Introduction and Review of Literature

INTRODUCTION AND REVIEW OF LITERATURE

The fungi which are included under the order Ustilaginales are commonly known as `smuts´. These fungi caused the severe diseases to the crop and many other plants, especially to the economically important cereals. Similarly, they also infect many grasses, ornamental as well as other wild plants. The daily source of bread of mankind is affected by these, the economically important crop plants get attacked by these fungi, hence it became the subject of research. It was believed that smuts were known to the ancient workers, but these were included under `mildews´ and `blasts´. Mc Alpine and co-workers (1910) had no word to separate or to express the various types of smuts in their respective language. Tillet (1837) was the first person who was able to distinguish between `La Carie´ i.e. stinking smuts and `La Carbon´ i.e. loose smuts. Later on number of Ustilaginologists studied the details of the life-cycle of these and have added a good number of taxa in literature. Important contributions were made by Brefeld (1883); Liro, (1915); Christensen and Lutmen (1910); Hanna and Holton (1968). Fischer, E. (1920); Fischer, G.W. (1938), Duran (1957), Mundkur and Thirumalachar (1952) and others (Vide, Fischer and Holton, 1957; Duran 1973 and K. Vanky, 1985, 1987) etc.

Unlike other fungi smuts have limited number of morphological characters which are suitable for taxonomic purposes. According to Duran (1973), more than 75 families members of Angiosperms from the world attacked by more than 35 genera and their 1100 species. In addition to this, some species are recorded on the non-angiospermic host plants. Now there are more than 1000 species belonging to 50 valid genera and 3000 synonyms parasitizing 4000 host plant (Vanky, 1985).

Duran and Fischer (1961) transferred a few species of *Neovossia* and *Tilletia* to some other species on account of their erroneous interpretations. Fischer (1953) emphasised this point when he cautioned that a purely morphological system of classifying smuts would lead inevitably to unrealistic `lumping' of morphological similar but obviously different species e.g. *Urocystis colchici* (Sechelsht) Robenh. and *Urocystis agropyri* (Perivis) Schroat, which caused onion and wheat flag smuts respectively.

Realising the importance of host specialisation in the taxonomy of smuts and later Fischer (1963); Fischer and Shaw (1953), proposed a species concept based principally on morphology and host specialisation at the host-family level. However, some of the morphological characters make their identification more difficult, for example in *Entyloma* de Bary, morphological similarity among the species makes their

identification difficult, frustating or impossible. Under this proposed concept *Entyloma* deBary would be considered as the different species, if each accured on a different plant families, even though they are morphologically similar. According to Duran and Fischer (1961) and Duran (1973) morphological characters and host specialisation both are important and interdependent and complimentary in the classification of the smuts.

About hundred and fifty years ago, the taxonomical studies on Indian Ustilaginales has been started, when Hooker collected a smut collections from Assam and Sikkim. Later, important additions in the field of Ustilaginales were made by Cunninagham (1924), Berkley (1874), Butler and many other workers. By describing new taxa, Mundkur and Thirumalachar (1952) and Pavgi (1962) also helped to make important contributions in the field of Ustilaginales. So far 209 taxa (205 spp. and 4 varieties) belonged to 21 genera have been reported from India [Mundkur and Thirumalachar, (1952); Butler and Bisby (1956); Vasudeva (1961); Mukerji and Juneja (1974); Sorbboy *et al.* (1975); Bilgrami *et al.*, 1979 and 1981; Bhide, Patel and Kamat, 1985 and Patil (1992)]. In the "**Fischers Mannual Of North American Smut Fungi**" (1953) and the book entitled "**The Ustilaginales of the World**" written by Zundel (1953) included the description of all species known upto 1953. Another mannual '**Taxanomic Treatias**' Savulescu (1957) the

work done on the smuts of Romania has been published in two volumes. Work on smuts of Poland has been published by Josef Kochman and Tomansz Mozewski (1973). The study of monographic work (smut fungi of Carpathian area) by Kalman Vanky (1985) which includes 234 species. In 1950 Gusztau moesz's posthumously published monograph of 'Carpathian Ustilaginales of Hangari, which contains 124 species.

Maharashtra State is quite rich about its fungal flora and so also the smut fungi. So far 16 genera with 45 species have been reported (Butler and Bisby, 1960; Vasudevan, 1965; Bilgrami *et.al.*, 1979 and 81; Bhide *et.al.* 1978). However, the knowledge of smuts of Maharashtra still remains far from complete on account of the lack of intensive survey (field work) and proper germination studies.

In the present work 31 species have been worked out. These collections were mainly from South Western part of the Maharashtra State. Detailed macroscopic and microscopic observations were made for identification; recent upto-date keys (Duran, 1973; Kalman Vanky, 1985) have been followed with slight modifications, wherever possible.

FAMILES OF THE ORDER USTILAGINALES

Depending upon the high degree to species concept, there are about 1000 species of the Ustilaginales

distributed in 50 valid gerera on 4000 species of the host plants (Vanky, 1985) with 3000 synonyms.

Since, L.R. and C. Tulasne (1847) the order Ustilaginales has generally been divided into two families on the basis of the teliospores germination. This important discovery has been firstly made by Prevost (1807), later on Fischer and Holton (1957) observed that teliospore germination occurred in water. The teliospore on germination give rise to short germ tube i.e. `promycelium´ and minute reproductive bodies i.e. `sporidia´ which was shown by Tulasne (1847). He named the germ tube as `Promycelium´ and small reproductive bodies as `sporidia´. Kuhn (1859) studied the development of smuts and discovered that sporidia which reinfect the plant. DeBary (1884) and Fischer (1953) also worked out the further details of the spore germination. However, many a times the spore germination was very difficult and uncertain or slow. (Brefeld, 1883). Brefeld tried the spore germination on manure extract as well as on soil extract. Later on Plowright (1889) (Vide Fischer and Holton, 1957) studied cell fusion in smut fungi and observed fusion of neighbouring cells of promycelium in *Ustilago tritici* and the terminal sporidia of *Tilletia* and *Entyloma*. Dangeard (1893, vide Fischer, 1953) has published his classical account of several species of genera with binucleate condition of the teliospores and subsequent fusion of the two nuclei. Sporidial fusion of several

species were also studied by Lutman (1910) and he traced the passage of the nuclei from one cell to the another cell.

Tulasne brother's (1847) two family system based on the mode of the teliospore germination included-Ustilaginaceae and Tilletiaceae. The first one is characterized by septate promycelium developing basidiospores laterally and terminally. The Tilletiaceae have non-septate promycelium with terminal basidiospores. A third family, Graphiolaceae, parasitizing palms, has by some mycologists been included in the Ustilaginales. But by some others being regarded imperfect fungi. Liou (1949) errected new family: `Yeniaceae'. This family is characterized by rudimentary promycelium which produces progressively 1-4, pedicelled, usually septate `basidiosporophores' which in turn produce either new pseudo-promycelia or basidiospores. Several saprophytic fungi showed an ontogenic cycle very similar to that of the smut fungi (Bandoni and Johri, 1972, Fell, 1974 and Moore 1980). For some of these a new family `Filobasidiaceae' Olive (1968) was created. Later on Ruben Duran addressed that who would advocated a third family and the species germinate directly must be considered themselves to the problem of variation in the heterobasidium attributable to sub-optimum condition, notably excess moisture. Roger (1934) has pointed out the heterobasidium under these conditions is capable of only of modification

attributable to ordinary mycelium. So establishing or proposal of a third family of smut fungi seems premature and undesirable. In many smut species, the germination is still unknown and repeated attempts to promote it have failed. In these cases they were included one of these families on the basis of analogs or similarities of the spores with other species, whose germination is already known. Finally all the smuts were included in a single family 'Ustilaginaceae' (Cunningham, 1924; Fischer G.W., 1953 and Lindeberg, 1959). This view was supported and also accepted by Vanky (1985). The germination is mainly dependent upon environmental conditions, humidity, temperature, pH, etc. Nilsen (1966) observed that *Ustilago avenae, U. hordei* and *U. nigra* have changed the germination pattern from sporidial to mycelial type. For these reasons, Cunningham, (1924), Fischer, G.W. (1953) and Lindeberg (1959) rejected to divide the order Ustilaginales into two families and considered all smuts belong to only one family viz. Ustilaginaceae, the same approch has been also followed by Kalman Vanky (1985) who studied the smuts of Eastern Europe (Carpathian).

Whereas, the distinction between families based on the spore germination, delimitation of genera is based on the characters of the sori together with the characters of the spores and their germination. But in the present study, two family system of classification is adopted, which is more

fundamental and spore germination, an ontogenic charactar is more realistic and practical one.

SYMPTOMATOLOTY

Although the classification of the families based on the teliospore germination, the genera and species have been classified principally on the basis of symptomatology, teliospore morphology as well as host range. A key for identification of genera of Ustilaginales has provided by Duran (1973). Recently, Kalman Vanky (1985) has also provoided the key to the genera of 'Carpathian Ustilaginales'. For the most of the part, the gross morphology of inflorescence, stem, leaf and root smuts showed great or wide variation. The examples of these are found in *Sphacelotheca, Sorosporium, Sporisorium, Ustilago, Urocystis,* and *Tilletia*. Majority of the members of the family Ustilaginaceae which developed the sori in the inflorescence including ovaries, ovules, anthers and petals, whereas, the sori on vegetative parts i.e. stems, leaves, axillary buds, roots etc. are developed mainly by *Doassansia, Entyloma, Melanotaenium, Jamesdicksonia, Georgefischeria* etc. The infected inflorescence completely or incompletely destroyed by the number of species of *Ustilago, Sphacelotheca Sorosporium, Sporosorium, Tolyposporium, Tilletia* and *Neovossia* etc. In these cases,

infection may cause hypertrophy or deformity in the floral structures. The species of *Ustilago* showed most striking variations. It may develop inconspicuous and loss hypertrophoid sori. So it is very difficult to find out the infected plants in the field during collection. In *Tilletia* Tul., the sorus formation occurse mostly in the ovaries. In the species of *Ustilago, Sphacelotheca, Sorosporium, Sporisorium, Urocystis* and *Tilletia* showed their common occurrence of sori in the inflorescence as well as in any part of the host plant.

In *Malanotaenium* deBary sori mostly occurred in the leaves and rarely even in roots. The sori may be long, elongated and irregular and hypertrophy may also developed. At maturity, the sori become erumpent to expose agglutinized spore mass. In some cases the galls are produced on leaves, roots and stems. In case of *Ustilago crussgalli* the large galls are produced on stem. The galls are partly made up of host tissue and partly of fungal tissue, the same is also found in head smut of maize to the inflorescence and *Entorhiza* of Cyperaceae and Juncaceae to roots.

SPORE MORPHOLOGY

During the classification of genera and species of the Order Ustilaginales, following spore morphological characters are used.

1. Spores single, in pair or in more or less persistent spore ball (which may consists only of spore or of spores, sterile cells or hyphae),

2. Colour,

3. The size,

4. The shape of the spore, spore balls and sterile cells where present,

5. The structure of the spore wall (layers, thickness etc.) and

6. The spore surface ornamentation.

In addition to these characters, gelatinous sheat, pigmentation are also important. The spore size has definite taxonomic value in smut fungi systematics. Single spores are found in many genera, e.g. *Anthracoidea* Bref., *Cintractia cornu*, *Entyloma* deBary, *Tilletia* Tul. In *Ustilago* Roussel, *Sphacelotheca* deBary, *Sporisorium* Ehrenberg Ex Link., *Sorosporium* Rud. and *Tolyposporium* Woron. spores are small. While larger spores are observed in genera like *Tilletia Tul.*, *Cintractia cornu*, *Neovossia Kornicke*. Colour of the spore mass is not a morphological character, but rightfully included under all description of the smut fungi. Although it varies in every species and considered as a constant

character for particular species. The pigments in the exospore wall is unevenlly distributed in some species. The colour of the spores of smut species varies from hyaline to light-violate, yellowish to dark reddish-brown; chestnut-brown to blackish-brown etc. In various species of *Ustilago* Roussel observed dark equatorial bands and light polar areas or caps on exospores along with an unevenl y distributed cluster of verruculation which gives the spore uneven surface colour pattern (Duran and Fischer, 1961).

SPORE BALLS

Roughly, half of the genera of the smut fungi produced their spores in clumps or aggregates are known as spore balls. The spore balls are entirely made up of fertile cells or it may be the combination of fertile and sterile accessory cells and /or sterile pseudoparenchymatous elements. Morphology of spore balls have played an extraemly important role in smut systematics.

In case of *Sorosporium* and *Tolyposporium* the spore balls are entirelly made up of the fertile spores. In *Tolyposporium* Woron. the special type of spore balls are observed. The spores are hold together by a curious net work of their interconnected folds and thickening on the spore walls. These spore balls are permanent or semipermanent. In case of *Urocystis* Robenh. the central fertile spores are

surrounded by outer wedge-shaped, sterile cells. These sterile cells are hyaline. The spore balls containing 1-20 brownish fertile spores. On the other hand, the spore balls of *Doassansia* are permanent and remain embeded in the host tissue. In this case, the inner fertile spores are surrounded by outer layer of sertile cells. Most of the smuts of fresh water aquatic plants, generally spore balls have been observed which is a special ecological adaptive feature for dispersal- a special group of aquatic smuts.

TELIOSPORES

Taxonomy of smut fungi is mainly based on teliospore morphology, mode of germination and origin (Ontogeny). Most species cannot be identified or adequately described totally on the basis of teliospore characters.

Teliospore characters include size, shape, colour, ornamentation and are used in smut systematics either alone or in combination.

(i) Size of the Teliospores

In the delimitation of many smut species the size of the spores play an important, often decisive role. The smut genera are characterized by certain average spore size. *Tilletia* Tul. species possess generally much larger spores than those of *Ustilago* species *Sorosporium*, *Sphacelotheca*,

Tolyposporium etc. The species of *Neovossia* and *Cintractia* are also producing large teliospores comparatively.

(ii) Colour of the Teliospores

The colour of the teliospores of the smut species varies from species to species from hyaline to light yellowish-brown, yellowish to dark reddish-brown, chestnut-brown to blackish-brown or light violet to dark brownish-violet. Spores or sorus colour alone is not sufficient to delimit a smut genera or species. However, a specific colour may be characteristic for smut taxon. The blackish-brown spores are observed in *Cintractia, Melanotaenium, Tolyposporium,* etc. Light, hyaline, yellow or pale yellowish-brown spores are typical for a smut genera *Entyloma*. Nannfieldt was covinced that smut with violet tinted spores are phylogenetically related to species which mostly in the genera viz. *Sphacelotheca* and *Ustilago*-which infect the members of the Caryophyllales.

(iii) Ornamentation

One of the most important criterion used for delimination of species in smut fungi by most of the Ustilaginologists is spore surface ornamentation. The ornamentation provides the most valuable taxonomic information in the Ustilaginales. The spores may be smooth,

reticulate, cerebriform, echinulate, verrucose, tuberculate etc. Echinulate and verriculate spores are generally described in terms of density. For most parts, they are so small that they can not be measured accurately. Generally, in case of *Ustilago* Roussel, *Cintractia* Cornu, *Doassansia* Cornu. the spores are smooth.

In the past, nearly each worker who worked intensively and indipendently, with the smut fungi, created his/her own classification for spore morphology. Yen (1937) recognised six surface type:

1. Pactue
2. Verruqueax
3. echinule
4. catanule
5. reticule
6. ruguex

Ohta (1966) assigned 22 species of *Ustilago* to five groups after an electron microscopic observation of spores:

1. lisse
2. verrucose
3. echinulate
4. flat-headed
5. panctate
6. crenelee
7. verrucose
8. verruquese
9. echinule
10. spinuleuse
11. epineuse
12. busselee
13. nodulease
14. catenulate
15. cristulee
16. cretee
17. costulee
18. ailee

19. inter-ruproeticulee 20. reticulee
21. atvealee 22. verniculee

Anisworth and Bisby (1953) have classified the spore surface of smut species as follows:

1. smooth 2. punctate
3. verrucose 4. chinulate
5. aculeate 6. tuberculate
7. reticulate 8. reticulate-narrow
9. reticulate-brood 10. potholate
11. covered by sterile cells

Govora and Azbukina (1958b) distinguished following types of spore wall ornamentation:

1. smooth 2. verrucose
3. echinate 4. reticulate
5. locunose 6. striate
7. pappilate 8. tuberculate

Kalman Vanky (1985) has classified the smut spore surface ornamentation into following 13 types.

1. smooth 2. punctate
3. verrucose (papilate) 4. tuberculate
5. foveolate 6. echinulate (spiny)
7. nail headed 8. reticulate

9. cracked (scaly)
10. striate
11. ridged
12. cerebriform, and
13. operculate

Vanky proposed that when two (or more) types of ornamentations are present on the same spore surface, the dominant type may be noted first and less conspicuous type(s) afterwards.

Type of spore surface ornamentation undoubtedly has definite function. Ornamentation may aid in spore protection or dissemination. In case of the genus *Urocystis* the empty sterile cells around the spores may function like air-sacs as in pollen grains facilitating dispersal by wind or as floats in water.

STERILE CELLS

At certain extent the characteristic of the sterile cells including size, shape and colour used for delimitation of the species. In some species of *Sphacelotheca* deBary the cellular components of sorus peridium gets disintegrated into fragment of individual cells, this is known as a sterile cells. In fact, sterile cells are found to have a greater taxonomic application in the species of *Tilletia* Tul. then those other genera. (Duran and Fischer, 1960). In the *Sporisorium* Ehrenberg ex Link. the sterile cells (=

partitioned cells) are present in groups or chains.

The exact role end origin of sterile cells are uncertain. Their morphology often affords a practical mean of helping to establish specific distinction among the species of *Tilletia* Tulasne

Holton (1941), working with the sterile cells of dwarf bunt organism, *Tilletia contraversa*, was able to germinate them. He put evidence that they are haploid bodies and their haploid nature can infect the host unless they are mated with other sexually compatible body. This indicates that the 'sterile cell' is misnomer. Since, they are viable and germinable, able to cause, infection and to perpetuate the species in the manner of primary sporidia, as demonstrated by Holton (1941). An alternative term might be 'haploid spores' or 'hyaline spores' be applied either, the sterile cells are not universly hyaline (Duran and Fischer, 1960)

fertile ?

The proportion of sterile cells to the mature spores varies with the species to species, the sterile cells may be a few or narrow or absent. The size of the sterile cells may also vary. They may be tinted or hyaline, thin or thick-wall, round or polyhydral and even contorted. They may contain homogenious or granular content or in some cases a gigantic gelatinous sheath as in *Tilletia patchyderma* (Duran and Fischer, 1961): wall may be striated or non-striated.

In sharpening delimitation of certain smut genera *Sorosporium*, *Sphacelotheca*, *Sporisorium* Langdon and Fullerton (1978) showed their importance in sorus ontogeny.

The sterile cells play and important role in the species delimitation. These cell found intermixed with the matured spores in case of *Tilletia* as well as *Sphacelotheca*, *Sporisorium*, *Sorosporium*, *Ustilago* etc. In case of *Tilletia* more importance has been given to the sterile cells in the classification. But in *Sphacelotheca* no special role in classification. These sterile cells are nothing but fragments of peridium tissue. In case of *Sporisorium* the sterile cells are present, which are in chain or in groups and called as partitioned cells (Vanky, 1985).

Thirumalachar and Mundkur (1962) also describe the presence of sterile cells in a few species of *Sorosporium*, *Sphacelotheca* and some times in *Ustilatgo*. But they didnot knew the exact role about them, i.e. either these were fungal cells or peridial cells or the host tissue.

Langdon and Fullerton (1975, 1978) studied the sori in a very ycung stage. They also studied and showed that the columella, when present, may be very different in structure and function. On this basis and taking into consideration also other characters they reinstated the old genus *Sporisorium* with the type species *Sporisorium sorghi*

The genus *Sporisorium* was erected by Ehrenberg (1825) accomodate *Spori. sorghi.* Later this species was transferred to the genus *Sphacetotheca* by Clinton (1902) and also described as new species of *Ustilago* by Passerini (1873). The generic name *Sporisorium* was forgotten. Recently Langdon and Fullerton (1978) in the study on the ontogeny of mature structure of the sori of *Sphacelotheca* conclude that *Sphacelotheca sorghi* and some other smuts of Graminae are markedly different from the type species of *Sphacelotheca* and should not be included in that genus: *Sporisorium* is available for these. Vanky had transferred species to this genus, which are graminicolous and described as *Sorosporium*, *Sphacelotheca or Ustilago* which belong certainly to *Sporisorium*.

This indicates that the smut taxonomy, even though more than 150 years old, is far from the perfection. The great variability of these characters opens the way for the speculation and subjectivism. This is the main reason for the numerous changes that are still taking place in the taxonomy of smuts and many new genera such as *Anthracoidea* and *Nannfeldtiomyces* have been established or rehabilitated from 35 to number 50 (Vanky, 1985).

The genus *Burrillia*, *Doassansia*, *Doassansiopsis*, *Pseudodoassansia* and *Tracya* are included in one natural group. The species are parasitic on aquatic or paludel

plants. The spore balls embeded in the host tissues.

The genus *Urocystis, Tuburcinia* and *Ginannidela* also form a natural group. The spore balls of which having one to several central spore surrounded by sterile cells. The number of spores and sterile cells in the spore balls served as a character for their generic classification (Ulbrich, 1940). Consequently most recent mycologist recognise only one genus i.e. *Urocystis.*

The genus *Sporisorium* Ehrenberg ex Link (1825) which has been recently reinstated. It was long forgotten genus. Langdon and Fullerton (1978) studied the ontogeny and natured structure of sorus of *Sphacelotheca* and concluded that *Sphacelotheca sorghi* and other smut genera infecting the Poaceae members are markedly different from the type species of *Sphacelotheca* and showd not be included in that genus. Vanky (1985) transferred many a species to this genus as a combination nova. Many species of the *Sorosporium, Sphacelotheca* and *Ustilago* shows the characteristics which are similar to *Sporisorium.* The *Sporisorium* shows following characteristic features:

1. Sori covered by a peridium formed by hyphae overlaid by host tissue.

2. A columella composed of host tissues permeated by hyphae which produce spores and sterile cells (= Partitioning

cells).

3. Spores in more or less loose spore balls, when mature often single, dark coloured.

4. Sterile cells in groups or chains, hyaline, intermixed with the fertile spores.

5. Germination of *Ustilago* type.