Topic: Temporary and Permanent Mounting
Lecture No: 3
Subject: Research Methods in Entomology
Class: MS (Replica)
Zoology Department, Lahore College for Women University, Lahore.
Course Instructor: Prof. Dr. Farkhanda Manzoor
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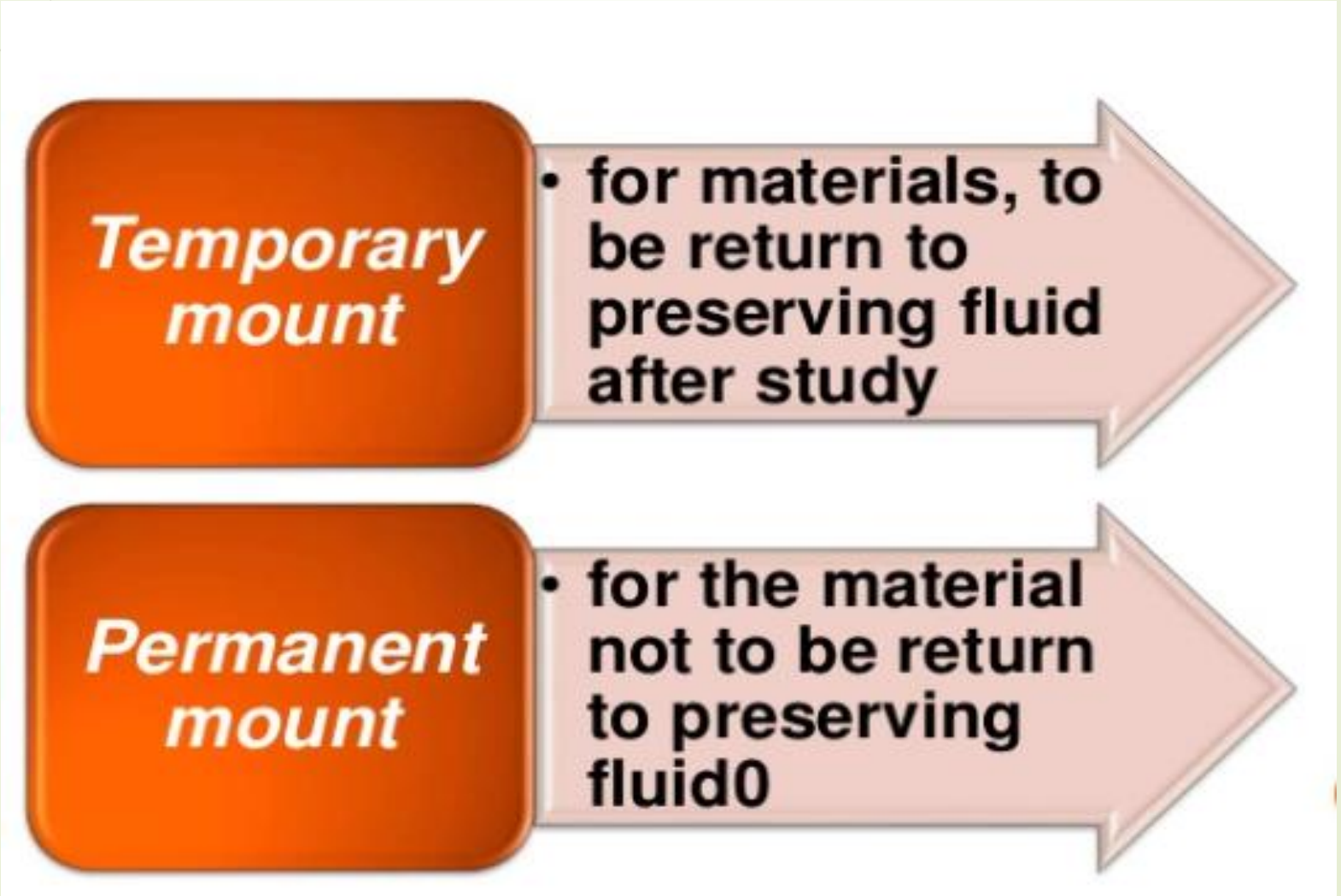
INTRODUCTION .....

- . Small arthropod ,generally soft bodied less then 1 mm i.e. Thrips mites etc are isolated and their body parts are only best studied when mounting is done on microscopic slides.

- The materials are transferred to a slide from the preserving fluid

Types of Mounts



Permanent Mounting

The following procedures are given for Permanent mounting specimens to be examined microscopically:

- Maceration
- Washing
- Bleaching.
- Dehydration.
- Staining
- Mounting.
- Drying Labelling

Materials Required

- **Preserving solution**

Hood's solution

- 70 – 80% ethyl alcohol 95cc, glycerin 5cc

Kahles solution

- 95 % ethyl alcohol 30cc, 40% formal dehyde 12 cc, water 60cc, rest glacial acetic acid

Maceration

- The aim of maceration is to eliminate external secretions, foreign matter, some organs, muscles, and fat bodies without damage to chitinous parts.
- This is accomplished by immersing the specimen in a suitable agent, such as a sodium hydroxide (NaOH) solution, lactic acid, or lacto phenol.
- These chemicals are strongly caustic and must be handled carefully to avoid damage to the skin and eyes.

Washing

- For the removal of the caustic agent used to macerate the specimen, ordinary tap water in a small dish, such as a small plastic bottle cover, will suffice.
- Distilled water is unnecessary. If the specimen is placed for at least a few minutes in plain water for manipulation it then will be ready for further treatment.
- Adding a drop of acetic acid (white vinegar) will guarantee that no caustic remains.

Cleaning Agent

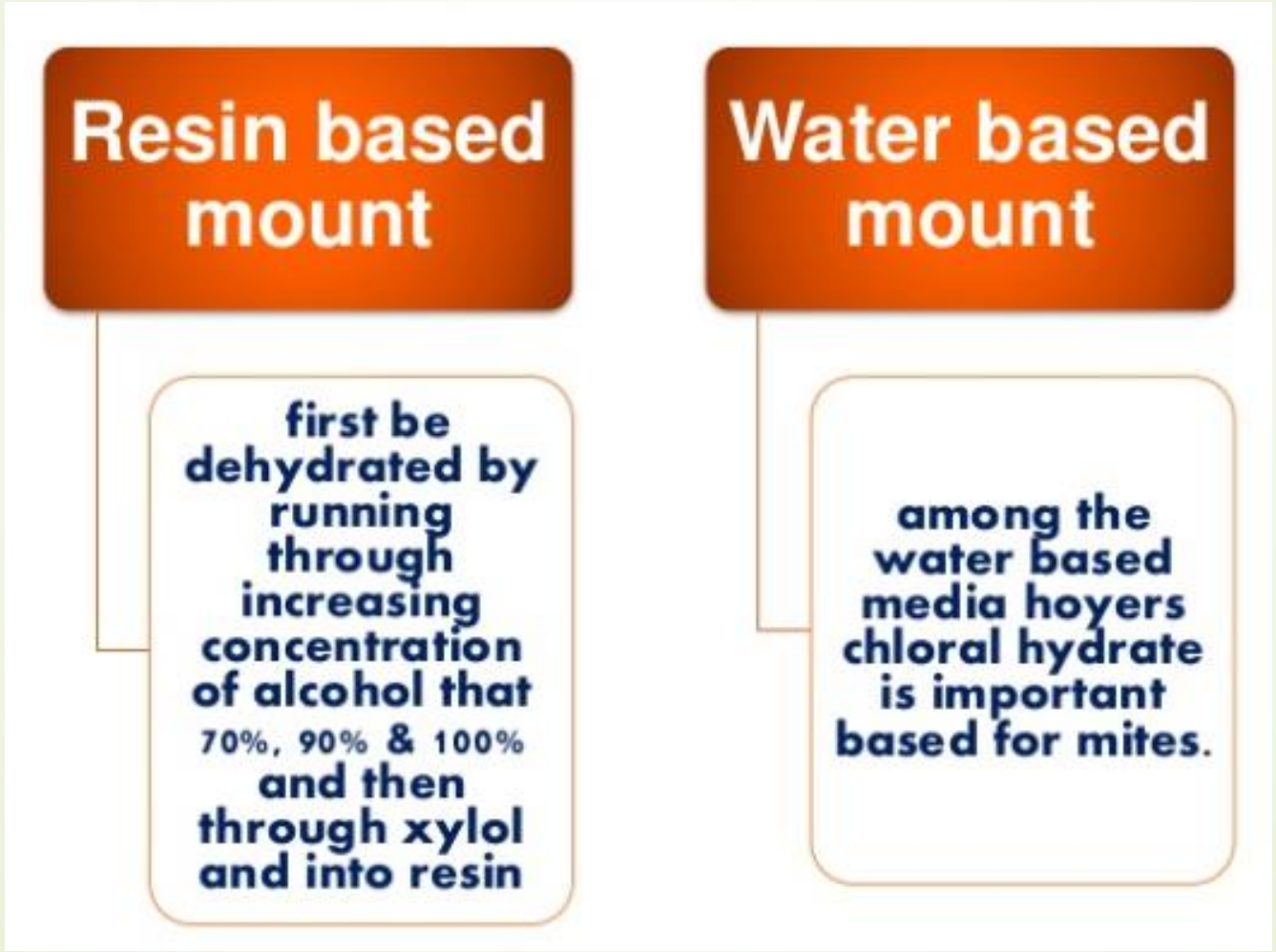
CLEANING AGENT ....

potassium hydroxide	• before mounting used to clean almost all arthropod. After cleaning washed in water with acetic acid.
Lactophenol-	• - used for mites specimen, lactic acid 50 parts phenol crystal 25 parts in water. Stored at room temperature
Andris fluid	• glacial acetic acid , chloride hydrate and water in equal proportion

Procedure

- The specimen are mounted on slides without support of glass.
- The thicker specimen mounted on a depression slide with some solution for support

Media for Permanent Mounting



Resin Based Mount

RESIN BASED MOUNT

A.CLEARING:

- Specimens or body parts which are large and/or thick should be cleared to allow transmitted light to pass through them. Clearing dissolves soft body parts, allowing the structure of the cuticle to be seen in its entirety once the specimen is slide mounted

Methods for Clearing

. There are two methods for clearing:

1)Cold Potassium Hydroxide (KOH) –

- Specimens in 10% KOH solution at room temperature overnight.
- After clearing with KOH, specimens should be returned to pH neutral water or alcohol before being passed through the alcohol series for dehydration.

2) Warm KOH –

- Specimens in 10% KOH can be heated on a double burner over an electric hot plate. This will simply speed up the clearing process.
- .After clearing, specimens should be returned to pH neutral water before being passed through the alcohol series

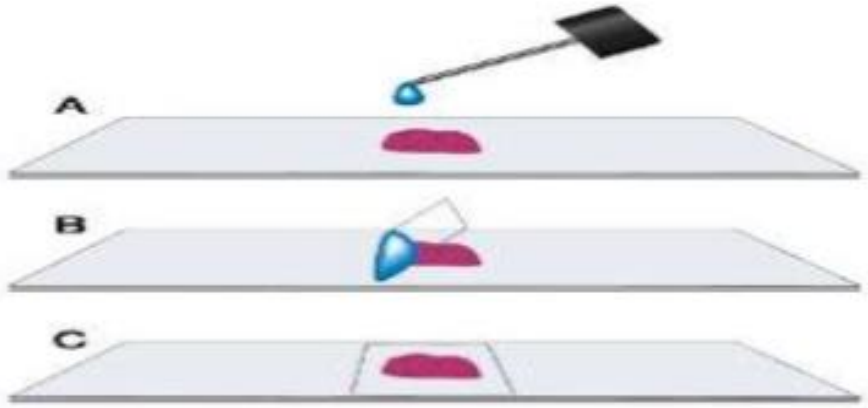
Staining

- After clearing and washing, specimens may be stained if necessary, although if a phase-contrast microscope is available, staining. Several kinds of stains are available from biological supply houses.
- Acid fuchsin is generally used for aphids, lice, and scale insects. Chlorozol black or mercurochrome generally are used for microlepidoptera.
- An easily obtained stain for the exoskeleton of insects is made by dissolving a small amount of Mercurochrome crystals in water.
- Specimens may be immersed in the stain solution for 1 minute or more, depending on the degree of staining needed, and then briefly rinsed in plain water.

Staining

B. STAINING:

- Two types of cuticle stains may be used: acid-fuschin and Harris hematoxylin.



- These can be added to your specimens while they are in 70% alcohol

. The specimen will become darker as time increases.

Some of the stain will be leached from the specimen in later stages of the dehydration series

Bleaching and Dehdration

- **Bleaching:** If specimens are too dark to reveal sufficient detail after maceration, they may be bleached in a mixture of one part strong ammonia solution to six parts hydrogen peroxide solution.
- The length of time the specimen is left in the ammonia-peroxide solution depends on the amount of bleaching needed.
- **Dehyrdation**. Specimens should be dehydrated (have the water removed) in alcohol.
- The length of time depends on the specimens, but 10-20 minutes is usually sufficient.

Dehydration

C. DEHYDRATION:

- Essentiality of dehydration

1. for the clear vision of desired characteris of of specimen

2. prevent the mounted specimen from bacterial infestation

(*which occurs in presence of water*)

Methods of Dehydration

METHODS OF DEHYDRATION

- Dehydration is accomplished by passing the insect through a series of increasingly concentrated grades of ethanol.
- After dehydrating in 100% ethanol, they should then be soaked in xylene before mounting on slides.
- The amount of time spent in each step depends on the thickness of the specimen. In general 15 minutes per stage is acceptable.
- The key is to watch the dehydrated specimen when it is in the final mounting medium
- . If clouding is visible, return the specimen to earlier stages in the dehydration series.

D. Drying Slide Mounted Specimens

- : After mounting, slides should be dried very slowly over very low heat.

- Leave them for several days on a proper slide drier or on a hot plate set at its very lowest temperature

Mounting

- **Mounting Media.**
- The standard medium for permanent mounts is Canada balsam.
- Balsam may yellow somewhat with age and this can make observation of characters and photography difficult; it can also be difficult to manipulate delicate specimens in it if it is not thinned properly.
- To place specimens in the medium, put one or more drops of the medium in the center of a 2.5- by 7.5-cm clean glass slide. The precise amount of medium to use will require some experience.

- Place the cleared and washed (also stained or bleached if necessary) specimen in the medium on the slide and make sure that it is well immersed and that air bubbles are absent.
- Arrange it in the desired position with a fine needle.
- If the specimen is thick, place at least three pieces of broken cover glass or plastic props around it to prevent undue crushing when the cover slip is applied.
- Then gently lower a cover slip onto the specimen with forceps, Apply gentle pressure with the forceps to fix the position of the specimen.

Water Based Mount

WATER BASED MOUNT

- Among the water based media **Hoyers chloral hydrate** is important based for mites.

- This is more permanent than resin based mount..

PREPARATION OF HOYERS MEDIUM...

PREPARATION...

1. clean gum arobic without extraneous material powered by mortar and pistel dissolved in distilled water
2. chloral hydrate crystal is added to above mixture in powered form
3. Glycerin is added to it with well stirring the medium is filtered through 2 – 3 layers of muslin cloth

4. . Filtration is repeted as long as dirt is found observed under microscope

5 • Thereafter medium should be kept in bottle with well fitted lid.

6 • Consistency of medium such that when taken out on a rod a drop would drop down after a few seconds. If consistency is found ropy then little water added

- Gum arobic shouldn't be used for preparation of media, sometimes required to stain some insects species.

- Canada balsam is generally used.

- Permanent mounting lasts for many years....

- https://www.slideshare.net/BhubanandaAdhikari/permanent-mounting-of-insect-by-bhubanananda-adhikari