Effects of growth hormones on seed germination and growth of seedlings.

Aim: To observe the effect of IAA, MH, and NAA on seed germination and on growth pattern of seedlings.

Requirements: Growth hormones like, IAA, MH & NAA; Seeds like ground nut (*Arachis hypogea*), castor (*Ricinus communis*) & Pea (*Pisum sativum*).

Theory: NAA, MH & IAA, are chemical factors acting as growth regulating substances. The effect of these substances under in vitro conditions will be in varying degrees depending on the mode of action of these growth regulators.

Procedure: Prepare 100 ppm (= 0.01%) solutions of the growth regulators such as NAA, IAA & MH. The seeds of the above mentioned species are treated with 10 ml of the 100 ppm solution for 24 Hours in separate embryo cups. The control seeds are treated with distilled water. The seeds are removed, washed thoroughly in distilled water and air dried at room temperature. The seeds are germinated in Petri plates with moistened filter papers, each according to its treatment. Observations are made at regular intervals. The filter paper should be kept moistened throughout the experiment. Tabulate the results.

Table . Effect of growth hormones on seed germination in Castor

S. No.	Growth Hormones	Germination percentage						
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
1.	Distilled water							
2.	IAA							
3.	NAA							
4.	MH							
5.								

Effect of removal of leaf primordial and terminal bud

Aim: T observe the effect of removal of leaf primordial and apical bud both on morphology and anatomy of the plant.

Requirements: Apically dominant plants, garden, blade, microscope, slides and cover glass.

Theory: Certain plants like *Ficus*, *Carica* and *Ricinus* are apically dominant plant and hence whenever the apical buds are removed they are capable of developing new bud and grow further. Again these plants are capable of developing new young leaves, whenever the young leaf primordium is removed.

Procedure: Proper plant with apical dominance is grown / selected. The plant should be free of disease. The apical bud of the plant is removed with the help of a razor blade. The changes, both in morphology and anatomy are noted and compared with the control. In the same way leaf primordia are removed and observations are made regularly.

Results:

No. Days	Growth pattern in apical bud removed plant		Growth pattern in leaf primordial removed plant	
	Control	Expt. plant	Control plant	Expt. plant
2.				
4.				
6.				
10.				
15.				
20.				

Results and Inference / Conclusion:

Effect of UV irradiation on seed germination and growth pattern of seedlings.

Aim: To observe the effect of UV irradiation on seed germination and growth pattern of seedlings.

Theory: UV is a physical factor which affects morphogenetic development of plant by acting as mutagen.

Requirements: Seeds like Castor (*Ricinus communis*), UV light source, Pots / Garden.

Procedure: Seeds are taken and exposed to UV at different time intervals (one set for 60 minutes and another for 90 minutes). One is kept as control. Untreated and treated seeds are growth in the field or pots and morphological and anatomical observations are made at regular intervals.

Results:

No. of days	Control	Treated (60 minutes)	Treated (90 minutes)
2.			
4.			
6.			
8.			
10.			
15.			

Results and Inference / Conclusion:

Effect of IAA and sugar on the apical bud:

Aim: To study the variation pattern in the morphology & morphology and anatomy due to the application of IAA and sucrose.

Theory: Growth substances like IAA and sucrose applied to the apical bud move downwards and cause differentiation of vascular elements below. The young leaf primordium of *Ricinus communis* also produces the growth hormone like IAA which helps the basipetal differentiation of vascular elements. The exogenous application of IAA and sucrose may stimulate rapid differentiation of vascular elements.

Procedure: *Ricinus communis* seeds are sown in the pots. After 20 days, the plants may grow to 15 cm height. 1 ppm IAA and 1 % sucrose are applied on the apical bud region. Morphological and anatomical observations are made at regular intervals.

Results:

No. of days	Control	Apical bud treated with IAA	Apical bud treated with sucrose
2.			
4.			
6.			
8.			
10.			

Results and Inference / Conclusion:

Effects of bisection on shoot apices:

Aim: To study the behaviour of shoot apices after bisection.

Theory: Since the apices are meristematic they are expected to either join and develop or develop separately after bisection.

Procedure: The shoot apex of the experimental plant is split into tow and is allowed to grow after it is tied with the thread. A control is maintained. Length of the splitting is 2 cm. Observations are made at regular intervals.

No. of days	Behaviour in control	Behaviour in bisected apex

Results and Inference / Conclusion:

Wound healing response in dicots and monocots

Aim: To study the wound healing response in dicot and monocot plants.

Theory: Wound healing is a process of dedifferentiation. New tissue is formed due to wound healing hormones.

Procedure: Make 'V' shaped wound on the stem surface of matured plants of dicot and monocot plants. Observe the morphological and anatomical changes at regular intervals.

Observation:

Plant type / species	Initial results	Final results after .....days
Dicot-Shrub		
Dicot-Tree		
Monocot		

Effect of sucrose and pH on pollen germination:

Aim: To study the effect of concentration of sucrose and pH on pollen germination.

Theory: After pollination, the pollen-grains germinate on the stigma where suitable nutrients and pH are available due to the secretion of several kinds of chemicals by the stigma. We can study the important factors such as the nutrient sucrose (chemical factor) and pH (physical factor) on pollen germination in artificial culture.

Procedure:

Pollen germination medium:

Sucrose	-	10%
Boric Acid	-	100mg / l
Calcium Nitrate	-	300mg / l

Boric Acid and Calcium Nitrate are added at required amount and mixed in 10% sucrose solution. Take three sets each of 50 ml or 100 ml of this basal medium and set the pH at 6.2, 7, 7.8. In another set, prepare the medium with at least three different concentrations of sucrose.

Take few drops of medium from each set in a cavity slide. Mix the pollen grains of Balsam in the medium. Put the cover glass on the cavity slide. Leave it for germination. Observe the percentage of pollen germination and also the rate of pollen germination by measuring the length of pollen tube. Compare the results with different pH and different concentrations of sucrose.

Table: Effect of pH and sucrose on pollen germination

S. No.	Duration	pH 5.8	pH 7	pH 7.8	Sucrose ….%	Sucrose ….%	Sucrose …%
1.	20 minutes						
2.	40 minutes						
3.	60 minutes						
4.	80 minutes						
5.	120 minutes						

Determination of pollen viability:

Aim: To determine the percentage of pollen viability.

Theory: All the Pollen grains – Microspores produced in the microsporangium are not viable due to several factors. For various purposes like tissue culture, plant breeding, reproductive biology etc, it is necessary to know the percentage of viable pollen grains. There are several methods and the simple method is by acetocarmine staining method. When the pollen grains are stained with acetocarmine stain, the viable pollen grains are well stained and the non-viable pollen grains are not stained well. Based on this principle the percentage of viable pollen grains is calculated.

Procedure: Collect pollen grains from mature undehisced anthers of different species like Rose, Papaya, Banana, Hibiscus, etc. on different micro-slides. Put one or two drops of acetocarmine stain. Put a covergalss and leave it for at least five minutes. The stained and unstained pollen grains per microscopic field are counted and the percentage of stained pollen grains is calculated.

Table: Pollen viability test-Observation

S.No.	Percentage of viable pollen grains			
	Species 1	Species 2	Species 3	Species 4 ... .
1.				
2.				
3.				
4.				

Determination of cell density in explant:

Aim: To determine the cell density in explant.

Theory/Principle: In *in vitro* tissue culture, particularly in cell suspension culture, number of cells in the explant plays an important role in the result. So the understanding of cell density of the explant is necessary. It can be done by macerating the tissues of the explant by particular chemical at particular concentration.

Procedure: Select the explant stem, root or leaf with known size/volume. Put the explant(s) separately in each test tube with .....% of ..... Incubate them in refrigerator for 24-48 Hrs. After incubation, shake the macerated tissue. Measure the volume of the solution with cell suspension. If necessary, dilution can be made. Take out know volume (microliter) of the suspension by using micropipette and drop it on a clean micro-slide. Put a cover-glass and observe under microscope. Count the number of cells in the microscopic area. (Take at least 5-10 readings). Measure the area of cover-glass by using the formula πr^2 for circular cover-glass and length x width for rectangle cover-glass. Calculate the cell density as follows:

Calculation of cell density:

Area (leaf) or volume (stem-root) of the explant = ... mm^2 / .....mm^3 (V)

Volume of the cell suspension = ... ml = ... µl (A)

Volume taken for cell count = ... microliter (B)

Area of the Microscopic field = ... μm^2 (C)

Number of cells per microscopic field area = ... μm^2 (D)

Area of the cover glass (in mm^2) = ... mm^2

Area of the cover glass (in μm^2) = 1000000 x..... mm^2(E)

Total number of cells in the total area of cover glass (F):

$$F = \frac{E}{C} \times D$$

*Number of cells in the taken volume of cell suspension is equivalent to the number of cells per cover glass area (F).

*Number of cells in the entire explant (G) = Number of cells in the entire volume of the suspension (A).

Total number of cells in the taken volume /area of the explant =

$$\frac{\text{Total volume of cell suspension. (µl). (A)}}{\text{Volume taken for cell counting (µl) (B)}} \times \text{Total number of cells in the total area of cover glass (F)}$$

Cell density (Number of cells per mm^2 or mm^3) =

$$\frac{\text{Total Number of cells in the taken volume / area of explant}}{\text{Total volume } (mm^2) \text{ or total area } (mm^3) \text{ of the explant}}$$

= ..... per mm^2 (or) mm^3