Materials & Methods

The specimens were collected during the extensive collecting programmes carried out under the All India Co-ordinated Project on Taxonomy, Ministry of Environment and Forest, Government of India, at various forest localities (given below) of western Maharashtra during the last three years. The specimens were dried, studied and were kept in labelled folders with details of locality, date, and name of the collector etc. All specimens were deposited in Ajrakar Mycological Herbarium (AMH), at Agharkar Research Institute in Pune.

Collecting Localities

Collections were mainly made in western Maharashtra where humidity is comparatively high, and the flora in these regions is diverse, compared to the other drier regions of Maharashtra for example Khandesh, Marathwada and Vidharbha, where the humidity is very low and are not suitable for the lichen growth and we often get material too scanty and insufficient to be studied and identified correctly.

Konkan


Deccan or Desh

Nasik district, Anjani fort, Brahmagiri, Chandwad ghat, Igatpuri, Nasik, Sapatashrini gad, Trimbakeshwar. Ahmednagar district, Bhandardara, Kalsubai, highest peak 1654 m, Harishchandra gad. Pune district, Amby valley, Baneshwar, Bhaja caves, Bhimashankar, Bhushri dam, Borna hills, Bor ghat, Diva ghat, Dongarwadi, Durgwadi, Fergusson hill, Junnar, Karla, Katraj ghat, Khandrada, Lonvala, Malavali, Mulshi, Purandar, Sinhagad, Tamhini ghat, Vetal hills, and Walwan Dam. Satara district, Ajinkyatora fort, Ambenali ghat, Arthur seat point, Bhos khind, Bombay Point, Dhobi
The recent collection and the specimens which were collected earlier by the lichenologists at Agharkar Research Institute (P. G. Patwardhan & his associates) during the last four decades and deposited in Ajrekar Mycological Herbarium (AMH) were carefully examined in the light of modern taxonomic criteria and with the help of advance techniques used in the taxonomy of lichens.

Morphological and Anatomical Studies

Morphological features like nature, colour, texture and size of the thallus; shape and size of lobes, lobules etc. presence or the absence of isidia, soredia, pruina, cilia, and rhizines; colour, shape and the size of ascomata were observed under stereo zoom binocular microscope (CITOVAL-2 Carl Zeiss JENA make). Photographs were taken by Nikon D100 Digital Camera.

Anatomical features were studied by taking dry handcut vertical sections of thalli and ascomata with a sharp razor blade. Sections were observed in water, 10% KOH (K) followed by Lugol’s solution (1g iodine and 2 g potassium iodine in 300 ml water), lactophenol cotton blue (LPCB), different colour reactions of the ascospores, asci and hymenium were observed. Sections were mounted in lactophenol for making permanent slides. All anatomical details were studied using transmission light microscope (Weswox Optik) having 15x ocular (eye piece) and trinocular research microscope CX 41 RF, Olympus having 10x X 20x ocular and having x5, x20, x45 and x100 (objective lenses) respectively. Measurements of thickness of the different layers of the thallus, epithecium, height of hymenium, subhymenium, hypothecium, size of asci and ascospores were taken.
under different magnification and measured in micrometer (μm). Drawings were made using prism type camera lucida.

**Determination of lichen substances**

Many lichenized fungi produce complicated organic compounds, 'lichen substances', which only rarely are produced by a fungus not associated with a green or blue-green photobiont. These substances crystallize on the surface of the hyphae of cortex and medulla, and sometimes also in apothecial tissue. As they are often diagnostic for a particular species or a group of related species, therefore they are of great practical value in identification. The lichen substances were determined with colour test, (Hale 1969) and by thin layer chromatography (TLC) using methods standardized for lichen products (Culberson & Kristinsson, 1970; Culberson, 1972; White & James, 1985; Orange et al., 2001).

**Colour tests**

Colour tests were carried out by the direct application of the reagents on a small piece of thallus, exposed medulla and ascomata, using following common reagents

- **K**: 10% KOH in H₂O
- **C**: a solution of calcium hypochlorite in water or commercial bleaching powder
- **KC**: reagent K quickly followed by C
- **P**: a saturated solution of para-phenylenediamine in 96% alcohol (ethanol).

The colour changes were noted with 10x lens.

**Thin Layer Chromatography (TLC)**

In order to identify lichen substances more sensitive methods were used, such as thin-layer chromatography using following solvent systems

- **BDA**: benzene/1,4-dioxane/acetic acid 180: 45: 5
- **TDA**: toluene/1,4-dioxane/acetic acid 180: 45: 5
- **TEF**: toluene/ethyl acetate/formic acid 139: 83: 8
- **HEF**: hexane/ethyl ether/formic acid 130: 100: 20
The solvent systems were allowed to stabilize and saturate after preparation for two hours for assuring the even running of the solvent front.

For thin layer chromatography E. Mercks precoated silica gel 60 F 254, 0.25 mm thickness aluminium plates were used (20 cm x 10 cm). The plates were marked with a base line about 1.5 cm from the bottom edge with a soft pencil and ruler. Eighteen spots were applied on the plates 9 mm apart. On every plate the first, ninth, and eighteenth positions were used for controls. The controls on the plate were spotted from the same solution to eliminate $R_f$ variation due to concentration.

A small fragment of lichen thallus was taken in a vial and extracted with acetone. The extracts were then spotted on the corresponding numbered point on each TLC plates. The loading of extract was done for about 20-30 times at each point on the plates.

The plates were then placed in the sealed tanks containing different solvent systems in such a way so that the base of plate was immersed in the shallow layer of a specific mixture of organic solvents and allowed to run till the top.

The spots were developed by spraying with 10% $H_2SO_4$ using mouth sprayer and then the plates were transferred to a preheated oven at 110$^\circ$C approximately for 10 minutes until the spots were developed. The TLC plates were also observed under long wave length (365 nm) UV light before and after spraying 10% $H_2SO_4$ and after heating the plate.

Identification of lichen substances was done using $R_f$ values and colours of standard substances or the specimens containing those substances which were loaded simultaneously along with the specimens.

$$R_f \text{ value} = \frac{\text{distance travelled by the substance front from base line}}{\text{total distance travelled by the solvent front}}$$

**UV Fluorescence**

The thallus of all the specimens were observed for their fluorescence under long wave (365 nm) UV light for the indication of the presence of various lichen substances like lichexanthone, zeorin etc.
Taxonomic Account

Study of over 2000 specimens collected from various forest localities in Maharashtra state has so far resulted in the identification of 84 species in 25 genera and 9 families. The genera have generally been placed in the family according to the circumscription by Eriksson and Hawksworth (1991, 1994). Lichen genera and species are artificially arranged in alphabetic order. Characterisation of these species based on the modern concepts along with the keys for their determination have been presented on the following lines:

1. Key to the macrolichen genera from western Maharashtra
2. Generic description
3. Key to the species
4. Name of species followed by citation and basionym
5. Description of species and chemistry
6. Habitat
7. Distribution in India and world
8. Discussion of specific features and relationships.
9. Specimens examined

Unfortunately several species, recorded earlier, have not been recollected in the present work, but they have been included in the thesis with detailed taxonomic description based on the original literature where they had been described from Maharashtra by the authors so as to give the information regarding all species at one place to make the work as comprehensive and useful as possible for the workers with the hope to find these species from this area in future and can be identified.