GERMINATING SEEDS ON GELATIN

DEVELOPERS:

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TOPIC AREA: Germinating Seeds

The following experiments with plants can be used progressively through the plant units in the K-6 science curriculum. They are all based upon growing seeds in a sterilized baby food or Snapple® jar filled with 3/4 inch of unflavored gelatin to which a few drops of liquid house plant fertilizer has been added for nutrients. This set-up provides an amazing environment for seed growth because: 1. It doesn't need to be watered. 2. All parts of the plant can be very easily observed. 3. Many conditions for growth can be tested simply by wrapping the jar in foil or plastic wrap, or turning the jar upside down.

TABLE OF CONTENTS:

PART 1: BASIC GELATIN JAR PREPARATION

GOAL:
Students will germinate seeds free of mold on a simple gelatin culture media using a modified sterile technique and observe the growth of plants under varying conditions.

OBJECTIVES:
1. Germinate a seed on gelatin so all parts of it can easily be seen as it grows.
2. Use this controlled environment to test the conditions necessary for a seed to germinate.
3. Perform a controlled experiment changing only one variable.
4. Have a chance to practice a simplified sterile technique in performing a scientific experiment.
5. Gain an awareness of the responsibility one must take in clean-up and disposal of contaminated materials when doing research.

BACKGROUND:
In industry, plant tissues are grown on a culture medium, usually agar, with all the necessary nutrients added. This is one way to greatly increase the number of plants with exactly the same genetic makeup in a very short time. In using this technique called tissue culture, the scientist must take every precaution to use absolutely sterile procedures, since only one mold spore or bacterium is all it takes to destroy a culture and with it perhaps many months of work.

In a science classroom where we are germinating seeds to watch them grow, we do not need to be quite so extreme in our sterile techniques. This method is a science room adaptation of industrial sterile tissue culture. The adaptations are noted as follows.

In industry, agar is used as a source of food and water as well as a solid substrate to support the seed or plant, instead of soil, but we will be using unflavored gelatin. In industry, agar can be made with many different combinations of nutrients added, but we will be using household liquid fertilizer added to the gelatin. In industry, the glassware, all apparatus and the agar must be sterilized under pressure in an autoclave for 15 minutes at 121°C. We will sterilize baby food, Snapple® or other jars in a dishwasher on the sanitary cycle and dry with heat. We will make unflavored gelatin (which is purchased at the grocery store instead of from a biological supply company) in the microwave but it can also be made in a pot on a stove or in a pot on a hot plate. Bean seeds need to have the seed coat softened by soaking in either sterilized water or tap water to stretch the coat so the disinfectant will reach all the crevices. In industry the seeds are then sterilized using a bleach solution and a rinse of autoclaved water, but we will sterilize the seeds in 3% hydrogen peroxide . Typically in industry, before each use, the forceps and stirring apparatus are dipped in 91% alcohol and passed through an open flame to burn off the excess alcohol. We will just soak the forceps or spoon in 91% alcohol. Industry would use a disinfected work area under a ventilated hood to provide germ-free air. We can wash our hands and the tables and cover containers with plastic wrap or replace a lid quickly to prevent microbial contamination. Since we are not protecting months of work from contamination, our methods can be more user-friendly.

VOCABULARY:

Agar -a polysaccharide made from algae, which is dissolved in hot water and then forms a gel when it cools. It is used as a support for culturing organisms such as bacteria and plants.

Germination -the beginning of growth in a seed.

Medium -a gelatinous substance containing the nutrients for the culture of microorganisms and the germination of seeds.

Microorganism -a general term for any microscopic organism, including bacteria and fungi such as mold.

Sterile Technique -procedures used to prepare cultures of plants while excluding other unwanted organisms such as mold or bacteria.

Toxin -a poisonous substance given off by certain organisms.

MATERIALS:

unflavored gelatin (such as Knox Unflavored Gelatine®)
3% hydrogen peroxide (usual strength right from the bottle)
91% isopropyl alcohol
liquid house plant food (such as Miracle-Gro®)
disinfecting hand soap
household disinfectant
tap water
seeds (such as radish, mung bean, lima bean, green bean, alfalfa, and corn)
clear, tall jars with lids (baby food, Snapple® or other taller jars)
2 or 4 cup measuring cup
forceps or tweezers
spoon, glass rod, or other stirring instrument
lid opener (bottle opener)
transparent tape
clear plastic wrap
paper towels

OPTIONAL MATERIALS:

plastic bags, 12" x 12"
rubber bands
aluminum foil
colored plastic wrap - red, blue, green, yellow, and clear (made by Reynolds)

TEACHER PROCEDURE: (Try "Recommendations" first!)

Sterilize Jars:

1. Sterilize jars and lids open end down in dishwasher on sanitary cycle and use heated cycle to dry. Wait until cool before opening door.
2. Transfer lids only to a cookie sheet face down and cover with a sheet of aluminum foil. Place in oven at 350^{o}F for 20 minutes to complete drying and sterilizing. Leave jars in dishwasher.
3. After they cool, quickly put lids on jars as they are removed from the rack to minimize the possibility of microbial contamination (i.e. mold). If you use baby food jars make sure jars and lids are cool or the gasket will form too tight of a seal.
4. Also if you use baby food jars, use a lid opener to pry out one side of the lid so it does not make an air tight seal.
5. Put tape across the middle of the jar for label. Fold one end of the tape over on itself ~1/2 inch to make a tab for easy removal.

Prepare Gelatin:

1. Put 3/4 cup cold tap water in a 2 cup glass measuring cup.
2. Sprinkle one 1/4 oz envelope of unflavored gelatin over the water.
3. Cover loosely with plastic wrap to prevent microbial contamination.
4. Let sit for about 2 minutes.
5. Microwave on high until it has fully boiled for about 1 1/2 minutes, watching constantly because it boils over very easily. Times will vary with the power of the microwave, but try about 3 1/2 minutes.
6. Wait until it cools so condensation doesn't form in the jars when you pour. You can speed up cooling by putting the measuring cup in the refrigerator for about 30 minutes with the cover on it.
7. Use a spoon or glass stirring rod to stir the gelatin before pouring. Wipe a clean spoon or other stirring instrument with a paper towel moistened with alcohol. Wiping helps remove microorganisms. Then give a final rinse, pouring the alcohol over the spoon and air drying. You can also soak the stirrer in a jar of alcohol. Or you can place a cleaned glass stirring rod or smooth heat resistant plastic spoon in the measuring cup when you microwave it.

   (Four envelopes of gelatin can be made at once using 3 cups of water in a 4 cup measuring cup. Time will vary but try heating on high for 7 minutes, again letting it boil for about 1 1/2 minutes.

   If a microwave is not available, gelatin can also be made in a pot over a stove or hot plate, although this provides more chances for contamination. First sterilize the pot and a metal spoon by boiling water in the pot. Then prepare gelatin in the same proportions as in the microwave. Gelatin has more of a tendency to erupt as it cooks on the stove so stir it often. **Safety glasses may need to be worn since the liquid tends to erupt as it boils.** Let it boil for about 2 minutes on medium heat to kill any microorganisms. **Caution: It will burn on high heat.** Cover with plastic wrap while it cools.)

8. Carefully lift up lids from sterile jars just long enough to pour in gelatin to a level of about 3/4 inch. (Three fourths cup of gelatin will make about 6 tall baby food jars or 4 Snapple® jars.)
9. Quickly replace lid.

10. Put 3 drops of liquid house plant fertilizer in each baby food jar or 5 drops in Snapple® jars. If you are doing an experiment without fertilizer, skip this step.
11. Replace lid, being careful not to contaminate and gently swirl to mix.
12. Let gelatin set up at room temperature until firm enough to turn jar upside down without it falling out. Time will vary with temperature of room and amount of water in mixture but it will set up eventually. Using 1 cup of water may take several days to set up in the summer. Using 1/2 cup water may take 6 hours to set up firm, but it will not provide as much water for the plant. Jars with larger diameters and more gelatin also take longer to set up. Many other size jars will work but keep in mind they will take more gelatin. **Do not place seeds on gelatin until it has gelled since they will sink below the surface and get stuck.**

    You may want to make the jars up 2 or 3 days ahead of time in warm weather. The reason is to allow plenty of time for the gelatin to gel and to make sure the gelatin was not contaminated when it was prepared, especially if you are doing an experiment to test for contamination.

Prepare Seeds:
To stretch the seed coat before disinfecting the seeds, soak them in tap water in a clean, covered baby food jar, according to the following schedule:

Lima Beans-	7 hours or until seed coat is smooth
Mung Beans-	2 hours or until seed coat is smooth
Green Beans-	2 hours or until seed coat is smooth
Corn-	does not need to be soaked
Radishes-	do not need to be soaked
Alfalfa Sprouts-	do not need to be soaked

If these soaking times are difficult to meet, an alternative method for lima beans, mung beans and green beans will also work. <u>Instead of soaking in water and then disinfecting, put lima beans, mung beans, or green beans in 3% hydrogen peroxide for 12 to 16 hours or overnight.</u> Then the students can use the seeds right from this jar without additional treatment.
With these soaking times, most seeds will germinate in less than 24 hours after being placed on gelatin. But there is usually a certain percentage, about 20%, that do not germinate.

STUDENT PROCEDURE:

Preparation:

(For best results)

1. Students should wash their hands with a disinfecting soap.
2. Disinfect desks with a household disinfectant or 91% isopropyl alcohol before working with the seeds.
3. Tie back long hair.
4. Close windows and turn off any fans, if possible, to prevent movement of microorganisms.

Disinfect Seeds:

1. Disinfect seeds in a **fresh** 3% solution of hydrogen peroxide in a clean baby food jar with a lid according to the following time chart:

Lima Beans-	15 minutes
Mung Beans-	15 minutes
Green Beans-	15 minutes
Corn-	16 hours or overnight
Radishes-	15 minutes
Alfalfa Sprouts-	15 minutes

2. Swirl jar frequently so all of the seed surfaces get treated.

Transfer Seeds:

1. To transfer seeds to gelatin jars, use clean forceps that have been soaking in a baby food jar of 91% isopropyl alcohol and will be re-dipped in that jar after each seed is touched. Gently tap the forceps against the edge of the jar to remove all excess alcohol before picking up the next seed. Alcohol contaminating the gelatin will dissolve it.
2. Lift lids and take seeds out of the jar of hydrogen peroxide one at a time and allow any excess liquid to drip back into the jar before dropping seed onto gelatin surface.
   Note: A few radish seeds may pop and shoot out of the jar as the seed coat splits open after soaking in fresh hydrogen peroxide about 12 minutes. You may want to have students use **safety goggles,** caution them not to get their eyes too close to the jar, or leave seeds in the disinfectant for 20 minutes before they get them.
3. Open and close gelatin jar lid briefly after each time a seed is dropped in to minimize the possibility of microbial contamination. Holding the lid over the jar at an angle like a roof will help. Do not breathe on the jar. Avoid touching the gelatin with the forceps.

4. When all seeds are placed on the gelatin surface, check jar lid to make sure it is on securely but that there is a crack for air exchange. Since baby food jars have a rubber gasket, the edge needs to be pried up just a little so the seal is not complete. Other jars without the gasket just need to be screwed on half way for air exchange and for this reason may be better to work with than baby food jars.
5. Place a piece of transparent tape over the lid and down the sides about one inch to keep the lid on while still allowing gas exchange.
6. Seeds that do not germinate tend to contaminate the gelatin. If you discover this soon enough, you can try to remove them quickly with forceps sterilized in alcohol.

TEACHER PROCEDURE:

Disposal of Contaminated Material:

If gelatin gets microbial growth, precautions must be taken to dispose of the contaminated material in a responsible way, just as must be done in industry.

1. Pour 1 to 2 teaspoons of a diluted bleach solution (made with 1/4 cup bleach and 1 cup of water) into each contaminated jar and replace the lid. You can also use 1 or 2 teaspoons of 91% isopropyl alcohol instead of bleach.
2. Gently swirl jar and let sit overnight.
3. Pour the contents of the jar through a strainer in the sink. A coffee can with holes in the bottom will work.
4. Wrap the solid, now disinfected, material in a paper towel or plastic bag, and place it in the trash, since it is harmless.
5. Wash hands after completion of the experiment.
6. Jars and lids can now be re-sterilized in the dishwasher on the sanitary cycle and used again. You may want to put them through twice or soak the empty jars in a 20% bleach solution overnight before washing to be sure all the microbial contamination is killed.

RECOMMENDATION: First try this quick setup!

1. Clean 6 or 8 baby food or Snapple® jars. For this quick setup you can wash jars and lids by hand with soap and water. Then swirl 91% alcohol around in the jar and wipe jar and lid with a clean, alcohol moistened paper towel. Let jar air dry upside down on the lid. You may get some microbial contamination, but you will also see how quickly you will get results with the seeds.

2. Mix 2 packages of gelatin with 1 1/2 cups of water in a 2 cup measuring cup and microwave it for 30 seconds after it starts to boil.
3. Let cool slightly and swirl the measuring cup to finish mixing.
4. Pour gelatin into 6 or 8 jars to a level of about 3/4 inch.
5. Add 3 drops of liquid fertilizer to each jar and swirl to mix it.
6. The gelatin will set up at room temperature in about 6 hours or overnight.
7. Disinfect radish seeds in 3% hydrogen peroxide for 15 minutes and place on the gelatin with forceps rinsed with alcohol.
8. Remember to open the jars only briefly for each seed as described earlier.

Seeds will germinate in less than 24 hours and stems will grow 1 inch in 2 or 3 days at a warm room temperature.
After using this simplified setup to get a feel for the process, the detailed directions for the sterile technique described earlier will be easier to follow. They can be used to perform many different kinds of experiments on seeds and plant growth. Some are described below, but the possibilities are endless.

PART II: EXPERIMENTS

EXPERIMENT 1: OBSERVING GERMINATION
What Do Seeds Look Like As They Germinate?

1. Place 6 or more seeds on the gelatin surface in a jar using the sterile transfer technique.
2. Observe the seeds every day to see the stages of growth, step by step.
3. Draw pictures of what appears first, second, etc., until students have a complete set of drawings, showing all the stages in the germination process. Have them identify the following parts: root, root hairs (especially in radishes), stem, seed coat, seed leaves or cotyledons (were originally inside the seed), and true leaves (first new leaves formed). Then give the students a set of drawings showing the plant at different stages and have them put the drawings in the correct sequence.

Questions:

1. What is the order of the appearance of the plant parts?
   (Roots, stem, leaves.)
2. What do the root hairs in radishes look like?
   (White fuzz.)

EXPERIMENT 2: HAIRY ROOTS

How Fast Do Roots Grow?

1. Germinate 6 or more radish seeds to observe the location of root hairs (they look like white fuzz) and how fast the roots grow.
2. Make a mark on the jar each day to mark the tip of the root so a comparison can be made the next day. Also point out that new growth in the root occurs at the tip, so try to observe that the older part of the root is in the same location it was in the day before. (Radishes will germinate in less than 24 hours in this setup and sometimes roots will grow 1/2 inch in 6 hours during the school day, so results can be seen very quickly. Root hairs will appear the second or third day.)
3. Older students can measure the daily growth of the root and graph the results. Y= length of root and X= number of days. If there are several plants, they can take an average, or pick one plant.

Questions:

1. Where does growth occur in the root?
   (At the tip.)
2. What is the function of root hairs?
   (Taking in water and minerals.)
3. Where do these plant get water and minerals?
   (From the gelatin.)
4. What is the average daily root growth?
   (Answers will vary.

EXPERIMENT 3: CONDITIONS FOR GROWTH

What Conditions Are Necessary For the Germination and Growth of Seeds?

Test the conditions for germination of seeds using a control and a single variable or factor to be tested. Have students make predictions before they perform the experiment. Let the students try to design a way to test their hypothesis. Have them make sure, in the design of their experiment, that there is only one variable so if they observe a difference, they know it is because of the tested variable and not because they set up the 2 jars differently. Here are some suggestions.

A. Light vs. Dark

1. Set up 2 jars with 6 seeds each on the gelatin.
2. Wrap 1 jar in aluminum foil but do not cover the lid with foil so air can be exchanged.
3. Do not wrap the other jar.
4. Place them side by side where they will have a warm temperature and indirect sunlight.
5. Check them each day to compare how and when they germinate. Make a chart to write in the daily observations or draw a picture. Radishes (and most of the other seeds listed) germinate in less than 24 hours so they will get results very quickly.

Once they have determined whether or not light is necessary for germination, they can continue to make observations for several days to see if the new plant needs light to continue to grow and make food. They should make daily observations of leaf color as well as stem length in each jar every day. Then record the results and take an average. They can make a graph of the data, Y= height of stem and X= number of days.

Questions:

1. Do seeds need light to germinate?
   (No.)
2. How can they germinate in the dark?
   (They use the food stored in the seed for energy to germinate. Keep in mind, it is dark where seeds are planted in the ground.)
3. Do seeds in the light and dark germinate at the same time?
   (Yes.)
4. Which stems grew the tallest?
   (The ones in the dark.)
5. Why do the plants in the dark grow faster than the ones in the light?
   (Plant cells that are in the dark are stimulated to grow fast to find light in a hurry, so they tend to grow taller faster and look lanky.)
6. Which plant had greener leaves?
   (The one in the light.)
7. Which plant can continue to grow well under its present conditions and why?

(The one in the light because it can make its own food from light.)

8. Do plants need light to grow healthy?
(Yes, without it, they cannot make food.)

B. Temperature

1. Set up 3 to 5 jars with 6 seeds in each.

2. Wrap each jar in aluminum foil (this is part of the identical treatment in a single variable experiment.)

3. Since temperature will be the only variable and all other conditions must be identical, place each jar in a location with a different temperature. Try to find places with temperature differences, such as 40°F (a refrigerator), 50°F (a refrigerator with the temperature turned up warmer), 60°F, 70°F, and 80°F, depending on what is available.

4. Record the temperature at each location.

5. Record observations by noting day or hour of germination.

6. Students can make a graph of their results, Y=temperature and X=number of days or hours to germinate. (The germination time difference will be in hours, near 24, for most temperatures except cold ones such as the refrigerator which takes about seven days.

The effects of temperature on germination and on plant growth after germination can also be tested in the presence of light. Place the plants in locations with different temperatures that also have light. Omit the aluminum foil. Take the temperature at each location. Record the day or hour of germination of each plant. Measure the height of the stem each day, since the plants have light. Make graphs of the average stem growth at various temperatures, Y= height of stem and X= number of days.

Questions:

1. As the temperature gets colder, what happens to the length of time it takes the seeds to germinate?
(It takes longer.)

2. What effect does temperature have on stem growth of the plant?
(The growth of the stem slows down as the temperature gets colder.)

C. Fresh Air vs. Stale Air

1. Set up 2 small jars with 6 seeds each on the gelatin. Results may be better with mung beans which are larger than radishes.
2. Seal the lid tight on one jar.
3. Adjust the lid on the other jar, either by prying a gap in the lid as in a baby food jar or screwing a loose lid down just slightly, so air can be exchanged.
4. Put tape across the lid to keep it from coming off when the jar is picked up.
5. Set both jars side by side at room temperature in indirect light.
6. Make daily measurements and observations of both plants and record them on a chart.

After a while in the jar that is sealed, one of the gases will be used up and there will be an excess of another. This can be tested at the end of the experiment by the teacher using a flame test. Light a candle, then quickly insert the candle into the jar as you open the jar and hold the jar upside down or sideways to prevent the gas from mixing with fresh air. If oxygen is the main gas present, the flame should glow brighter. If carbon dioxide is the main gas present, the candle should go out.
Caution: Use safety goggles when performing this test.

Questions:

1. What do plants use carbon dioxide for?
   (Plants use a lot of carbon dioxide for making food during photosynthesis.)
2. What do plants use oxygen for?
   (Plants use a small amount of oxygen to burn the food they make which supplies the energy for growth.)
3. When do plants give off oxygen?
   (During photosynthesis.)
4. Do plants give off carbon dioxide?
   (Yes, when they burn food to get energy, just like animals do.)
5. Which gas was there more of in the jar at the end of the experiment and why?
   (Oxygen, because plants make more food than they burn. The food that they do not burn, they store. They give off more oxygen from photosynthesis than they do carbon dioxide from respiration.)

D. Amount of Fertilizer

1. Liquid house plant fertilizer is added to the gelatin when it is made up. To test for the need of fertilizer, make up some jars without adding any fertilizer.
2. Then make up some jars with different amounts of fertilizer, such as 1 drop, 2 drops, 3 drops, etc., or a lot more.
3. Add the same number and kind of seeds, disinfected the same way.
4. Place the jars in the same location so they have the same growing conditions.
5. Record the daily length of the stem and observe the color of the leaves. The average stem length can be graphed for each number of drops of fertilizer, Y= height of stem and X= number of days.

Questions:

1. What is the reason for putting the same number of seeds in each jar?
   (So each jar has the same number of plants using the fertilizer.)
2. Is the volume of gelatin important to consider in this experiment? Why?
   (Yes, because different volumes of gelatin provide different amounts of nutrients and water.)
3. How much fertilizer is the optimum amount to add to the gelatin?
   (Answers will vary.)
4. What nutrients are in the liquid fertilizer?
   (See label on fertilizer container.)
5. Why is fertilizer added to the gelatin?
   (To provide nutrients for the plant.)

E. Crowded vs. Roomy

1. Take 2 jars with gelatin.
2. In 1 jar give the seeds plenty of room to grow. If seeds are large, just use 1 seed.
3. In the other jar, place many seeds.
4. Have the students observe the size and shape of the plants as well as how fast the level of the gelatin goes down. This can be difficult to observe, but look at the gelatin from different angles.
5. You can also try different sized jars as the variable and use the same number of seeds.

Questions:

1. Does the level of the gelatin stay the same? Why?
   (No, the plants use the water and nutrients in the gelatin for growing.)
2. What happens to the plants when they are crowded?
   (The roots grow close to each other and they compete for space and nutrients. The stems and leaves compete for light.)
3. What happens when the plants grow in larger jars?
   (They have more room and their shapes are not affected by the closeness of other plants. They also grow larger because there is more food and space.)

EXPERIMENT 4: GROWTH AND CONSERVATION OF MATTER
Where Does the Plant Get the Materials to Make It Grow so Big?

1. Weigh the jar with seeds on the day it is set up.
2. Then weigh it each day as it grows to see if there is any change in mass of the system.
3. After the experiment is finished, weigh a seed and the grown plant with the gelatin removed, Compare the mass.

Questions:

1. Is there any change in the mass of the closed jar from day to day?
   (No.)
2. Does the plant grow in size?
   (Yes.)
3. Where does the plant get the materials to increase its size?
   (It used the materials in the gelatin and the air to make a new product, the plant.)
4. What happens to the volume of the gelatin?
   (It decreases.)
5. Does the difference in the mass of the seed and the plant equal the mass of the gelatin that is used up?
   (Yes, approximately, since the air used has very little mass. According to the Law of Conservation of Matter, matter is neither created nor destroyed during a chemical change.)

EXPERIMENT 5: UPSIDE DOWN PLANTS

What Effect Does Gravity Have on Germination of a Seed?

1. Turn a jar upside down as soon as the seeds stick to the gelatin surface to see if the roots grow up towards the gelatin or down with the force of gravity.
2. Also observe which way the stem grows.

Questions:

1. Which way do the roots grow?
   (They grow down toward the pull of gravity.)
2. Which way does the stem grow?
   (Up.)
3. Can the plant can grow for very long this way?
   (No.)
4. What will make it stop growing?
   (It will not be able to get water or nutrients.)
5. Is the stem able to penetrate the gelatin to grow into it?
   (Sometimes, but it can't grow as tall as it normally does.)
6. How can you be sure the results were due to gravity and not light?
   (You can't without doing a similar experiment to test the reaction to being upside down in the dark.)
   Since light and gravity are both variables in this experiment, you may want to do the experiment a second way and try wrapping both jars in foil to test the effect of gravity in the dark.

EXPERIMENT 6: CROOKED PLANTS

What Effect Does Changing the Position of the Plant Several Times Have on the Growth of the Plant?

1. After a stem (radish works well) has grown about 3/4 inch, turn the jar on its side to see which direction the stem grows. Make sure the gelatin is from a firm batch (3/4 cup of water or less per envelope.)
2. Make a mark on the tape label on the side of the jar to indicate the side that is down so it can be returned to the same position if it is moved.
3. The next day put the jar right side up and keep alternating these positions each day.
4. Draw the plant each day.

Questions:

1. How does the stem grow each time the position is changed?
   (Up.)
2. What makes the stem change the direction it is growing each time its position is changed?
   (Either gravity or light.)
3. What test can you do to determine if the reaction is due to gravity or light?
   (Perform the same experiment with the jars wrapped in aluminum foil to eliminate light as a variable.)

EXPERIMENT 7: COLORED LIGHTS

What Color of Light Is Best for Growing Plants?

1. Set up five tall jars with seeds and wrap each jar twice around in a different color of plastic wrap (red, green, blue, yellow, and clear).
2. Place them in the same location and observe for about 10 days.
3. Determine which of these colors of light plants need for photosynthesis by observing the height and color of the plants grown in the different colors of light.

Questions:

1. Which color of plastic wrap represents the control?
   (Clear.)
2. Plants grown under which color of light are the greenest?
   (Results will vary depending on the type of plant and it's most active type of chlorophyll. The color of plastic wrap is the color of light the plant receives. All other colors are filtered out by the plastic.)

EXPERIMENT 8: THE POINT OF LIGHT

How Does a Plant Respond to Light from Only One Direction?

1. Wrap a jar with radish seeds in aluminum foil.
2. Cut a circle about 3/4" in the foil on one side of the jar and draw this circle on the jar with a marker to mark the location of the hole.
3. Face this side of the jar towards light to observe the reaction of a plant to light coming from only one direction instead of from all around.
4. Remove the foil for observations but replace it making sure that the hole in the foil is lined up with the mark on the jar.

Questions:

1. How does the plant respond to the direction of light?
   (It grows towards the light. The side of the stem away from the light grows faster than the side of the stem facing the light. This makes the stem curve and grow towards the light.)
2. How does a plant grown this way compare to one grown on a window sill?
   (Both stems curve and grow towards the source of light.)

EXPERIMENT 9: MONOCOTS AND DICOTS OR CORN AND BEANS

What Is the Difference in the Germination and Growth of Monocots and Dicots?

1. Germinate Monocot seeds (such as corn which has only one embryonic leaf in the seed) and Dicot seeds (such as beans and radishes which have two embryonic leaves in the seed and the seed splits into two halves).
2. Observe their growth for several days.
3. Make a table comparing observations of the two types of seeds as they grow. Include shape of leaves, number of leaves, location of seed as the plant grows, shape of roots, and appearance of stem.

PART III: HELPFUL HINTS

1. Radish seeds are the easiest and quickest to germinate.
2. Putting the gelatin jars in the refrigerator makes them set up faster but it does not help this technique because they only melt again when put at room temperature. They set up when they would have anyway.

3. Direct sunlight or heat source will melt the gelatin in the jars.
4. Mung bean and alfalfa seeds can be purchased at nutrition stores.
5. Label all the jars from each batch of gelatin in case it needs to be identified as a source of microbial contamination. Make a gelatin control for each batch by putting a small amount of media in a jar and labeling it control, no seeds.

6. 91% isopropyl alcohol dries faster than 70% isopropyl alcohol because it has less water in it. Either can be used, but 91% may reduce contamination better.

7. Lima beans purchased from the grocery store food shelf do not germinate as well as lima beans from seed packages.

8. When plants grow too tall for the container, you can remove the lid and cover the top of the jar with a 12" x 12" clear plastic food storage bag secured with a rubber band. This will give them about 10 inches of space to grow taller. They will eventually die when the gelatin is consumed or overcome with microbial contamination.

9. The plants can be transplanted to soil. All the gelatin must be gently rinsed off the roots with warm water and pulled off between your fingers. Be careful not to pull off the roots! If any gelatin is left, microorganisms in the soil will have a feast and eventually destroy the plants.

10. Although plastic jars are great for safety with younger students, some brands of jars may warp on even the top shelf of the dishwasher. They may also warp if the gelatin is too hot when poured. Jars which are not affected by the heat have a PETE 1 recycling symbol on them.

11. Students will be more successful when they transfer the seeds to the gelatin if they have had a chance to practice the sterile technique first. They can simply use seeds soaked in water, forceps standing in a jar of water and a jar with water, instead of gelatin. Make sure they also practice with lids on what would be the gelatin jar and the disinfectant jar. The forceps jar does not get a lid.

12. If mold contamination appears in the gelatin, it is from the preparation process if it is <u>below</u> the surface. It is airborne contamination if it is on the surface.

PART IV: SOLVING A PROBLEM

Using Household Disinfectants to Eliminate Microorganisms on Seeds.

GRADE LEVEL: Sixth

GOAL:
The student will have an opportunity to test the effects of various disinfectants on microorganisms and observe the results.

OBJECTIVES:

1. Observe the effect of a disinfectant on the growth of microorganisms on a seed germinating in a gelatin culture medium.
2. Learn to read product labels to obtain information about a product's ability to disinfect.
3. Develop an appreciation for sterile technique and for how easy it is for contamination to occur in a culture medium.
4. Gain an awareness of the responsibility one must take in toxicity of materials used in an experiment.

BACKGROUND:
In designing this technique for germinating seeds on gelatin, one of the major obstacles to overcome was the problem of microorganisms contaminating the system. They would either kill the plant or turn the gelatin into a liquid, due to the toxins given off by the microorganisms. Many different household disinfecting products were tested for varying times and at varying concentrations, both with and without soaking the seeds, as in the case of the beans. Some of the products killed all the microorganisms but stunted the growth of the plant. Some required that the seeds be treated for a long period of time to be effective, and some containing alcohol reacted with the gelatin, turning it into a liquid. To be an effective disinfectant for the seeds, the product must prevent contamination of microorganisms without affecting the growth of the plant or the consistency of the gelatin. To be suitable for young children to use, it should not contain bleach or other strong chemical.

PROBLEM:
Which household disinfecting agent is the best product to use to disinfect radish seeds in 15 minutes of treatment time without killing the seed or stunting the growth of the plant?

DISCUSSION:

1. Discuss with the students the problem involved in growing radish seeds in the jar of gelatin. Since the gelatin medium contains nutrients for growth, if any mold spores or other microorganisms contaminate the gelatin, they will grow very rapidly and quickly outgrow the seed and kill it. In setting up the gelatin jar there are many opportunities for contamination (cleaning the jars, making the gelatin, pouring the gelatin into the jar) which have to be controlled using a sterile technique. If a scientist were doing a project involving months of work growing a plant on a culture medium, one mold spore could contaminate the culture and destroy months of work. So scientists are extremely precise in how they perform sterile technique. Most science classrooms do not have the equipment required for industrial sterile techniques, but we can make use of some of their practices in solving our problem.
2. Describe or demonstrate the preparation of the gelatin jars, emphasizing the precautions that were taken to prevent contamination. Show a jar with gelatin in which radish seeds were planted without being treated to illustrate the problem with microbial contamination.
3. The students now have the problem of figuring out how to treat the seeds to kill the microorganisms and not the seed. Have them search their shelves at home for disinfecting products. Have them look at the labels for words to identify the product as a disinfecting agent, such as, antibacterial, mold or mildew control, disinfectant, antiseptic, anti-infective, and germicidal. Also have them read the labels to see if the products have any ingredients which would be harmful to the environment if they were used on a large scale and then required special disposal. Two examples are mercury (Mercurochrome®) and petroleum distillates (Lestoil®). This is an important consideration in responsible product evaluation in industry. One resource for this information is the software package on hazardous materials listed in the references.
4. After reading labels, the students can discuss in groups and select several products they think have the most potential to be effective in killing the microorganisms. Suggested products are not limited to the following:

91% isopropyl alcohol
3% hydrogen peroxide (usual strength right from the bottle)
Lysol® Basin, Tub and Tile Cleaner
Lysol® Disinfectant (diluted 1/8 tsp. in 1/2 cup water)
Lysol® Disinfectant Spray (diluted 1 tsp. in 3 tsp. water)
Lysol® Deodorizing Cleaner (diluted 1/8 tsp. in 1/2 cup water)
Liquid Dial® Antibacterial Soap (diluted 1/4 tsp. in 1/4 cup water)
Spic and Span® (diluted 1/8 tsp. in 1/2 cup water)
Listerene® (diluted 50%)

MATERIALS:

disinfecting products to be tested
safety goggles
gelatin jar setup (one per student)
clean baby food jars and lids for seeds (one per product to be tested)
91% isopropyl alcohol in a jar to soak forceps (one per team)
forceps (or tweezers)
radish seeds
clock

EXPERIMENT:

1. Divide the class into teams, for example, 6 (four member) teams. Each member of the team will test the same product. One student per group will be the control and just soak their seeds in water. So the experiment does not get too unmanageable, have the class select the 6 most promising products to test. Seat all members of the same product team together for testing. (For increased reliability, the test results from other classes can be combined if more than 1 class does the experiment)

2. Follow the steps for preparation of work conditions described in the Student Procedure Section of Part I.

3. Give each student a jar prepared with gelatin and fertilizer that has gelled. Be sure to label all jars made from the same batch so that they can be checked if batch contamination becomes a possibility.

4. Give each team a clean baby food jar with lid and the product they wish to test. Add to the empty jar the disinfectant or a dilution of the disinfectant calculated from the use directions on the container.

   Caution: Goggles should be worn while working with these products since most of their labels warn that they are harmful if they get in the eyes. Also they may want to wear some type of protective covering (lab coat or apron or old shirt).

5. When the test solution is prepared, drop enough seeds in it for all the students on the team to place about 6 seeds in each jar. Cover jar with a lid.
6. Time the treatment for 15 minutes.
7. Each team should also have one jar of seeds soaked in water for the control.
8. Then to transfer seeds, use forceps that have been soaking in a jar of 91% isopropyl alcohol and will be returned to the jar after each time a seed is touched.
9. Take the seeds one by one out of the disinfectant jar with forceps and allow all excess alcohol to drip off.
10. Briefly open the gelatin jar and close it after each seed is dropped in. Decide how many seeds each student should test (about 6-8).
11. Each student should label his or her jar with name, date, product tested, and dilution. The batch number should already be on the label.

OBSERVATIONS:

Decide what observations will be important in determining the best product. Make daily records of these observations. Students will want to look for the appearance of microorganisms (i. e. mold) and when it forms. They should also look at plant characteristics such as time of germination, daily growth of stem and roots, leaf color, and overall health of the plant and roots. They can measure the stem lengths each day, average the data and graph the results.

ANALYZE DATA:

Compare the results of each member on the team and then of all the teams to decide which product was the best. If several products allow no microorganisms, then graph each group's stem length data on identical (xeroxed from the same blank master graph) overhead

transparencies, Y=stem length and X=number of days. They can be laid on top of each other and compared that way on an overhead projector.
If all jars get microbial contamination, then gather information on how the jars were prepared to see if the contamination resulted from sources other than the seed.

At the end of the experiment, the contaminated material must be disposed of using a 20% bleach solution or 91% isopropyl alcohol as described in Part I under Disposal of Contaminated Material.

QUESTIONS:

1. Is one product clearly the best?
   (Answers will vary, depending on products tested.)
2. If the experiment gives definite results, does it seem logical, based on the ingredients or the claims on the product label?
3. Is the most effective product the most efficient to use?
4. Is the best product environmentally friendly, that is would disposal of it on a large scale harm the environment?
5. Does it harm the growth of the plant?
6. What other problems does this experiment lead you to try to solve?

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