LABORATORY II
Allelopathy in Plants
An Introduction to the Scientific Method and Data Analysis

LEARNING OBJECTIVES

When you complete this lab, you will be expected to:
1. Use a flow chart to describe the scientific method.
2. Use the scientific method and properly design a controlled experiment.
3. Define independent and dependent variables.
4. Define biological replicates and discuss their importance in an experiment.
5. Discuss the importance of a well-designed control group.
6. Effectively present the results of an experiment using a table and/or graph.
7. Discuss the importance of a statistical test and interpret the result.
8. Effectively describe the conclusions of an experiment based on the results.

Each student must complete the PRE-LAB EXERCISE: EXPERIMENT DESIGN found on pages 14-16 BEFORE coming to the lab period on week 1.

OVERVIEW

The following lab will be carried out over **2 lab periods** (2 weeks). During period 1, students will work in teams of 2 to perform 1 set of trials of the experiment. During period 2, students will collect their data, calculate descriptive statistics on pooled trial data from a group, and present this data in a table and graph. The data will then be used to carry out a *t*-test (statistical test). The results of the experiment will be interpreted and conclusions drawn.

INTRODUCTION

Scientific Method

Science is a body of knowledge based on **observations** and **experiments**. While observations help to describe life, biological scientists attempt to explain life in terms of **hypotheses**. They make **predictions** from these hypotheses and test them using carefully designed **experiments**. If confirmed, a hypothesis is retained with greater confidence. If falsified, it is either rejected outright as false, or modified and retested. Alternatively, it might be decided that the experiment was not a valid test of the hypothesis. Nearly all scientific research deals with testing of small-scale hypotheses. The flow chart in Figure 1 illustrates a general model of the scientific method. A **theory** is based on a collection of hypotheses supporting it. It covers a range of natural phenomena – a larger-scale idea than a hypothesis.

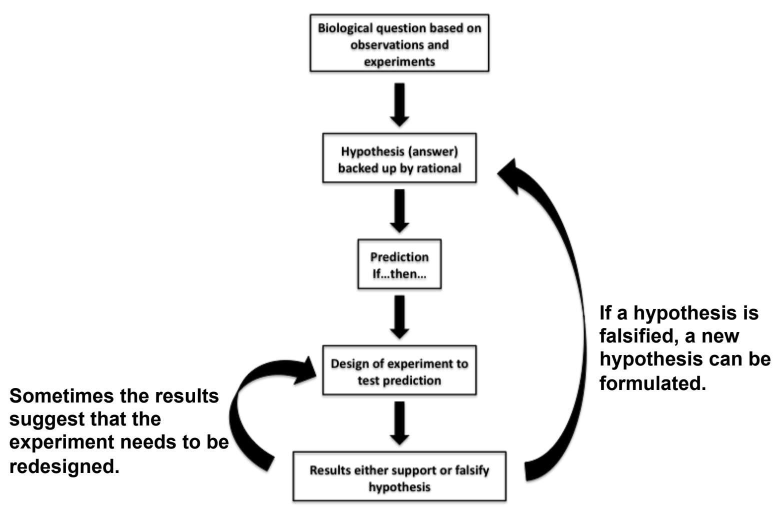


Figure 1. The Scientific Method

One fundamental rule applies to the scientific method and hypothesis testing: ***No hypothesis can ever be accepted or rejected with 100% certainty.*** **Statistical tests** are important tools in data analysis and allow us to quantify the ***probability of an erroneous conclusion***, but we are nevertheless left in a position of never being 100% certain that we have rejected all relevant alternative hypotheses, nor 100% certain that our decision to reject some alternative hypotheses was correct. However, despite these problems, experimental science has yielded and continues to yield many important findings.

An Experiment in Ecology: Allelopathy in Plants

During the next two lab periods, you will carry out an experiment to answer the question:

Does an onion plant (Allium cepa) produce allelochemicals?

Plants can be complex chemical factories. They may produce scents and nutrients to attract pollinators. They may produce and store poisons in their tissues to discourage potential herbivores and parasites. Plants also produce chemicals to *compete* with other plants for sunlight, soil, nutrients, and water. Since they do not have the option of avoiding **competition** by moving away (like motile organisms) they use *chemical warfare* termed **allelopathy** . The chemicals, termed **allelochemicals**, can be released by the plant into the environment where they *inhibit* some aspect of reproduction: seed germination, development, growth of neighboring plants competing for the same resources. Plants use a wide variety of mechanisms to disperse their allelochemicals. Some plants release them into the soil from roots or decomposing leaves, or directly into the air (volatile chemicals), where they can be deposited on the soil or the surface of other plants when dew forms (Duke, 2003). As with all competition between individuals in an ecosystem, allelopathy can be against plants of the same species (**intraspecific** competition) or plants of a different species (**interspecific** competition). See extracts from the ***garlic*** **plant** (*Allium sativum*) is known to produce allelochemicals and an extract of the plant has been shown to *inhibit* pea plant (*Pisum sativum*) seed germination (Abou El-Ghit, 2016). Garlic plant extract inhibited radish plant (*Raphanus raphanistrum*) seed germination by almost 100% in our lab (Fig. 2).

This natural form of competition is an important topic in the study of **community ecology**. Allelopathy may also have potentially important ***applications*** **for agriculture**. For example, when planning a crop rotation schedule, it is important to know if certain crops leave chemicals in the soil that may inhibit growth of future crops. Asparagus is known to have such effects, especially on future asparagus growth [*Is this an example of intraspecific or interspecific allelopathy?*]. Allelopathy is also of interest as an alternative method of weed control. Instead of traditional herbicides, plants such as rye that produce allelochemicals could be used as cover crops to control weed growth. Alternatively, allelochemicals could potentially be identified and used as ‘natural herbicides’.

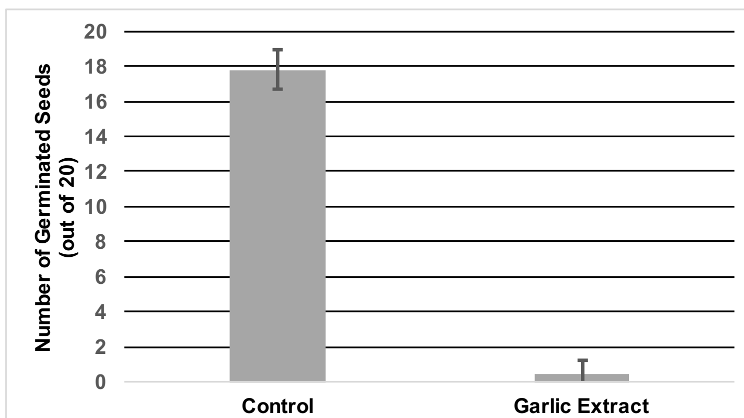


Figure 2. Germination of radish seeds in a garlic plant extract. Each bar in the graph represents the mean number of seeds that germinated (out of 20) in 10 independent trials. Error bars represent the standard deviation for the trials of each treatment group.

MATERIALS AND METHODS

MATERIALS

- 2 Petri dishes with filter paper
- Jar of radish plant seeds
  N.B. The seeds are coated in a green-colored fungicide. The fungicide will prevent fungi growth in our plates but can also be toxic to us! Use forceps to handle the seeds, do not use your hands.
- Forceps
- 3 substances:
  A. Distilled Water B. Garlic bulb extract in distilled water (0.14 g / mL) C. Onion bulb extract in distilled water (0.14 g / mL)
- 2 X 10 mL graduated cylinder (one for each solution so that each solution stays separate and no mixing occurs)
- Pre-cut strips of Parafilm ® (paraffin film) for sealing dishes

PROCEDURE

Overview

As a class:

a) **Discuss** your hypotheses for the experimental question and back it up with a rationale.
b) **Design** an experiment that will allow you to test possible hypotheses to this question. To do this, discuss your responses to the pre-lab questions and ideas.
c) **Execute** an experiment and collect samples to be analyzed next week.

Guidelines for designing your experiment

1. Your experiment must allow you to test two opposing hypotheses:
   ***A. Onion plants PRODUCE allelochemicals*.**
   ***B. Onion plants DO NOT PRODUCE allelochemicals*.**
2. Your experiment design must include:
   - **TWO (2) *experimental* treatments**
   - **ONE (1) *control* treatment**
   - **ONE (1) *dependent* variable** that is **QUANTITATIVE** (*not qualitative*)
3. **Replication must be carried out to create treatment *groups*.** Each team of two (or three) from the lab class will be in charge of a **set of trials**: **ONE trial** of the ***control* treatment** and **ONE trial** of ***experimental* treatment 1** and **ONE trial** of ***experimental* treatment 2**.

   N.B. ***All trials for a particular treatment must be carried out on separate specimens but in an identical manner in order to be considered replication!***

   Your instructor will assign your team of 2 (or 3) a letter identifier.

   List your team letter here → My Team: _____

Next week, you will collect your data and analyze the results from the ***separate* trials** conducted by your class.

Experimental Procedure Worksheet

Use the space provided below to describe the details of the experimental procedure for a set of trials to be performed by each team and make important notes or diagrams.

Names: __________________ __________________ **Lab Section:** _____

LAB REPORT SECTION

A. Introducing your Experiment

The introduction will describe the goal of the experiment, what is known about the question you are trying to answer, your hypothesis, and rationale. Use your instructor's guidelines to write an Introduction section of your experiment report.

B. Describing your Material and Methods

The materials and methods section will describe important details about what equipment was used and how the experiment was carried out. Use your instructor's guidelines to write a Material and Methods section of your experiment report.

C. Recording Data in a Table

For each of your two plates, you will be counting the **number of germinated seeds (out of total number)**. Germinated seeds will be cracked and you will see green shoot tissue and white root tissue (Fig. 3). Sometimes it is difficult to tell whether a seed is germinated or not. *Establish guidelines with your instructor and classmates as to what you will consider a germinated vs. a non-germinated seed so that your results are consistent.* These criteria are essential to make sure that data obtained in independent trials are comparable. A **stereoscopic microscope** can be used to better view your specimens.

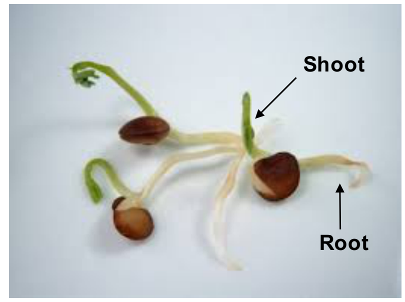


Fig. 3 Three (3) germinated radish seeds. N.B. *Our seeds have a green fungicide covering the brown seed*

Complete Table 1

1. Provide a descriptive title next to the table identifier below.
2. Use a *term* to describe/differentiate the treatments (fill-in the blank at the top of the column).
3. List the measured (dependent) variable in the appropriate location in the table and include units (if applicable).
4. Record your team's data in the correct row and then the class' data. There are enough rows for recording 12 trials, *use the number of rows that you need to cover the total number of trials from your group*.
5. Calculate the **mean** of the measured variable collected for each treatment group.
6. Calculate the **standard deviation** of the measured variable collected in each treatment group. Your teacher will give you instructions on how to calculate the standard deviation.

Table 1. ____

	Trial	***Treatments***		
		CONTROL ________	***EXPERIMENTAL 1*** ________	***EXPERIMENTAL 2*** ________
Measured Variable: ________	**1 (Team A)**			
	2 (Team B)			
	3 (Team C)			
	4 (Team D)			
	5 (Team E)			
	6 (Team F)			
	7 (Team G)			
	8 (Team H)			
	9 (Team I)			
	10 (Team J)			
	11 (Team K)			
	12 (Team L)			
	Mean			
	Standard Deviation (S.D.)			

D. Displaying Results in a Graph

In most cases, the results of an experiment are best summarized and communicated to a reader using a figure such as a **graph**. There are many different types of graphs and the type used will depend on the independent and dependent variables used in an experiment. In this case, a **bar chart** is appropriate to display our results (see Fig. 4).

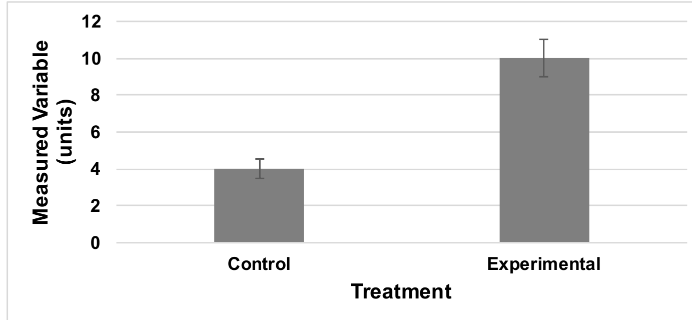


Figure 4. ***[Descriptive Title]. [Figure Information].***

Each bar in a bar graph usually represents the ***mean*** variable measurement for the specimens for one treatment group. **Error bars** are also included in bar charts. Usually these error bars represent **standard deviation** or another **measure of dispersion** of the data collected among the specimens within a treatment group. When constructing any type of graph, all of the information necessary for a reader to understand the data must be present. This includes a descriptive title and well-labeled axes. In addition, figures generally require a short ***description*** (or sometimes a legend) ***under the graph*** to deliver all of the necessary information to a reader.

In this case, two items of information that ***must*** be included as information in the figure description are:

1. How many specimens (number of replicates) were included in each group and represented by the mean?
2. What measure of dispersion do the error bars represent?

Other notes when completing a bar graph by hand: 1. Use a ***complete* scale** (no scale brake) for the *y*-axis 2. Write in ***all values*** for your intervals on the *y*-axis (not just the ones close to the value of your bars) 3. Use a ruler to draw bars and error bars to be neat and precise.

Using the guidelines above, make a bar chart using your team's data.

Figure 5.

E. Using a Statistical Test

The mean proportion of germinated seeds in each treatment are likely different from each other. However, how do you know if this difference is a ***real difference*** (i.e. an effect of your independent variable) or simply due to ***chance*** because of ***natural variation*** in seed germination among the plates? The answer is *you can never know for sure*. However, statistical tests can be used to test whether the difference in the dependent variable between two treatment groups is ***statistically* significant** and are very useful tools in analyzing data.

A ***statistically* significant difference** means that the difference is ***unlikely* to be due to chance** and so it leads us to believe that the difference in the dependent variable is due to our manipulation of the independent variable. In other words, **the independent variable had an effect on the dependent variable**.

A **difference that is *not* statistically significant** means that the difference is **likely to be due to chance** and leads us to believe that the difference in the dependent variable is *not* due to our manipulation of the independent variable. In other words, **the independent variable had *no* effect on the dependent variable**.

A statistical test involves two hypotheses:
1. The **null (no effect) hypothesis (H_0)** of the test. The H_0 states that the **difference in the dependent variable** between the two treatment groups is ***not* statistically significant**.
2. The **alternative (effect) hypothesis (H_A)** of the test. The H_A states that the **difference in the dependent variable** between the two treatment groups is **statistically significant**.

P-value and Significance Level

Statistical tests examine the **probability of obtaining the difference you observed (or greater) between treatment groups if the H_0 IS TRUE**. This probability is calculated as a ***p*-value** that ranges from 0 (0%) to 1 (100%). To carry out a statistical test, you must decide on a significance level to add meaning to what you mean by “significant”. Your significance level is essentially your ***threshold*** for what you consider not very likely. The lower the p-value, the less likely that you would obtain this difference by chance and so you don’t believe the H_0 (you reject it) and you believe the H_A. Usually a significance level of 0.05 is chosen for a significance test. A significance level of 0.05 means that if there is less than a 5% chance (1 out of 20 times) of getting these results (or a larger difference) when the H_0 is true, then the H_0 is too unlikely to be true!

Interpreting the Results of a Statistical Test

Compare the *p*-value to your significance level:
a. If the *p*-value is ≥ 0.05, then you fail to reject the H_0: The difference IS *NOT* statistically significant.
b. If the *p*-value is < 0.05, then you reject the H_0 and accept the H_A: The difference *IS* statistically significant.

Some Important Notes on Statistical Tests

Statistical tests are useful tools for data analysis and drawing conclusions about the results of your experiment, but **they are not the *result* of an experiment**. Many students mistakenly think that finding a statistical significance between two treatment groups means that the experiment was a success and that their experimental hypothesis is 100% supported.

However, this is simple not the case! *It is still the responsibility of the researcher to put meaning to this difference and draw appropriate conclusions for an experiment.*

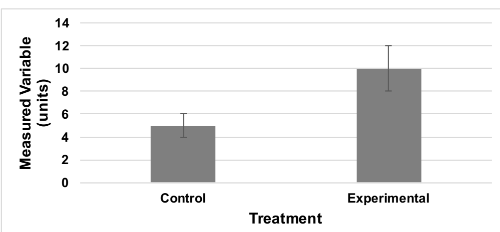
Things to consider:
1. A difference between treatment groups might be significant but *small* and scientifically *boring*.
2. A statistical test does not look at how you carried out the experiment and cannot identify a *false positive* result because of an unwanted variable.
3. A statistical test cannot look at other limitations of the experiment in testing a hypothesis.

Using a *t*-Test

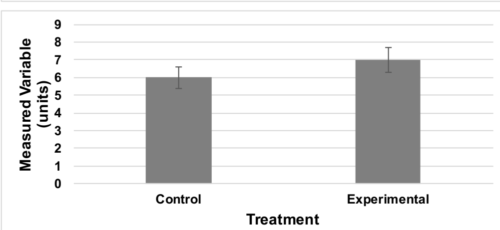
A *t*-test is an appropriate statistical test to use to test the difference between two (2) treatment groups when quantitative data is collected. A *t*-test essentially takes into account two (2) factors to judge whether a difference is statistically significant or not:
1. The size of the difference in the measured variable BETWEEN treatment groups.
2. The variation in the measured variable WITHIN each treatment groups.

The **LARGER** the ***difference*** and the **SMALLER** the ***scatter*** of the data within each group, the more likely that the difference is statistically significant.

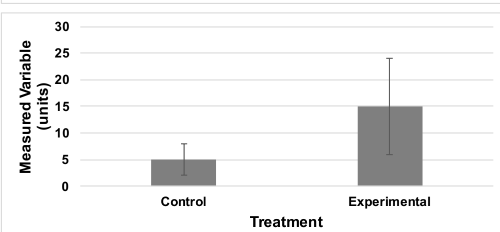
Consider the following three results demonstrated by bar graphs. Which of the three is likely a *real* difference (statistically significant)?



A. Medium Difference / Medium Scatter



B. Small Difference / Low Scatter



C. Large Difference / High Scatter

Figure 6. Three sets of results that vary in the size of the difference in the mean dependent variable between treatment groups and the scatter within these groups.

Explain your response.

____

____

1. **Before carrying out a *t*-test on your data, use your EYE-TEST for fun! Do you think the difference between your treatment groups is statistically significant? Why? Hint: Use the guidelines on the previous page.**

____

____

____

2. **State the GENERAL hypotheses of ANY t-test (don't use information specific to your experiment)**

Null hypothesis:

____

Alternative hypothesis:

____

3. **Which of these hypotheses listed above supports your specific EXPERIMENTAL hypothesis stated in the pre-lab exercise? Briefly explain.**

____

____

4. **Carry out a series of *t*-tests to determine whether any difference observed in the mean dependent variable between treatments is statistically significant (P<0.05). Your teacher will give you instructions on how to carry out this statistical test. Use the results to complete Table 2.**

Table 2. Results of a *t*-test to compare the statistical significance of the difference between your treatment groups.

Treatment Group Comparison	***P*-Value**	***t*-test Result** **Do you:** **A. Fail to reject the H_0 OR** **B. Reject the H_0 and accept the H_A ?**	**1. Statistical Interpretation** ***Was the difference in the dependent variable measured between treatment groups statistically significant?*** **2. Experimental Interpretation** ***Did the independent variable have an effect on the dependent variable?***
Experimental 1 vs. Control			
Experimental 2 vs. Control			
Experimental 1 vs. Experimental 2			

F. Describing the Results of an Experiment

In a CONCISE paragraph (make your sentences as compact as possible), describe the results of the experiment. The goal is to summarize the data obtained, highlight the *difference* in the mean dependent variable between treatments and whether the difference is considered statistically significant by a given statistical test. N.B. *Do not include your conclusions here.*

Things to include:

a. Compare the mean dependent variables and standard deviation for the treatment groups. Make sure to include what the *treatments* and *dependent variable* were and the *number of replicates* in each treatment group.
   Example: *The mean heart rate in a group of 51 women that received the placebo (control) treatment was 91 beats per minute (S.D. = 12 beats per minute) while the mean heart rate in a group of 39 women that received the drug (experimental) treatment was 108 beats per minute (S.D. = 23 beats per minute).*

b. A description of the *difference* in the mean dependent variable between the treatment groups.

- The size of the difference
- Whether the difference was found to statistically significant.
- The statistical test used.
  Example: *Mean heart rate was on average 17 beats per minute higher (1.2 times increase in heart rate) in the drug treatment group when compared to the placebo treatment group. This difference was found to be statistically significant by a t-test ($P<0.05$).*

G. Making Conclusions

Considering your EXPERIMENTAL hypothesis, what do the results of the experiment *suggest*?

H. Discussing the Results

1. **LIMITATIONS: All experiments have limitations and can NEVER *PROVE* that a hypothesis is *right* or *wrong*. Describe TWO (2) such limitations of this experiment. N.B. Limitations are not mistakes! Do not describe mistakes here.**

Limitation 1:

Limitation 2:

2. **FUTURE DIRECTION: Briefly describe what your next goal would be to better understand the biological question under study (or a related question) and summarize the design of an experiment to carry it out.**

3. **IMPACT: What impact does such an experiment have on 1. Our understanding of Biology and/or 2. Applications in research, medicine, or industry? *In other words, why is this research important?***

Names: ________________ ________________ **Lab Section: _____**

EXPERIMENT DESIGN (Pre-Lab Exercise)

1. **Complete the *Design* exercise BEFORE coming to the lab.**
2. **Refer to the *experiment guidelines* on page 3.**
3. ***You must complete this exercise by hand using a PENCIL so that it can easily be modified.***

Design an experiment to answer the question:

Does an onion plant produce allelochemicals?

4. **State your *HYPOTHESIS*:**

5. **Give a *RATIONALE* for your hypothesis:**

6. **Briefly describe your *TREATMENTS* and the results that you predict for each treatment:**

A. *EXPERIMENTAL* Treatment 1:

Result Predicted:

B. *EXPERIMENTAL* Treatment 2:

Result Predicted:

B. *CONTROL* Treatment:

Result Predicted:

7. Importance of a CONTROL treatment in an experiment.

a) In general, what is a *control* treatment in an experiment?

b) What information can be determined from the data obtained for this treatment?

c) Why is this information essential to know when analyzing the results of an experiment?

8. Describe the *INDEPENDENT* variable in this experiment?

9. Describe the *DEPENDENT* variable in this experiment? Is this variable *qualitative* or *quantitative*?

10. List 5 *specific* and *distinct CONTROLLED* variables (i.e. variables held constant between treatments) in your experiment.

A. ______________________________
B. ______________________________
C. ______________________________
D. ______________________________
E. ______________________________

11. What is your *UNIT* of study? N.B. The easiest way to identify your unit of study is to think about your dependent variable. *What is the specimen used to make ONE independent measure of your dependent variable?*

12. How many *REPLICATES* (number of these units) do you have in *each* group?

***Experimental* Group 1: ______**

***Experimental* Group 2: ______**

***Control* Group: ______**

13. Importance of REPLICATION in an experiment.

a) In general, what is *replication* in an experiment?

b) What information can be determined from the data obtained when replication forms treatment *groups*?

c) Why is this information essential to know when analyzing the results of an experiment?

REFERENCES

Abou El-Ghit, HM. 2016. Physiological allelopathic effect of aqueous extract of cucumber, carrot, onion, and garlic seeds on germination and growth of pea. *Journal of Pharmaceutical, Chemical, and Biological Sciences*, 4(1): 13-19.

Duke, SO. 2003. Ecophysiological aspects of allelopathy. *Planta*, 217(4): 529-539.

Jones A, Reed R, Weyers J. 2007. Practical Skills in Biology. 4th ed. London, England: Pearson Education Limited. 496 p.

Pechenik JA. 2010. A Short Guide to Writing About Biology. 7th ed. London, England: Longman. 304 p.