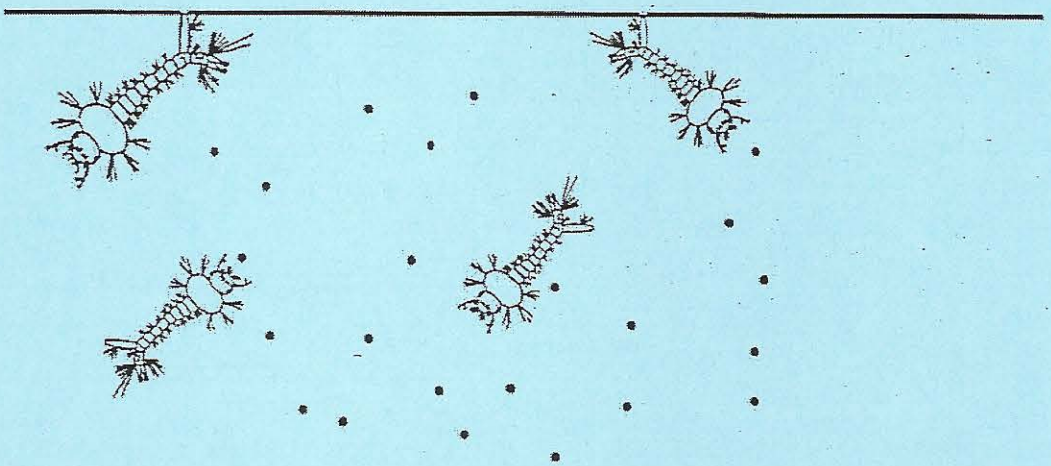
Name _____

Period _____

Date _____

Instructions for Determining the **Level** of **Toxicity** of ***Bacillus sphaericus*** to ***Culex*** **Mosquito Larvae**



Introduction

In the past, concern has been raised over the widespread application of pesticides in natural areas. The effects of many of these pesticides on wildlife have been evaluated. Some pesticides, such as DDT, have been banned in the United States. However, the need for pesticides has not been eliminated. Pesticides play a vital role in food production and public health. Efforts are now under way to find new pesticides which produce fewer effects on the environment. In many instances, no pesticides are required for pest management. It is sometimes possible to use natural or biological agents to control pests. In mosquito control various "biological" agents have been studied and found to be effective under certain circumstances. These organisms may be predators such as mosquito fish and dragonfly nymphs or pathogens such as fungi and bacteria.

In this test you will discover the level at which a bacteria, *Bacillus sphaericus*, will effectively control mosquito larvae. This test is called a **bioassay**. Bioassays are tests used to determine the toxicity of a substance to an organism. *Bacillus sphaericus* is not toxic to humans or most other animals.

Bacillus sphaericus Bioassay

Vocabulary

1. adult mosquito - mature stage of a mosquito's life; it has three body regions, six legs and two wings.
2. adulticide - (v) to kill adult mosquitoes; (n) a pesticide that kills adult mosquitoes.
3. agar plate - a Petri dish filled with a jello-like nutrient material used to grow bacteria upon.
4. *Bacillus sphaericus* - a bacteria used to biologically control mosquito larvae.
5. bioassay - a test to determine the toxicity of a substance to an organism.
6. Bti - *Bacillus thuringiensis israelensis*, a commonly used bacteria to control mosquito larvae.
7. Diptera - the order of insects that includes flies, gnats, and mosquitoes.
8. instar - a developmental phase of larval mosquitoes, four instars in all.
9. larva - the aquatic feeding stage of immature mosquitoes.
10. larvicide - (v) to kill mosquitoes in the larval stage; (n) a pesticide that kills larval mosquitoes.
11. metamorphosis - the four stages, egg, larva, pupa and adult, in the mosquito life cycle.
12. molt - shedding of the exoskeleton as part of the growth process.
13. organochlorines - a class of insecticides which contain carbon, chlorine, and hydrogen. Some of the more persistent insecticides are organochlorines such as DDT and Chlordane.
14. organophosphates - a class of insecticides which contain phosphorus. They are initially toxic but degrade quickly when exposed to air and light. Malathion and Abate are organophosphates.
15. pipet - a dropper-like piece of equipment used to accurately measure small amounts of liquid.
16. pupa - the nonfeeding stage in insect metamorphosis between larva and adult.
17. vector - an insect capable of transmitting a disease.

Bacillus sphaericus
Bioassay

Lab Preparation

Work in teams of four.

Materials

You will need one of each of the following items.

prepared agar petri dish
1 ml pipet
test tube of *B. sphaericus* culture
pencil
label
pi pump

Procedure

1. Obtain materials necessary to conduct this lab.
2. Be sure to keep the lid on the petri dish until ready for use, or it may become contaminated.
3. Use the 1 ml pipet to add 3/10 ml of *B. sphaericus* bacterial culture to the petri dish. Close the lid and swirl gently to coat the agar medium evenly with the bacterial solution.
4. Do not set the used pipet on the counter or table. Place it in the container provided by your teacher.
5. Stick a label on the petri dish lid; write the date, the names of each student in your group, and the class period on the label.
6. Place the petri dish upside down in an incubator maintained at 30°C for 24 hours.

When you are finished...

Be sure to take time to read over and familiarize yourself with the vocabulary from page 2. Read the lab procedures from pages 4 and 5. Make sure you understand what you will be investigating and how you are going to go about collecting the data. Keep a list of questions you come up with and important or related points you think about as you conduct the bioassay.

Bacillus sphaericus
Bioassay

Lab Activity

Materials

1 – legal size clip board with dilution sheet
1 – treated agar petri dish
1 – clean tongue depressor
10 – 3 oz. paper cups
distilled water
1 – 50 ml graduated cylinder
9 – 5 ml pipets
1 – pi pump
2 – eye dropper
100 – *Culex quinquefasciatus* mosquito larvae

Procedure

1. Examine your petri dish. Notice the growth of bacteria on the agar. Use the tongue depressor to carefully scrape off the bacteria, being careful not to dislodge the agar medium. Give the tongue depressor with bacteria to your teacher. This will be mixed with distilled water to make a bacterial slurry.

2. Place the Dilution sheet on a legal size clipboard. This will make moving the samples easier.

3. Number the sides of the 3 0z. paper cups. The first cup will be #1 and the last cup being #10.

4. Using a clean 50 ml graduated cylinder, accurately fill each of the ten paper cups with 27 ml of distilled water. Place the cups of distilled water on the circles on the Dilution sheet.

5. Using a clean pipet and the pi pump, get 3 ml of the prepared bacterial solution from your teacher and add to cup #1. Stir well, using the pipet. Cup #1 will now have three parts of the *B. sphaericus* solution to 30 parts of distilled water or a ratio of one part bacteria solution to ten parts water. Do not reuse this pipet. Place it immediately in the container provided by your teacher.

6. Using a clean pipet and the pi pump, get 3 ml of solution from cup #1 and add to cup #2. Stir well with the pipet then place the pipet in the container provided by your teacher. Cup #2 will have one part bacteria solution to 100 parts distilled water. Discard the pipet.

7. Using a clean pipet and the pi pump, add 3 ml of solution from cup #2 to cup #3. Stir. Discard the pipet.

8. Using a clean pipet and the pi pump, add 3 ml of solution from cup #3 to cup #4. Stir. Discard the pipet.

9. Using a clean pipet and the pi pump, add 3 ml of solution from cup #4 to cup #5. Stir. Discard the pipet.

10. Using a clean pipet and the pi pump, add 3 ml of solution from cup #5 to cup #6. Stir. Discard the pipet.

11. Using a clean pipet and the pi pump, add 3 ml of solution from cup #6 to cup #7. Stir. Discard the pipet.

12. Using a clean pipet and the pi pump, add 3 ml of solution from cup #7 to cup #8. Stir. Discard the pipet.

13. Using a clean pipet and the pi pump, add 3 ml of solution from cup #8 to cup #9. Stir. Discard the pipet. The concentration in this cup will be one part bacterial solution to one billion parts water.

14. Cup #10 will be your control. It should contain 27 ml of distilled water with no bacteria. There will be no bacteria in cup #10.

15. Using the eyedropper, add ten mosquito larvae to each cup. Start with the control. Then add larvae, starting at the cup with the weakest solution to the strongest solution. Add exactly ten, being careful not to count molts as live larvae. **Do not let the eyedropper come in contact with the solution in any of the paper cups as these contain bacteria and will contaminate your eyedropper.**

16. Read the results of the bioassay in 24 hours and record the results on page

Bacillus sphaericus Bioassay

On the table below, enter the data from your bioassay sheet.
Use the following formula to determine the percent mortality.

% mortality = (# of dead larvae / total # of larvae) x 100

Cup #	Concentration	# Dead Larvae	Total # Larvae	% Mortality
1	1:10			
2	1:100			
3	1:1,000			
4	1:10,000			
5	1:100,000			
6	1:1,000,000			
7	1:10,000,000			
8	1:100,000,000			
9	1:1,000,000,000			
0	control			

When scientists want to find out just how poisonous or hazardous a substance is they do tests very much like the one you did on mosquito larvae. They do the tests to find out what concentration of the substance will kill half of the population of test subjects (in this case, mosquito larvae). The concentration that results in 50% mortality is called the LC_{50}. The LC_{90} is the concentration that kills 90% of the subjects. LC stands for lethal concentration. The number tells you what percent were killed. Determine which concentration of *B. sphaericus* resulted in the LC_{50} and the LC_{90}. Enter your data in the chart below. Use data from the rest of the class to complete the chart.

Groups	Concentration for LC_{50}	Concentration for LC_{90}
your group		

Bioassay Graph

Names _____

_____

_____

_____

Period _____

Date _____