (1963). The medium used consisted of Sabouraud's dextrose agar containing

C. Penicillin	1000 units/ml.
Streptomycin sulfate	.1 mg/ml.
Cycloheximide	.5 mg/ml.

Penicillin and Streptomycin were dissolved in sterile distilled water, while Cycloheximide was dissolved in acetone. SDA was autoclaved at 120°C for 10 minutes, cooled to 48°C, above antibiotics were added and distributed in about 9 ml amount in autoclaved 15 x 150 mm tubes.

The samples of wood, leaves, sphagnum moss, barks, straw, wood, and rat droppings were inoculated directly on SDA slants containing Penicillin, Streptomycin, and Cycloheximide. Soil samples were moistened with sterile water containing antibiotics in concentrations as in medium and then inoculated on the above medium.

The cultures were incubated at 25°C and examined daily for any growth. The growth thus appeared and was identified based on colony character, gram staining, and lactophenol cotton blue preparation. The fungi not fitting into S. schenckii on colony characters and microscopic examination were not attached to any further importance and were diagnosed only on these two criteria. The growth resembling S. schenckii was further processed. It was incubated at 37°C in Brain heart infusion broth at pH 5.6 and examined daily for turbidity. When growth was observed, smears were prepared, gram stained, and looked for yeast-like cells, which are characteristics of S. schenckii grown at 37°c.

RESULTS

Weight of pooled S. schenckii growth - 1.940 gm.

TABLE I - Major Lipid Constituents of S. Schenckii

Constituents	mg/gm of wet weight
Total Lipids	18.56
Total Phospholipids	10.36 ± .25
Triglycerides	3.10 ± .26
Total Cholesterol	1.00 ± .08

Values are mean ± S.D. of 6 observations.

TABLE II - Percentage of Major Lipid Constituents of S. Schenckii in total Lipid

Constituents	% of total lipid
Total Phospholipids	55.8
Triglycerides	16.7
Total Cholesterol	5.4
Others Lipids	22.1

Values are mean ± S.D. of 6 observations.

Table 1 depicts the amounts of major lipid constituents of S. schenckii expressed on the wet weight basis. It is evident that Phospholipids constitute (10.36 ± 25 mg/gm wt.) the significant portion of lipids. Cholesterol contents (1.00 ± .08) of S. schenckii are the lowest of all lipid classes investigated. Triglycerides fall in the intermediary range (3.10 ± .26).

Percentage of Phospholipid, Triglycerides, and Cholesterol in total lipids are shown in Table II. It is observed that Phospholipids, the major lipid fraction of S. schenckii, constitute 55.8% of the total lipids, followed by Triglycerides (16.7%) and Cholesterol (5.4%). Other lipids (22.1%) were scattered in multiple small bands on thin layer chromatography. The fractions were too small to be quantitated. Hence these fractions were considered collectively.

TABLE III - Cholesterol Fractions of S. Schenckii

Constituents	mg/gm weight	Percent of total Cholesterol	Percent of total Lipid
Free Cholesterol	.73 ± 08	73.2	3.94
Esterified Cholesterol	.27	26.8	1.45

Values are Mean ± S.D. of 6 observations.

TABLE IV - Phospholipid Constituents of S. Schenckii

Constituents	mg/gm wet weight	Percent of total Phospholipids	Percent of total Lipid
PE	2.82 ± .12	27.2	15.2
PC	3.68 ± .11	35.5	19.8
Sph	1.43 ± .07	13.8	7.7
PI + PS + LPC	2.44 ± .17	23.5	13.1

Values are Mean of 6 observations.

PE - Phosphatidylethanolamine

PC - Phosphatidylcholine

Sph - Sphingomyelin

PI - Phosphatidylinositol

PS - Phosphatidylserine

LPC - Lysophosphatidylcholine.

Out of cholesterol fractions of S. schenckii, free cholesterol is the main component, followed by esterified cholesterol, as depicted in above Table - III.

The percentage distribution of free and esterified cholesterol in total cholesterol show 73.2% and 26.8%, respectively.

The percentage of free and esterified cholesterol in the total lipid content of S. schenckii is shown in Table III. Free cholesterol constitutes 3.94% and esterified Cholesterol 1.45% of total lipids.

Individual phospholipid fractions are shown in Table IV. Phosphatidylcholine (PC) constitutes the significant amount, followed by Phosphatidylethanolamine (PE), Phosphatidylinositol (PI), Phosphatidylserine (PS), and Lysophosphatidylcholine (LPC) combined fraction and sphingomyelin (Sph).

The percentage distribution pattern of various phospholipid constituents in total phospholipids of S. schenckii depicts Pc as the principal constituent, which accounts for 35.5%, followed by PE (27.2%) and Sph (13.8%). PI, PS, and LPC constitute 23.5% of the total phospholipids of S. schenckii. Among the total lipids, S. schenckii has PC 19.8%, PE 15.2%, Sph 7.7%, and PI, PS, and LPC (combined) contribute 13.1%.

TABLE V - Fungi Isolated

Fungus Isolated	No. of samples
Aspergillus spp.	59 (*A.niger* - 39, *A.fumigatus* - 11, *A.flavus* - 9)
Trichophyton spp.	43 (*T.rubrum* - 8, other species - 35)
Penicillium spp.	32

Alternaria spp.	14
Mycelia Sterilia	11
Mucor spp.	7
Hemispora spp.	6
Nigrospora spp.	5
Candida spp.	3
Cladosporium spp.	3
Rhizopus spp.	2
Monontospora spp.	2
Fusarium spp.	2
Helminthosporium spp.	1
Trichothecium spp.	1
Microsporum spp.	1

Multiple fungus were grown in a single sample in many samples.

Table VII depicts 16 types of fungi isolated from a total of 120 samples. Maximum isolations were of *Aspergillus* spp. (59) followed by *Trichophyton* spp. (43), *Penicillium* spp. (32) and *Alternaria* spp. (14). These four fungi constituted 77% of the isolates. Other fungi belonging to 12 different species made up only 23% of the isolates. *Helminthosporium* spp., *Trichothecium* spp., and *Microsporum* spp. were isolated only in one sample each.

TABLE VI - Fungi Isolated from Different Samples

Samples	No. studied	Fungi grown
1. Leaves	27	*Aspergillus* spp. (12), *Trichophyton* spp. (9), *Penicillium* spp. (8), Mycelia Sterilia (3), *Nigrospora* spp. (2), *Heimspora* spp. (2), *Cladosporium* spp. (2), *Microsporum* spp. (1), *Alternaria* spp. (1), and *Rhizopus* spp. (1).
2. Wood	23	*Aspergillus* spp. (15), *Trichophyton* spp. (8), *Penicillium* spp. (6), Mycelia Sterilia (2), *Alternaria* spp. (2), Monontospora spp. (1), *Mucor* spp. (1), *Rhizopus* spp. (1), and *Helminthosporium* spp. (1).
3. Potting soil	17	*Aspergillus* spp. (9), *Trichophyton* spp. (8), *Penicillium* spp. (3), Mycelia Sterilia (3), *Alternaria* spp. (3), *Hemispora* spp. (2), *Cladosporium* spp.(1), and *Nigrospora* spp. (1).
4. Sphagnum moss	16	*Aspergillus* spp. (7), *Penicillium* spp. (7), *Trichophyton* spp. (4), Mycelia Sterilia (1), *Trichothecium* spp. *Monontospora* spp. (1), *Candida* spp. (1), *Hemispora* spp. (1), and *Alternaria* spp. (1).
5. Bark	15	*Aspergillus* spp. (8), *Trichophyton* spp. (5), *Penicillium* spp. (3), *Alternaria* spp. (2), Mycelia

		Sterilia (1), *Candida* spp. (1), *Hemispora* spp. (1), and *Nigrospora* spp. (1).
6. Straw	13	*Trichophyton* spp. (6), *Aspergillus* spp. (4), *Alternaria* spp. (4), *Mucor* spp. (3), *Penicillium*, spp. (2), and *Fusarium* spp. (2).
7. Thorn	7	*Aspergillus* spp. (3), *Trichophyton* spp.(3), *Penicillium* spp. (2), *Mucor* spp. (2), Mycelia Sterilia (1), *Candida* spp. (1), and *Nigrospora* spp. (1).
8. Rat dropping	2	*Aspergillus* spp. (1), *Penicillium* spp. (1), and *Alternaria* spp. (1).

Various types of fungi were isolated from different samples, as depicted in above Table - VIII. *Aspergillus* spp. showed maximum isolation in all the samples. Most of the other fungi were isolated in almost all samples, with the exception of Helminthosporium found in only one of the wood samples and *Trichothecium* spp. in one of the moss samples only. Similarly, *Fusarium* spp., *Rhizopus* spp., *Monontospora* spp., and *Cladosporium* spp. were isolated in a few samples only, as shown in the table.

TABLE VII - Fungal Flora of Different Places

Place	No. studied	Fungi grown
1. Central Potato Research Institute, Shimla - 171 002	30	*Aspergillus* spp. (16), *Penicillium* spp. (11), *Trichophyton* spp. (9), Mycelia Sterilia (4), *Alternaria* spp. (3), *Hemispora* spp. (3), *Microsporum* spp. (1), *Candida* spp. (1), *Helminthosporium* spp. (1), *Mucor* spp. (1), *Cladosporium* spp. (1), *Nigrospora* spp. (1), and

		Fusarium spp. (1).
2. Indra Gandhi Medical College, Shimla - 171 001	30	*Aspergillus* spp. (13), *Penicillium* spp. (11), *Trichophyton* spp. (9), *Alternaria* spp. (4), Mycelia Sterilia (3), *Candida* spp. (4), *Hemispora* spp. (1), *Monontospora* spp. (1), *Mucor* spp. (1), *Nigrospora* spp. (1), and *Rhizopus* spp. (1).
3. Institute of Advanced Studies, Shimla - 171 005	25	*Aspergillus* spp. (13), *Trichophyton* spp. (13), *Penicillium* spp. (3), *Mucor* spp. (3), *Nigrospora* spp. (2), Mycelia Sterilia (2), *Alternaria* spp. (1), *Hemispora* spp. (1), *Monontospora* spp. (1), and *Rhizopus* spp. (1).
4. Central Fruit Research Centre, Mashobra, Shimla - 171 007	20	*Aspergillus* spp. (10), *Trichophyton* spp. (8), *Penicillium* spp. (5), *Alternaria* spp. (3), *Mucor* spp. (2), *Cladosporium* spp. (1), *Hemispora* spp. (1), *Fusarium* spp. (1), *Nigrospora* spp. (1), and Mycelia Sterilia (1).
5. Wild Flower Hall, Charabra, Shimla - 171 007	15	*Aspergillus* spp. (7), *Penicillium* spp. (6), *Trichophyton* spp. (3), *Cladosporium* spp. (1), *Candida* spp. (1), *Hemispora* spp. (1), *Mucor* spp. (1) and *Trichothecium* spp. (1).

Table - IX depicts various fungi isolated from different places in and around Shimla. Samples from all these places showed maximum isolations of *Aspergillus* spp. Although the samples were collected from widely scattered areas, the fungal flora pattern was almost similar.

TABLE VIII - Fungi Isolated from Various Samples

Type of Sample and No. Studied

Fungi isolated	Decaying Leaves (27)	Decaying Wood (23)	Potting Soil (17)	Sphagnum Moss (16)	Tree Bark (15)	Straw (13)	Thorn (7)	Rat Dropping (2)	Total (120)
Aspergillus spp. + *Monontospora* spp.	-	-	-	1	-	-	-	-	1
Aspergillus spp. + *Candida* spp.	-	-	-	-	1	-	-	-	1
Aspergillus spp. + Mycelia Sterilia	-	1	1	1	1	-	-	-	4
Trichophyton spp. + *Penicillium* spp.	-	2	1	2	-	-	-	-	5
Trichophyton spp. + *Mucor* spp.	-	-	-	-	1	1	1	-	3
Trichophyton spp. + Mycelia Sterilia	1	-	-	-	-	-	-	-	1
Trichophyton spp. + *Nigrospora* spp.	1	-	-	-	-	-	-	-	1
Aspergillus spp. + *Penicillium* spp.	4	2	1	1	1	1	1	1	12
Aspergillus spp. + *Hemispora* spp.	1	-	-	2	1	-	-	-	4
Aspergillus spp. + *Mucor* spp.	-	1	-	-	-	1	-	-	2
Aspergillus spp. + *Alternaria* spp.	-	-	1	-	-	-	-	-	1
Aspergillus spp. + *Helminthosporium* spp.	1	1	-	-	-	-	-	-	2
Trichophyton spp. +	1	-	-	-	-	-	-	-	1

Hemispora spp.									
Trichophyton spp. + *Cladosporium* spp.	1	-	-	-	-	-	-	-	1
Trichophyton spp. + *Fusarium* spp.	-	-	-	-	-	1	-	-	1
Trichophyton spp. + *Alternaria* spp.	1	1	-	-	1	-	-	-	3
Penicillium spp. + Mycelia Sterilia	1	-	-	-	-	-	-	-	1
Penicillium spp. + *Hemispora* spp.	-	-	1	-	-	-	-	-	1
Penicillium spp. + *Mucor* spp.	-	-	-	-	-	1	-	-	1
Penicillium spp. + *Candida* spp.	-	-	-	1	-	-	-	-	1
Mycelia Sterilia + *Cladosporium* spp.	-	-	1	-	-	-	-	-	1
Nigrospora spp. + *Hemispora* spp.	-	-	1	-	-	-	-	-	1
Penicillium spp. + *Candida* spp.	-	-	-	1	-	-	-	-	1
Mycelia Sterilia + *Mucor* spp.	-	-	-	-	-	-	1	-	1
Alternaria spp. + *Fusarium* spp.	-	-	-	-	-	1	-	-	1
Aspergillus spp. + *Penicillium* spp. + *Rhizopus* spp.	-	1	-	-	-	-	-	-	1
Trichophyton spp. + *Penicillium* spp. + *Candida* spp.	-	-	-	-	-	-	1	-	1

DISCUSSION

The present study of S. schenckii was divided into two parts. The fungus under study was analyzed, and various lipid fractions were quantitated. The ecology of S. schenckii was studied by processing samples collected from soil, leaves, wood, straw, moss, bark, thorn, and rat droppings in and around Shimla; this constituted the second part of the study.

The purified lipid extract of S. schenckii was further subjected to thin layer chromatography on silica gel, revealing several fractions. Amongst Phospholipid constituents, Phosphatidylcholine, Phosphatidylethanolamine, and Sphingomyelin were estimated, and their concentrations were in descending order. Phosphatidylinositol, Phosphatidylserine, and Lysophosphatidylcholine were estimated as a combined fraction because individual constituents were too small to be estimated separately. The cholesterol content of S. schenckii was constituted by free and esterified cholesterol.

Lipids constitute a considerable fraction of various fungi, but this aspect has been studied to a limited extent. Phospholipids of Saccharomyces cerevisiae yeast have been investigated by Letters (1966). It showed that Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidylserine were the most abundant phosphatides. In the present study, the results were comparable with that of Letters (1966) as far as the significant phospholipid constituents, Phosphatidylethanolamine and Phosphatidylcholine, were concerned. However, Sphingomyelin content was more in S. schenckii (13.8%) as compared to that in Saccharomyces cerevisiae (2.4%).

In studies of the lipid profile of Microsporum gypseum and Epidermophyton floccosum revealed that Phosphatidylcholine, Phosphatidylserine, and Phosphatidylethanolamine were the major lipid constituents. Chromatography of acetone soluble lipids indicated the presence of free and esterified cholesterol, triglycerides, diglycerides, monoglycerides, and free fatty acids (Khuller, 1978; Khuller, 1979). Similar fractions were identified in the present study, but diglycerides and monoglyceride fractions were too small to be quantitated. As far as lipids are concerned, the results in the present study were also in accordance with the study conducted by Sawyer et al. (1975), showing Phosphatidylethanolamine, Phosphatidylcholine, Phosphatidylinositol, and Phosphatidylserine as the phospholipid fractions of Trichophyton terrestre.

There is hardly any literature concerning lipid fractions of S. schenckii. However, as compared to other fungi, in the present study also, the Phospholipids were the significant lipid fraction (55.8%) of total lipids of S. schenckii It was followed by triglycerides (16.7%) and cholesterol (5.4%). The major phospholipid constituents included Phosphatidylcholine,

Phosphatidylethanolamine, Sphingomyelin, Phosphatidylinositol, Phosphatidylserine, and Lysophosphatidylcholine.

In the second part of the study, 120 samples from the environment were processed to study the prevalence of S. schenckii in the environment. The samples subjected to the study were potting soil, decaying leaves, decaying wood, straw, moss, tree bark, thorns, and rat droppings. None of the samples showed growth of S. schenckii. However, all the samples were positive for one or the other fungus. Out of 192 isolations, the maximum was the common contaminant fungi. In 59 cultures, *Aspergillus* spp. were identified, with *Aspergillus niger* (39) heading the list. It is the commonest *Aspergillus* spp. present in environmental flora (Emmons, 1977). *Trichophyton* spp. were found in 43 cultures, and *Penicillium* spp. in 32 cultures. In 48 samples, there were single isolations, whereas the remaining samples revealed the presence of 2 or more fungi in each of them. There are reports in the literature indicating the saprophytic nature of S. schenckii. Isolations have been reported outside India from Sphagnum moss (D'Alessio et al., 1965), potting soil (Kenyon et al., 1984), and dead and decaying organic matter. These isolations of S. schenckii from environmental substrates have been from sources and places which were supposed to be the source of infection. S. schenckii could not be isolated in the present study involving 120 samples scattered over the places. However, the endemicity of the disease in the area points toward the existence of the fungus in the area. Moreover, the prevalence of the disease in humid climates points to more studies to be undertaken to find lipid as an antigen for diagnosis.

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