# Biology

# Investigatory Project

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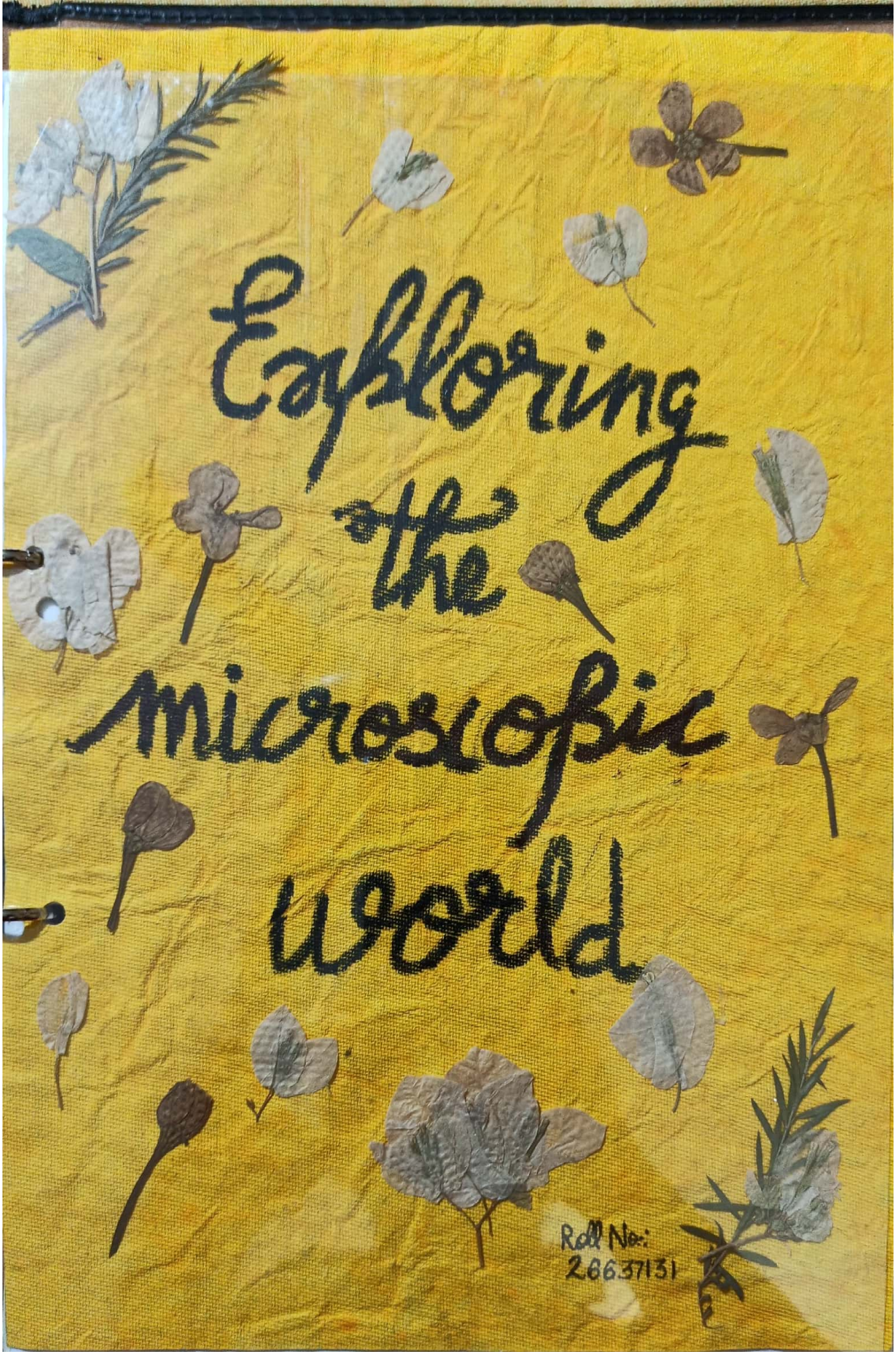

K.R. MANGALAM
World School
Vikaspuri

Together
Towards
TOMORROW

# CERTIFICATE

This is to certify that
**Manan Suri**
Of Class XII- A has successfully completed his
Biology Investigatory Project:

*"Exploring the Microscopic World"*

This report is the result of his/her efforts. It is completed under my guidance and supervision in partial fulfillment of the requirements for appearing in A.I.S.S.C.E. 2020.

INTERNAL EXAMINER

# Acknowledgement

Behind every great endeavour of us as students, lies the motivation and guidance of our wellwishers who strive to help us. This project was no different from the above postulate.

I would like ~~to~~ bestow my expressed gratitude to my biology teacher, Ms. Trista Sablok. Ma'am has given me guidance every step of the way. With every artefact I collected and every specimen I observed, Ma'am went over and beyond in helping me identify, catalogue and learn. Ma'am has truly been a mentor to me in all senses.

I would like to thank Sanjay Sir, our lab technician who has been very resourceful and helpful to me in executing this project. My gratitude is also due to the foldscope community, 'Microcosmos', specifically Dr. Lakshminarayan Iyer who has not only inspired my journey with foldscope but helped me understand and identify the specimens.

Lastly, I would like thank my parents and my sister ~~sis~~ for supporting me in all my endeavours.

Aman Suri

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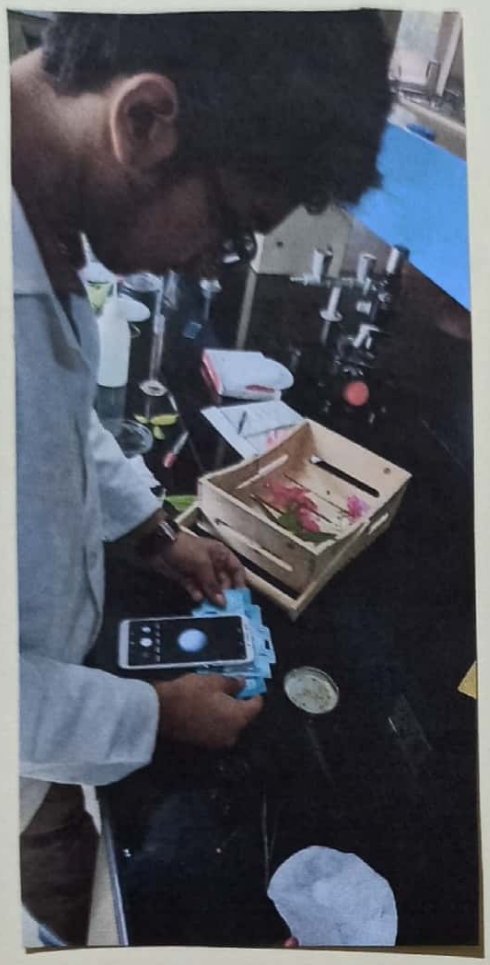


fig 1: Analysing sample using foldscope with smart phone

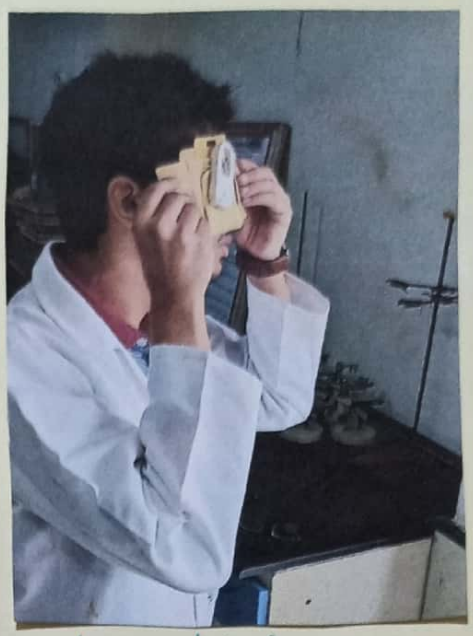


fig 2: dirctly viewing the sample

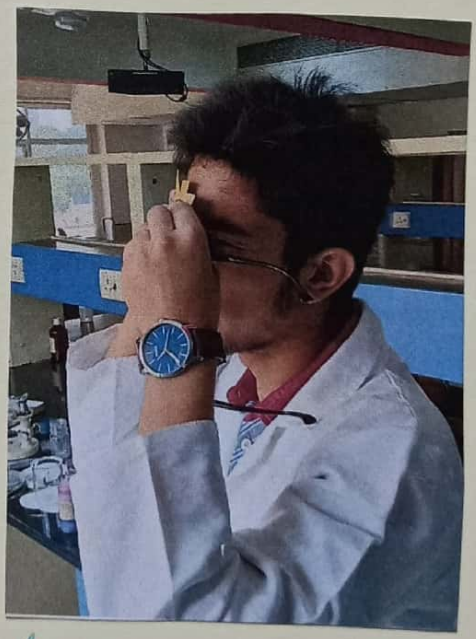


fig: 3 viewing sample through foldscope

fig 4: Preparing sample for a slide

# Statement of Purpose

I always had a curiosity to explore the microscopic world, glimpses of which I had seen at my school's lab. When I found out through a TED Talk that an inexpensive, yet efficient 'microscope' had been invented, I set out on my journey to explore the microscopic world.

As my interest grew, I was able to collect and observe specimens as more than a hobby. With the encouragement I received from my biology teacher, I decided to organise my explorations and embark on new ones, which culminated into this project.

As I started this project, my aim was also to learn new techniques, and methods as well as perfect the existing methods I have been using.

Human Puri


fig 5: Foldscope Deluxe Kit

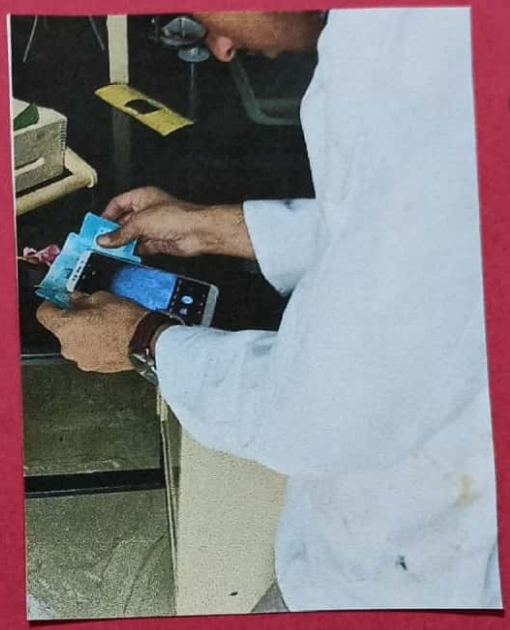


fig 6: using foldscope to view a slide

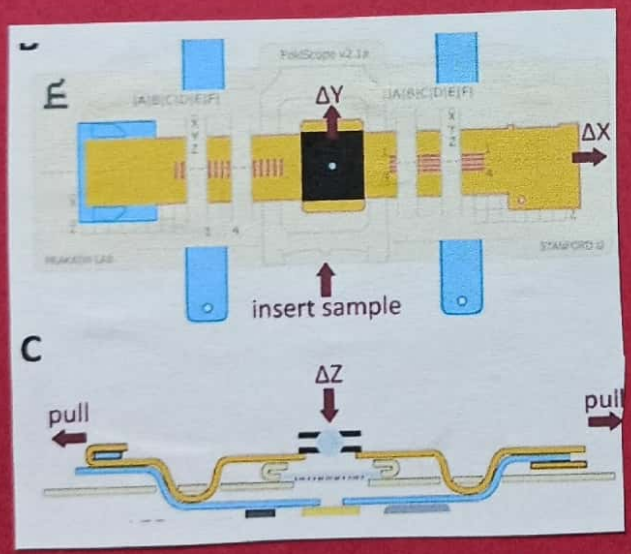



fig 7: components of the instrument

# About Foldscope

→ For a few centuries now, the world has been revolutionising the way we percieve the microscopic universe around us.
The basic principle of using a small spherical lens held close to the eye dates back to Antonie van Leeuwenhoek who was the first to see the microscopic organisms (living) using a lens..
We have come a long way since then, and the foldscope is one such landmark.

→ A foldscope is an optical microscope that has been assembled using simple components, cardstock and a spherical glass lens. It offers magnification of 140X, and a resolution of 2 microns.

→ Foldscope was developed by Dr. Manu Prakash and his Ph.D student at Stanford, Jim Cybulski. It is cost effective and a part of the 'frugal science' movement.

→ Using different tools and techniques, different configuration like bright field, darkfield, polarised microscopy etc.

→ A foldscope can be used in different ways:
- → Direct eye to lens viewing
- → Viewing via a camera phone
- → Projection on a wall.

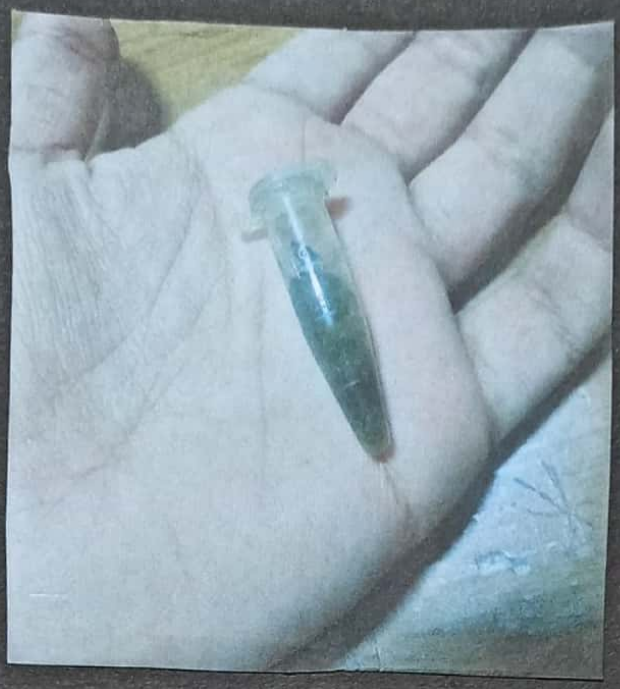


fig 8: moss sample hydrated and stored in eppendorf tube

fig.9: paramoeba in red circle squirming in moss debris

# Hydrated Moss

About hydrated moss ecosystem:
Mosses are small flowerless plants that typically form a mat or dense green clumps in damp and shady location. They are a part of the division bryophyta.
Mosses serve as a habitat for invertebrates as:
- → They have airspaces in network of plant tissue
- → They hold water within these spaces
- → They provide insulation from rapid temperature, humidity changes

Procedure: A small clumps of green moss was taken from backyard and water was added to it in an eppendorf tube. The supernatant is put in a cavity slide with moss tissue and viewed under foldscope.

Organisms observed:

1. **Peranema:** Domain: Eukaryota
   Phylum: Euglenozoa
   Class: Euglenoidea
   Order: Euglenales
   Genus: Peranema.

fig. 10 : magnified view of peranema

fig. 11

fig. 12

fig. 13

fig 11, 12, 13 → nematodes in moss debris

Peranema is a genus of free living phagocytotic euglenoids. Although they belong to the class euglenoidea and morphologically similar to green euglena, they lack chloroplasts and hence don't conduct autotrophy.
Since the cell is pear shaped, it's name is derived from pyronema.
It has a gliding motion due to it's flagella.
It also has a squirming motility due to helical microtubules

## 2. Nematode:

Kingdom: Animalia
Phylum: Nematoda

Nematodes are members of the phylum Nematoda They are amongst the most abundant animals on earth. They may be free living or parasitic They are round worms, bilaterally symmetrical and tapered near the ends
Nematode had a distinct movement under the foldscope, where they were moving by flexing parts of the body at a time, curling. This explained by the presence of longitudinal muscles and absence of circular muscles. There movement was thrashing back and forth. The alimentary canal was visible in some parts by the granular texture.

fig. 14 : Artificial lotus pond at 'Herbal Garden', Panjabi Bagh

fig. 15: storage of ~~water~~ sample in glass jar

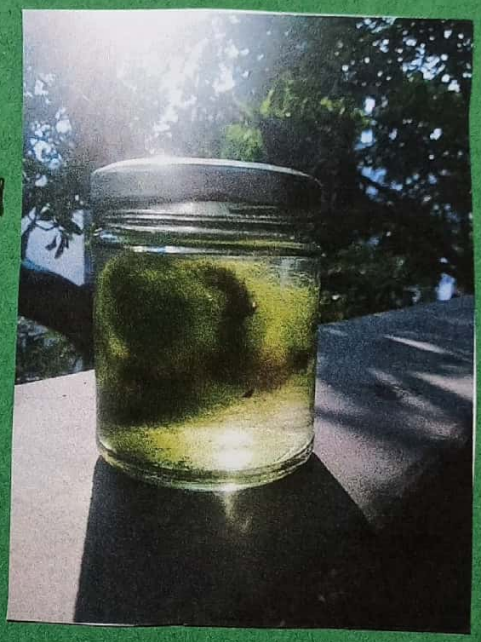


fig. 16: tuft of filamentous algae

# Pond Water

About pond water ecosystem,

Sample: The sample of pond water was collected from an artificial lotus pond from MCD Herbal Garden, Punjabi Bagh.

Pond Description: The artificial lake was swamped by lotus leaves, as well as weeds and algae. Amongst these algae, organisms like snails, certain worms were visible.

Collection and Storage: The sample was collected by wearing sterile rubber gloves in a jar. Syringe and forceps were used for assistance. Water was collected from regions adjacent to algal blooms. Algae was collected.

As an ecosystem, pond water is very dynamic because it provides a habitat to a wide array of organisms, which interact and depend on eachother.

Several samples observed included:
- algae
- diatoms
- flagellates
- ciliates
- water flea.
- rotifer

fig.17:

fig.18:

fig.20:

fig.19:

fig.21:

fig.17 → Algal filament with a bursting cell

fig.18, 19 → sessile ciliate on algae

fig.20 → pattern of chloroplasts in algae, as well as an achinete

fig.21 → terminal achinete

# 1. Algae

Algae are chlorophyll bearing, simple, thalloid, autotrophic and largely aquatic organisms

The algae sample included a mix of various filamentous species from the class chlorophyceae and class cyanophyceae. The algae were filamentous.

Algae serve as a very essential component in the slides that have been prepared. This is because of the following reasons:

(i) Several organisms hide between algal filaments as they serve as habitat, food. These include rotifers, ciliates, flagellates. Several sessile organisms like certain diatoms were found to be anchored onto the algae.

(ii) The slide is supported mechanically as a cushion of algae prevents loss of water. By capillary action, protects organisms

fig. 22

fig 23

fig 24

fig 28

fig 26

fig. 22 → variety of algal filaments; Chloroplasts are visible distinctly

fig. 23 → species may be Pithophora

fig. 24 → the grey 'Blobs' represent certain organisms living amongst the algae

fig. 28 → A diatom present amongst algae

fig 26 → terminal achinete

getting crushed by cover slip.

One dominant genus in the sample was found to be Lithoflora.
Identification was based on coarse cotton ball like texture, presence of dark specks. It had little frills coming off from it.

The specks were representatives of akinetes. The akinetes are resting stage cells. They are formed at the end of a cell, seperated by a membrane. The contents of the cell are thicker, darker and the cell is swollen. Akinetes were observed mainly in two stages:

(i) terminal akinetes → swollen end of algal filament.

(ii) akinetes which had given rise to cells of vegetative nature.

Notably, branched akinetes were absent.

The algae were also useful in studying different morphology of chloroplasts, eg. in fig. 25, 22.

Ciliates were observed like in fig. 33 as sessile forms.

Diatoms were also found, both motile (fig. 25) and

fig 27: Algal filament supporting a large number of diatoms ←

→ fig. 28: Diversity of diatoms present in sample

This figure sheds light into the two theca of diatoms ←

→ fig. 30 → diatoms; the circled one may be in a stage of cell division

sessile (fig. 27).

# 2. Diatoms

Diatoms: Kingdom: Plantae
Superphylum: Heterokonta
Phylum: Ochrophyta
Class: Bacillariophyceae

Diatoms are unicellular, eukaryotic microalgae found in freshwater and marine environments. They are usually photosynthetic.

Diatoms can be colonial, like fig. 27 as observed as dwelling on algae, or solitary as in fig. 25, fig. 29.

Diatoms have a unique feature that they are constituted by two thin overlapping shells, as in fig. 29.

fig. 28, 29 also makes a characteristic hard appearance of silica deposits, which makes their cells virtually indestructible. These special cell walls are called frustules which have structural coloration

fig. 31: telotroch phase of a vorticella; cell is quite transparent, but distinguishable

Diatoms contribute in 20% of oxygen produced on earth each year.

Several of these diatoms were visible as 'wine glass' like structures.

# 3. Vorticella (Telotroch)

Kingdom: Protista
Phylum: Ciliophora
Class: Oligohymenophorea
Sub Class: Petrichia
Order: Sessilida
Family: Vorticellidae
Genus: Vorticella

Vorticella is a genus of bell shaped ciliates that usually use a stalk to attach themselves to a substrate. They use their beating cilia near the cell's anterior region to produce a vortex.

Their bodies have globulous, oval shape when contracted


fig. 32: motile ciliate; species not identified

fig. 33: sessile ciliate; movement of band of cilia was observed

Vorticella usually have a transparent protoplasm. This was clearly observed. They also tend to have pellicle which may be characterised by observation of pustules or warty projection.

One interesting thing about the vorticella observed was that it was in it's blotroch phase.

A telotroch is the free-swimming stage of order Sessilidia. A sessile individual loses it's stalk to migrate to a better place.

# 4. Ciliates

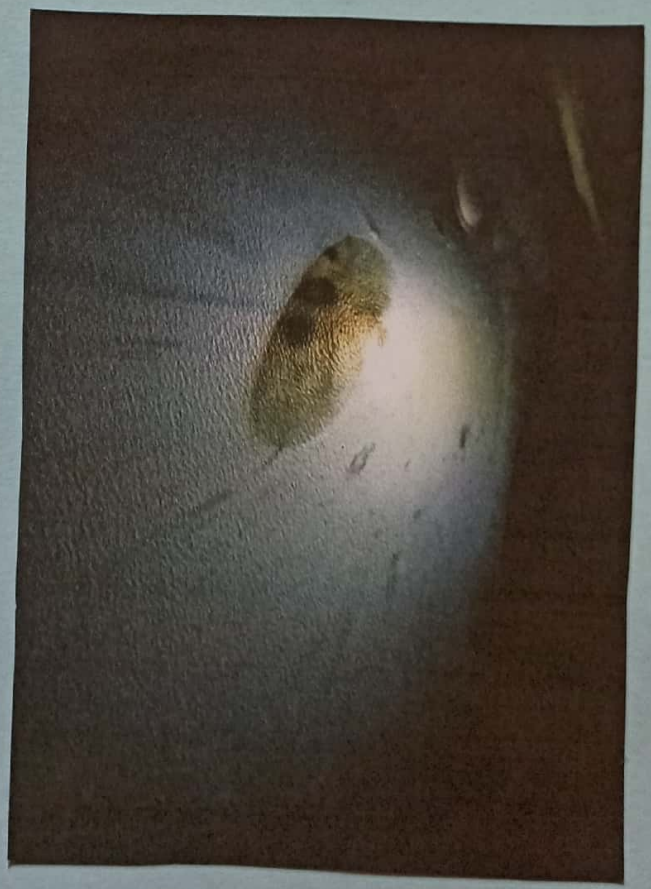
Kingdom: Protista
Phylum: Ciliophora

Ciliates are protozoans characterised by presence of hair like organelle's — cilia.
Cilia are used for swimming, crawling, attachment, feeding and sensation.

Two different types of ciliates were observed by the foldscope:

fig. 34 → entire water flea under brightfield mode with identifiable features

fig. 35 → slide projected onto a wall using foldscope



- sessile individuals:

These belong to the order Sessilidea. The individual observed was fixed to an algal filament, with a row of cilia near it's mouth.

- non sessile individual:

Cilia were covering the entire cell. They had a quick and rapid motion both in the plane as well as along it's own axis. The oral groove was visible.

# 5. Water Flea

Kingdom: Animalia
Phylum: Arthropoda
Sub phylum: Crustacea
Class: Branchiopoda
Subclass: Phyllopoda
Order: Cladocera

A water flea is a crustacean that is commonly found in freshwater habitats. It is considered as a kind of zooplankton.

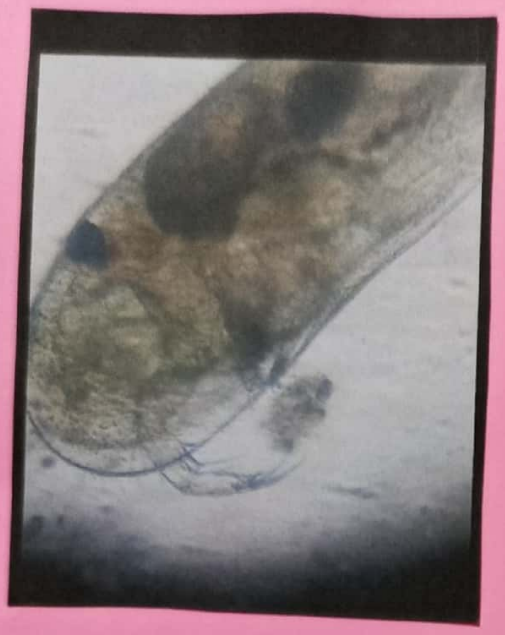


fig.36: frontal portion of water flea; notice how clear the mouth parts are; also observed is the detritus

fig.37: Hind portion of the water flea; certain appendages are observed

Water flea was observed to be in a jerky, hopping motion.

Most of the water fleas are 0.2-6 mm, a single median compound eye, a carpace (shell like structure) covering the thorax and abdomen.

The water flea bears two pairs of antennae, used for oltfactory sense and for locomotion.

The mouth parts consist of unpaired labrum, a pair of mandibles, pair of maxillia and unpaired labium.

The water flea was found to be feeding on detritus.

The thorax was found to have a number of appendages, with a number of hair/setae.

Several structures in the back portion may have been developing eggs. Most species show cyclical parthenogenesis but occasionally supplement it with sexual reproduction.

fig. 38 : Rotifer eating chlorella ; mastax is distinguishable

fig. 39 : Rotifer has bent itself near it's foot

fig. 40 : motile rotifer

fig 41 : rotifer amongst algal filaments

# 6. Rotifer

Kingdom: Animalia
Phylum: Rotifera

Rotifers, also called "Wheel Animalcule" is a species belonging to the phylum Rotifera that are aquatic, microscopic and pseudocoelomate.

Rotifers are multicellular animals that have specialised organ systems and a complete digestive tract. The name rotifers is derived from latin "wheel bearer" due to the wheel like arrangement of cilia which creates a vortex to feed substrates.

Rotifers are dioecous and may reproduce sexually or parthenogenetically. They exhibit eutely, i.e. no. of cells is constant

Rotifers can fix themselves on surfaces or move using their toe like structure

The mastax which is a chewing organ was visible along with the corona (row of cilia) and stomach. The toe was visible in certain motile individuals

fig 42 → alimentary canal of polychaete; parapodia

fig 43: → posterior and anterior ends of worm

fig 44: → dead polychaete worm; segmentation is easily visible

# 7. Polychaete Worm

Kingdom: Animalia
Phylum: Annelida
Class: Polychaeta

Polychaetes are segmented worms, that are bilaterally symmetrical, triploblastic, coelomate.

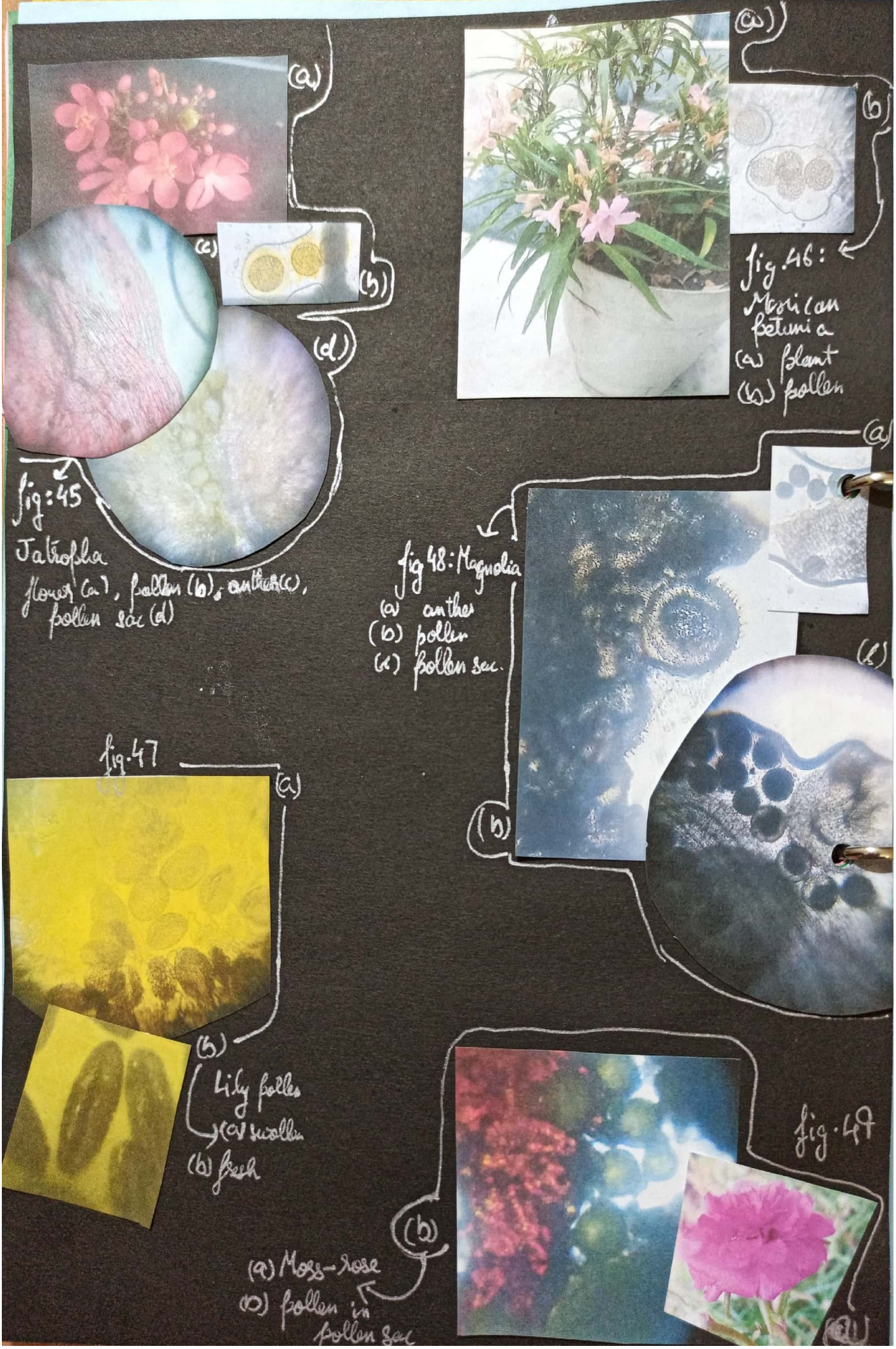
Each segment of a polychaete has a pair of protrusions known as parapodia which bear bristles on them.

In the observed individuals, the head was distinctly visible. The digestive tract along with it's movements was visible as well.

Part of a dead polychaete worm shed light on segmentation (fig. 44).

The polychaetes were found wriggling in a rapid 'S' shaped motion amongst tufts of algae.

There are 168 species of freshwater polychaetes and total 8000 marine polychaetes



# Plant Samples

## 1. Anther, Pollen

**Pollen:** Pollen represents the male gametophyte in flowering plants. Its size ranges from 15 to 200 μm.

Pollen are produced in the microsporangia. In angiosperms, these microsporangia are part of the anther.

Each pollen grain consists of a vegetative cell and a generative cell. This might be evident in magnolia pollen (fig. 48).

Often, the pollen swells in presence of water, like in lily pollen (fig. 47).

The pollen grain has two cell wall layers- the intine which is cellulosic and the exine. Exine is made of sporopollenin, which causes variation in patterns. Germ pores are present to allow growth of pollen tube.

**Anther:** Anther refers to the terminal, bilobed structure of stamen in angiosperms which consists of microsporangia and their wall layers.

A typical angiosperm bilobed, with each lobe having two thecae, i.e. dithecous. A longitudinal groove separates the thecae.

Fig. 50 : Spider plant
(a) plant
(b) Pollen along line of dehiscence; wall layers
(c) Pollen

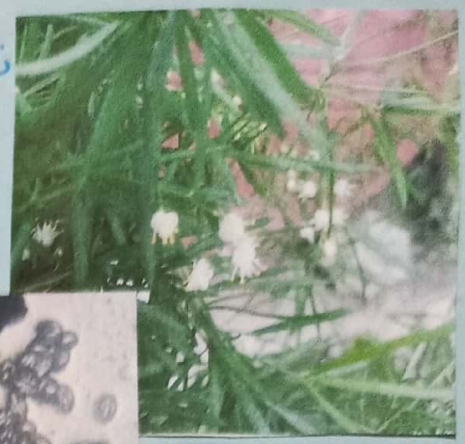


Fig. 51
Sprenger's Asparagus
(a) flowers; plant
(b) pollen

Fig. 52
Chrysanthemum
(a): wall layers
(b), (c): pollen

Each lobe has two microsporangia which develops into pollen sac. These 4 microsporangia have different wall layers: epidermis, endothecium, middle layers and tapetum.

The following specimen were observed:

Jatropha integerrima

Ruellia simplex ; Mexican Petunia

Magnolia

Lilium candidum ; Lily

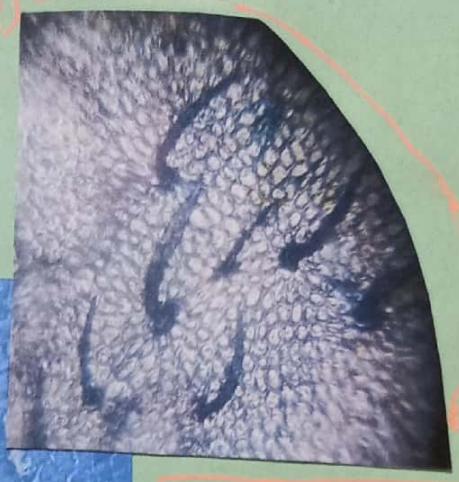
Chlorophytum comosum ; Spider plant

Asparagus aethiopicus ; Sprenger's asparagus

Chrysanthemum

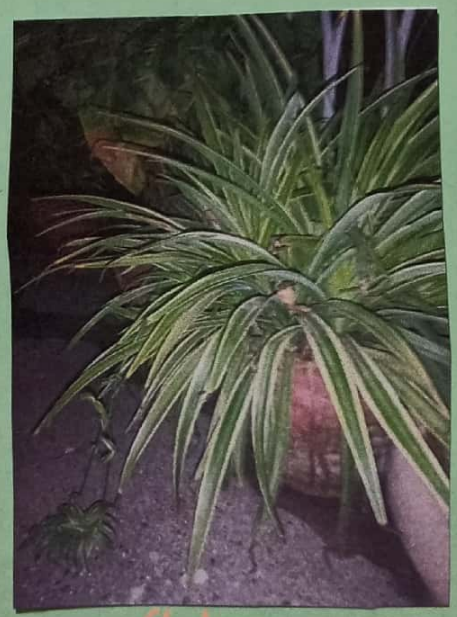
Portulaca grandiflora ; moss-rose

20.



(a)
(b)
(c)
(d)
(e)

Fig. 53 (Butterfly pea)
(a) pea flower
(b) Keel petal
(c) Vexillary petal
(d) dried vexillary petal with trichomes
(e) trichomes on keel petal



(a)
(b)
(c)

Fig. 54 (Spider plant)
(a), (c) Translucent petal with stomata
(b) plant

# 2. Petals

Petals are modified leaves which surround the reproductive parts of flowers. They are often brightly coloured to attract pollinators. The whorl of petals is known as the corolla.

Petals of following flowers were studied:

## Clitoria ternatea

This plant is popularly known as butterfly pea and is a member of the family fabaceae. The flowers had vexillary arrangement.

Standards: the standard petals were pigmented blue and cells were mostly cuboidal in shape. presence of leaf trichomes was observed.

Keels: Petals were unpigmented. The cells were irregular and jigsaw shaped.

## Chlorophytum comosum

Chlorophytum comosum is commonly known as spider ivy. The petals were unpigmented. cells were elongated. The petal had stomata as well.

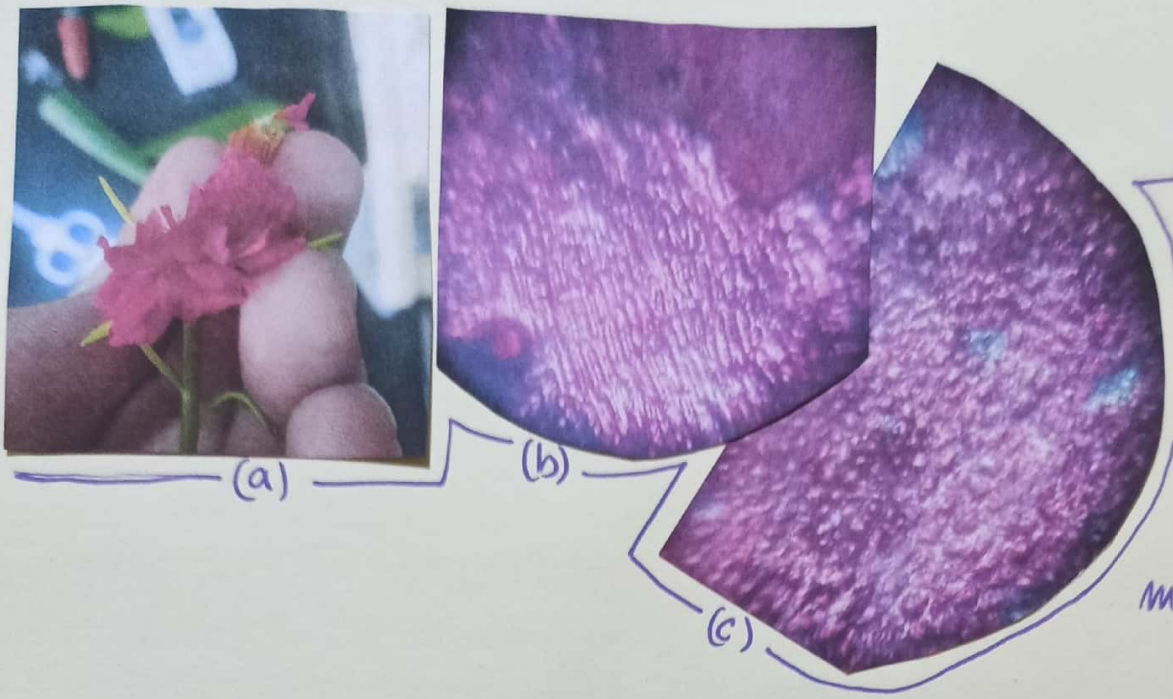



fig. 55
(a) flower
(b) longitudinal cells
(c) circular cells of moss-rose

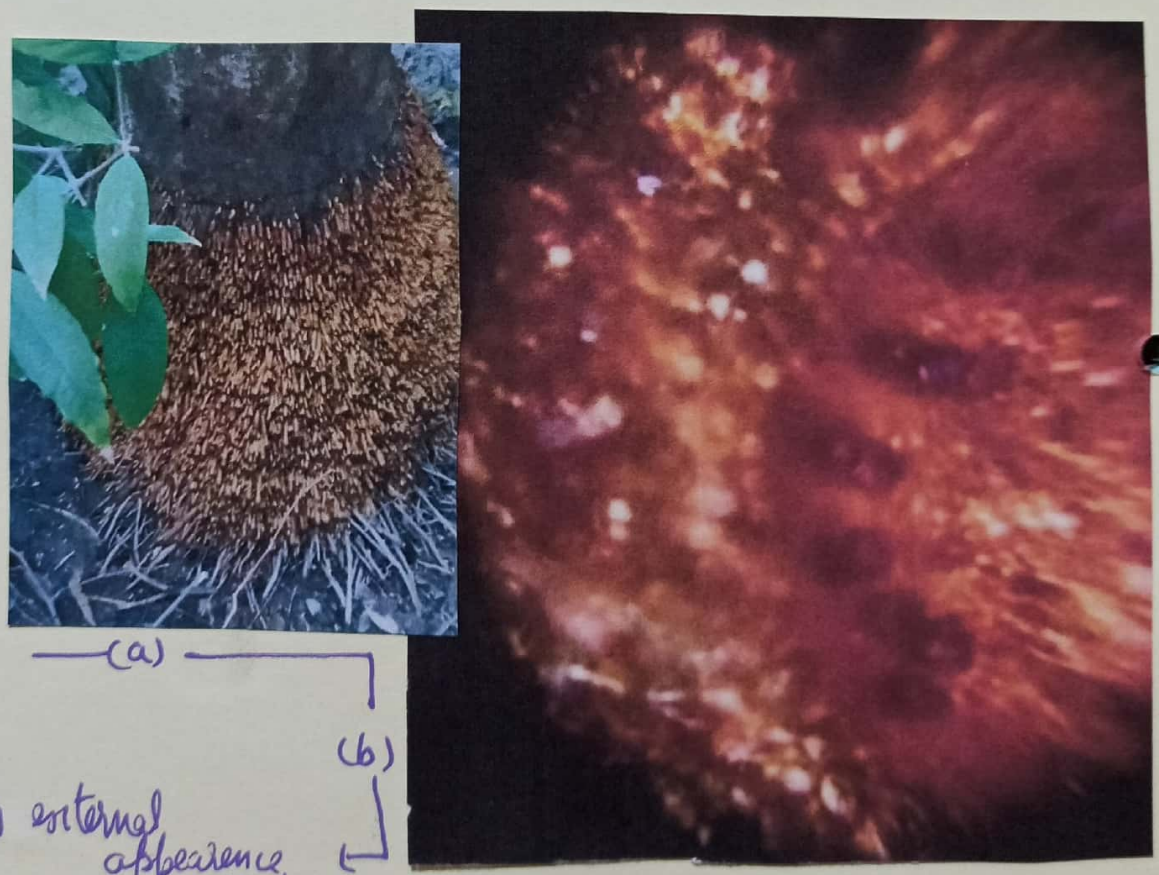
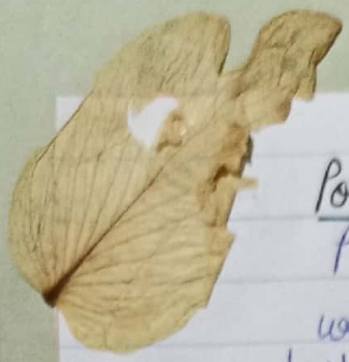



fig 56 (a) external appearance of root
(b) root cross-section



Portulaca Grandiflora

Pink petals with two different type of cells were observed:

- cuboidal cells near the basal part of the petal
- elongated cells petal's tip region.

# 3. Cross-section of Monorot Root

Cross-section of a monocot root was taken. It was stained with safranin orange.

A ~~dicot~~ monocot root has:

- Epidermis
- Cortex
- Endodermis
- Pericycle
- Phloem
- Protoxylem
- Metaxylem
- Pith

The stele was more darkly stained than the cortex. Xylem vessels were distinct. Pith was well developed.

→ fig. 57 → cells of stigma stained by pollen; a few pollen near the bottom

→ fig. 58 → cross-section of stigma

fig. 59 → pollen tubes ←

# 4. Stigma

The stigma of a *Lilium candidum* was cut and appressed on the glass slide the following features were observed:

- Irregularly arranged cells
- Pollen grains were found. Adjacent cells to pollen grain had acquired yellow tint.
- In other regions, cells were unpigmented.
- Prominent nucleus were present.
- Cells were easily ~~torn~~ tearing apart from eachother
- Pollen tubes were seen emerging from certain region.

# 5. Epidermis

Epidermis in botany is the outermost, protoderm derived layer of cells covering the stem, root, leaf, flower, fruit and seed of the plant.

It provides a barrier against mechanical injury, water loss and infection

(a)

(b)

→ fig 60 (a) epidermis of aloe
(b) stomata

→ fig 61: money plant upper epidermis; chloroplasts are visible.

(a)

(b)

fig 62
(a) Tradescantia epidermis with stomata
(b) Leaf impression on transparent nail polish

The following specimens were observed:

Aloe Vera

- Cell walls were distinct.
- Stomata were present, but in fewer density.
- Cells had granular appearance. My hypothesis is that they may be representative of the cellular machinery required to synthesis aloe gel, like ER, golgi etc.
- Stomata were closed.

Epipremnum aureum (Money plant).

- Upper epidermis was observed
- Cells were cuboidal but irregular. Observed slide had more than a single layer of cells.
- Green coloured chloroplasts were observed as tiny sac like structures inside the cells.

Tradescantia

- Since leaf was variegated, cells were found to be purple, green, unpigmented.
- Stomatal apparatus was distinct: stomatal pore, guard cells, subsidiary cells.
- Nail polish impression of epidermis showed three dimensional structure of the cells.

fig 63 → wings of thrip

appendages ← fig. 64


fig 65 → (a): antenna
(b) compound eye

thorax ← fig 66

# Arthropods

## 1. Thrips

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Thysanoptera

Thrips are minute, slender insects with fringed wings and unique mouth parts. Different thrips species feed mostly on plants by puncturing and sucking up the contents.

The thrips was fixed by immersing the thrips in alcohol, and nail polish was used as a mountant.

Compound eyes were identified, with distinct ommatida. The fringes of the wings were found to be due to hair like structures. Body is segmented. A pair of antenna, with hair like sensory cells present

Thrips pose much economic losses as they feed on valuable fruit and serial crops. Several species act as vectors of plant viruses.

fig. 67 → head of larva with antennae, compound eye

thorax of larval mosquito; heart like structure, spiracles ← fig. 68

fig. 69 → abdomen; spiracles, digestive canal

# 2. Mosquito Larva

A mosquito larva was collected from a groups of larvae that were found near an empty flower pot.

A cavity slide was used with water as temporary mounting media

The mosquito larva had a well developed head with mouth brushes used for feeding, and developing compound eye.

Inside the thorax, mosquito's developing heart was beating. The segmented abdomen had a digestive canal (gut) which ends in an anus. The anus is surrounded by anal gills. The anal gills are the only part of the larvae, permeable to water.

The larva breathes using a siphoning tube. This tube attaches itself to the interface between water and air. This feature is often used in controlling mosquito populations

Based on identification keys, the larvae may belong to the genus Aedes.

Fig. 70: hind portion showing siphon at air-water interface

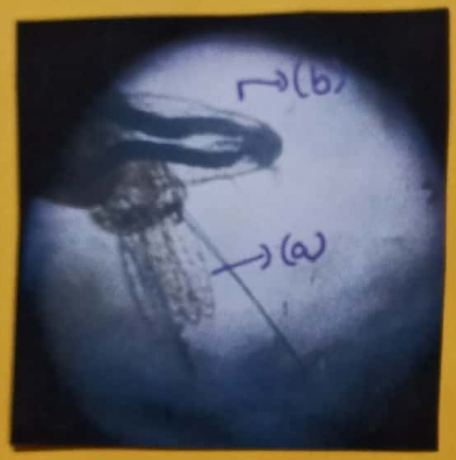



Fig. 71: anal gills (a) and siphon (b)

Fig. 72: details of anal gills

Based on that key thus, the classification is as follows:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Culicidae
Genus: Aedes

The mosquito larvae were killed by putting dishwashing soap in the collected water. They create bubbles which block the siphoning tube. Moreover, dishwashing soap also has chemical toxicity to the larvae.

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