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Research Article

A mounting and permanent slide preparation technique for Cryptogams

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Abstract

A technique for 'whole mounting' for permanent slide preparations of lower cryptogams have been developed. Pieces of fresh thermocol dissolved in xylol (100 ml) up to saturation and transferred in oven for filtration through Whatman paper no. 1 at constant temperature 30^0C for one hour so as to evolve air bubbles. This filtrate serves as a mounting medium to make permanent slide preparations of lower cryptogams (epiphytic algae, and bryophytes; leaf associated saprophytic fungi, sooty moulds and black mildew fungi). This medium is also useful for obtaining stomata and hydathode impression peels for angiosperm leaves fern leaflets respectively. The method for obtaining entire thalli of epiphytic algae and bryophytes; colonies of ectophytic fungi from leaves (sooty moulds & black mildews); stomatal peel is found useful for measuring stomatal density. For studying structure of hydathodes, peel from fern leaflets is useful. The peels are obtained for getting entire, undamaged structures of them and do not require scraping (as scraping damages of structures to be studied).

INTRODUCTION

Before any beginner botanist can take keen interest in systematic studies in cryptogams, he needs to acquire a general knowledge of collection, preservation, deposition techniques respectively applied. The most satisfactory way to do this is to have knowledge about common species of the group in which he is interested and so that taxonomic research of any group needs systematic collection of specimens and their technique of mounting for further studies. It may not be possible to identify the members collected from any group in the field, hence he needs a systematic collection and a skill for "whole mounting" of the specimens without damaging or disturbing their original structures that help in their identification. A very simple technique is developed and discussed in detail.

During field explorations, plant twigs with leaves associated with/infested with epiphytic algae, bryophytes; saprophytic fungi, ectophytic sooty moulds and black mildews; and fern fronds are collected in separate polythene bags, brought to the laboratory and kept for drying in blotting papers; or used when fresh for slide preparations by using thermocol solution made in xylol and are used for identification. A fresh thermocol sheet is made into pieces and dissolved in 100 ml of xylol by stirring the mixture slowly using glass rod. These pieces are dissolved in xylol up to saturation. This mixture is filtered through Whatman paper no. 1 through funnel by keeping it in oven at 30^0C for one hour which help in eliminating air bubbles for the mixture and filtrate is stored in amber color bottle. This transparent solution is served as whole mounting medium for making permanent slides of certain lower cryptogam specimens.

While making the temporary slide preparations of fresh cryptogams epiphytic algae, bryophytes, fungi, microscopic observations for their thalli structure may damage or injured lifting from hosts so that their structures may not remain intact and such damage structures may not be useful for identifications of the respective taxa while scraping. Therefore, for mounting epiphytic algae and bryophytes; ectophytic sooty moulds and black mildews from angiosperm host, a drop of this mounting solution is applied using a glass rod, on leaf surfaces, and solution is spread uniformly so as to form a thin film (Fig. 1). Where the respective thalli get embedded in the solution and this preparation is allowed to dry. And a very thin transparent film of peel solution is formed (Fig. 2). Without touching the centre of film, from margins film of peel is lifted with the help of forceps and mounted in a drop of same solution on a glass slide. Again one more drop of this solution is kept on the film of peel and cover glass is applied (Fig. 3) then very little pressure is applied on a cover glass to remove excess solution. The slides are kept in oven (at 30^0C temp.) for 5 minutes and allowed to evolve air bubbles from embedded structures. After evolving the bubbles, slides are removed from oven, again little pressure is applied on cover glass and slides are allowed to dry which are ready for microscopic observations. However, for studying stomatal density from dicotyledonous and monocotyledonous leaves and for studying structure of hydathodes in ferns, a drop of solution is applied as mentioned earlier. After one hour dried film peels are mounted in a drop of glycerin on a slide and cover-glass is applied.

Fig. 1: Application of mounting solution.

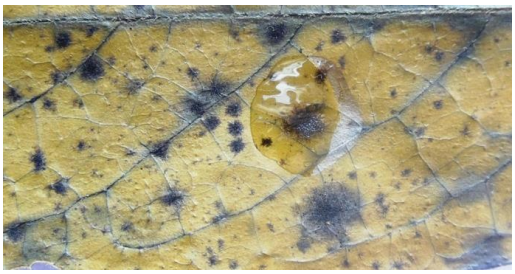

Fig. 2: Dried thin film of mounting solution.

Fig. 3: Slide preparation.

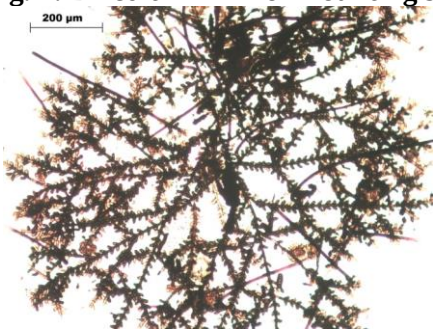

Fig. 4: Intact structures of black mildew.

When specimens are brought to the laboratory, their examination starts with macroscopic features visible to the eyes, hand-lens and microscope. There were several methods adopted for mounting algae, bryophytes, fungi specimens and also for stomatal peels. For algae, glycerin jelly method; for bryophytes for mounting Berlese's fluid is used. For sooty moulds and black mildews; celloidin solution (Gaillard, 1892) is used. Stevens (1961) modified Gaillard's solution for their mounting, Ellis (1950, 1960) used necol, and Hansford (1961) applied Celloidin acetone drops to obtain films; Hosagoudar and Kapoor (1958) used colourless nail-polish Hosagoudar and Mohanan (1985) used thermocol isobutyl methyl ketone solution for getting flips from affected surfaces of leaves; for stomatal density, peels are obtained using nail polish or diluted fevicol solution. Usually the slides are prepared by using routine solutions but there were other methods e. g. Nail-polish application, Celloidin in acetone (Hansford, 1961), cellulose acetate NaCl mounting technique (Ellis 1950, Dring 1971) and cello tape application to strip off the thalli of ectophytic fungi; algal specimens smeared in a drop of 10% glycerin and made semi-

permanent with glycerin jelly. While bryophyte specimens are mounted in 50 % aqueous glycerin and sealed with paraffin, these are mounted in 'gum chloral' mounting medium (Berlese fluid). Thus such methods were used for getting peels by applying different mixtures and peels are mounted in their glycerin, glycerin jelly, and lacto phenol depending on type of cryptogam. The routine methods used for obtaining thalli from leaf surfaces, is by scraping them with a blade but it may damage them and this may results difficulty in identification of specimens as the entire and intact structures of these thalli are not obtained for microscopic observations, so that thermocol xylol solution serve as a good peeling solution and for permanent slide preparation. The application of this solution on leaf surface forms thin transparent films. After drying, it can be stripped off easily.

Authors have modified the technique for getting better peels for studying epiphytic alga, bryophytes, ectophytic sooty moulds and black mildews (Fig. 4) as well as for determining stomatal density and studying hydathode structures in ferns. This technique may found better useful for them and describe below. The thalli in this film remain intact and unbroken and become useful for taxonomic identifications. The benefits of using this medium are as follows:

(1) In case of leaf associated Dematious hyphomycetes, the nature of thalli, type of conidiogenous cells, arrangement of hyphae etc. characters are important for identification of taxa. This solution is useful to expose these structures very intact in a peel.
(2) In ectophytic sooty moulds and black mildews, colony size, origin of mycelia setae and its arrangement, branching pattern of mycelia hyphae, hyphopodia arrangement and their angles are very important for identification of taxa. The application of this solution is very useful for exposing entire structures that help in identification of taxa.
(3) In epiphytic bryophytes and algae especially from leaf surfaces, the size, shape, nature of thalli, type and arrangement of filaments, reproductive structures remain intact in peel for taxonomic identification.
(4) Hyperparasites on black mildews in peels embedded intact that help in their correct identification.
(5) The peels gives impression of hydathode structures from fern leaflets
(6) A direct peeling of leaves may have leaf tissue which may become obstacle for determination of stomatal density. This medium lifts entire peels.
(7) The colour of algae and fungi thalli remains unchanged which may be helpful in their identification.
(8) The fruiting bodies and their dehiscence pattern, reproductive structures, hyphae branching etc. remain intact in peels as on host surfaces.
(9) Technique is useful to entomologists for identification of insects (secreting sugary substances) on host leaves on which sooty moulds grow.
(10) The technique help in trapping leaf associated saprophytic fungi.
(11) The entire peels of respective specimens can be mounted in glycerin or lacto phenol and slides can be made semi-permanent by sealing with wax.
(12) The slides can be made permanent by mounting respective peels in a drop of same peeling solution.

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