Impact Factor – 6.625 ▪ Special Issue - 325 ▪ June 2023 ▪ ISSN – 2348-7143

INTERNATIONAL RESEARCH FELLOWS ASSOCIATION'S

RESEARCH JOURNEY

Multidisciplinary International E-research Journal

PEER REFREED AND INDEXED JOURNAL

Two Day Interdisciplinary National Conference On

INDIA @75

... Guest Editor ...
Dr. O. J. Rasal
Mr. S. S. Patel

... Chief Editor ...
Dr. Dhanraj T. Dhangar

... Executive Editors ...
Mr. H. S. Shaikh
Mr. C. M. Gangawane

... Jointly Organized by ...
Pravara Medical Trust's,
Arts, Commerce & Science College, Shevgaon
Dist. Ahmednagar – 414502

Printed by : Academic Book Publications, Jalgaon

मराठी

ISSN : 2348-7143
June 2023
RESEARCH JOURNEY Internationl Multidisciplinary E-Research Journal
Impact Factor (SJIF) - 6.625 | Special Issue 325 : India@75

In vitro Germination of Pollen Correlation Between Viability (TTC staining) Germinability (In-vitro) of Flowering Plants.

Rahul Bandu Kharat
Department of Botany,
PMT'S Arts Commerce and Science College, Shevgaon.

Abstract :

In Vitro germination of pollens germination and there viability help to understand the reproductivity of flowering plants. Pollen germination stimulated by presence sucrose help to bring pollen germination n, pollen viability affect various factors such as temperature, humidity, moisture content and other in this Study help to check pollen viability using chemical 2,3,5 Triphenyl terta azolium chloride (TTC). Pollens germination percentage detect by using different concentrations of sucrose solutions.

Key words : In-vitro germination Pollen viability, pollen germination.

Introduction :

Acanthaceae are members of plants that are found all over the tropics and temperate regions. A total of 222 genera and 3565 species belong to the family Acanthaceae according to by A.L.de.Jussieus. and According to the report titled "State of the World's Plants", released by researchers at the Royal Botanic Gardens, Kew, in the United Kingdom, there are about 391,000 species of vascular plants currently known to science. About 369,000 species (or 94%) are flowering plants Of World

To study theirs in vivo pollen germination, pollen viability, and pistil receptivity which help us to understand the following aspects of plants belonging to the family Acanthaceae study the pollen viability I had to check the how many pollen grains are viable and how many are not viable and also check the if have some factors are affecting the pollen viability or not for e.g. temperature, moisture, humidity, presence or absence of light which affects or not.

Pollen germination test studies how different concentrations of sucrose solution affect the germination rate and percentages .pistil receptivity studies the capability of the pistil to receive the pollen whether the pollination happens successfully or not.

The main objective of this investigation, therefore, was to measure the pattern of pollen viability, pollen germination, and pistil receptivity during the pollination period under natural conditions, and to evaluate the effect of these factors on seed production.

PLANT DESCRIPTION :

1. Barleria cristata L.

Scientific classification :

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Barleria*
Species:	***B. cristata***

Description :

Morphology

General Habit :

Subshrub ; cystoliths present.

Leaves :

Elliptic, ovate to lanceolate, base and apex are attenuate, surfaces hairy.

Reproductive morphology Flowers :

Flowers are 2–5-flowered unilateral cymes, single-flowered in top of leaf axils; flowers are bracteoles linear-lanceolate,size up to 8.5–16 mm long, apex is spine-tipped.

Flowers Calyx :

Calyxare form their tububar, initially green or tinged purple, turning scarious, anterior and posterior lobes ovate-trullate, the former 14.5–19 × 6.5–8.5 mm, the latter somewhat longer, margins spinulose-toothed; lateral lobes lanceolate, 6–8 mm long.

Flowers Corolla :

Corolla blue, mauve or white, tube cylindrical towards the base, campanulate above attachment point of stamens, 31–43 mm long; the limb of 5 lobes, abaxial lobe splitting from the tube before remaining 4 lobes, each 11–22 mm long.

Flowers Androecium Stamens :

Stamens 2, exserted, bithecous, thecae at equal height; staminodes 3, lateral pair with small antherodes.

Ecology :

A native of tropical and subtropical Asia. Widely and commonly cultivated in the tropics.

Vernacular :

Philippine Violet.

2. *Barleria prionitis L.*

Scientific classification :

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Barleria*
Species:	***B. prionitis***

Description :

Morphology :

General Habit :

Subshrub; cystoliths present.

Leaves :

Leaves elliptic, ovate or lanceolate, base and apex attenuate or acute, surfaces strigose mainly on veins beneath.

Reproductive Morphology Flowers :

Flowers held in contracted 2–5-flowered unilateral cymes, or single-flowered in upper leaf axils; bracteoles linear-lanceolate, 8.5–16 mm long, apex spine-tipped.

Flowers Calyx :

Calyx conspicuous, initially green or tinged purple, turning scarious, anterior and posterior lobes ovate-trullate, the former 14.5–19 × 6.5–8.5 mm, the latter somewhat longer, margins spinulose-toothed; lateral lobes lanceolate, 6–8 mm long.

Flowers Corolla :

Corolla yellow, tube cylindrical towards the base, campanulate above attachment point of stamens, 31–43 mm long; the limb of 5 lobes, abaxial lobe splitting from the tube before remaining 4 lobes, each 11–22 mm long.

Flowers Androecium Stamens :

Stamens 2, exserted, bithecous, thecae at equal height; staminodes 3, lateral pair with small antherodes.

Ecology :

A native of tropical and subtropical Asia. Widely and commonly cultivated in the tropics.

3. Pseuderanthum laxiflorum.L :

Scientific classification :

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms

Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Acanthaceae
Genus:	*Pseuderanthemum*
Species:	***P. laxiflorum***

Description :

Habitat : Terrestrial, evergreen

Habit : shrub

Stem : herbaceous, aerial weak, cylindrical, branched, fistular, hairy

Leaves : cauline and Ramal, alternate, spiral, exstipulate, simple, shortly petiolate, lanceolate with entire margin, the apex is acute, rough provided with small hairs on surface, faintly veined with a prominent midrib unicostate, green colour

Inflorescence : racemose

Flower : ebracteate, pedicellate, complete, bisexual, actinomorphic, pentamerous, cyclic, pink colour.

Calyx : 5sepals, polysepalous valvate.

Corolla : 5 Petals, Polypetalous, Imbricate.

Androecium : 5 Stamens, free, basifixed, dithecous.

Gynoecium : Monocarpellary, inferior, trilocular, axile.

4. Hymenocallis littoralis (jacq.) salibs.

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Monocot
Order:	Asparagales
Family:	Amaryllidaceae
Subfamily:	Amaryllidoideae
Genus:	*Hymenocallis*
Species:	***H. littoralis(***jacq.)

Description :

Habitat : Terrestrial, evergreen.

Habit : Is a bulbous peren.

Stem : herbaceous, short weak, cylindrical, unbranched, fistular, smooth surface.

Leaves : cauline,arise from the base of the stem , alternate, spiral, exstipulate, simple, sessile , lanceolate with entire margin, the apex is acute, leaves surface is thin membranous and shiny prominent midrib with parallel venation , green colour

Inflorescence : solitary.

Flower : ebracteate, pedicellate, complete ,bisexual, actinomorphic , pentamerous, cyclic ,white colour.

Calyx : 5sepals, gamosepalous formation of tubular structure.

Corolla : 5 Petals, all petals are free form a tail like structure.

Androecium : 5 Stamens, free, basifixed, dithecous, yellow colour.

Gynoecium : Monocarpellary, inferior, trilocular, axile.

5. Cassia fistula L. :

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideae
Genus:	*Cassia*
Species:	**C. fistula**

Description :

Morphology :

General Habit : Tree

Leaves : Leaves glandular; leaflets 3–6(–8) pairs, acute or bluntly pointed, minutely tuberculous beneath

Inflorescences ; Racemes lax, pendulous, 1–3 together, 15–60 cm long; bracts caducous

Flower :

Corolla : Petals yellow, c. 2–3 cm long

Fruits : Pods cylindrical, 20–50 x 1.5–2.5 cm, black, indehiscent, with hard woody walls.

Distribution : At least in S2 and S3 native of tropical Asia.

Vernacular : Golden shower, Indian laburnum (English).

MATERIALS AND METHODS :

Collection of plant materials :

All plants used for the present study were collected from Savitribai Phule Pune University Campus, Pune. Barleria prionitis which are collected the near the Jaykar Library, Barleria cristata are also collected from the front of the Department of the Botany, and other Pseuderanthemum species are collected from the University gate road to the department of Botany building

Preparation of chemicals :

Chemicals :

Sucrose, Boric acid, 2,3,5- triphenyl tetrazolium chloride (TTC), KOH.

Prepare a sucrose solution of different concentrations :

Solution (100ml of 10% sucrose) : 100ml DW containing 10gm sucrose, 30mg calcium nitrate, and 10mg boric acid.

Solution (100ml of 15% sucrose) : 100ml DW containing 15gm sucrose, 30mg calcium nitrate, 1Omg boric acid.

Solution (100ml of 20% sucrose) : 100ml DW containing 20gm sucrose, 30mg calcium nitrate, 1Omg boric acid.

Solution Staining : TTC (2,3,5- triphenyl tetrazolium chloride) 1% TTC (0.2 g. TTC and 12 g sucrose dissolved in 20 mL distilled water)

10% KOH Solution : 10 gm KOH dissolved in 100mL distilled water

Pollen viability Tests : A drop of the TTC solution will be placed on a microscope slide and the pollen grain will be spread with a slim brush and covered with a coverslip. Pollen viability counts by observing the dark stained and faintly stained pollen after 1 hour. Pollen grains stained that orange or bright red colour will be counted as viable pollen.

In vitro Pollen Germination Test : A drop of the different concentrations of sucrose solutions solution will be placed on a microscope slide and the pollen grain will be spread with a slim brush and covered with a coverslip. Pollen germination counts by observing the pollen germination tube and there percentage after 1 hour. Pollen grains swollen or germinated will be counted as germinated pollen.

RESULTS AND DISCUSSION :

Pollen Viability Test :

Pollen Viability percentage will be determined by:

Pollen Viability Percentage = Number of pollen grains Stained X100

Number of pollen grains observed

Sr. No.	Species Name	Viable Pollen	Total No of Pollen	Viability Percentage %
1.	*Earlier cristata*	20	33	60.60%
2.	*Barleria prionities*	70	90	77.77%
3.	*Pseuderanthemum laxiflorum*	35	70	50%
4.	*Hymnocallis littoralis*	11	23	47.82%
5.	*Cassia fistula*	55	88	62.05%

$$\text{Pollen Viability Percentage} = \frac{\text{Number of pollen grains Stained}}{\text{Number of pollen grains observed}} \times 100$$

1. Barliaer cristata= 22/33 x100 = 60.60%
2. Barleria prionities= 70/90 x100 = 77.77%
3. Pseuderanthemum carrythersii= 35/70 x100 = 50%
4. Pseuderanthemum laxiflorum= 11/23 x100 = 47.82%
5. Pseuderanthemum maculatum= 55/88 x100= 62.05%

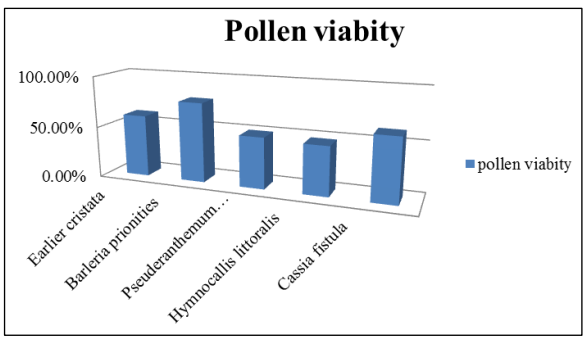


Fig. Pollen viability Test using TTC

Pollen Germination Test :

Pollen germination percentage will be determined by :

$$\text{Pollen Germination Percentage} = \frac{\text{Number of pollen grains germinated}}{\text{Number of pollen grains observed}} \times 100$$

Sr. No.	Species Name	Germination Percentages %		
		10% Sucrose	15% Sucrose	20% Sucrose
1.	*Barliaer cristata*	76.00%	80.00%	83.00%
2.	*Barleria prionities*	25.64%	40.00%	84.00%
3.	*Pseuderanthemum laxiflorum*	48.50%	73.68%	76.00%
4.	*Hymnocallis littoralis*	28.57%	41.66%	45.83%
5.	*Cassia fistula*	54.83%	79.72%	91.85%

1. *Barleria cristata* L :
For 10% sucrose solution = 60/78 x 100 =76%
For 15% sucrose solution = 102/127 x 100 = 80.31%
For 20% sucrose solution = 15/18 x 100 = 83%

2. *Barleria prionities :*
For 10% sucrose solution = 20/78 x 100 =25.64%
For 15% sucrose solution = 12/30 x 100= 40%
For 20% sucrose solution = 42/50 x 100= 84%

3. *Pseuderanthemum laxiflorum :*
For 10% sucrose solution = 22/48 x 100 =45.80%
For 15% sucrose solution = 28/38 x 100 =73.68%
For 20% sucrose solution = 38/50 x 100=76%

4. *Hymnocallis littoralis :*
For 10% sucrose solution = 8/28 x 100 = 28.57%
For 15% sucrose solution = 15/36 x 100 = 41.66%
For 20% sucrose solution = 11/24 x 100 = 45.83%

5: *Cassia fistula:*
For 10% sucrose solution = 102/186 x 100 = 54.83%
For 15% sucrose solution = 118/148 x 100 = 79.72%
For 20% sucrose solution = 124/135 x 100 = 91.85%

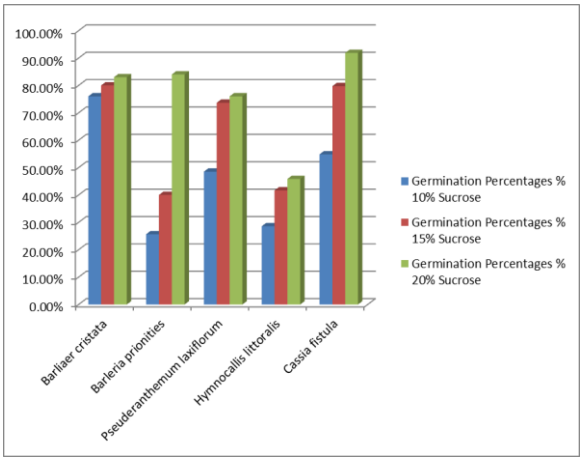
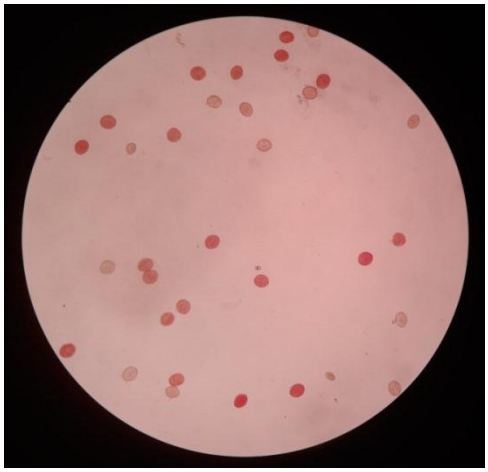
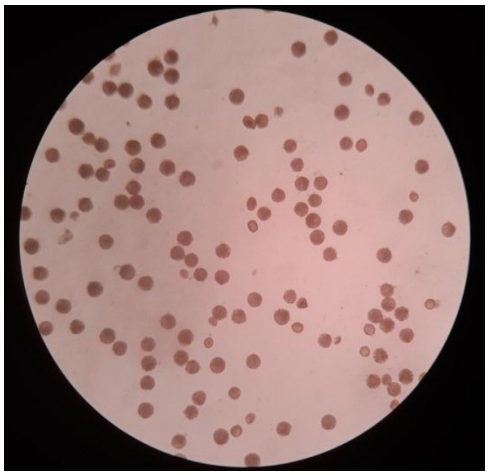


Fig. In Vitro Germination of Pollen using Different concentration of Sucrose solution.

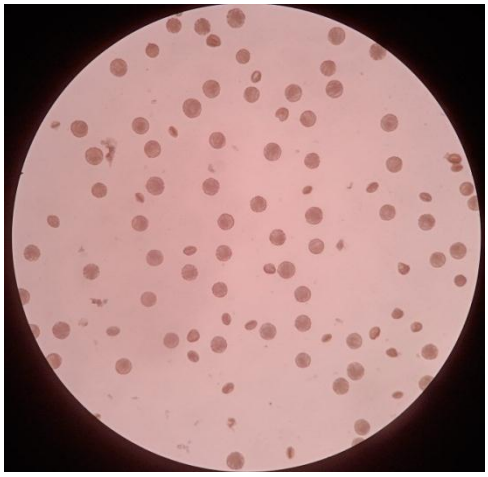


viability pollen slide 1

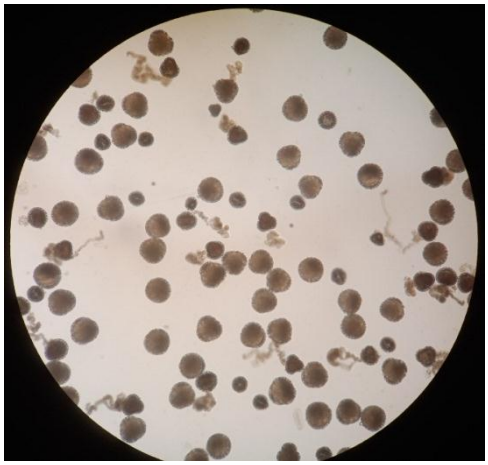


10 % pollen germination slide 1

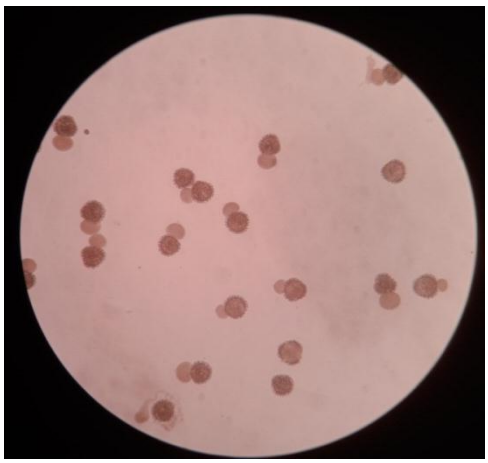
ISSN : 2348-7143
June 2023



15% pollen germination slide

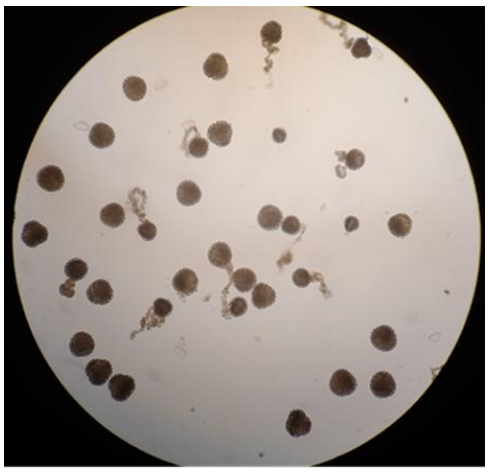


10% pollen germination slide

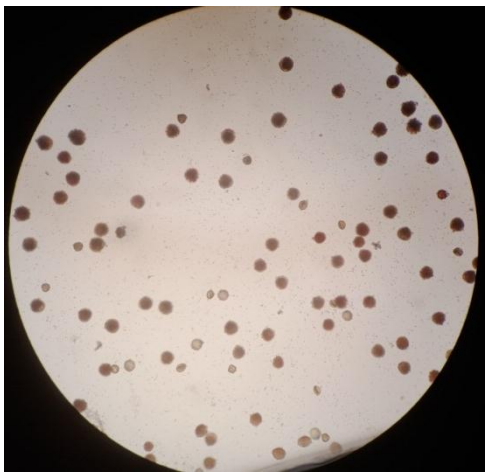


20% pollen germination slide 1

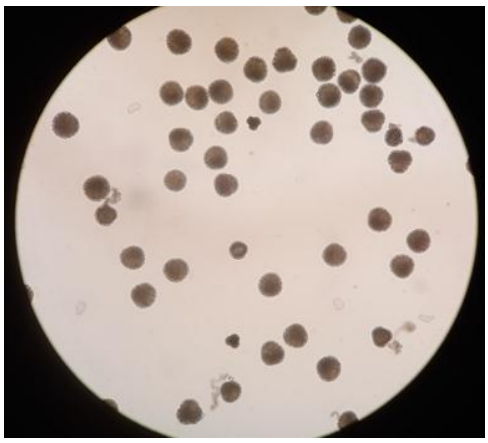
Barlria cristata L.



15% pollen germination slide

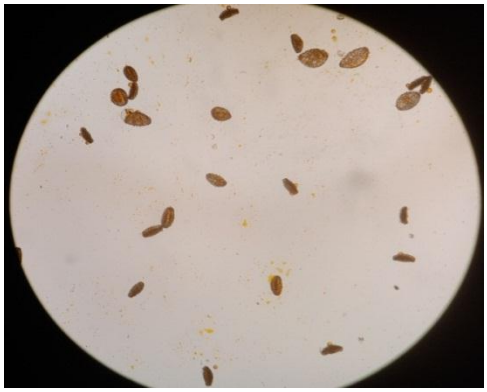


pollen viability slide

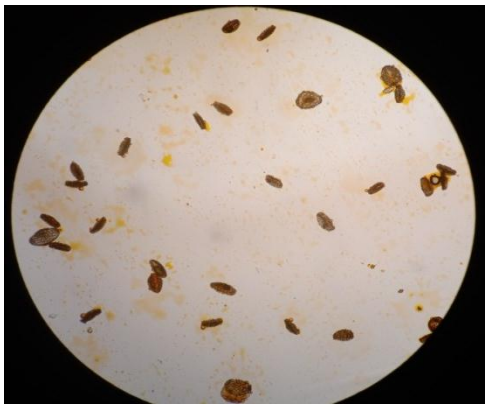


20% pollen germination slide

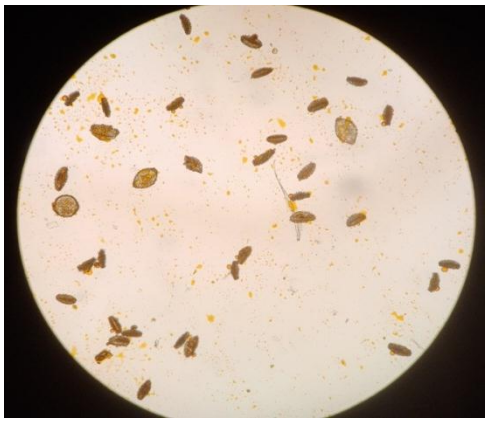
Barleria prionities L.



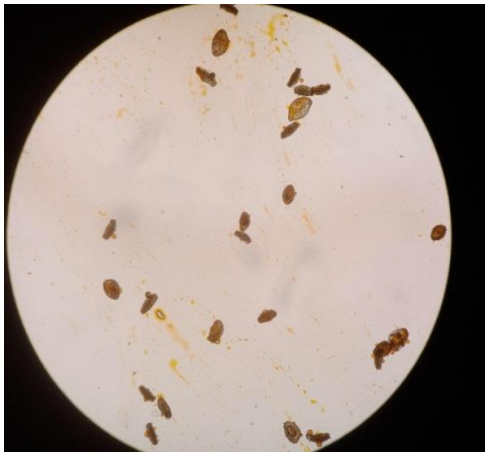
Pollen viability slide



10% pollen germination slide

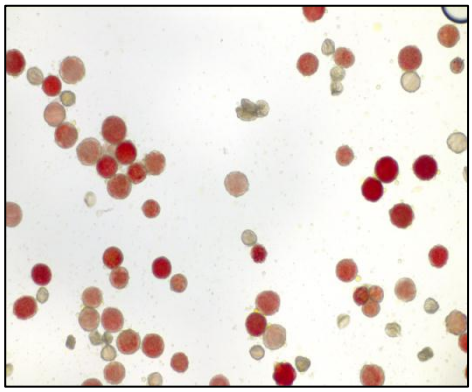


15% pollen germination slide

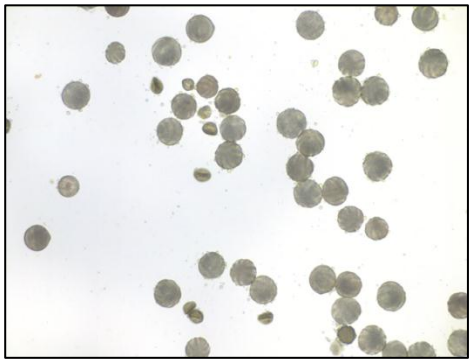


20% pollen germination slide

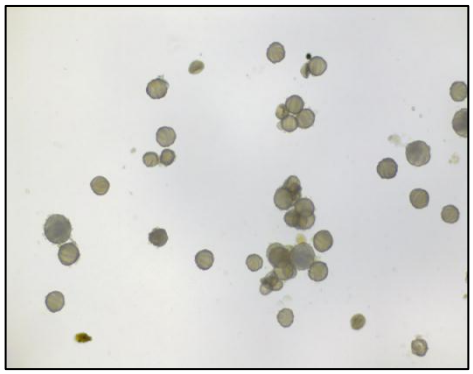
Hymnocallis littoralis



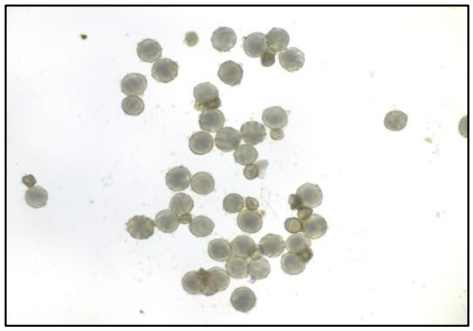
pollen viability slide



10% pollen germination slide

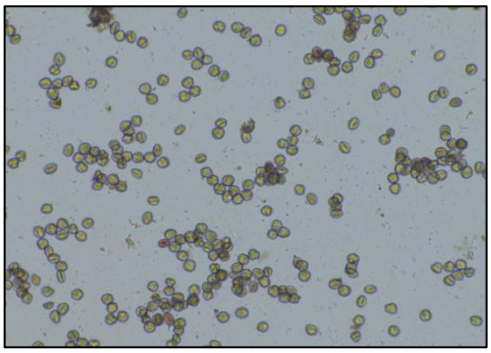


15% pollen germination slide 1

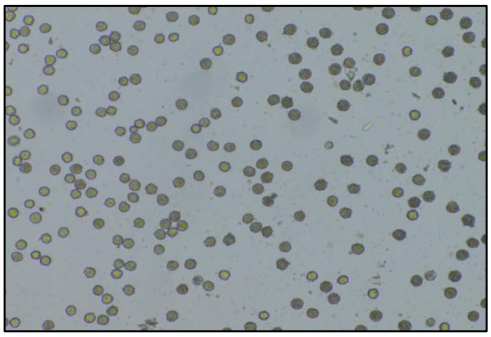


20% pollen germination slide

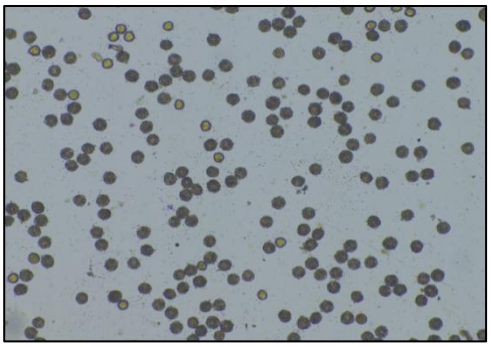
Pseuderanthemum laxiflorum



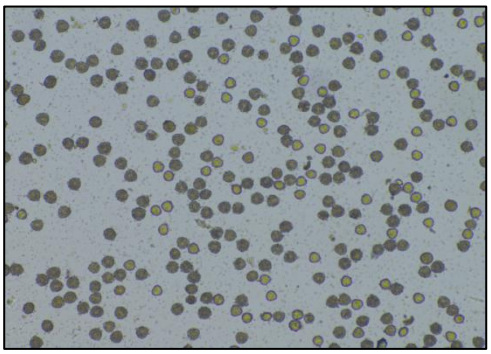
pollen viability slide



10% pollen germination slide



15% pollen germination slide 1



20% pollen germination slide 1

Cassia fistula

Conclusion :

Above experiment, I had concluded that the following points.

1. **Pollen Viability** : The pollen grain is treated with the solution of 1% TTC solution which gives red colour pigmentation due to the reduction of 2,3,5 triphenyl tetra azolium chloride (TTC) converted into tetrazine pigment pollen appears the red colon
2. **Pollen germination** : The pollen grain is treated to the different concentrations of sucrose solution concluding that the sucrose concentration affects the germination percentages. A high concentration of sucrose help to germinate the pollen grain and boric acid also help to increase the rate of germination and there. In this experiment, I had observed the 20% sucrose solution germination percentage is higher than the 10%,15% of sucrose solution
3. **Pistil receptivity** : The pistil is treated with the 10% KHO solution and macerated, boil the pistil in a test tube for 10 minutes and transfer to the clean water and later stain by

using safranin stain observed the stigma surface under the microscope to see some pollen grain are trapped or received on the stigma surface

References :

i. Zehao Huang, Jinmao Zhu, Xijin Mu and Jinxing L.2003.
ii. Pollen Dispersion, Pollen Viability, and Pistil Receptivity in Leymus Chinensis
iii. Aleemullah M, Haigh AM, Holford P. 2000. Anthesis, anther dehiscence, pistil receptivity, and fruit development in the Longum group of Capsicum annuum. Australian Journal of Experimental Agriculture 40: 755±762
iv. Gabriella da Silva Saab, Vidal de Freitas Mansano, Anselmo Nogueir,
v. Isabele Carvalho Maia, Pedro Joaquim Bergamo,and Juliana Villela Paulino.2021. A sophisticated case of division of labour in the trimorphic stamens of the *Cassia fistula*
vi. (Leguminosae) flower
vii. Tomas Rodriguez-Riano · Amots Dafni, A new procedure to asses pollen viability.1999
viii. Chichiricco G, Caiola MG (1982) Germination and viability of the pollen of Crocus sativus L. G Bot Ital 116:167–173
ix. Oberle GD, Watson R (1953) The use of 2,3,5-triphenyl tetrazolium chloride in viability test of fruit pollen. Am Soc Hortic Sci 61:299–303
x. Hatum S, Flowers TJ (1995) The estimation of pollen viability inrice. J Exp Bot 46:151–154
xi. Dafni A, Firmage D. 2000.Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Systematics and Evolution 222: 113±132
xii. Nepi M, Pacini E. 1993.Pollination, pollen viability and pistil receptivity in Cucurbita pepo. Annals of Botany 72: 527±536
xiii. https://powo.science.kew.org/about

***

Organized by

Pravara Medical Trust's,

Arts, Commerce & Science College,

Shevgaon, Dist. Ahmednagar, (M.S.) India

Publisher & Printer

Academic Book Publications

Dyandeep Apartment, Plot No. 2, Chaitanya Nagar,
Opp. Progressive English Medium School, Jalgaon - 425 001.
Ph.: (0257) 2235520, 2232800. **Mob.:** 93092 60560

E-mail : academicbooksjalgaon@gmail.com

Price : ₹ 1000/-