A PROJECT REPORT

ON

"Measurement of Size of Pollen Grains of Different Flowers Using the Technique of Micrometry"

SUBMITTED BY

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SUBMITTED TO :

VIVEKANAND COLLEGE, KOLHAPUR

DEPARTMENT OF BOTANY

FOR PARTIAL FULFILLMENT OF

BACHELOR OF SCIENCE IN BOTANY

(2024-2025)

UNDER THE GUIDANCE OF

DR. A. R. KASARKAR

(Department of Botany)

DECLARATION

I hereby declare that the project work entitled "MEASUREMENT OF SIZE OF POLLEN GRAINS OF DIFFERENT FLOWERS BY USING TECHNIQUE OF MICROMETRY" submitted to the Vivekanand College, Kolhapur for the award of the degree of "Bachelor of Science in Botany" is the result of bona fide work carried out by me under the guidance of Prof. Mr. A. R. Kasarkar.

I further declare that the results presented here have not been the basis for the award of any other degree.

Place: Kolhapur

Date: 04/03/2025

MISS MUSHFIRA SHAIKH

MISSVANSHITA MURTI

Shri Swami Vivekanand Shikshan Sanstha'S
Vivekanand College, Kolhapur, (Autonomous)
Department of BOTANY

certificate

This is to certify that Vanshita Murti and mushfira Shaikh . Exam Number has satisfactorily carried out his project report as per the syllabus prescribed by Department of, Botany. Vivekanand College (Autonomous) for B.Sc.- III Botany. This project report represents his/her bonafied work during academic year 2024-2025.

Place: Kolhapur

Date: 05-03-2025

Kasarkar 05.03.25

(Dr. Abhijeet R. Kasarkar)

HEAD
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(EMPOWERED AUTONOMOUS)

Acknowledgment

This project work is a successful outcome of the contribution and guidance of others, for which I express my deep gratitude.

I also express our thanks to Prof. Dr.A.R Kasarkar , Department of Botany , Vivekanand College, Kolhapur, for providing me with the laboratory facilities of the Botany Department to carry out the experiment work.

I also express our gratitude towards my guide Dr. A.R. Kasarkar, for their guidance, encouragement, and support throughout the course of study, which enabled me to complete my project work.

I also wish to express my gratitude to the laboratory staff for completing the project work. Lastly, I express my gratitude to my parents, all our friends, and classmates for their support and cooperation.

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MISS MUSHFIRA SHAIKH **MISS VANSHITA MURTI**

Index

INTRODUCTION : POLLEN GRAIN

Pollen grains are microscopic structures , which bear the androecium male reproductive organ of the flower . pollen grains are tiny Particles that carry the male productive cells in flowering plants . they contain special cells called microgametophytes ,which develop into sperm cells during fertilization the study of pollen & its various characteristics known as palynology

Pollen grains are essential for pollination, the process of transforming the male part of the flower to the female part for successful reproduction in many flowering plants. Pollen grains in flower structure help ensure genetic diversity & the continuation of plant species .

- **Structure of pollen grains is the main three components :**

    1. **Living cytoplasm :-** this is the inner part that contains the cell's.
    2. **Intine :-** this inner layer of the cell wall is made of cellulose & pectin.
    3. **Exine :-** this is the tough outer layer composed of sporopollenin.

- **The Size , Shape and colour of pollen grains :**

    1. **Size :-** Although pollen grains are microscopic , their size varies widely among species , typically ranging from about 3 micrometers to 200 micrometers.

2. **Shape :-** pollen grains can appear round , oval , triangular . disc like . or even beans – shaped the same have smooth surfaces . while others have a spiky texture.
3. **Colour :-** most pollen grains are naturally white , but certain species produce in shades of yellow , orange and cream .

- **Types of pollen grains :-**

Different types of pollen grain can be classified based on factors like their shape , surface texture & the number of pores or furrows on their exine for instance , same grains have multiple furrows on their surface others may have just one or two tense variations help botanists. Identify and differentiate pollen grains belonging to different plant species.

- **Importance of pollen grains :-**

1. Fertilization pollen grains are produced in the anther , which is part of the stamen.
2. **Pollen tube formation** - after landing on the stigma a tub cell within the pollen grains forms the & release sperm cells for fertilization.
3. **Biodiversity & survival** – successful transfer of pollen ensures genetic variation and the survival of pollen grains plants , contributing to a healthy ecosystem.

Pollen grains are typically measured using microscopy techniques, primarily by visually assessing their size under a microscope. Light microscopy to measure basic dimensions like polar length and equatorial diameter, scanning electron microscopy (SEM) for detailed surface features, and image analysis software to automatically measure large quantities of pollen grains with high precision.

- **Basic measurements :-**

    1. **Polar length** :- The length from the pole to the equator of pollen grains.
    2. **Equatorial diameter** :- The width of the pollen grains at its widest point.
    3. **Aperture size** :- The diameter of the openings on the pollen grains surface.

- **Microscopy techniques** :-

    1. **Light microscopy** :- Most commonly used for basic measurements due to its accessibility , but can be limited by resolution.
    2. **Scanning electron microscopy (SEM)** :- Provides high - Resolution image of the pollen grain surface, allowing detailed analysis of surface structure and features.
    3. **Transmission electron microscopy (TEM)** :- used for detailed internal structures of pollen grains.

- **Image analysis software :- automated measurements -**

    1. Software can automatically measure pollen grain dimension from digital images captured under a microscope , significantly increasing efficiency.
    2. **Morphological analysis :-** can also be used to identify pollen grain shape and surface features.

- **Sample preparation :-**

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Sample preparation :- Pollen grain needs to be properly prepared for analysis often involving suspension in liquid medium and mounting on a microscope slide.

- **Calibration :-**

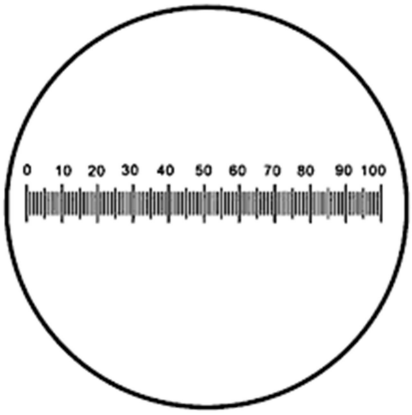
microscope objectives and image analysis software must be calibrated to ensure accurate measurements.

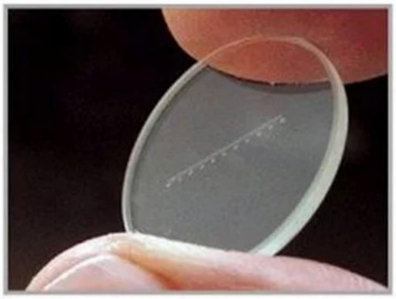
- **Statistical analysis :-**
- When measuring a large number of pollen grains, statistical analysis is necessary to assess variation and identify trends.

INTRODUCTION : MICROMETRY

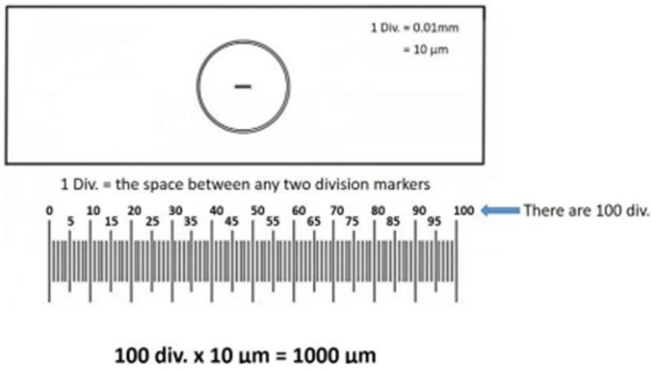
Micrometry is a technique used to measure the size of microscopic objects. Principle: Calibration of the ocular micrometer using the stage micrometer. When an ocular micrometer is set correctly inside the eyepiece, it should be enabling to see a sample and a micrometer scale simultaneously when we look through a microscope, by determining single pitch length of a micrometer scale, we will be able to measure the actual sample size Ocular micrometer

Ocular Micrometer



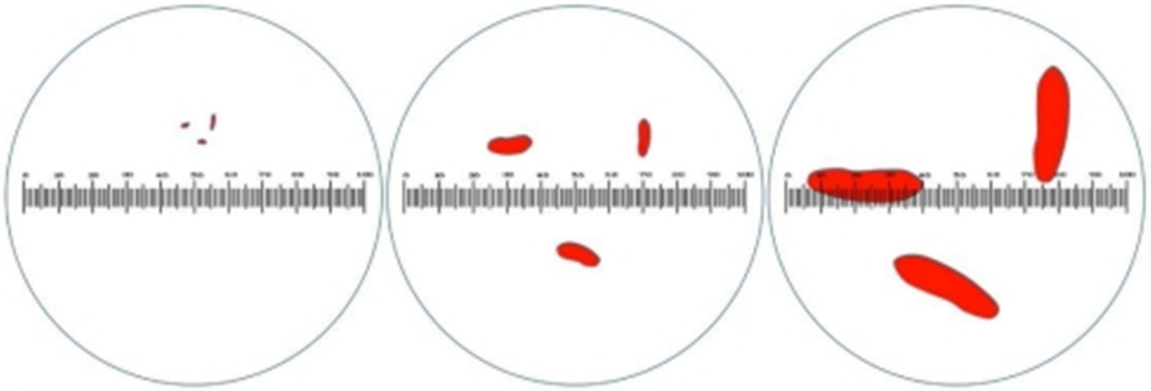


Stage micrometer



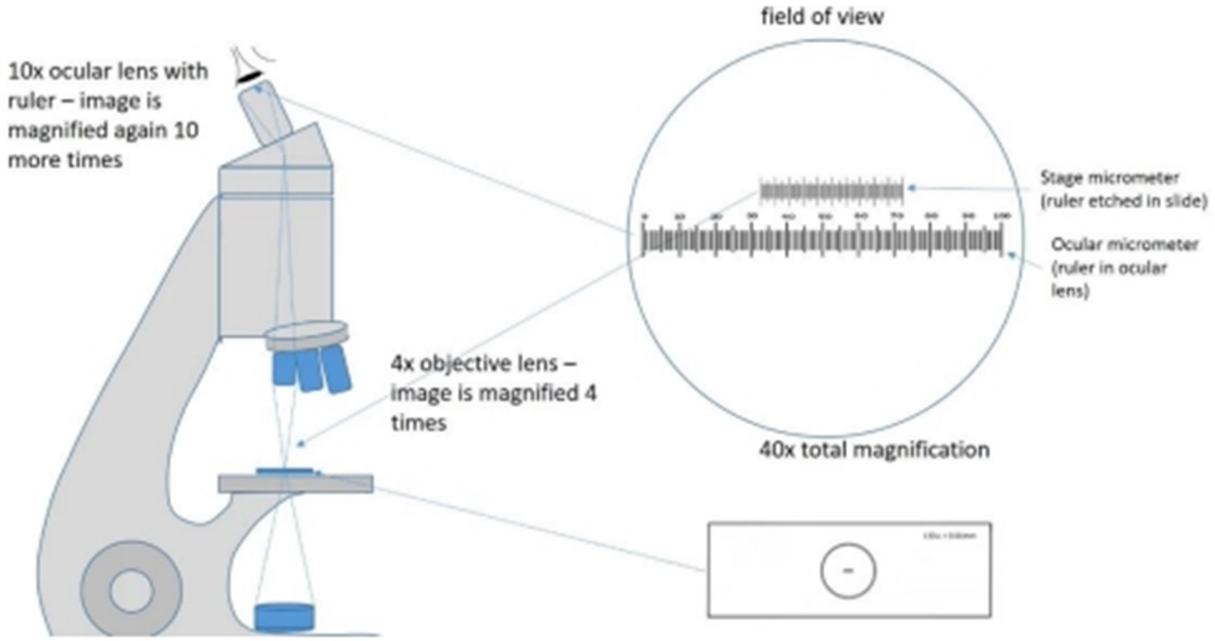
100 div. x 10 µm = 1000 µm

We can measure the size of pollen grains in the following manner



Ocular Lens	**10X**	**10X**	**10X**
Objective lens	**4X**	**10X**	**40X**
Specimen's Image	**4X x 10X = 40X**	**10X x 10X = 100X**	**40X x 10X = 400X**

Calibration



AIM AND OBJECTIVE

AIM:

The aim of this project is to measure the size of pollen grains from different flowers using the technique of micrometry.

OBJECTIVES:

1. **Measurement of Pollen Grain Size**:
    - Use micrometry techniques to accurately measure the size of pollen grains from various flowers. This involves calibrating the microscope, preparing pollen samples, and measuring the dimensions of the pollen grains under different magnifications.
2. **Comparison of Pollen Grains**:
    - Compare the measured characteristics of pollen grains from different flowers to identify variations and commonalities. This could involve statistical analysis to understand the range and distribution of sizes and other features.
3. **Evaluation of Micrometry Technique:**
    - Assess the effectiveness and accuracy of the micrometry technique in measuring and characterizing pollen grains. This includes discussing any limitations, potential sources of error, and suggestions for improving the methodology.
4. **Documentation and Reporting:**
    - Compile the findings into a comprehensive report, detailing the methods used, results obtained, and conclusions drawn. This report will serve as a reference for future studies and provide insights into the use of micrometry for pollen analysis.

REVIEW OF LITERATURE

1 .Miroslwa Chwil – 2015 – Micromorphology of pollen grains of fruit trees of the genus *prunus* ,Acta sci. pol. Hortorum cultus, 14(4) ,115-119.The micromorphological characteristics of the pollen grain sculpture is one of the most important diagnostic traits in plants. Pollen grains in various species of the sub family Prunoideae are characterized by variations in size, shape and exine sculpture. In the present work, for the first time, the micromorphology of pollen grains of fourteen cultivars from five fruit tree species of the genus *Prunus* was compared. Morphometric observations and analysis of pollen grains were performed using light and electron scanning microscopy. In terms of size, the pollen grains studied were classified as medium sized and large. The obtained results on the exine sculpture of pollen grains can be used in the taxonomy of species of the genus *Prunus.*

2. U.G. Basarkar -2017 -light microscopic studies of pollen grains by acetolysis method J R B A T ,vol.v (3) 1-10. Total 30 types of pollen grains from different plants collected. Out of these pollen types 7 belonging to Asteraceae, 3 from Malvaceae, 3 from Convolvulaceae, 3 from Fabaceae, 2 from Euphorbiaceae and one from Amaranthaceae, Plumbaginaceae, Verbenaceae, Caesalpinaceae, Brassicaceae, Sapinadaceae, Zygophylacaeae, Boraginaceae, Acanthaceae, Steraceae, Oxalidaceae and Gentiaceae family respectively.

3. SHAISTA JABEEN .ETAL-2024 –MICROMETER INSIGHTS INTO NEPETA GENUS :POLLEN MICROMORPHOLOGY UNVEILED ,MICRON VOL 177 .103574 .This study provides a comprehensive pollen micromorphology within the *Nepeta* genus, revealing intricate details about the pollen grains' structure and characteristics. The findings shed light on the evolutionary and taxonomical aspects of this plant genus, offering valuable insights for botanists and researchers studying *Nepeta* species. The pollen grains of 18 Nepeta species were studied using scanning electron microscopy (SEM) and light microscopy (LM) in Northern Pakistan. At the microscale, pollen quantitative measurements, qualitative traits, and diverse sculpturing patterns were reported and compared. Significant differences in pollen size, shape, ornamentation, and sculpturing patterns were discovered among the Nepeta species.

4.BRADLEY D.E.- 1958 ,THE STUDY OF POLLEN GRAINS THE STUDY SURFACES IN THE ELECTRON MICROSCOPE10.1111/j.1469-8137.1958.tb05308Citations: 13 Pollen grains cannot be examined directly in the instrument because of their size and consequent density to electrons, and even in the case of acetolysed grains the remaining wall is still far too thick to allow the passage of electrons through it. It is therefore necessary to employ either thin sections or surface replicas. The first method has been used extensively by Mühlethaler (1953), Afzelius, Erdtman and Sjöstrand (1954) and Afzelius (1950) in studies of pollen and spore walls. Surface replicas have been employed by Mühlethaler (1955) who used the carbon replica method (Bradley, 1954a). The present author has also made some preliminary studies using this technique (Bradley, 1956). This paper is concerned with the potentialities of the carbon replica in the study of pollen grain surfaces.

MATERIALS

Following are the materials used:

Materials	Plant material Ocular micrometer Stage micrometer.
Glassware	Glass slides Watch glass Brush
Chemicals	Stain - Safranin
Equipment	Microscope

METHODS

Plant Collection and Identification:

- **Collection:** The flowers of various plants were meticulously collected from the college campus. Each flower was chosen with care to ensure that the plant material was free from any disease or damage. Collecting disease-free samples is crucial to obtaining accurate and uncontaminated results in subsequent analyses.
- **Identification:** The collected plant materials were identified using standard botanical literature. This process involved referencing authoritative botanical texts and guides to accurately determine the species and characteristics of each plant. Proper identification is essential to ensure the validity and reliability of the study.

Extraction of Plant Material & Preparation of Pollen Grains

- **Washing and Preparation:**
    - The collected flowers were thoroughly washed with clean water to remove any dust, dirt, or contaminants. This step ensures that only the pure plant material is used for further analysis.
    - After washing, the pollen grains were carefully removed from the flowers using a brush. This delicate process requires precision to avoid damaging the pollen grains.
- **Staining:**
    - The extracted pollen grains were stained using safranin, a biological stain commonly used in microscopy. Safranin stains the pollen grains, making them more visible under a microscope.

This staining process highlights the details of the pollen grains, such as their size, shape, and structure.

- **Slide Preparation:**
  - Slides were prepared by placing the stained pollen grains onto a clean microscope slide. A cover slip was carefully placed over the pollen grains to create a flat and even surface for observation.
  - These prepared slides were then observed using a calibrated microscope. Calibration of the microscope is crucial to ensure accurate measurements and observations. The microscope's calibration involves adjusting the magnification and focusing mechanisms to provide clear and precise images of the pollen grains.

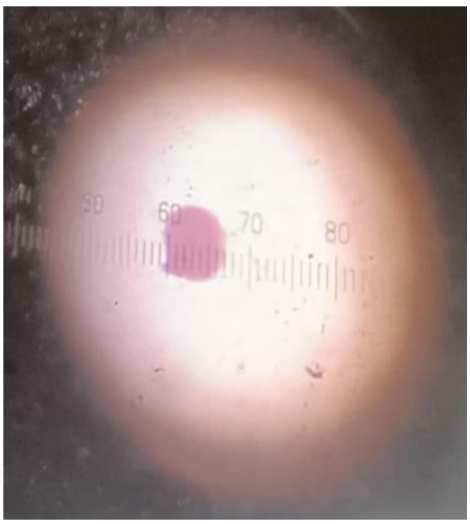
- **Observation and Analysis:**
  - Under the microscope, the stained pollen grains were observed in detail. The size, shape, color, and other morphological characteristics of the pollen grains were recorded. This analysis helps in understanding the variations and specific features of pollen grains from different flowers.

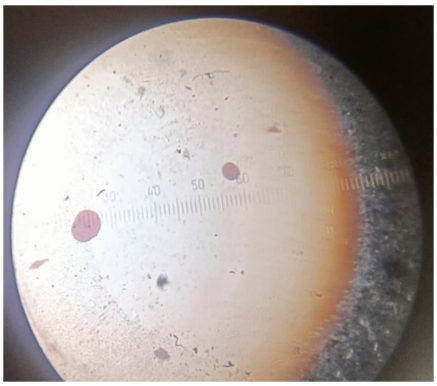
RESULT

Sr.No.	Name of the plant	Family	Pollen size at low power	Pollen size at high power
1.	*Cardia subestena*	Baraginaceae	6µm	12µm
2.	*Codiaeum variegatum*	Euphorbiaceae	7µm	14µm
3.	*Impatiens balsamina*	Balsaminaceae	5µm	12µm
4.	*Plumbago auriculata*	Plumbaginaceae	7µm	14µm
5.	*senna barronfieldii*	Fabaceae	4µm	8µm
6.	*Clerodendrum splendens*	Lamiaceae	5µm	12µm
7.	*Ixora*	Rubiaceae	6µm	11µm
8.	*Euphorbia milii*	Euphorbiaceae	5µm	12µm
9.	*Jatropha integrrima*	Euphorbiaceae	8µm	16µm
10.	Areaceace (palm)	Areaceace	3µm	8µm


cardia subestena l.


codiaeum variegatum





CONCLUSION

Examining pollen germination on a slide isn't just a fascinating scientific activity it's like peeking into the intricate connections among flowers, fruits, and the basis of our food chain. It underscores the fragile harmony of nature and the amazing influence of life's tiny marvels. pollen grain project highlights the crucial role of these microscopic structures in plant reproduction, showcasing their diversity, structure, and ecological significance, including pollination and potential applications. Pollen grains are small particles that contain androecium a flower's male reproductive organ. The center of the pollen grain comprises cytoplasm as well as the tube cell, which changes into a pollen tube, and the generative cell, which releases the sperm nuclei.

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