Agri 201 Bio-assay Lab

MATERIALS:

Tomato Seeds	Sterile water
Perennial rye seeds	Cheese Cloth
Germination paper (blue)	Petri Dishes (sterile plastic)
8 Syringes 39ml	1,5,10,50,100,500, 1000 ppm 2,4Dichlorophenoxy acetic acid
Syringe filters .2um	5% Bleach solution
Scissors	PROBIT plot graph paper
Forceps	Aluminum foil
Disposable gloves	Tape
Timer	Waste container

PURPOSE: To demonstrate the usefulness of bioassay in the determination of biologic activity of various compounds.

Note: Do this experiment with another group. Each group will take one species i.e. tomato or rye. The two groups should place the seeds in the drawers at the same time.

METHOD:

1. Autoclave 500 ml of DI water, forceps wrapped in foil and germination paper wrapped in foil.

2. Soak tomato seeds (200) and fescue seeds (200) in 5% bleach for 5 minutes. Rinse with sterile water three times. Use cheesecloth to help strain seeds.

3. Using sterile forceps, place sterile germination paper into 16 separate petri dishes

4. Using a syringe with a syringe filter attached, apply 6ml of one of the 2,4,D solutions to each of two petri dishes (one petri dish for the tomatoes and one for the annual rye. Repeat until all 8 solutions have been applied i.e. 0 control, 1,5,10,50,100,500,1000 ppm 2,4,D)

5. Use sterile forceps to add 25 seeds to each petri dish.

6. Label each dish with concentration, seed type, your initials and the date.

7. Store in Agri 201 cabinet in Extraction room i.e. cabinet closest to the door.

8. Check germination in five days and then each day there after. When no new seeds are germinating, (usually 5 to 8 days), record number of seeds germinated. Write a description of each petri dish. (Root length, shape etc., shoot length, shape etc.,)

9. Obtain the germination data from the other two groups in your class and combine the results e.g. if one group had 15 rye seeds germinate at 100 ppm 2,4D and another had 16 seeds, there would be a total of 31 rye seed that germinate at 100 ppm 2,4,D.

10. Plot data i.e. adjusted germination % vs. concentration, on log normal (log PROBIT) paper. Adjusted Germination % = (germinated seeds in treatment/ germinated seeds in control) x 100 Example: If 15 seed germinate for the 500 ppm solution and 20 seeds germinate for the control then the adjusted germination % is 15/20 =75%. See example in Insturment room.

Questions

1. Discuss results, what other types of data from this experiment could be expressed on this type of plot?
2. What is the resolution and detection limit of this procedure?
3. What is the LD 50 for tomato? What is the LD 50 for fescue?

Note: All waste should be place in proper container.

2,4 D Safety information –(HEALTH - 2 MODERATE, FLAMMABILITY - 1 SLIGHT, REACTIVITY - 1 SLIGHT, CONTACT - 2 MODERATE)