

## **II. MATERIALES AND METHODS**

"Black Mildew" fungi are very conspicuous and predominant in nature as parasites of higher plants. The collection of these fungi and their preservation are relatively simple processes as with some other groups of fungi. 'Black Mildews' are predominantly foliicolous and rarely infect the soft stems, tender shoots and fruits. These are shade and moisture loving and prolonged drought inhibits their growth. Hence 19-30°C temperature, 50-65% relative humidity and 40-200 mm rainfall generally favour their growth. Different fungi grow as parasites in different seasons. The material was collected by repeated visits to different localities throughout the year, especially to the different places with sufficient quantity of good quality along with its host if it is flowering, fruiting. While collecting the infected plant parts, field notes were made regarding their pathogenicity, nature of the colonies, locality, altitude, date etc. Infected parts of plants were collected in the polythene bags or paper envelopes along with the hosts if flowering or fruiting to identify the host perfectly.

*what if not so? how hosts are identified then?*

These collections were brought into the laboratory and pressed and dried well in blotting papers. Each material was then carefully studied by care inspection macroscopically as well as microscopically in both respects to study the primary pathogen i.e. meliolaceous one and also the mycoparasites associated or overgrowing on the colonies.

Careful study of the collected materials in large quantity from each locality was made. So these materials were observed with unaided eye, with hand lense also under dissecting microscope or even compound microscope if infection is very inconspicuous or sparse. Each part of host which was infected was noted i.e. leaves, stem, petioles and fruits. In case of leaves the infection was noted whether it is epiphyllous, hypophyllous or amphigenous. The nature of infection i.e mild, heavy or moderately with spots, swelling, yellowing etc. are produced or not as well as nature of the colonies : distinct, spreading, coalescing, small, big, round, regular black, brown, yellow thick or sparse were observed because these characters are used during correct identification of species. Careful search of each colony was also made to find <sup>if</sup> the hyperparasites <sup>are</sup> overgrowing on these colonies or not.

For routine examination of the materials, scrapes or whole mounts were taken from infected host and mounted in water and observed under microscopes. Scrapes or whole mounts taken directly from infected hosts and mounted in lactophenol.

*Sealed* ? Using cotton blue as stain for staining. After gently warming to remove air bubbles and excess stain blotted out. A new drop of lactophenol was added. Sealing was done with the help of 52°C paraffin wax or nail polish. Cotton blue was used for staining the hyperparasites as well as to make septa visible,

lactophenol was used as mounting medium and clearing agent.

For the microscopic study, scrapes were taken directly from the infected host and mounted in 10 % KOH solution. After 30 min. KOH was replaced by lactophenol. KOH solution worked well as swelling <sup>agent for the</sup> material in case of dry materials and also <sup>a clearing</sup> acts as agent. Maximum number of micropreparations were prepared of different colonies of different parts of infection to give the range of variations and also the hyperparasites growing or associated with the colonies for further study.

While making whole mounts of the black mildew fungi, several methods have been adopted to study them. Gaillard (1892) was the first to use "celloidin solution drops". In this method a drop of celloidin solution placed on the colonies, allowed to dry, to form a thin film over it, in which fungal colonies firmly embedded. The composition of "celloidin solution drop" is as follows -

Composition of "Celloidin Solutin Drop"

i)	Celloidin	... 04 ml
ii)	Alcohol	... 10 ml
iii)	Ether	... 32 ml
iv)	Castor oil	... 02 ml
v)	Lactic acid	... 02 ml

The colonies were removed and placed on slide.

The celloidin film was dissolved in ether alcohol solution and free colonies were mounted in glycerin jelly. This technique is useful for studying the branching pattern of mycelia, nature of hyphopodia without disturbing the natural colonies.

Stevens (1916) used Gaillard's celloidin solution excluding castor oil and lactic acid; and the flip was dehydrated by absolute alcohol and xylol and mounted in xylol-canada-balsam. Ellis (1950, 1960) used "necol" or any similar cellulose preparation for these fungi. Placing the necol drops on colonies, thinned down, peeled off the flips and after drying mounted in lactophenol to dissolve the necol.

Hansford (1961) suggested that "celloidion-acetone drops" by the usual procedure 'flip' was prepared. Then those flips were mounted in lactophenol with coverslips were sealed with double layers of nail polish. He also suggested the use of canada balsam in place of lactophenol for making the slides permanent.

Butlar & Mann (1959), Bretz & Berry (1964) Flagal (1980) have suggested to use the 'adhesive cellopane tape' for mounting the pathogenic epiphyllous fungi.

Hosagoudar & Kapoor (1985) <sup>1985</sup> have used the application of thin layer of "natural colour nail polish" on the selected colonies. After drying the apple rose coloured flip was mounted in D.P.X. (or canada balsam) for preparing the permanent slides.

Hosagoudar & Mohanan (1985) further suggested and used "Thermocol isobutyl methyl ketone" solution for mounting the meliolaceous fungi.

Composition of "Thermocol isobutyl methyl ketone":

- |     |                                 |           |
|-----|---------------------------------|-----------|
| i)  | Pure white thermocol            | ... 2.5 g |
| ii) | Isobutyl Methyl Ketone Solution | ... 10 ml |

Above quantity of thermocol dissolved in 10 ml of isobutyl methyl ketone solution. The former readily dissolved in the latter. A thin layer of this solution has been applied on the selected colonies to form flips. These flips were then mounted in D.P.X. for making the slides permanent.

Patil (1990) has modified the Hosagoudar & Mohanan's (1985) formula for the whole mount preparation of black mildew fungi as follows :

The white thermocol was cut into small pieces and were dissolved in 20 ml of rectified xylol solution upto saturation. The thermocol readily dissolved and produced vigorous effervescences in xylol. The solution was stirred and kept open in oven at 30°C for 15 min. to eliminate air bubbles. Then it was removed from oven and immediately filtered through clean muslin cloth and cooled. The transparent solution was stored in an air tight bottle. A thin layer of this solution was applied on the selected colonies to form flips. These flips were then

mounted in D.P.X. The flip immediately dissolved in D.P.X. xylol solution. Excess D.P.X. was then removed after drying.

In the present work polyvinyl alcohol (PVA) was used as mounting medium instead of lactophenol for making the slides permanent.

Composition of Polyvinyl Alcohol (PVA) :

i)	Polyvinyl alcohol	... 11 g
ii)	Distilled water	... 100 ml
iii)	Glycerin	... 10 ml
iv)	Distilled phenol	... 20 to 25 drops
v)	HgCl <sub>2</sub>	... 0.1 mg

The polyvinyl alcohol powder was slowly added to cold water with constant stirring, warming on water bath and 20-25 drops of distilled phenol, 10 ml glycerine was added with constant stirring. Also 0.1 % HgCl<sub>2</sub> was added to check microbial growth. This solution was stored in amber coloured bottle. The technique is useful for making permanent slides as well as to study undisturbed colonies along with their hyperparasites.

Black midlews fungi are ectoparasites and so that all these above techniques are useful for their study. These techniques are found quite superior to scrapping and mounting which do not disturb the colony as well as the hyperparasites associated and give good results as well as the problem of drying semipermanent micropreparations was also avoided by using

traditional methods.

All these micro preparations or slides which were prepared as semi-permanent or permanents were numbered and properly labelled of each collection and stored. Then these slides were studied critically with the help of compound microscopes to observe all the morphological details. All the structures i.e. hyphae, setae, hyphopodia, appendages, perithecia, asci and ascospores were measured with the help of "metric units". Microscopic measurements were made by calibrated "Ernst Leitz Wetzlar" ocular by using 6X, 10X, 15X eye pieces and 10X, 45X, 100X objectives. Choice of the objectives and eye pieces were according to the size of fungal structure. These measurements have great taxonomical significance to identify the taxa.

Camera lucida drawings have been drawn with the help of "Erma" mirror type at stage level, using proper combinations of eye pieces and objectives. The pencil sketches were transferred on the ivory sheet in proper sequence and were inked by rotoring pens using rotoring ink and plates were prepared, numbered and scaled.

Photomicrography of the semipermanent slides were taken by using "Olympus PM-G Unit" with the help of proper combinations of objectives and eye pieces. The infected materials were also photographed of certain taxa with the help of Asahi Pentax.



All collected materials and micropreparations were properly labelled and deposited in the Mycological Herbarium, Department of Botany, Shivaji University, Kolhapur under the code number WIF (Fungi of Western India) and new taxa, taxa new to India, State (M.S., K.S., Kerala), new hosts have been also deposited in Herbarium Indiae Cryptogamae Orientalis (HCIO) at New Delhi, India and their accession numbers are inserted while describing the taxa during the present work.

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Table. 1

**STATISTICAL SUMMARY OF THE TAXA STUDIED.**  
**A) FOR MELIOLACEOUS FUNGI :-**

Class	Order	Family	Genus	Species	Variety	Host & Family	Remarks
Pyrenomycetes	Meliolales	Meliolaceae	<u>Amazonia</u>	<u>Peregrina</u> H. & P. Syd.	--	<u>Mussa indica</u> (Roxb) dc. (Myrsinaceae)	New to M.S.
			<u>Appendicula</u>	<u>gloriosa</u> (Doidge) Hansf.	--	<u>Celastrus paniculata</u> willd. (Celastraceae)	New to India
			<u>Armatella</u>	<u>balakrishna-</u> <u>anii</u> Hosagoudar	--	<u>Cinnamomum</u> <u>zeylanicum</u> L. (Lauraceae)	New host with diff mode of germination
			<u>A.</u>	<u>gymnosporae</u> Patil	--	<u>Gymnosporia rothiana</u> Laws (Celastraceae)	Doubtful species
			<u>Asteridiella</u>	<u>atricha</u> (Speg.) Hansf.	--	<u>Eugenia</u> sp. (Myrtaceae)	New to India
			<u>A.</u>	<u>ohiana</u> (stev.) Hansf.	major K. & C.	<u>Eugenia</u> sp. (Myrtaceae)	New to K.S. & new host
			<u>A.</u>	<u>tremae</u> (speg.) Hansf.	--	<u>Trema orientalis</u> Bl. (Ulmaceae)	New to India & new host
		*	<u>A.</u>	<u>wendlandiae</u> sp. nov.	--	<u>Wendlandia notoniana</u> wall. (Rubiaceae)	New species
			<u>Irenopsis</u>	<u>leeae</u> Hansf.	--	<u>Leea indica</u> (Brum.) merr. (Leeaceae)	New to M.S.
		*	<u>I.</u>	<u>ligustri</u> sp. nov.	--	<u>Ligustrum neilgherrense</u> clerk (Oleaceae)	New species
		*	<u>I.</u>	<u>nephali</u> sp. nov.	--	<u>Nephali longon</u> Lour (Sapindaceae)	New species
		*	<u>I.</u>	<u>rubi</u> sp. nov.	--	<u>Rubus moluccense</u> L. (Rosaceae)	New species
			<u>Meliola</u>	<u>deithops</u> gacc	--	<u>Acacia auriculiformis</u> A. cunn. and <u>Acacia longifolia</u> A. cunn. (Mimosaceae)	New to K.S. & Kerala New to K.S. Kerala & new host
			<u>M.</u>	<u>atalantiae</u> Hosagoudar	--	<u>Atalantia wightii</u> Tanaka (Rutaceae)	New to M.S.
			<u>M.</u>	<u>atylosiae</u> Hosagoudar	--	<u>Atylosia lineata</u> wight. & Arn. (Fabaceae)	New to K.S.
			<u>M.</u>	<u>bengalensis</u> Hansf. & Thirumalachar	--	<u>Ficus bengalensis</u> Linn. (Moraceae)	New to Kerala

✓ = 9.

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Class	Order	Family	Genus	Species	Variety	Host & Family	Remarks
			M.	<u>capensis</u> (K.&C.)Theiss	malay- ensis Hansf. ord	<u>Nephalium longan</u> Lour. (Sapindaceae)	New to M.S.
			M.	<u>carissae</u> Doidge	indica Hansf. ord	<u>carissa carandas</u> L. (Apocynaceae)	New to M.S.
			* M.	<u>celtidiae</u> Yates	indica var. nov.	<u>Celtis cinnamomea</u> Lind. (Ulmaceae)	New Variety
			* M.	<u>dimorphocha-</u> <u>eta</u> sp.nov.	--	<u>Tylophora tenuis</u> Bl. (Asclepiadaceae)	New Species
			M.	<u>ervatamiae</u> Hosagoudar	--	<u>Ervatamia heyneana</u> T.cooke(Apocynaceae)	New to M.s. & K.S.
			M.	<u>ixorae</u> Yates	macro- pora Hosago udur	<u>Ixora</u> SP. (Rubiaceae)	New to M.s.
			M.	<u>longiseta</u> V. Hohnel	--	<u>Psychotria</u> sp. (Rubiaceae)	New to India
			M.	<u>malabarensis</u> Hansf.	--	<u>Olea dioica</u> Roxb. (Oleaceae)	New to M.s.
			M.	<u>petrakii</u> stev. and Rold.	--	<u>Melica composita</u> wild.(Meliaceae)	New to K.S. and new host
			* M.	<u>puerariae</u> sp.nov.	--	<u>Pueraria tuberosa</u> DC. (Fabaceae)	New species
			M.	<u>santalacea -</u> <u>rum</u> Budathoki usha et al.	--	<u>Osyris arborea</u> wall. (Santalaceae)	it is not new species but not studied
			M.	<u>stenospora</u> Wint.	major Hansf.	<u>Piper hookeri</u> mig. (Piperaceae)	New host
			M.	<u>tenella</u> pat.	atalan- tiae (Pat.) Hansf.	<u>Atalantia monophylla</u> L.(Rutaceae)	New to M.S.
			M.	<u>tylophora</u> Hosagoudar	--	<u>Tylophora cappari-</u> <u>diolia</u> wight & Arn. (Asclepiadaceae)	New to M.S.
			M.	<u>vitis</u> Hansf.	--	<u>Vitis</u> sp. (Vitaceae)	New to India.

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B1 FOR HYPERPARASITES.

Class	Order	Family	Genus	Species	Fungal Host.	Angiospermic Host.	Remarks
Pyrenomyces	Sphaerialiales	Ophioglossaceae	Ceratocystis	angusticollis wright & Griffin	Meliola memecyli H. & p. sydow	Memecylon umbellatum Brum (Melastomataceae)	New to India & New host
Loculomycetes	Dothideales	Dimeriaceae	Dimerina	parasitica (Sacc) Hansf.	Meliola canthi Hansf. & M. Puerariae sp. nov.	Canthium umbellatum (Rubiaceae) Pueraria tuberosa (Fabaceae)	New to India & New host
			Dimerium	mellicola	Meliola memecyli H. & P. syd.	Memecylon umbellatum (Melastomataceae)	New to India & new host
			Phaeodimeriella	cantareirensis (P. Henn) Hansf.	Asteridiella atricha (speg.) Hansford Meliola mappiae patil	Eugenia sp. (Myrtaceae) Mappia foetida Miers (Oleaceae)	New to India & new host
			Phragmieriella	clavatispora SP. nov.	Meliola memecyli H. & P. syd.	Memecylon umbellatum Brum. (Melastomataceae)	New species
			P.	Fusiformis sp. nov.	---"--- and Meliola dispyri H. & p. syd	---"--- and Diospyrus montana.	New species
			P.	pachydermus sp. nov.	Meliola memecyli H. & P. syd	Memecylon umbellatum Brum (Melastomataceae)	New species
			P.	quadripata sp. nov.	Meliola memecyli H. & P. syd.	Memecylon umbellatum Brum (Melastomataceae)	New species
Coelomycetes			Cicinnobella	Parodiellae P. Henn.	Asteridiella gymnosporae syd and Meliola buteae Hafiz Azmatalla & Kaf.	Gymnosporia rothiana Laws (Celastraceae) Butea monosperma (Lam) kuntze (Fabaceae)	New to India
			Ectosticta	bignoniicola sp. nov.	Meliola mappiae Patil M. Puerariae sp. nov. M. canthi Hansf.	Mappia foetida Miers (Icacinaeae) Pueraria tuberosa DC (Fabaceae) Canthium umbellatum Wt. (Rubiaceae)	New to India
Deuteromycetes	Hyphomycetales	Moniliaceae	Acremonium	melicola F.L. Stevens	Meliola holigrahnae stevens	Holigrahna grahamii (Wight) Kunze (Apocynaceae)	New to India
		Dematiaceae	Attractilina	parasitica (Wint.) Deighton & Pirozynski	Meliola memecyli H. & p. syd	Memecylon umbellatum Brum (Melastomataceae)	New to India

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Class	Order	Family	Genus	Species	Fuengal Host.	Angiospermic Host.	Remarks
			<u>Isthmospora</u>	<u>spinosa</u> stevens.	<u>Irenopsis wendl</u> <u>andiae</u> patil, <u>Meliola</u> <u>carissae</u> Doidge var. <u>indica</u> Hen- sf. <u>Mcrescentiae</u> stev. <u>mgrotens</u> H. syd. <u>M. nothope-</u> <u>giae</u> Hansford, <u>M.</u> <u>memecyli</u> syd. & syd.	<u>Wendlandia notoniana</u> wall. (Rubiaceae) <u>carissa cardndae</u> L. (Apocynaceae) <u>Heterophragma roxburg</u> <u>hii</u> Dc. (-Bignoniaceae) <u>Mussa indica</u> wall. (Myrsinaceae) <u>Nothopegia colebrook</u> <u>iana</u> wright (Anacardiaceae) <u>Memecylon</u> <u>umbellatum</u> Brum.	New to ks.
			<u>Spiropes</u>	<u>armatellae</u> Ellis, M.B.	<u>Armatella</u> <u>litseae</u> (P. Henn) Theissn & syd.	<u>Litsea</u> sp. (Lauraceae)	New to India
			S.	<u>capensis</u> (Thum.) Ellis, M.B.	<u>Meliola</u> <u>atalantiae</u> Hosagoudar	<u>Atalantia wightii</u> Tanaka (Rutaceae)	New host
			S.	<u>clavatus</u> (Ellis & Martin) Ellis, M.B.	<u>Armatella</u> <u>litseae</u> (P. Henn. Theiss. & syd. <u>Meliola listsea</u> syd. & syd. <u>M. nothopegiae</u> Hansf.	<u>Litsea</u> sp. (Lauraceae) and <u>Nothopegia colebrook-</u> <u>iana</u> wight (Anacardiaceae)	New to India & New hosts.
			S.	<u>dorycarpum</u> (mont.) Ellis M.B.	<u>Meliola bengalo</u> <u>rensis</u> Honsk. & Thirum.	<u>Ficus bengalensis</u> L. (Moraceae)	New to host
			S	<u>effusis</u> (pat.) Ellis. M.B.	<u>Meliola</u> <u>scelopinae</u> var. <u>zeylanicum</u> Hans <u>M. memecyli</u> syd. & syd. <u>M. puerariae</u> sp. nov <u>Ophioiorenina</u> <u>theae</u> Sawa Yamamo.	<u>Flacourtia montana</u> Grah. (Flacourtiaceae) <u>Memecylon umbellatam</u> Burm. f. (Matace- ae) <u>Pueraria tuberosa</u> Dc (Fabaceae) <u>Thea sinensis</u> . (Theaceae)	New to India & New hosts
			S.	<u>guareicola</u> (stev.) eif	<u>Meliola atalan-</u> <u>tiae</u> Hosagoudar <u>M. ixorae</u> yates var. <u>macrospora</u> Hosagoudar <u>M. nothopegiae</u> Hansf.	<u>Atalantia wightii</u> Tanaka (Rutaceae) <u>Ixora polyantha</u> wt. (Rubiaceae) <u>Nothopegia colebrook-</u> <u>iana</u> weight. (Anacardiaceae)	New hosts
			S.	<u>japonicus</u> (P. Henn) gil, s.M.B.	<u>Meliola canthi</u> Hansf.	<u>Canthium umbellatum</u> wt. (Rubiaceae)	New host.