CHAPTER-II

- MATERIALS AND METHODS

II. MATERIALS AND METHODS

Rust and Smut fungi normally appear and show their luxurient growth from mansoon to late winter season in this part of the country. Almost all specimens, on which this work is based were collected paying frequent visits to different places like Radhanagari, Satara, Mahabaleshwar, Koyananagar, Ambaghat, Gavase, Panhala, Gaganbawda etc. Rusts and Smuts occur both on dicotyledonous as well as on monocotyledonous host plants. The family Poaceae supported large no. of genera of rusts and smuts in the present work. The specimens were collected in polythene as well as in paper bags and brought into the laboratory for further investigation. During collections, field observations, such as locality, date, season, host and its association etc. were also noted. Host associations are obviously of great importance in the study of heteroecious rusts, because, repeated associations provide clues to probable alternate hosts which may be confirmed experimentally. Along with the infected plants, flowers and fruits were also collected. These materials were brought to the laboratory for the identification of the host. The hosts were identified with the most up-to-date and recent taxonomic literature like Blatter and McCann (1934), Bor (1960), Cooke (1903) and then confirmed. The fresh collections collected from the field in a large quantity in the paper bags or polythene bags or

bottles (Smuts) carefully and separately and were dried by keeping them in blotting papers with press along with its flowering or fruiting material if available for identification of its host.

Critical observation of the infected materials collected from the field was made in the laboratory in respects of the nature of infection i.e. heavily or moderately, site of infection i.e. to the root, or stem, or leaf, or inflorescence or flower or carpel or fruit. If it is on the leaf then on lower or upper or on both sides. The mode of pustule formation i.e. crowded or separate or coalesced, then the effect of infection whether produced swelling, hypertrophy or yellowing or chlorosis or defoliation etc. were observed. The collected specimens were first examined under dissecting binocular microscope. The rust fungi were critically examined under microscope by usual laboratory methods. The spores were mounted in water by usual method for details of ornamentations. Though the mounts in lactophenol are not permanent, the rust and smut spores in lactophenol can quickly be brought to full turgor and become thoroughly cleared. Thus, it is possible to study the ornamentation as well as the dimensions of the spores in detail. A very small drop of lactophenol was placed in the middle of the slide. Spores from the several sori were picked up with a tip of a fine needle, or scraped with an edge of the blade slightly moistened with lactophenol

and floated them in the central drop of lactophenol. Free hand sections were also taken and stained with 1% cotton blue in lactophenol and mounted in lactophenol.

Some of the slides of the specimens like Aecidium Pers., Uromyces (Link.) Unger, Melampsora Cast., Melanotaenium deBary, Ustilago (Pers.) Roussel., Tolyposporium Woron. etc. were made permanent. First the fresh infected material collected from the field was cut into small pieces and then fixed in F.A.A. for 24-48 hours and then washed well twice or thrice so as to remove the traces of formalin and acetic acid and then stored in 70% alcohol for further use. Special care was taken to prepare the micro preparations of the smut material while mounting. The spores or spore balls were taken from the unbroken sori so as to avoid the mixing of the spores. Many collections of the same species on the same host collected from different localities if occurred in different time were used to prepare the micro-preparation and all these collections and their micro-preparation were studied very carefully so as to see the range of variations.

For carrying out double staining procedure for microtomy sections, the fresh materials were fixed in fixative (F.A.A.) for 24 hours. After 24 hours they were washed in 70% alcohol and preserved. The dehydration procedure was followed by using different grades of alcohol like 80%, 90%,

absolute alcohol, then combination of alcohol and xylene like 3:1, 1:1 and 1:3 respectively. Infilteration was followed by adding wax of 56-58°C M.P. in the specimen bottles and storing them in oven at 60°C. After complete infilteration and removal of xylene blocks of the material were prepared. They were cut, treammed well and mounted neatly on the block holder and sections (10-15 μm thick) were cut by using rotary microtome (Lipshaw 45, Michigan). The straight ribbons of the length 2-3 cms were mounted on glass slides smeared with egg albumin as adhesive. The ribbons were spread on hot plate in water flooding. The slides were drained and then dewaxing procedure was carried by keeping the slides in coupling jars containing xylene. The slides were kept in each xylene jar for 3 to 4 hours. Then dehydration procedure was adopted by using different combinations of xylene and alcohol and then different alcohol grades such as absolute alcohol, 90%, 80%, 70%, 50%, 30% upto water. The slides were stained with aqueous haematoxyline. Excess stain removed by picric acid treatment. Then upgradation procedure was carried by passing the slides through the alcohol grades such as 30%, 50%, 70%, 90%, 100% xylene, clove oil. The slides were counter stained by orange G washed again in clove oil and then xylene and mounted in Canada balsam (Johansen, 1940). Excess Canada balsam was removed after drying the slides & were well labelled, numbered and critically observed.

All the semipermanent and permanent sections were critically observed under microscope to study the morphological details carefully. Detailed sketches were made with camera lucida 'Erma Tokyo' camera lucida at stage level using 10 X, 15 X, 45 X and 100 X (oil immersion) objectives; choice of the combination of eye piece and objective varied according to the size of the structures. Measurements were made by 6 X 'E. Leitz Wetzlar' occular and 10 X, 45 X and 100 X objectives.

Photomicrographs of the slides were taken by Leitz. Photographic unit and photographs of the habits were made from Pentax camera at desire magnifications so as to reveal the details.

The morphology of the fruiting structures (in case of rusts) i.e. pycnia, aecia, telial and uredinial sori mixed or separate were studied in details and then measurements were taken. Then the spores-pycniospores, aeciospores, urediniospores and teleutospores along with the sterile structures like paraphyses or sterile cells or sheaths were also observed very carefully. Colour of the spores especially of urediniospores and teleutospores were observed by mounting the spores in the water as mounting medium to give its natural shades. Ornamentation of the spores especially of the teleutospores in smuts and

urediniospores and aeciospores of the rusts was observed under the oil immersion to give the clear cut nature which is one of the diagnostic feature in these fungi. Germ pores and its ornamentation is being of the diagnostic feature in the urediniospores of the rust which was critically and repeatedly observed to see its range.

Identification of the rusts to their respective genera, species and varieties were made on the basis of morphology, dimensions, ornamentations if any and host specificity with the help of standard keys provided e.g. by Cummins (1971) for graminicolous rusts. If host is not known definitely or doubtful then the species were confirmed with the keys based on morphology and dimensions of the spores-groups. A group system based on uredinial stage especially for all rusts of grasses based on 9 groups mostly applicable to Puccinia Pers., Uromyces (Link.) Unger and Uredo Pers. also given by Cummins (1971) for the graminicolous genera of the rust fungi. If only uredinial or aecidial stage only occurred then these collections were placed tentatively under these form genera viz. Uredo Pers. and Aecidium Pers. respectively.

The identification of the Smuts to their respective genera and species were made on the basis of sori in leaves, stems or inflorescence endorsed by a thin pseudomembrane or sori with a columella in the centre. Spores single or united

into spore balls, germinating by a septate promycelium bearing terminal and lateral sporidia or rarely a single sporidium; infection hyphae often formed without the formation of sporidia, but usually following the conjugation of sporidia.

Uptodate literature have been used to confirm the identification of the genera, species and varieties of the rusts and smuts during this work. So far in this taxonomical study of rust and smut fungi the view adopted here is some what conservative.

Identifications of these fungi and their reports were confirmed with the help of following literature. Cummins (1959, 1971), Thirumalachar and Mundkur (1949, 1950, 1952), Laundon (1965, 1973), Butler and Bisby (revised by Vasudeva 1960), Vasudeva (1962), Sarbhoy et al. (1975), Ainsworth and Bisby (1971) and Bilgrami et al. (1979), Bhide et al. (1987).

All the materials properly labelled, slides (semi-permanent and permanent) sketches, camera lucida drawings have been deposited in the Mycologiyal herbarium, Deptt. of Botany, Shivaji University, Kolhapur under code WIF Nos. (Fungi of Western India).