EXTENDED ESSAY

Subject: Biology

The phytotoxic effects of horticultural insecticides; malathion, cypermithrin, and imidacloprid on photosynthesis in *Elodea canadensis*

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WORD COUNT: 3986

Abstract

In agriculture, insecticides are used for eradicating or controlling pests that cause harm to plants. However some insecticides can also have adverse effects on plant photosynthesis by interfering with the electron movement down the electron transport chain from photosystem II to photosystem I. Research has shown that highly toxic insecticides inhibit photosynthesis, but insecticides with lower toxicity were not examined. The aim of the essay is to investigate **the phytotoxic effects of the horticultural insecticides malathion, cypermithrin, imidacloprid on *Elodea canadensis.*** *Elodea canadensis* is a aquatic plant with thin leaves and relatively large organelles making it ideal for observing phytotoxic effects and for chloroplast isolation. The insecticides used were from three different classes that are commonly used in agriculture: organophosphates, pyrethroids, and neonicotinoids.

In the experiment *Elodea was* exposed to different concentrations of the insecticides for 3 days and then the leaf was examined under the microscope in comparison to a control plant exposed to no insecticide. The chlorophyll content of the plants after exposure to the insecticides was also measured. The rate of the electron transport was then monitored using the artificial electron acceptor, 2,6-dichlorophenolindophenol (DCPIP) on the photosynthesis of isolated chloroplast from the *Elodea*. The reduction of DCPIP results in a color change from a blue to a colorless, which was measured spectrophotometrically at 600 nm. The rate of reduction of DCPIP was used to calculate the rate of electron transport in the chloroplast, during photosynthesis.

From the investigation as the concentration of insecticide increases, the plant's leaf became more bleached, the chlorophyll content decreased, and the rate of reduction of DCPIP also decreased. The insecticide inhibited the rate of photosynthetic electron transport. Malathion caused the most severe phytotoxic effect, and the highest inhibition, followed by cypermithrin, then imidacloprid.

Word count: 292

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1. Introduction

Agricultural research is a fundamental aspect of food manufacture and technological advances, which aim to increase efficiency and to maximize crop yield. With numerous factors affecting the availability of food, minimizing problems that cause inefficiency of food production has become vital. The presence of crop eating insects has many adverse effects on field crops. There are over 10,000 species of crop eating insects and near to 700 species cause harm to agricultural crops, in the field or in storage[1]. Some insects cause up to a shocking 66% decrease in crop yield[2]. These pests gravely distress the agricultural industry, prompting the need for insecticides to eliminate the problem[3].

Figure 1: Crop damage by the insect, *Trichoplusia ni* [3]

[1] Ware and Whitacre: AN INTRODUCTION TO INSECTICIDES." *Radcliffe's IPM World Textbook | CFANS | University of Minnesota.* Web. 03 Nov. 2009. <http://ipmworld.umn.edu/chapters/ware.htm>.

[2] "Thrips Tabaci (English) - IPM/CIIFAD." *Cornell University's New York State Agricultural Experiment Station*. Web. 08 May 2010. <http://www.nysaes.cornell.edu/ent/hortcrops/english/thrips.html>.

[3] "Cabbage Looper." *TELUS Internet Services - Member Services.* Web. 08 May 2010. <http://www3.telus.net/conrad/insects/cabloop.html>.
Crop damage by cabbage looper" (1981) © Agriculture and Agri-Food Canada. Published by the Government of Canada. This reproduction has neither been endorsed by, nor produced in affiliation with, the Government of Canada.

Insecticides are agents of chemical or biological origin used to control insects or pests. Control is achieved by killing the insects, or preventing them from inflicting mass destruction to crops[4]. In agriculture they are used in order maximize crop yield. However research has found that they may have adverse effects on plant photosynthesis by decreasing photosynthetic efficiency to a substantial extent. The once very commonly used organochlorine insecticides exhibited phytotoxic effects on photosynthesis[5]; these insecticides were eventually substituted by organophosphate insecticides[5]. Methyl parathion, a more toxic form of this class of insecticides inhibited photosynthesis by 50% at 47 μM concentration[5]. This would mean that the use of this insecticide would be ineffectual; instead of protecting the crops it could act like a herbicide and cause a decrease in the rate of photosynthesis. This would lead to a worsening in the rate of crop growth and thus food production. Furthermore the use of insecticides has many other risks. In agriculture, more than 98% of insecticides used reach destinations other than the targeted species, including non-target species, air, water, silt, and food[6]. This can be extremely threatening to humans and the environment due to the toxicity of insecticides [7].

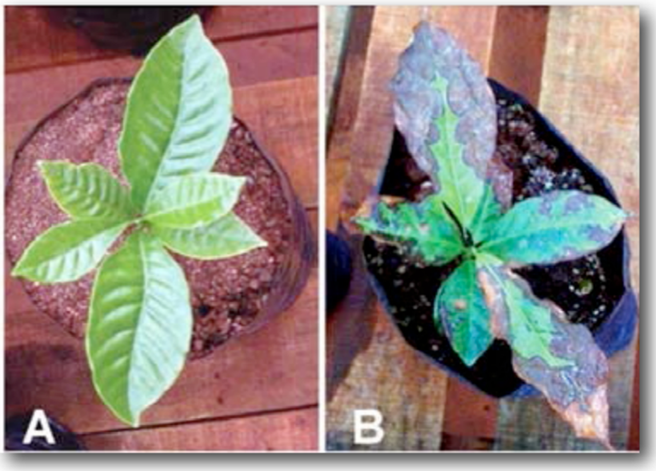

Figure 2: Coffee plant before (A) and one week after (B) being sprayed by herbicide [7]

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[4]"Ware and Whitacre: AN INTRODUCTION TO INSECTICIDES." *Radcliffe's IPM World Textbook | CFANS | University of Minnesota.* Web. 03 Nov. 2009. <http://ipmworld.umn.edu/chapters/ware.htm>.

[5]P. R. ANBUDURAI, R. MANNAR MANNAN, and SALIL BOSE. "The inhibition of photosynthetic electron transport by methyl parathion." *J. Biosci* Vol. 3.Number 1 (March 1981,): 23-27.

[6] Miller GT (2004), *Sustaining the Earth*, 6th edition. Thompson Learning, Inc. Pacific Grove, California. Chapter 9, Pages 211-216

[7] Ribas, Alessandra Ferreira. "Genetic Transformation of Coffee." *Brazilian Journal of Plant Physiology* 18.1 (2006). Web.
Ribas Alessandra Ferreira, Pereira Luiz Filipe Protasio, Vieira Luiz Gonzaga E.. Genetic transformation of coffee. 2006, Braz. J. Plant Physiol. 18(1): 83-94.

The rationale for using insecticides thus becomes questionable. If insecticides cause harm to the species it protects from insects, they not only lose their purpose but also become an unnecessary hazard. The extent of this harm must be investigated to weigh the cost of using insecticides. Hence the purpose of this research is to;

I. Observe the phytotoxic effects of different horticultural insecticides on plant morphology

II. Calculate the percentage inhibition these insecticide have on plant photosynthesis

The aim of this research project is to find out the extent of the phytotoxic effects of certain insecticides used in agriculture on plant photosynthesis, and to compare the damaging effects these insecticides have on the plants. It will also provide a better understanding on the mechanism by which insecticides work. The results from this research will be useful to farmers, insecticide-producing companies, or other people in agriculture related field. It will inform them about the harmful effects of the insecticides and recommend the appropriate concentrations that should be used if insecticide use is still deemed necessary. It would also give a better idea on which type of insecticide is best to use and to weigh the opportunity costs to maximize crop yield. Finally this research paper will provide a better insight on the adverse effects of insecticides on the environment.

2. Literature Review

2.1 Insecticides

The insecticides used for this study are classified to be low in toxicity and are commonly used in agriculture. They were chosen as they are the primary insecticides used in Singapore, and are commercially available. The insecticides were malathion, cypermethrin, and imidacropid. Malathion is an organophosphate insecticide that is non-systemic, broad-spectrum, and disrupts the nervous system function in insects[8]. Cypermethrin is a synthetic pyrethriod that is broad spectrum, non-cumulative and is a fast-acting neurotoxin[9]. Imidacloprid is a neonicotinoid that is systemic and works by interfering with he transmission of stimuli in the insect nervous system[10]. Insecticides that have phytotoxic effects on crops have been found to inhibit electrons transport in photosystem II of the light-dependent reactions during photosynthesis[11] [12] [13].

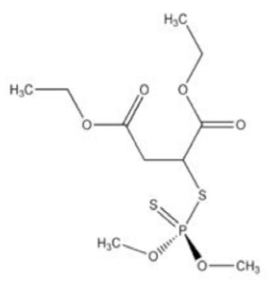
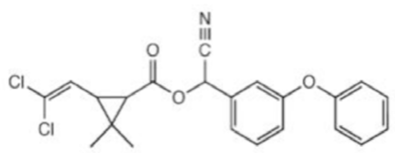
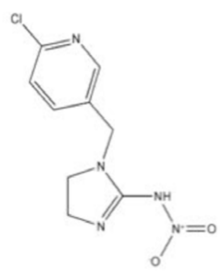
Malathion	Cypermithrin	Imidacloprid
		

Figure 3: Insecticides used for experiment[13]

[8]Oregon State University,, Weniger Hall. "Malathion (Technical Fact Sheet)."

[9] "Cypermethrin (PDS)." IPCS INCHEM. 10 Jan. 2010 <http://www.inchem.org/documents/pds/pds/pest58_e.htm>.

[10] "Imidacloprid." PMEP Home. 10 Jan. 2010 <http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/imidacloprid-ext.html>.

[11] Yukimoto, Mineko. "Effect of Organophosphorus Insecticides on Hill Reaction." *Pesticides* 8 (1983): 63-68. *Ministry of Agriculture*.

[12] P. R. ANBUDURAI, R. MANNAR MANNAN, and SALIL BOSE. "The inhibition of photosynthetic electron transport by methyl parathion." *J. Biosci* Vol. 3.Number 1 (March 1981,): 23-27.

[13] *Pesticide Action Network North America | Advancing Alternatives to Pesticides Worldwide*. Web. 03 May 2010. <http://www.panna.org/>.

2.2 Photosynthesis

Photosynthesis is a biological process which converts light energy to chemical energy as carbohydrates and other organic compounds. It consists of a sequence of chemical reactions, which involve carbon dioxide, water and storing chemical energy in the form of sugar. Photosynthesis occurs in the chloroplast and is a complicated process consisting of both the light-dependent and light-independent reactions [14].

Image removed for copyright reasons

Figure 4: Chloroplast[14]

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Figure 5: light-dependent and light-independent reactions[14]

[14] Campbell, Neil A., and Jane B. Reece. BIOLOGY. Eighth ed. San Fransisco: Pearson Benjamin Cummings, 2008.

2.3 Light-dependent reactions

During the light-dependent reaction, photons of light from the sun strike a pigment molecule in photosystem II (PSII), causing an electron to excite to a higher energy state. The photo-excited electron passes down an electron transport chain (ETC). The electron then enters another photosystem (PSI) and passes through another ETC. The final electron acceptor of the process is NADP+ and it forms NADPH. The whole process can be seen in **figure 4** below.[15] Past investigations have shown that Insecticides interfere with electron transport at the PS II during these reactions.[16]

Image removed for copyright reasons

Figure 6: light-dependent reactions[15]

[15] Campbell, Neil A., and Jane B. Reece. BIOLOGY. Eighth ed. San Fransisco: Pearson Benjamin Cummings, 2008.

[16] P. R. ANBUDURAI, R. MANNAR MANNAN, and SALIL BOSE. "The inhibition of photosynthetic electron transport by methyl parathion." *J. Biosci* Vol. 3.Number 1 (March 1981,): 23-27.

2.4 Photosystem II [17]

Photosystem II (PSII) is located in the chloroplast of the thylakoid membrane. It consists of a light harvesting complex (LHC), two proteins known as the D1 and D2 proteins, a P680 reaction center, and two electron carriers. The electron carriers, plastoquinone-A (PQA) and plastoquinone-B (PQB) help transport electrons from PSII to PSI. During the process of photosynthesis the LHC passes excitation energy to P680. This causes charge separation and absorption of an electron by the reaction center. The electron is transported to the PQA in the D2 protein and then binds to the PQB in the D1 protein. The PQB is attached to the D1 protein by two hydrogen bonds. When it accepts electrons from PQA these bonds are broken and the PQB leaves the D1 protein as a reduced PQB. Another unreduced PQB immediately substitutes it, and the process continues [18].

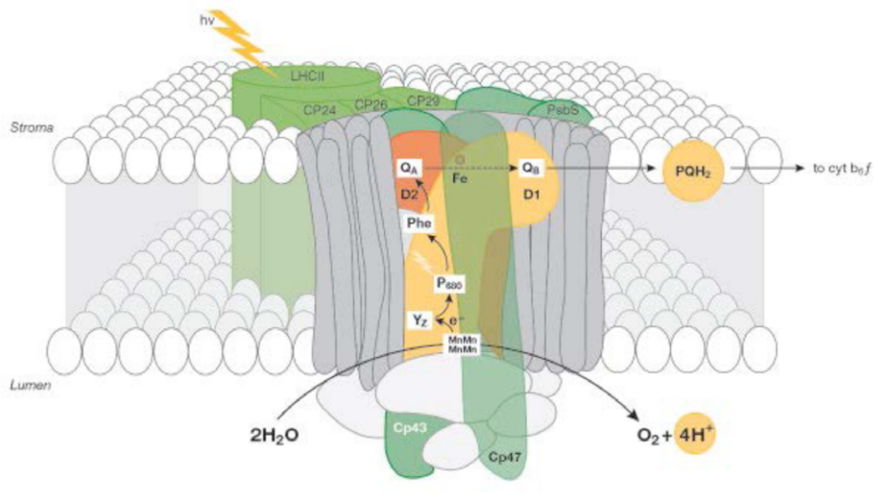

Figure 7: Photosystem II reactions [18]

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[17] Thayaril-Santahakumar, Nischanth. "Mechanism of Herbicide Resistance in Weeds." *Plant & Soil Science*. Web. 26 Apr. 2010. <http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF>.

[18] "Light and Oxygenic Photosynthesis: Energy Dissipation as a Protection Mechanism against Photo-oxidation." *Nature Publishing Group : Science Journals, Jobs, and Information*. Web. 09 May 2010. <http://www.nature.com/embor/journal/v6/n7/fig_tab/7400460_f1.html>
Nield J, Orlova EV, Morris EP, Gowen B, van Heel M, Barber J (2000) 3D map of the plant photosystem II supercomplex obtained by cryoelectron microscopy and single particle analysis. Nat Struct Biol 7: 44–47. Reproduced with permission.

Insecticides act as non-reducible analogs of PQB in the PSII. They bind to the D1 proteins in the PSII and due to their higher affinity with the proteins they cannot be replaced by the PQB, thus inhibiting electron transport[19].

2.5 *Elodea canadensis*

The organism used for the investigation was *Elodea canadensis.* It is an aquatic plant that has wide but thin leaves and relatively large organelles clearly visible under the microscope[20]. *Elodea* was used for the experiment as its traits are essential in examining the phytotoxic effects as the plant. As it is aquatic it can constantly be exposed to the insecticides. This helped in showing the full extent of the effects of the insecticides. Also the characteristics of the leaves allow the changes in the morphology and histology to be clearly visible. Its large chloroplast is also ideal for chloroplast isolation[21].

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Britton, N.L., and A. Brown. 1913. Illustrated flora of the northern states and Canada. Vol. 1: 105

Figure 8: *Elodea canadensis*[19]

[19] Thayaril-Santahakumar, Nischanth. "Mechanism of Herbicide Resistance in Weeds." *Plant & Soil Science*. Web. 26 Apr. 2010. <http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF>.

[20] "Aquatic Plants." *Untitled Document*. Web. 07 Feb. 2010. <http://www.botgard.ucla.edu/html/botanytextbooks/lifeforms/aquaticplants/fulltextonly.htm

[21] "Brazilian Elodea or Egeria." *Department of Plant Pathology, Physiology and Weed Science | Virginia Tech*. Web. 19 May 2010. <*http://www.ppws.vt.edu/scott/weed_id/eldde.htm*>.

3. Research question

What are the phytotoxic effects of the horticultural insecticides malathion, cypermithrin, imidacloprid on *Elodea canadensis?*

In order to answer the research question, it was separated into two parts.

1. What are the phytotoxic effects of the insecticides on the *Elodea canadensis*'s morphology?
2. What is the percentage inhibition of the insecticides on photosynthesis in isolated chloroplast of *Elodea canadensis*?

4. Materials and methods

4.1 Experimental design

The experiment was separated into two parts:

In the first part *Elodea canadensis* was exposed to five concentrations of malathion, cypermethrin, imidacloprid and water (control) for three days. The physical changes as well as the cellular changes of the plants were examined after exposure to the insecticide. The chlorophyll content of each plant was then calculated to quantify the changes. This part of the experiment would enable the investigation of the phytotoxic effects of insecticides on a whole plant.

In the second part of the experiment, the rate of the photosynthetic electron transport of isolated chloroplast exposed to the insecticides was measured spectrophotometrically. An artificial electron acceptor 2,6-dichlorophenolindophenol (DCPIP) was used to accept electrons from the splitting of water to become reduced in isolated chloroplasts. The reduction of DCPIP from blue to colorless was measured over time by a spectrophotometer at 600nm wavelength. The extent of decolorization of DCPIP is theoretically proportionate to the rate of the photosynthetic electron transport in the isolated chloroplast. This was thus used to calculate the percentage inhibition of photosynthetic electron transport by the insecticides.

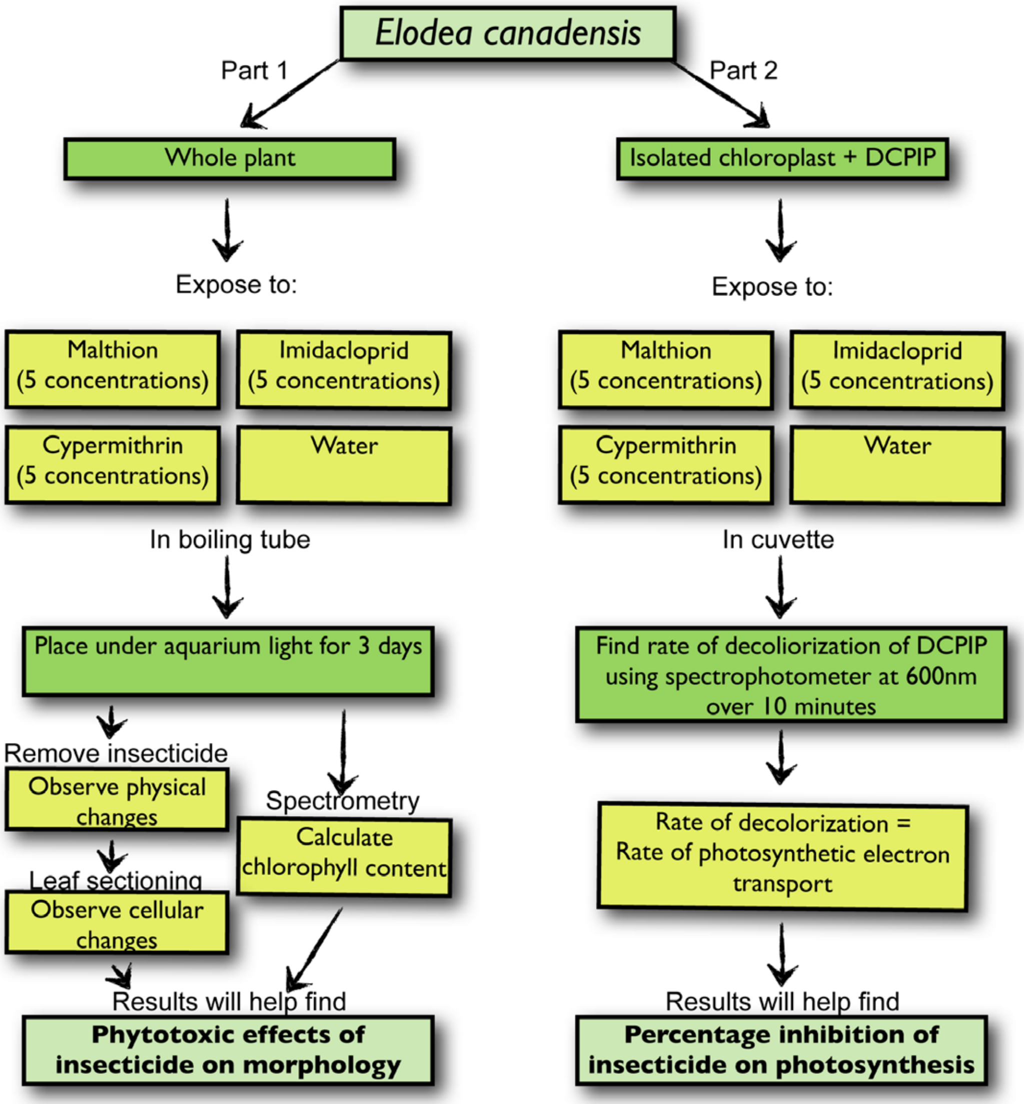


Figure 9: Flow chart of the experimental design

4.2 Materials

Table 1: Table of Materials used

Part 1: Phytotoxic effects of Insecticides on the morphology of *Elodea canadensis*	
a. Exposing of Elodea to insecticide	
Chemicals/Organism	Apparatus
84.3% Malathion insecticide	Boiling tube holder
15% Cypermethrin	Knife
1% Imidacloprid	$50cm^3$ measuring cylinder ($\pm 1.0cm^3$)
Distilled water	Micropipette ($\pm 0.01cm^3$)
Elodea canadensis	Boiling tube holder
-	16x boiling tube
-	Aquarium lamp
-	2x Retort stand
-	Ruler ($\pm$ 0.05cm)
-	Light Microscope
-	Slide
-	Cover slip
b. Chlorophyll content calculation	
Chemicals/Organism	Apparatus
Elodea canadensis (control and exposed to insecticide)	$100cm^3$ measuring cylinder ($\pm 1.0cm^3$)
Distilled water	16x $100cm^3$ beakers
90% acetone	Pestle and motar
-	4x Cheese cloth
-	Strainer
-	Centrifuge
-	6x $15cm^3$ centrifuge tubes
-	Micropipette ($\pm 0.01cm^3$)
-	2x cuvette
-	UV1240 Shimadzu Spectrophotometer (absorbance 600nm $\pm$ 0.005)

Part 2: Measuring percentage inhibition on photosynthetic electron transport	
Chemicals/Organism	Apparatus
Elodea canadensis	$100cm^3$ measuring cylinder ($\pm 1.0cm^3$)
Ice cold homogenizing buffer (0.02 M Tris, pH 8, containing 0.01 M NaCl and 0.4 M sucrose)	$50cm^3$ measuring cylinder ($\pm 1.0cm^3$)
Ice cubes	$10cm^3$ measuring cylinder ($\pm 1.0cm^3$)
84.3% Malathion insecticide	16x $100cm^3$ beakers
15% Cypermethrin	5 x $500cm^3$ beaker
1% Imidacloprid	4x Cheese cloth
Distilled water	Strainer
DCPIP Powder	Centrifuge
	6x $15cm^3$ centrifuge tubes
	Micropipette ($\pm 0.01cm^3$)
	6x cuvettes
	UV1240 Shimadzu Spectrophotometer (absorbance 600nm ± 0.005)
	Electronic mass balance ($\pm 0.01g$)
	Pestle and mortar
	Droppers
	Stop watch ($\pm 0.01s$)
	Parafilm
	Glass rod
	Aluminium foil

4.3 Methods

4.3.1 Measuring the phytotoxic effects of Insecticides on the morphology of *Elodea canadensis*

This procedure was seperated into two different parts: **a**, the exposing of the plant to the insecticide, and **b,** the calculation of the chlorophyll content. The experiment was set up as shown in **figure 10**.

Figure 10: Experimental setup for test on phytotoxicity of Insecticide on *Elodea canadensis*

a. Exposing of *Elodea canadensis* to insecticide

Elodea canadensis plants weighing 5g were cut from the purchased plant using a knife and a weighing balance. Each plant was then placed in boiling tubes containing specific concentrations of insecticide solutions as shown in the three figures below. The Boiling tubes were then placed in a boiling tube holder under an aquarium light for 3 days as shown in **Figure 11** below.

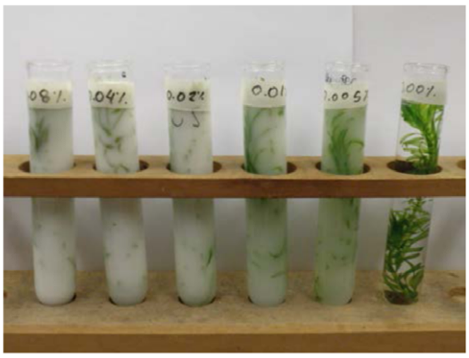
	Malathion Insecticide. Concentration from left to right: 0.28%, 0.14%, 0.07%, 0.035%, 0.0175%, Control (water)
	Cypermithrin Insecticide. Concentration from left to right: 0.20%, 0.10%, 0.05%, 0.025%, 0.0125%, Control (water)
	Imidacloprid Insecticide. Concentration from left to right: 0.08%, 0.04%, 0.02%, 0.01%, 0.005%, Control (water)

Figure 11: *Elodea canadensis* in boiling tube exposed to each insecticide at different

Comparison of morphology of the plant was made after 3 days of being submerged in the solutions. Each *Elodea canadensis* plant exposed to the different concentration of insecticides was then examined under the light microscope. A leaf sectioning was done using a potato and a blade to make the cells as thin as possible, in order to obtain a clear image of the cell histology. The sectioned leaf was then examined under the light microscope at 400x magnification and the same light intensity. The histology of the leaves was then compared between the control and the varying insecticide solutions, as well as the different insecticides. The figure below shows the histology of an *Elodea canadensis* before its use in the experiment.

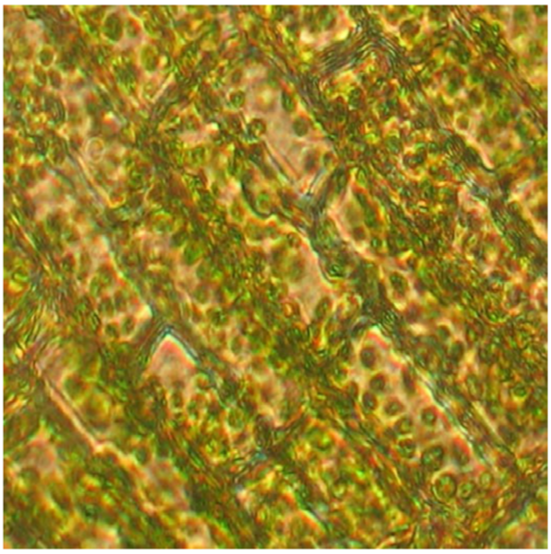


Figure 12: *Elodea Canadensis* leaf after sectioning at 400x magnification

b. Measurement of chlorophyll content (adapted from Arnon[22])

Chloroplast was first isolated from the plants (adapted from Edward A. Funkhouser, and Donna E. Balint)[23]. Each plant was washed under the sink to remove all insecticide before the experiment was conducted. It was then grounded in 10 cm^3 homogenizing buffer using a mortar and pestle. The homogenate was strained through 4 layers of cheesecloth. The filtrate was then centrifuged at 1000xg for 5 minutes to remove whole cells. The supernatant was centrifuged at 3000xg for 3 minutes to spin down the chloroplast. The supernatant containing cell debris was decanted. The pellet was re-suspended into 5 cm^3 of buffer.

An absorbance reading of each chloroplast suspension was then taken. 1.0 cm^3 of the isolated chloroplast solution of each insecticide was mixed with 5.0 cm^3 of 90% acetone in a centrifuge tube. The solution was then centrifuged at 1300xg for 3 minutes. The absorbance of the supernatant was read at 652 nm (A_{652}) using a blank containing 5.0 cm^3 of 90% acetone. The chlorophyll concentration was calculated by the following formula[24].

Formula
$\frac{mgChlorophyll}{ml} = A_{652} \times \frac{100}{34.5}$
Example
For example, if A_{652} = 0.125 then, $\frac{mgChlorophyll}{ml} = 0.125 \times \frac{100}{34.5} = 0.362$ ∴ The chlorophyll content of the plant would be 0.362 mg ml^{-1}

[22] Arnon, Daniel I. "COPPER ENZYMES IN ISOLATED CHLOROPLASTS. POLYPHENOLOXIDASE IN BETA VULGARIS." *PLANT PHYSIOLOGY* 24 (1949): 1-15.

[23] Edward A. Funkhouser, and Donna E. Balint. "The Hill Reaction: In Vitro and In Vivo Studies."

[24] 34.5 is constant

Experimental Variables	
Dependent variable	The morphology of the plant will change after exposure to the insecticides. It is directly affected by the independent variables. It was measured qualitatively, by comparing the whole plants exposed to each insecticide with the varying concentrations to the whole plant that was not exposed to any insecticide (control). The cells of the plants exposed to each insecticide with the varying concentrations were also compared to the cells of the plant that was not exposed to any insecticide (control).
	The chlorophyll content of the plant after exposure to the insecticide will change. It is directly affected by the independent variables. It was measured quantitatively, by isolating the chloroplast form each plant and using Arnons method toy calculate the chlorophyll. The chlorophyll content of the plants exposed to each insecticide with the varying concentrations was compared to the cholorophyll content of the plant that was not exposed to any insecticide (control).
Independent variable	The type of horticultural insecticide used for the experiment. Different insecticides have different molecular structure and thus different chemical properties. This would thus make them have varying phytotoxic effects on the plants. The three insecticides that were used for the investigation are malathion, cypermethrin, and imidacropid. Each of which are from different classes of insecticides. Malathion is an organophosphate insecticide, cypermethrin is a synthetic pyrethriod insecticide, and Imidacloprid is a neonicotinoid insecticide.
	Different concentrations of insecticides will have different phytotoxic effects on the plant. The higher the concentration the more adverse the phytotoxiic effects. The concentration of insecticide solutions was determined from the box of each insecticide. That is the recommended concentrations given by the insecticide companies. The five concentrations were malathion: 0.28%, 0.14%, **0.07%**, 0.035%, 0.0175%, cypermethrin: 0.20%, 0.10%, **0.05%** 0.025%, 0.0125%, and imidacloprid: 0.08%, 0.04%, **0.02%,** 0.01%, 0.005%. (bolded are the recommended concentrations). 0.00% was used as a control.
Controlled variable	Type of plant: *Elodea canadensis.* The plant was used as it had the traits that are essential in examining the phytotoxic effects of the insecticides.
	Mass of *Elodea canadensis.* The mass of the plant was made equal, 5 g, to ensure that the cell number in all the plant is approximately the same.
	Exposure to aquarium light. Plant photosynthesis can only occur in the presence of light, thus all the leaves need to be exposed to light. In order to ensure that all the leaves are exposed, the experiment was set-up in to ensure that this happened. The set-up can be seen in the methodology.
	The period of time which the *Elodea canadensis* is exposed to the insecticide solution. In the experiment it was three days. This time was chosen as it compliments the time insecticides are usually present on agricultural plants before its carried away or decomposed by some means or the other.

4.3.2 Measuring percentage inhibition on photosynthetic electron transport

The degree of inhibition was measured by using the spectrophotometer to quantify the rate decolorization of DCPIP by the isolated chloroplast exposed to the insecticides. 50 g of *Elodea canadensis* was used to isolate chloroplast. This was mixed with different concentrations of insecticides and DCPIP in a cuvette. The concentrations used for all the insecticides were 0.001%, 0.002%, 0.003%, 0.004%, 0.005% and 0.000% as the control. The DCPIP solution was made by mixing 0.1 grams of DCPIP with 400cm^3 of distilled water to make 0.025% DCPIP. 1.5 cm^3 of insecticide and 1.5cm^3 chloroplast suspension were added to a cuvette. Using parafilm as a cover, the cuvette was inverted to mix the solution. This was placed in the first slot of the spectrophotometer and used as the blank. 1.5cm^3 of DCPIP was then added to the cuvette and inverted again to mix the solution. The Absorbance readings were then taken and retaken every minute for ten minutes. The same step was repeated for the control and all other concentrations for each insecticide. This experiment was carried out three times.

The data was used to find the cumulative change of absorbance ($A_{Cumulative}$) for each minute. This was calculated according to the following formula at a specific concentration[25].

Formula
$A_{cumulative}$ at time X min= $\lvert A_{600}$ at time X min $- A_{600}$ at time 0 min$\rvert$
Example
For example, if A_{600} at 10 minutes= 0.036 , and at 0 minutes = 0.125 $A_{cumulative}$ at time 10 min= $\lvert 0.037 - 0.125 \rvert = 0.089$ ∴ The cumulative absorbance at 10 minutes would be 0.089

[25] The negative sign was ignored since it only represents the decrease in absorbance, which would be common to all the samples.

The cumulative data was plotted and the gradient was used to calculate the rate of photosynthetic electron transport and thus percentage of inhibition. This was calculated by the following formulas:

Formula
$\text{Rate of electron transport} = \frac{\Delta A_{600} / \text{min}}{\varepsilon} \times 10^6 \, \mu\text{mol mol}^{-1} \times \text{V}$
Where $\Delta A_{600} / \text{min}$: The slope of the line in the $A_{cumulative}$ versus time graph.
ε : The extinction coefficient for DCPIP at 600nm, 16,000 liters per mol^{-1}
V : The volume of the reaction mixture, in liters, 0.0045
Example
For example, if $\Delta A_{600} / \text{min}$ of the graph = 0.0049
$\text{Rate of electron transport} = \frac{0.0049}{16000} \times 10^6 \times 0.0045 = 0.00138$
∴ The rate of electron transport would be at 10 minutes would 0.00138 $\mu\text{mol min}^{-1}$

Formula
$\text{Percentage inhibition} = 100 - \left(\frac{A_{cumulative} \text{of concentration Y at 10 minutes}}{A_{cumulative} \text{of control at 10 minutes}} \right) \times 100$
Example
For example, if $A_{\text{cumulative}}$ at 10 minutes of 0.001% malathion = 0.077 and $A_{\text{cumulative}}$ at 10 minutes of control= 0.0092
$\text{Percentage inhibition} = 100 - \left(\frac{0.077}{0.092} \right) \times 100 = 16.3$
∴ The percentage of inhibition of 0.001% malathion on photosynthesis would be 16.3%

Experimental Variables	
Dependent variable	Absorbance reading at regular intervals of 1 minute for 10 minutes, for every concentration of insecticide tested. Spectrophotometers help measure the absorbance of a specific wavelength of light by a solution. According to different wavelength and solutions this absorbance value would change differently. The rate of photosynthesis can be measured by the rate chloroplast takes to reduce DCPIP from blue to colorless, which is also the rate of decrease in the absorbance value of the solution.
Independent variable	The type of horticultural insecticide used for the experiment. Different insecticides have different molecular structure and thus different chemical properties. This would thus make them have varying phytotoxic effects on the plants. The three insecticides that were used for the investigation are malathion, cypermethrin, and imidacropid. Each of which are from different classes of insecticides. Malathion is an organophosphate insecticide, cypermethrin is a synthetic pyrethriod insecticide, and Imidacloprid is a neonicotinoid insecticide.
	Different concentrations of insecticides will have different phytotoxic effects on the plant. The higher the concentration the more adverse the phytotoxic effects. The five concentrations used were 0.001%, 0.002%, 0.003%, 0.004%, 0.005% and 0.000% as the control. These concentrations of insecticide solutions were chosen as isolated chloroplast are very sensitive, and if the concentrations were too high the chloroplast would not function.
Controlled variable	Type of plant: *Elodea canadensis*. The plant was used, as it was the same plant used in the earlier experiment. Chloroplast was isolated from the plant.
	Light wavelength. Plant photosynthesis can only occur in the presence of light. The wavelength used in spectrophotometer was 600nm, which is red light. It was chosen as plants can photosynthesize under this wavelength.
	Concentration of chloroplast suspension. The concentration of the chloroplast has to be maintained the same to ensure that the same number of chloroplast are used for each insecticide, and concentration in the experiment. Stirring the isolated chloroplast suspension well, before use, ensures this.
	Reaction mixture composition. The reaction mixture contained 1.5cm^3 0.025% DCPIP, 1.5 cm^3 of a specific concentration of one insecticide and 1.5cm^3 chloroplast suspension.
	Rate of reduction of DCPIP of control. A control solution with 0.000% insecticide was used to measure the rate of reduction of DCPIP. This can thus be used to compare with the solution containing the insecticides in order to see the degree of inhibition it has on photosynthesis.
	Total exposure time in light. The time by which chloroplast is exposed to light must be the same, in order to ensure that the same amount of photosynthesis has occurred for all the different solutions in the investigation. The time used was 10 minutes.

5. Results

5.1 Phytotoxic effects on plant Morphology

In order to investigate the phytotoxicity of the insecticides on the whole plant, the morphology of each plant exposed to the different insecticides was compared to that of the control.

The effect of the insecticide on the whole plant can be seen in **figure 10**. In all the plants exposed to the insecticide, there was a clear decolorization of the plant leaf and stem as the concentration of insecticide increased compared to that of the control. The point at which the decolorization visually starts is however different for each insecticide. For malathion decolorization can visually be seen to start from 0.0175% concentration onwards. For cypermithrin the decolorization can be visually seen to start from 0.025% concentration onwards. For Imidacloprid the decolorization can visually be seen to start from 0.02% concentration onwards. The degree of the decolorization at the highest concentration of each insecticide is also different. The plant exposed to the highest concentration of malathion was observed to be the most decolorized, followed by cypermithrin, then imidacloprid. However all the plants showed phytotoxic effects at concentrations recommended by the box of insecticide or lower.

The morphology of the cells sampled from each plant also showed a similar trend. This can be seen in **Figure 11**. The decolorization of the whole plant can be seen to be due to the bleaching of chloroplast. It is unclear as to where the decolorization begins, however as the concentration of insecticide increases, the green color of the chloroplast becomes more bleached. Chlorophyll gives the chloroplast its green color; thus, there is a decrease in content of chlorophyll as the insecticide concentration increased. The cells exposed to malathion were observed to be the most bleached followed by cypermithrin and finally Imidacloprid.

5.1.1 Phytotoxic effects on whole plant

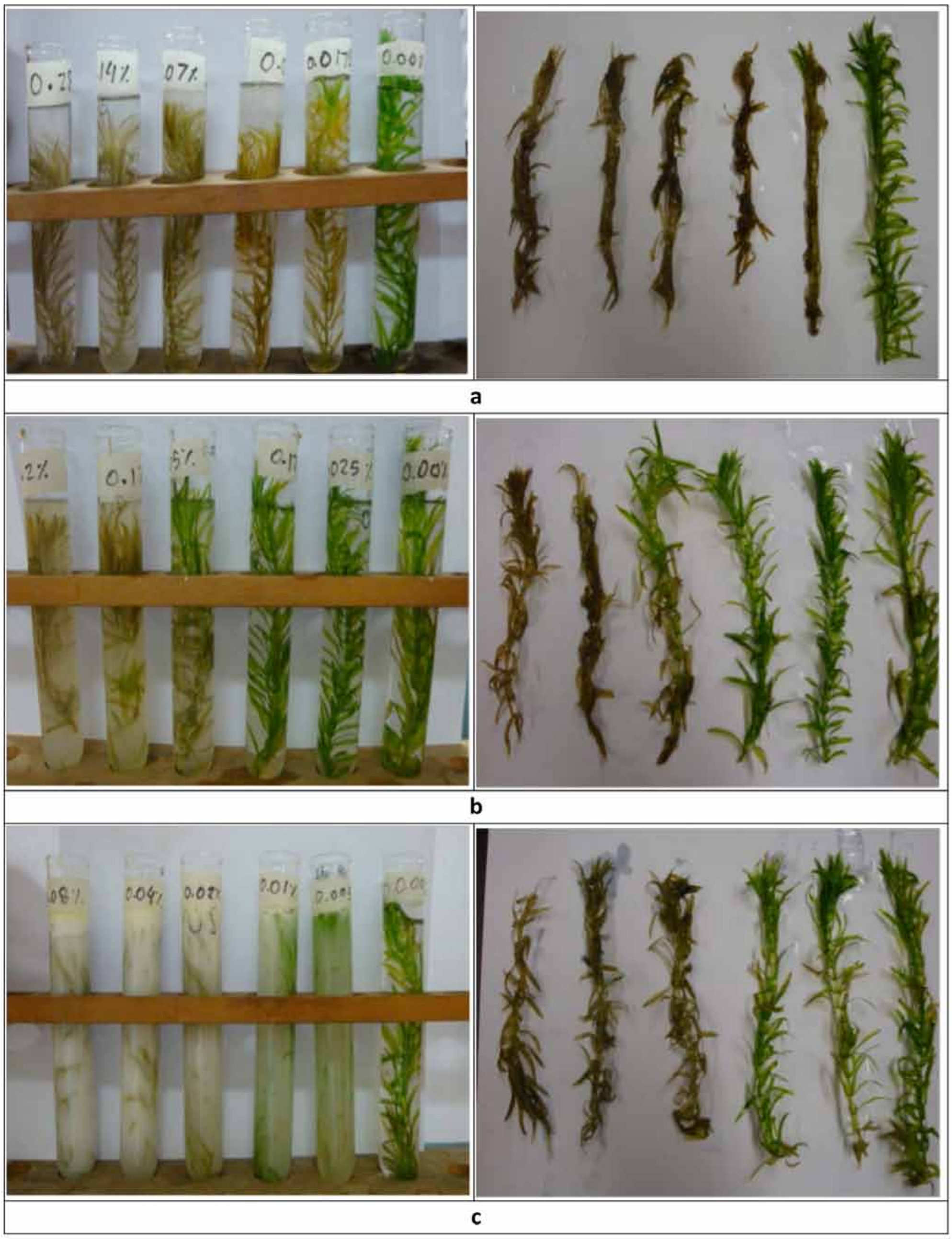


Figure 13: (a) Elodea after exposure to malathion Insecticide. Concentration from left to right: 0.28%, 0.14%, 0.07%, 0.035%, 0.0175%, 0.00% (control) **(b)** Elodea after exposure to cypermithrin Insecticide. Concentration from left to right: 0.20%, 0.10%, 0.05%, 0.025%, 0.0125%, 0.00% (control) **(c)** Elodea after exposure to imidacloprid Insecticide. Concentration from left to right: 0.08%, 0.04%, 0.02%, 0.01%, 0.005%, 0.00% (control)

5.1.2 Chlorophyll content

It was seen that the phytotoxic effects of the insecticide led to a decrease in chlorophyll content. In order to quantify this phytotoxic effect, the chlorophyll content of each plant used in the earlier experiment was measured. The fall in chlorophyll concentration corresponded with the qualitative results shown earlier.

As the concentration of insecticide increased there was a general decrease in the chlorophyll concentration of the plants exposed to all the insecticides, as can be seen in **Graph 1**. There is a significant decrease in concentration of chlorophyll in the plant exposed to malathion from 0.0175% concentration onwards. For cypermithrin the drop can be seen at 0.025% concentration onwards. For imidacloprid the drop is can be seen to start from 0.02% concentration onwards. The final chlorophyll concentration of imidacloprid was highest followed by cypermthrin then malathion.

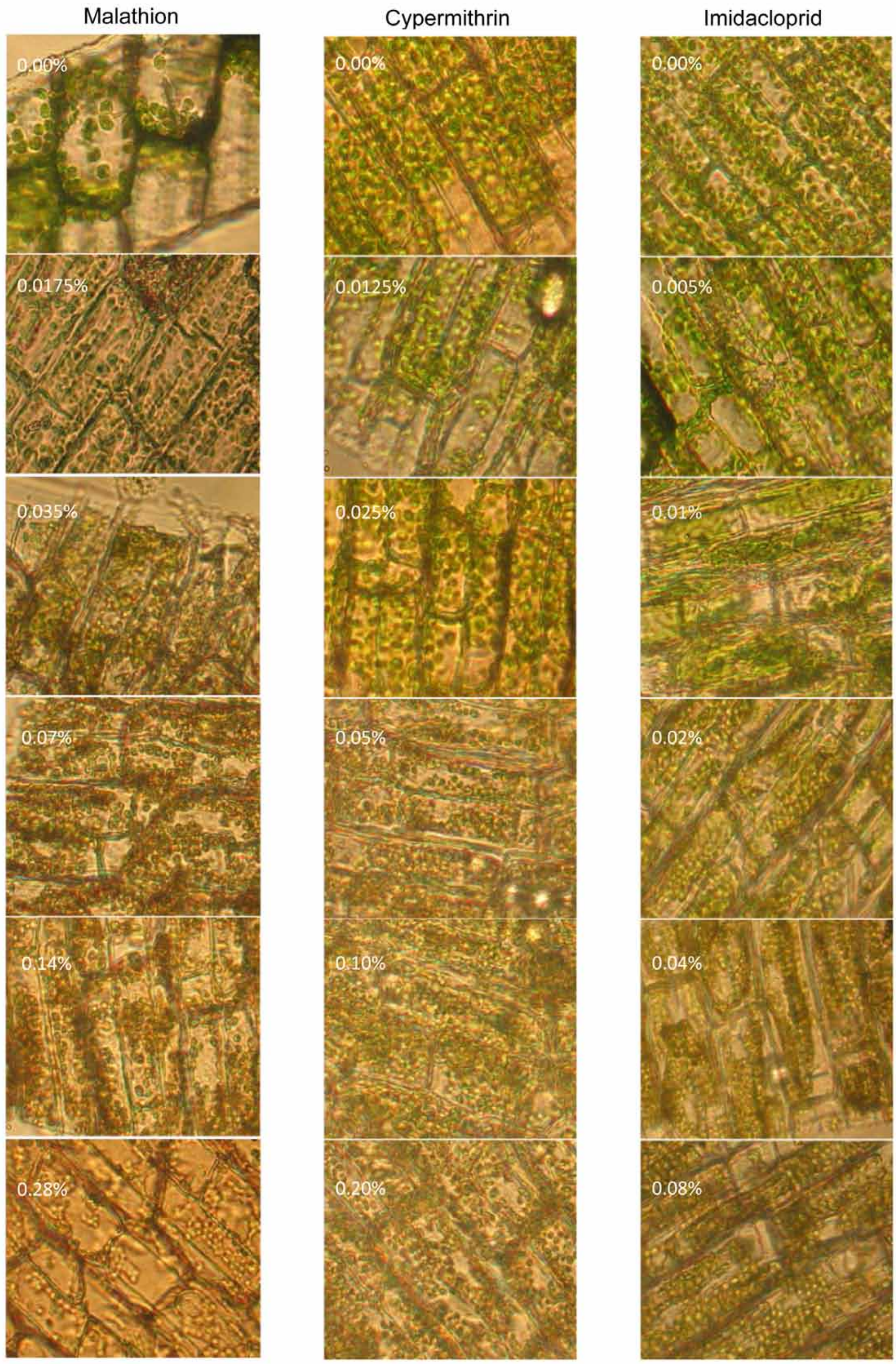
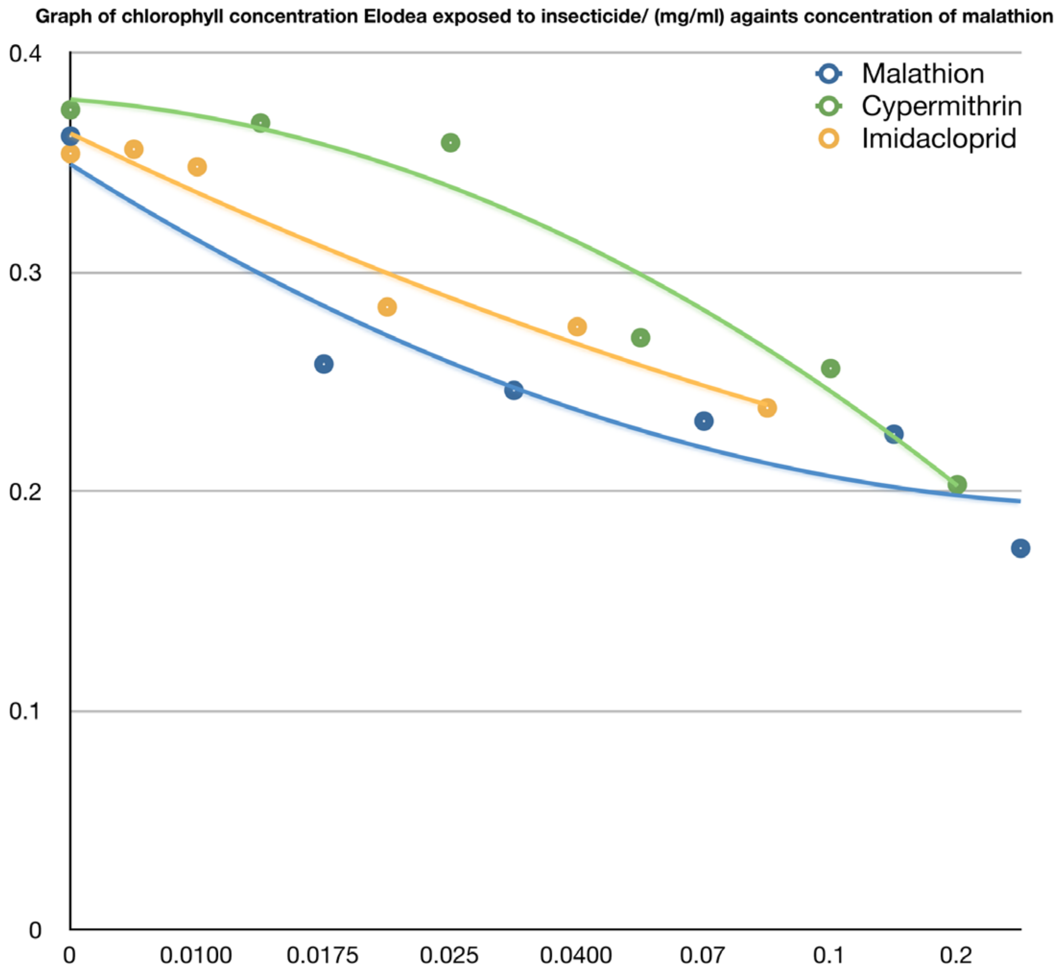


Figure 14- Exposed Plant cells at 400x magnification



Graph 1: Graph of Chlorophyll concentration of Elodea exposed to Insecticide/ (mg/ml) against concentration of Insecticide/%

5.2 Percentage inhibition on electron transport

5.2.1 Rate of photosynthetic electron transport

The results from the earlier experiments indicated that the insecticides affect the chloroplasts in the plants. Thus the rate of decolorization of DCPIP by the isolated chloroplast exposed to the insecticides was measured. This reflects the rate of photosynthetic electron transport during photosynthesis in the chloroplast. The DCPIP was reduced when the chloroplast began photosynthesizing, resulting in the decolorization of the solution as time passed. The absorbance values showed the degree of decolorization of DCPIP at specific times.

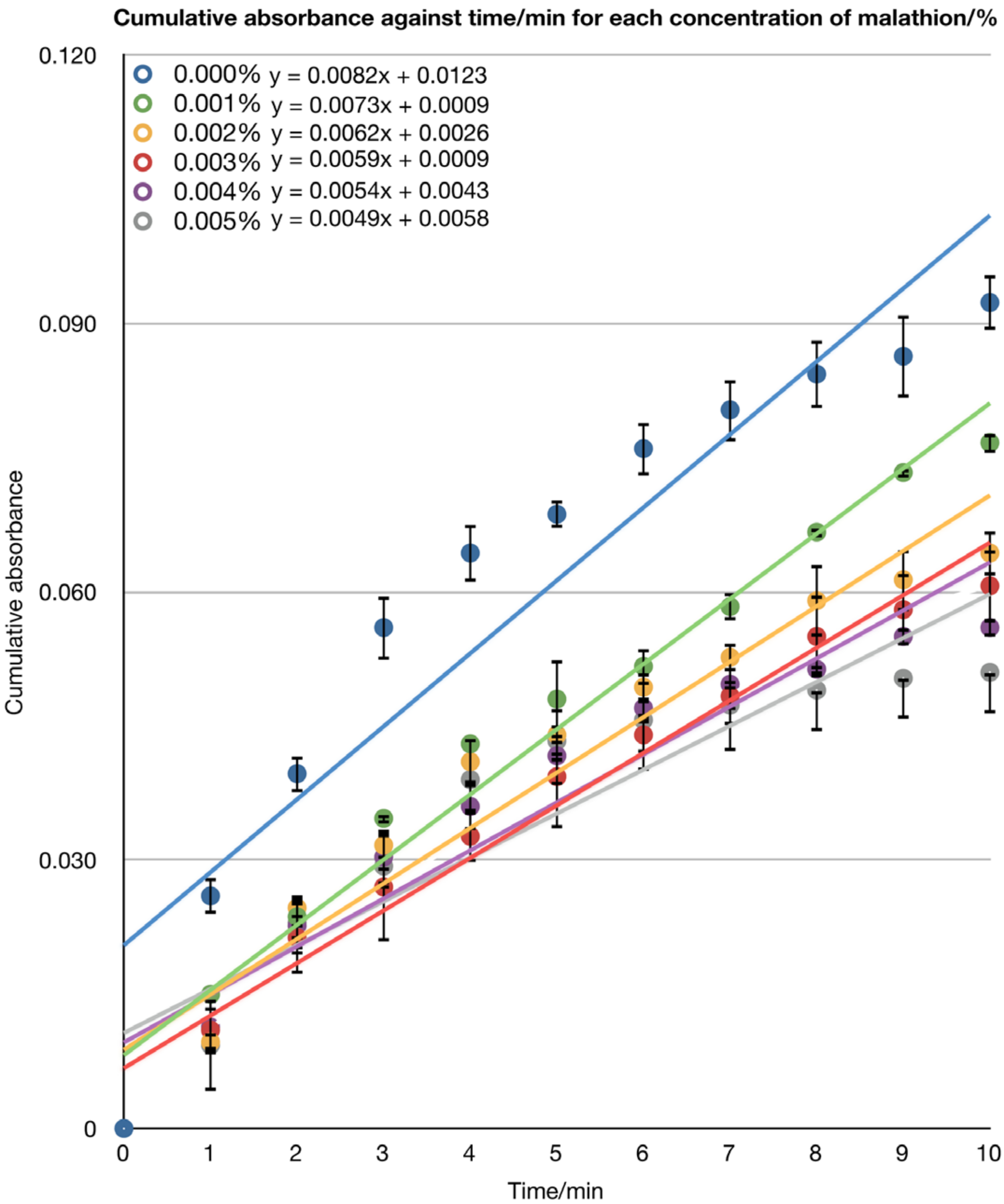
The results showed a general decrease in the average absorbance for all insecticides[26]. However the absorbance value at 0 minutes was different for each concentration of insecticide. It was thus necessary to calculate the cumulative change in absorbance. This was done by subtracting the absorbance reading at a particular time point from the initial absorbance reading at time 0. These values were plotted as a graph.

From **Graph 2** it can be seen that the $A_{Cumulative}$ values increased as time increased. As the concentration of malathion insecticide increased there was a decrease in all the values of the absorbance relative to that of the control. The gradient of the best-fit lines plotted from these results indicate the rate of decrease of absorbance for the insecticide. At 0.000% (control) the gradient of the graph was 0.008, at 0.001%- 0.007, at 0.002%- 0.006, at 0.003%- 0.005, at 0.004%- 0.005, and at 0.005%- 0.004. Similar observations were seen for the other two insecticides cypermithrin and imidacloprid[27]. The gradients were used to plot **Graph 3.**

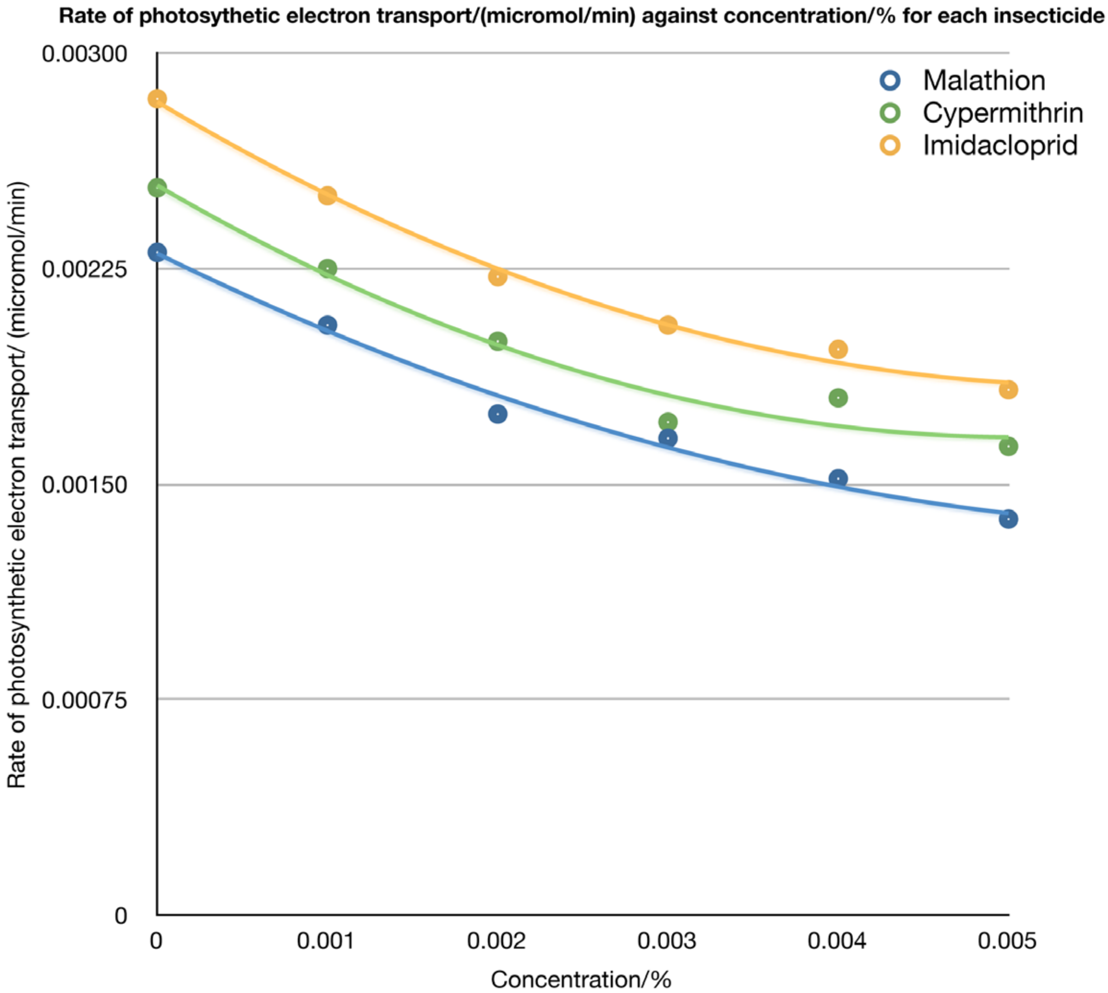
From **Graph 3**, it may be observed that as the concentration of insecticide increased, the rate of photosynthetic electron transport decreased for all insecticides. This indicates that the increase in concentration resulted in an increase in the inhibition of the photosynthetic electron transport.

[26] Raw data in the appendix

[27] Can be seen in **Graph 5**, and **Graph 6** in the appendix



Graph 2: Graph showing Cumulative absorbance against time/min for each concentration of malathion/%

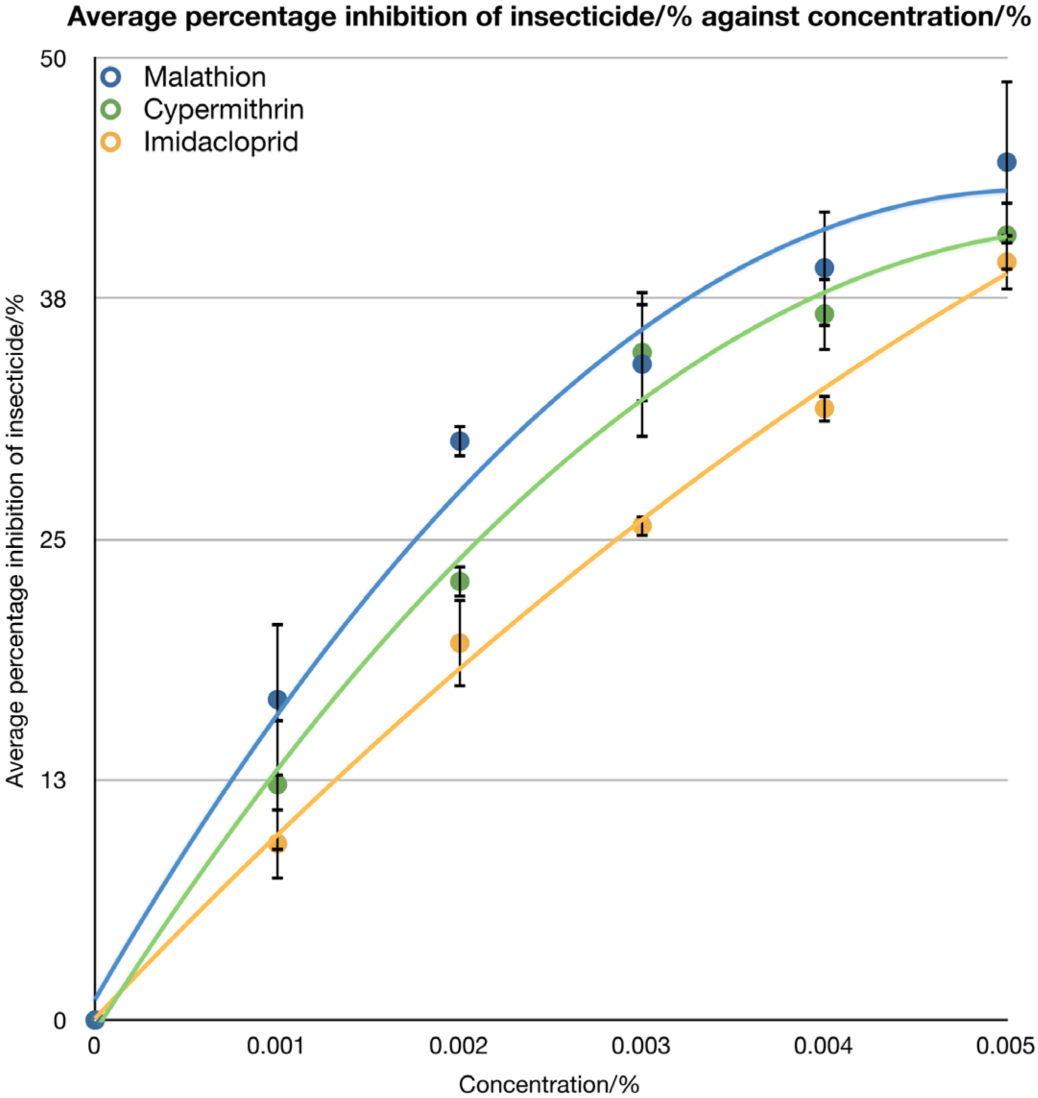


Graph 3: Graph showing rate of photosynthetic electron transport/(micromol/min) against concentration/% for each insecticide

5.2.2 Percentage inhibition

Graph 3 showed that as the concentration of insecticide increased, the inhibition of the insecticide increased, but it does not show the degree of inhibition of each insecticide on the photosynthetic electron transport. It shows that malathion insecticide has the most adverse effects on the plants. Thus the cumulative values were used to calculate the percentage inhibition of each insecticide at a specific time, and the values were plotted as a graph.

From **Graph 4** it can be seen that the percentage inhibition of photosynthetic electron transport increases as the concentration of the insecticide increases. All the values of the percentage inhibition of malathion were larger then that of cypermithrin. All the values of cypermithrin were larger then that of imidacloprid. At 10 minutes the percentage inhibition of malathion was 45%, of cypermithrin was 41%, and of imidacloprid was 39%. All insecticides inhibit photosynthesis by a very high percentage, malathion being the most inhibiting followed by cypermithrin, then imidacloprid.



Graph 4: Graph showing percentage inhibition of each insecticide/% against Insecticide concentration/%

6. Discussion

Insecticides are known to interfere with electron transport at the PS II during the light reactions of photosynthesis. They act as non-reducible analogs of plastoquinone-B (PQB) in photosystem II (PSII). They bind to the D1 proteins in the PSII and due to their greater affinity to the proteins they cannot be replaced by the PQB, thus inhibiting electron transport.[28] The aim of this investigation was to investigate the phytotoxic effects of the horticultural insecticides malathion, cypermithrin, imidacloprid on *Elodea canadensis.* The results obtain showed that all the insecticides had an acute phytotoxic effect on *Elodea canadensis*.

The insecticides had severe phytotoxic effects on the morphology of *Elodea canadensis* at high concentrations, and varying effects at lower concentrations. There was a general decrease in the chlorophyll content as the concentration of each insecticide increased, proving that the phytotoxic effects of the insecticides are linked to the chloroplast. All the insecticides inhibited the photosynthetic electron transport, and the percentage of inhibition increased as the concentration of insecticide increases. Different insecticides however have different phytotoxic effects, as some bind more easily to the D1 proteins due to more available oxygen atoms with lone pairs for hydrogen bonding. From the results it was seen that malathion exhibit the most phytotoxic effect followed by cypermithrin then imidacloprid. How these results answer the research question are discussed below.

6.1 What are the phytotoxic effects of the insecticides on *Elodea*'s morphology?

On whole plants the insecticide caused a decolorization of the plant leaf and stem. When examined under the microscope it could be seen that the green color of the chloroplast becomes more bleached. This is due to peroxidation of the chloroplast membrane. The binding of the insecticide molecules to the D1 proteins causes the production of triplet chlorophyll molecule

[28] Thayaril-Santahakumar, Nischanth. "Mechanism of Herbicide Resistance in Weeds." *Plant & Soil Science*. Web. 26 Apr. 2010. <http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF>.

along with oxygen radicals. These molecules get reduced by electrons from the lipids in membranes causing the chloroplast membrane to lose its integrity. This results in the leakage of chlorophyll and other contents of the chloroplast[29]. Therefore as the concentration of insecticide increases, more triplet chlorophyll molecule and oxygen radicals would be present in the system to undergo peroxidation, and more chlorophyll leaked from the chloroplast.

The decolorization of the plant could only be seen to start when it was exposed to certain concentrations of insecticide. This was because at that concentration there are enough radicals undergoing peroxidation to cause disruption of the chloroplast membrane. Once this occurred the chlorophyll would leak out of the chloroplast, and there would be visible bleaching of the plant color.

The chlorophyll concentrations calculated quantified the qualitative results discussed above. There was a significant decrease in concentration of chlorophyll in the plant exposed to malathion from 0.0175% concentration onwards, for cypermithrin 0.025% onwards and for imidacloprid from 0.02% onwards. As mentioned this was due to the disruption of the chloroplast membrane. The total drop in chlorophyll content of the plant however confirmed which insecticide was the most phytotoxic to the plant. Malathion caused a 0.188 $mgml^{-1}$ drop, cypermithrin a 0.171 $mgml^{-1}$ drop, and imidacloprid a 0.116 $mgml^{-1}$. Malathion was the most phytotoxic insecticide. This would be because the malathion molecule has a higher tendency to bind with the D1 protein compared to the other insecticides due to presence of more oxygen atoms available for hydrogen bonding.

6.2 What is the percentage inhibition of the insecticides on photosynthesis?

For all insecticides there was a general decrease in the rate of decrease in cumulative absorbance. As the concentration of insecticide increased there were more insecticide molecules available to bind to the D1 protein and inhibit electron flow. The rate of electron transport decreased, as there

[29] "Lipid Peroxidation Definition." *Department of Biochemistry, Molecular Biology, and Cell Biology - Weinberg College of Arts and Sciences - Northwestern University*. Web. 26 Apr. 2010. <http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-L/lipid_peroxidation.html>.

were fewer pathways where PQB was present to accept electrons from PQA. This was confirmed by the measure of the percentage inhibition of photosynthetic electron transport. It increased as the concentration of the insecticide increased. The values of the percentage inhibition of malathion after 10 minutes was 45%, for cypermithrin was 41%, and for imidacloprid was 39%. All insecticides inhibited photosynthesis by a very high percentage, malathion being the worst followed by cypermithrin, then imidacloprid.

7. Evaluation

The experiment conducted for this investigation had some limitations. As it was the light dependent reactions that was being observed, stray light could affect the absorbance readings. In order to prevent this from occurring the entire experiment should be conducted in a relatively dark laboratory. Also as DCPIP is sensitive to light it must be prepared in absence of light. However due to constraints light entering the experiment could only be minimized using aluminium foil.

Another limitation to experimental procedure was leaving out the pH measurement of all the buffer and sample mixtures. As pH could upset the integrity of the isolated chloroplast, change in pH could affect the results significantly. Any mixture with too high or too low a pH could inhibit all the reactions by large extent by disrupting the chloroplast. Measurement of pH for each sample mixture could also give a good indication of the pH value that becomes inhibiting to the light dependent reactions.

8. Relevance to practical applications

The results showed that the insecticides had drastic phytotoxic effects on whole plants even at concentrations recommended by insecticide companies. The severity can be seen by the percentage inhibition of the insecticides on photosynthetic transport. Thus the usage of these insecticides should be minimized. Not only does it have adverse effects on the environment, but also the species that is trying to protect the pest from. If it is vital for crop growth, insecticides such as imidacloprid should be used as it has a lower phytotoxic effect, and percentage inhibition on plants. Farmers should be informed of this information, as well as insecticide producing companies.

Further investigation could also be done on the inhibitory effects of other insecticides on plant photosynthesis. There are several other classes of insecticides that weren't studied. Extending this investigation to all the insecticides can help determine the most appropriate insecticides that can be used for agriculture. These experiments could be done on agricultural crops in the field to give a better indication of the phytotoxic effects of the insecticides on crops. By doing all this agricultural sectors can use insecticides effectively in improving the quantity and quality of crop yield.

9. Conclusion

In the investigation, the inhibitory effect of insecticides was examined at a macroscopic and a microscopic level. The three horticultural insecticides, from different classes, malathion, cypermithrin and imidacloprid, were used to test its phytotoxic effects on a whole plants, and its inhibitory effects on photosynthesis of isolated chloroplast. Chlorophyll content of the plant after exposure to insecticide was done to establish a link between the phytotoxic effects, and the photosynthetic electron transport inhibition. On whole plants the phytotoxic effects were that insecticides disrupted to the chloroplast membrane causing chlorophyll to leak out from the organelle. This lead to the decolorization of the plant and eventually death. Malathion showed to have phytotoxic effects from 0.0175% concentration onwards, cypermithrin from 0.025% and imidacloprid the 0.02%. The insecticides inhibited the photosynthetic electron transport by a significantly large percentage. Malathion inhibited the process by 45%, cypermithrin by 41%, and by imidacloprid is 39%. This makes malathion the most phytotoxic insecticide to plants.

10. Bibliography

1. Arnon, Daniel I. "COPPER ENZYMES IN ISOLATED CHLOROPLASTS. POLYPHENOLOXIDASE IN BETA VULGARIS." *PLANT PHYSIOLOGY* 24 (1949): 1-15.
2. "Aquatic Plants." *Untitled Document*. Web. 07 Feb. 2010. <http://www.botgard.ucla.edu/html/botanytextbooks/lifeforms/aquaticplants/fulltextonly.htm
3. Bracha P, M Luwish, N Shavit 1972 Thiadiazoles of herbicidal activity. In AS Tahori, ed, Proceedings Second International IUPAC Congress of Pesticide Chemistry, Vol 5. Gordon and Breach, New York, pp 141-151
4. "Brazilian Elodea or Egeria." Department of Plant Pathology, Physiology and Weed Science | Virginia Tech. Web. 19 May 2010. <http://www.ppws.vt.edu/scott/weed_id/eldde.htm>.
5. "Cabbage Looper." *TELUS Internet Services - Member Services*. Web. 08 May 2010. <http://www3.telus.net/conrad/insects/cabloop.html>.
6. Campbell, Neil A., and Jane B. Reece. BIOLOGY. Eighth ed. San Fransisco: Pearson Benjamin Cummings, 2008.
7. "Cypermethrin (PDS)." IPCS INCHEM. 10 Jan. 2010 <http://www.inchem.org/documents/pds/pds/pest58_e.htm>.
8. Fest, D. C. (1977) Recent developments of organophosphorous pesticides, Paper presented at the I Int. Congress on Phosphorous Compounds, Rabat.
9. "Imidacloprid." PMEP Home. 10 Jan. 2010 <http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/imidacloprid-ext.html>.
10. Kannan, N. and Jayaraman, J. (1980) *Pesticide residues in the environment in India,* Proc. Symp., Bangalore, November 1978, UAS Tech. Series No. 32, Paper No. 52, p. 244.
11. "Light and Oxygenic Photosynthesis: Energy Dissipation as a Protection Mechanism against Photo-oxidation." *Nature Publishing Group : Science Journals, Jobs, and Information*. Web. 09 May 2010. <http://www.nature.com/embor/journal/v6/n7/fig_tab/7400460_f1.html>
12. "Lipid Peroxidation Definition." *Department of Biochemistry, Molecular Biology, and Cell Biology - Weinberg College of Arts and Sciences - Northwestern University*. Web. 26 Apr. 2010. <http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-L/lipid_peroxidation.html>.
13. MACKINNEY, G. Absorption of light by chlorophyll solutions. Jour. Biol. Chem. 140: 315-322. 1941.
14. Marrs, T. C. Malathion. In *Pesticide residues in food, Joint FAO/WHO Meeting on Pesticide Residues, Evaluations 1997: Part II - Toxicological and Environmental*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1998; pp 189-219.

15. Miller GT (2004), *Sustaining the Earth*, 6th edition. Thompson Learning, Inc. Pacific Grove, California. Chapter 9, Pages 211-216

16. Oregon State University,, Weniger Hall. "Malathion (Technical Fact Sheet)."

17. *Pesticide Action Network North America | Advancing Alternatives to Pesticides Worldwide*. Web. 03 May 2010. <http://www.panna.org/>.

18. P. R. ANBUDURAI, R. MANNAR MANNAN, and SALIL BOSE. "The inhibition of photosynthetic electron transport by methyl parathion." *J. Biosci* Vol. 3.Number 1 (March 1981,): 23-27.

19. Ribas, Alessandra Ferreira. "Genetic Transformation of Coffee." *Brazilian Journal of Plant Physiology* 18.1 (2006). Web.

20. Thayaril-Santahakumar, Nischanth. "Mechanism of Herbicide Resistance in Weeds." *Plant & Soil Science*. Web. 26 Apr. 2010. <http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF>.

21. "Thrips Tabaci (English) - IPM/CIIFAD." *Cornell University's New York State Agricultural Experiment Station*. Web. 08 May 2010. <http://www.nysaes.cornell.edu/ent/hortcrops/english/thrips.html>.

22. Ware and Whitacre: AN INTRODUCTION TO INSECTICIDES." *Radcliffe's IPM World Textbook | CFANS | University of Minnesota.* Web. 03 Nov. 2009. <http://ipmworld.umn.edu/chapters/ware.htm>.

23. World Health Organization. *Organophosphorus Insecticides: A General Introduction*, Environmental Health Criteria, 63, Geneva, Switzerland, 1986.

24. York AC, CJ ARNTZEN 1979 Photosynthetic electron transport inhibition with buthidazole. Abstr Weed Sci Soc Am 19: 103 (Abstr No 218)

25. Yukimoto, Mineko. "Effect of Organophosphorus Insecticides on Hill Reaction." *Pesticides* 8 (1983): 63-68. *Ministry of Agriculture*.

26. ZAWA S, DR ORT 1974 Photooxidation of ferricyanide and iodide ions and associated phosphorylation in NH20H-treated chloroplasts. Biochim Biophys Acta 357: 127-143

11. Appendices

11.1 Raw data appendix

Concentration of insecticide solution/%	A_{652} (0.005) of Elodea exposed to malathion
0.0000	0.125
0.0175	0.089
0.0350	0.085
0.0700	0.080
0.1400	0.078
0.2800	0.060

Table 3: Table of Absorbance of isolated chloroplast from the plants exposed to each malathion at each concentration at 652 nm (A_{652}).

Concentration of insecticide solution/%	A_{652} (0.005) of Elodea exposed to cypermithrin
0.0000	0.129
0.0125	0.127
0.0250	0.124
0.0500	0.093
0.1000	0.089
0.2000	0.070

Table 4: Table of Absorbance of isolated chloroplast from the plants exposed to each cypermithrin at each concentration at 652 nm (A_{652}).

Concentration of insecticide solution/%	A_{652} (0.005) of Elodea exposed to imidacloprid
0.000	0.122
0.005	0.123
0.010	0.120
0.020	0.098
0.040	0.095
0.080	0.084

Table 5: Table of Absorbance of isolated chloroplast from the plants exposed to each imidacloprid at each concentration at 652 nm (A_{652}).

Concentration of malathion solution/%	Concentration of chlorophyll/(mg/ml)of Elodea exposed to malathion
0.0000	0.362
0.0175	0.258
0.0350	0.246
0.0700	0.232
0.1400	0.226
0.2800	0.174

Table 6: Table of chlorophyll concentration of Elodea exposed to malathion at each concentration of malathion in mg/ml

Concentration of cypermithrin solution/%	Concentration of chlorophyll/(mg/ml)of Elodea exposed to cypermithrin
0.0000	0.374
0.0125	0.368
0.0250	0.359
0.0500	0.270
0.1000	0.256
0.2000	0.203

Table 7: Table of chlorophyll concentration of Elodea exposed to cypermithrin at each concentration of cypermithrin in mg/ml

Concentration of imidacloprid solution/%	Concentration of chlorophyll/(mg/ml)of Elodea exposed to imidacloprid
0.000	0.354
0.005	0.356
0.010	0.348
0.020	0.284
0.040	0.275
0.080	0.238

Table 8: Table of Chlorophyll concentration of Elodea exposed to imidacloprid at each concentration of imidacloprid in mg/ml

Time taken/ min (± 0.01s)		Absorbancy at 600nm (± 0.005)					
		Concentration of insecticide solution (malathion)					
		0.000% (control)	**0.001%**	**0.002%**	**0.003%**	**0.004%**	**0.005%**
0	T1	0.125	0.123	0.130	0.119	0.125	0.124
	T2	0.127	0.122	0.131	0.117	0.125	0.120
	T3	0.128	0.125	0.132	0.125	0.123	0.128
	A	0.127	0.123	0.131	0.120	0.124	0.124
1	T1	0.101	0.108	0.121	0.109	0.113	0.116
	T2	0.099	0.107	0.120	0.108	0.111	0.115
	T3	0.102	0.110	0.123	0.111	0.115	0.113
	A	0.100	0.108	0.121	0.109	0.113	0.114
2	T1	0.086	0.099	0.106	0.100	0.101	0.101
	T2	0.085	0.101	0.105	0.098	0.100	0.100
	T3	0.090	0.099	0.108	0.099	0.104	0.102
	A	0.087	0.100	0.106	0.099	0.102	0.101
3	T1	0.073	0.089	0.100	0.095	0.094	0.095
	T2	0.070	0.087	0.099	0.094	0.092	0.093
	T3	0.069	0.090	0.099	0.091	0.096	0.096
	A	0.071	0.088	0.099	0.093	0.094	0.094
4	T1	0.063	0.080	0.090	0.088	0.088	0.086
	T2	0.064	0.079	0.087	0.086	0.087	0.084
	T3	0.060	0.082	0.093	0.089	0.090	0.085
	A	0.062	0.080	0.090	0.088	0.088	0.085
5	T1	0.058	0.078	0.086	0.083	0.084	0.083
	T2	0.057	0.076	0.090	0.081	0.083	0.080
	T3	0.059	0.072	0.085	0.079	0.081	0.079
	A	0.058	0.075	0.087	0.081	0.083	0.081
6	T1	0.052	0.072	0.082	0.077	0.079	0.080
	T2	0.051	0.072	0.080	0.073	0.078	0.079
	T3	0.049	0.071	0.083	0.079	0.075	0.076
	A	0.051	0.072	0.082	0.076	0.077	0.078
7	T1	0.048	0.066	0.079	0.073	0.075	0.078
	T2	0.047	0.064	0.078	0.070	0.076	0.077
	T3	0.044	0.065	0.078	0.073	0.073	0.075
	A	0.046	0.065	0.078	0.072	0.075	0.077
8	T1	0.045	0.057	0.075	0.068	0.074	0.076
	T2	0.040	0.055	0.072	0.063	0.073	0.075
	T3	0.042	0.058	0.069	0.065	0.072	0.074
	A	0.042	0.057	0.072	0.065	0.073	0.075
9	T1	0.044	0.050	0.072	0.061	0.070	0.074
	T2	0.038	0.048	0.070	0.063	0.069	0.074
	T3	0.039	0.052	0.067	0.063	0.069	0.073
	A	0.040	0.050	0.070	0.062	0.069	0.074
10	T1	0.036	0.045	0.068	0.059	0.068	0.074
	T2	0.034	0.046	0.067	0.060	0.069	0.073
	T3	0.033	0.049	0.065	0.060	0.068	0.072
	A	0.034	0.047	0.067	0.060	0.068	0.073

Table 9: Table of Absorbance of isolated chloroplast from the plants exposed to malathion at each concentration in % at 600 nm (A_{600}).

Time taken/ min ($\pm$ 0.01s)		Absorbancy at 600nm ($\pm$ 0.005) Concentration of insecticide solution (cypermithrin)					
		0.000% (control)	**0.001%**	**0.002%**	**0.003%**	**0.004%**	**0.005%**
0	T1	0.134	0.130	0.135	0.138	0.131	0.140
	T2	0.132	0.129	0.134	0.136	0.129	0.141
	T3	0.131	0.126	0.132	0.140	0.125	0.138
	A	0.132	0.128	0.134	0.138	0.128	0.140
1	T1	0.110	0.114	0.123	0.125	0.123	0.131
	T2	0.108	0.116	0.125	0.126	0.120	0.130
	T3	0.111	0.114	0.121	0.124	0.124	0.127
	A	0.110	0.115	0.123	0.125	0.122	0.129
2	T1	0.094	0.100	0.107	0.117	0.113	0.129
	T2	0.092	0.103	0.105	0.116	0.112	0.130
	T3	0.091	0.102	0.104	0.120	0.110	0.125
	A	0.092	0.102	0.105	0.118	0.112	0.128
3	T1	0.082	0.093	0.100	0.110	0.104	0.116
	T2	0.080	0.090	0.099	0.112	0.102	0.118
	T3	0.080	0.092	0.098	0.109	0.103	0.114
	A	0.081	0.092	0.099	0.110	0.103	0.116
4	T1	0.071	0.086	0.094	0.105	0.101	0.114
	T2	0.070	0.088	0.093	0.104	0.101	0.112
	T3	0.072	0.082	0.092	0.106	0.099	0.111
	A	0.071	0.085	0.093	0.104	0.100	0.112
5	T1	0.062	0.075	0.087	0.100	0.094	0.106
	T2	0.060	0.078	0.083	0.101	0.092	0.105
	T3	0.063	0.079	0.085	0.098	0.095	0.108
	A	0.062	0.077	0.085	0.100	0.094	0.106
6	T1	0.056	0.069	0.083	0.095	0.086	0.100
	T2	0.055	0.066	0.082	0.092	0.084	0.099
	T3	0.053	0.070	0.080	0.093	0.085	0.102
	A	0.055	0.068	0.082	0.093	0.085	0.100
7	T1	0.052	0.062	0.076	0.088	0.080	0.093
	T2	0.051	0.064	0.075	0.089	0.081	0.091
	T3	0.049	0.068	0.078	0.087	0.082	0.094
	A	0.051	0.065	0.076	0.088	0.081	0.093
8	T1	0.045	0.060	0.069	0.082	0.074	0.089
	T2	0.043	0.059	0.070	0.080	0.073	0.090
	T3	0.045	0.061	0.067	0.083	0.070	0.087
	A	0.044	0.060	0.067	0.082	0.072	0.089
9	T1	0.041	0.049	0.064	0.078	0.070	0.086
	T2	0.039	0.050	0.066	0.077	0.069	0.088
	T3	0.037	0.052	0.062	0.080	0.067	0.085
	A	0.039	0.050	0.064	0.080	0.069	0.086
10	T1	0.036	0.043	0.059	0.075	0.067	0.084
	T2	0.032	0.039	0.058	0.073	0.067	0.082
	T3	0.034	0.045	0.057	0.074	0.065	0.079
	A	0.034	0.042	0.058	0.074	0.066	0.082

Table 10: Table of Absorbance of isolated chloroplast from the plants exposed cypermithrin at each concentration in % at 600 nm (A_{600}).

Time taken/ min (± 0.01s)		Absorbancy at 600nm (± 0.005)					
		Concentration of insecticide solution (imidacloprid)					
		0.000% (control)	**0.001%**	**0.002%**	**0.003%**	**0.004%**	**0.005%**
0	T1	0.132	0.641	0.652	0.659	0.665	0.649
	T2	0.134	0.640	0.653	0.660	0.663	0.648
	T3	0.131	0.643	0.653	0.658	0.661	0.647
	A	0.132	0.641	0.653	0.659	0.663	0.648
1	T1	0.107	0.616	0.628	0.637	0.646	0.634
	T2	0.104	0.620	0.622	0.637	0.642	0.632
	T3	0.105	0.615	0.630	0.635	0.645	0.635
	A	0.105	0.617	0.630	0.636	0.644	0.634
2	T1	0.092	0.602	0.616	0.627	0.637	0.626
	T2	0.090	0.603	0.613	0.626	0.635	0.630
	T3	0.087	0.601	0.617	0.629	0.636	0.631
	A	0.090	0.602	0.615	0.627	0.636	0.629
3	T1	0.079	0.594	0.608	0.618	0.629	0.618
	T2	0.076	0.596	0.606	0.616	0.629	0.620
	T3	0.078	0.592	0.604	0.619	0.627	0.617
	A	0.078	0.594	0.606	0.618	0.628	0.618
4	T1	0.068	0.583	0.599	0.608	0.620	0.611
	T2	0.066	0.581	0.600	0.610	0.622	0.609
	T3	0.067	0.584	0.597	0.611	0.621	0.608
	A	0.067	0.583	0.599	0.610	0.621	0.609
5	T1	0.059	0.573	0.589	0.600	0.610	0.605
	T2	0.058	0.575	0.588	0.602	0.613	0.607
	T3	0.058	0.577	0.589	0.601	0.614	0.606
	A	0.058	0.575	0.589	0.601	0.612	0.606
6	T1	0.049	0.567	0.583	0.596	0.605	0.600
	T2	0.047	0.566	0.580	0.594	0.603	0.602
	T3	0.048	0.564	0.581	0.593	0.603	0.599
	A	0.048	0.566	0.581	0.594	0.603	0.600
7	T1	0.042	0.565	0.580	0.591	0.602	0.595
	T2	0.044	0.564	0.579	0.590	0.600	0.593
	T3	0.041	0.566	0.577	0.592	0.599	0.591
	A	0.042	0.565	0.579	0.591	0.600	0.593
8	T1	0.037	0.559	0.575	0.587	0.598	0.592
	T2	0.035	0.558	0.573	0.589	0.597	0.590
	T3	0.034	0.556	0.571	0.587	0.595	0.589
	A	0.035	0.558	0.573	0.588	0.597	0.590
9	T1	0.029	0.549	0.571	0.583	0.594	0.584
	T2	0.027	0.547	0.570	0.582	0.592	0.583
	T3	0.028	0.550	0.569	0.584	0.593	0.580
	A	0.028	0.549	0.570	0.583	0.592	0.582
10	T1	0.023	0.543	0.567	0.579	0.590	0.582
	T2	0.024	0.542	0.565	0.578	0.589	0.581
	T3	0.022	0.542	0.563	0.577	0.587	0.583
	A	0.023	0.542	0.565	0.578	0.589	0.582

Table 11: Table of Absorbance of isolated chloroplast from the plants exposed imidacloprid at each concentration in % at 600 nm (A_{600}).

Time taken/ min (± 0.01s)	Cumulative Absorbancy (± 0.005)					
	Concentration of insecticide solution (malathion)%					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.027	0.015	0.010	0.011	0.011	0.010
2	0.040	0.023	0.025	0.021	0.022	0.023
3	0.056	0.035	0.032	0.027	0.030	0.030
4	0.062	0.043	0.041	0.032	0.036	0.039
5	0.069	0.048	0.044	0.039	0.041	0.043
6	0.076	0.051	0.049	0.044	0.047	0.046
7	0.081	0.058	0.053	0.048	0.049	0.047
8	0.085	0.066	0.059	0.055	0.052	0.049
9	0.087	0.073	0.061	0.058	0.056	0.050
10	0.093	0.076	0.063	0.060	0.057	0.051

Table 12: Table showing cumulative Absorbance overtime for each concentration of malathion insecticide.

Time taken/ min (± 0.01s)	Standard deviation					
	Concentration of insecticide solution (malathion)%					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000
1	0.002000000	0.000000000	0.001154701	0.002645751	0.003055050	0.005131601
2	0.002081666	0.002516611	0.001154701	0.004041452	0.003214550	0.003000000
3	0.003605551	0.000577350	0.001527525	0.006082763	0.003055050	0.002516611
4	0.003214550	0.000000000	0.002645751	0.002886751	0.002645751	0.003605551
5	0.001527525	0.004358899	0.003000000	0.005773503	0.000577350	0.004932883
6	0.003000000	0.002081666	0.001527525	0.002000000	0.001000000	0.005686241
7	0.003511885	0.001527525	0.001527525	0.003214550	0.000577350	0.005131601
8	0.003785939	0.000577350	0.004000000	0.004582576	0.000577350	0.004582576
9	0.004618802	0.000577350	0.003511885	0.004000000	0.001000000	0.004509250
10	0.003055050	0.001154701	0.002516611	0.004041452	0.001000000	0.004582576

Table 13: Table showing the standard deviation cumulative Absorbance overtime for each concentration of malathion insecticide.

Time taken/ min (± 0.01s)	Cumulative Absorbancy at 600nm (± 0.005)					
	Concentration of insecticide solution (cypermithrin)/%					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.022	0.013	0.011	0.013	0.006	0.011
2	0.040	0.026	0.029	0.020	0.016	0.012
3	0.051	0.036	0.035	0.028	0.025	0.024
4	0.061	0.043	0.041	0.034	0.028	0.028
5	0.070	0.051	0.049	0.038	0.034	0.034
6	0.077	0.060	0.052	0.045	0.043	0.040
7	0.081	0.063	0.058	0.050	0.047	0.047
8	0.088	0.068	0.067	0.056	0.056	0.051
9	0.093	0.078	0.070	0.058	0.059	0.054
10	0.098	0.086	0.076	0.064	0.062	0.058

Table 14: Table showing cumulative Absorbance overtime for each concentration of cypermithrin insecticide.

Time taken/ min (± 0.01s)	Standard deviation					
	Concentration of insecticide solution (cypermithrin)%					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000
1	0.002309401	0.002081666	0.001527525	0.003000000	0.004358899	0.001154701
2	0.000000000	0.003055050	0.000577350	0.000577350	0.001527525	0.001154701
3	0.000577350	0.002516611	0.000577350	0.003511885	0.002886751	0.000577350
4	0.002081666	0.001732051	0.000577350	0.001000000	0.002000000	0.001527525
5	0.002309401	0.004000000	0.002081666	0.003511885	0.004041452	0.003055050
6	0.000577350	0.003605551	0.000000000	0.002081666	0.002886751	0.003055050
7	0.000577350	0.005131601	0.002886751	0.003000000	0.004041452	0.003000000
8	0.001732051	0.002886751	0.001000000	0.000577350	0.001000000	0.000000000
9	0.000577350	0.003605551	0.001527525	0.000577350	0.001527525	0.000577350
10	0.001527525	0.004582576	0.000577350	0.001732051	0.002000000	0.001732051

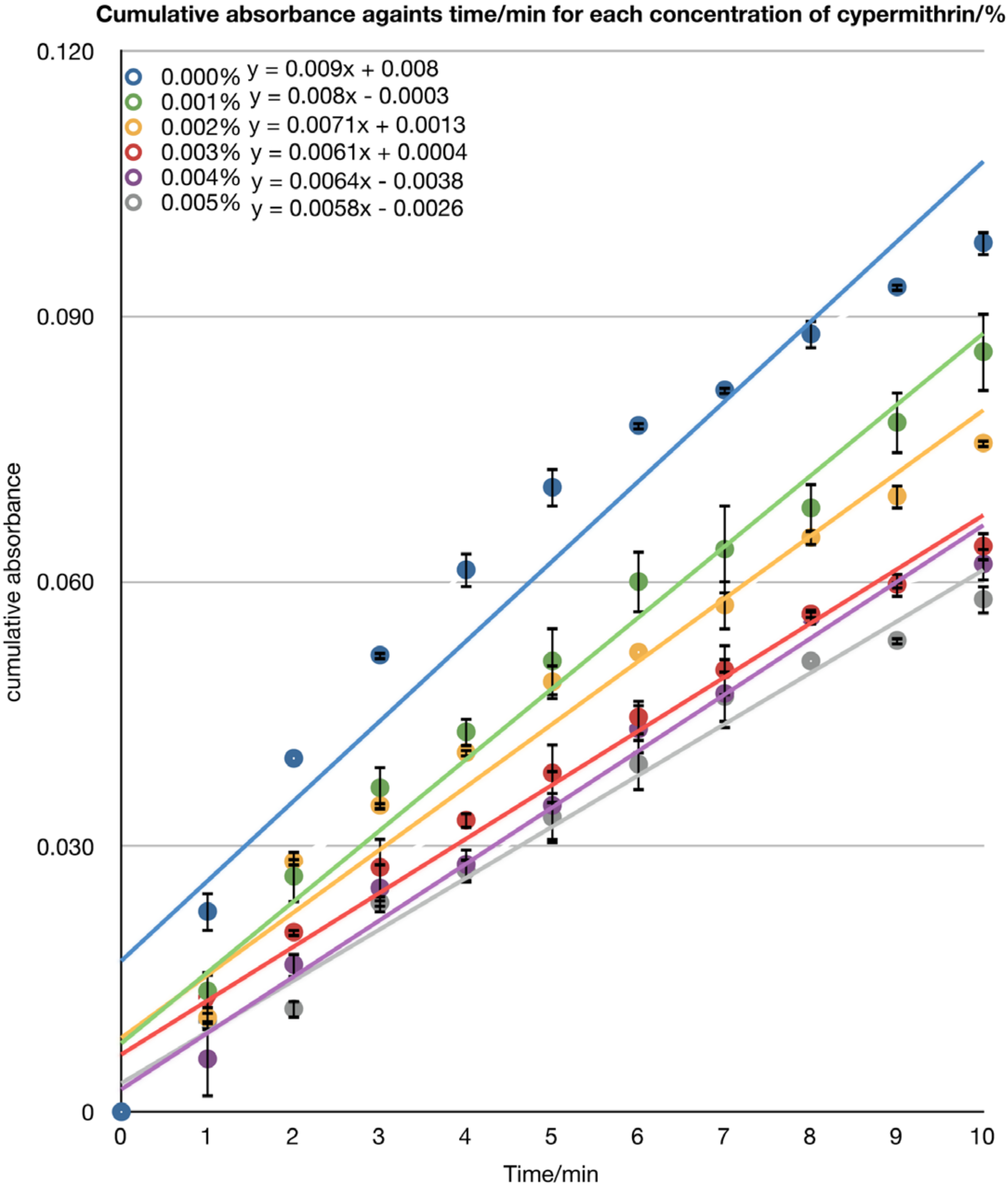
Table 15: Table showing the standard deviation cumulative Absorbance overtime for each concentration of cypermithrin insecticide.

Time taken/ min (± 0.01s)	Cumulative Absorbancy at 600nm (± 0.005) Concentration of insecticide solution (imidacloprid)					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.027	0.024	0.024	0.023	0.019	0.014
2	0.042	0.039	0.038	0.032	0.027	0.019
3	0.054	0.047	0.047	0.041	0.035	0.030
4	0.065	0.058	0.054	0.049	0.042	0.039
5	0.074	0.066	0.064	0.058	0.051	0.042
6	0.084	0.075	0.072	0.065	0.060	0.048
7	0.090	0.076	0.074	0.068	0.063	0.055
8	0.097	0.083	0.080	0.071	0.066	0.058
9	0.104	0.092	0.083	0.076	0.071	0.067
10	0.109	0.099	0.088	0.081	0.074	0.067

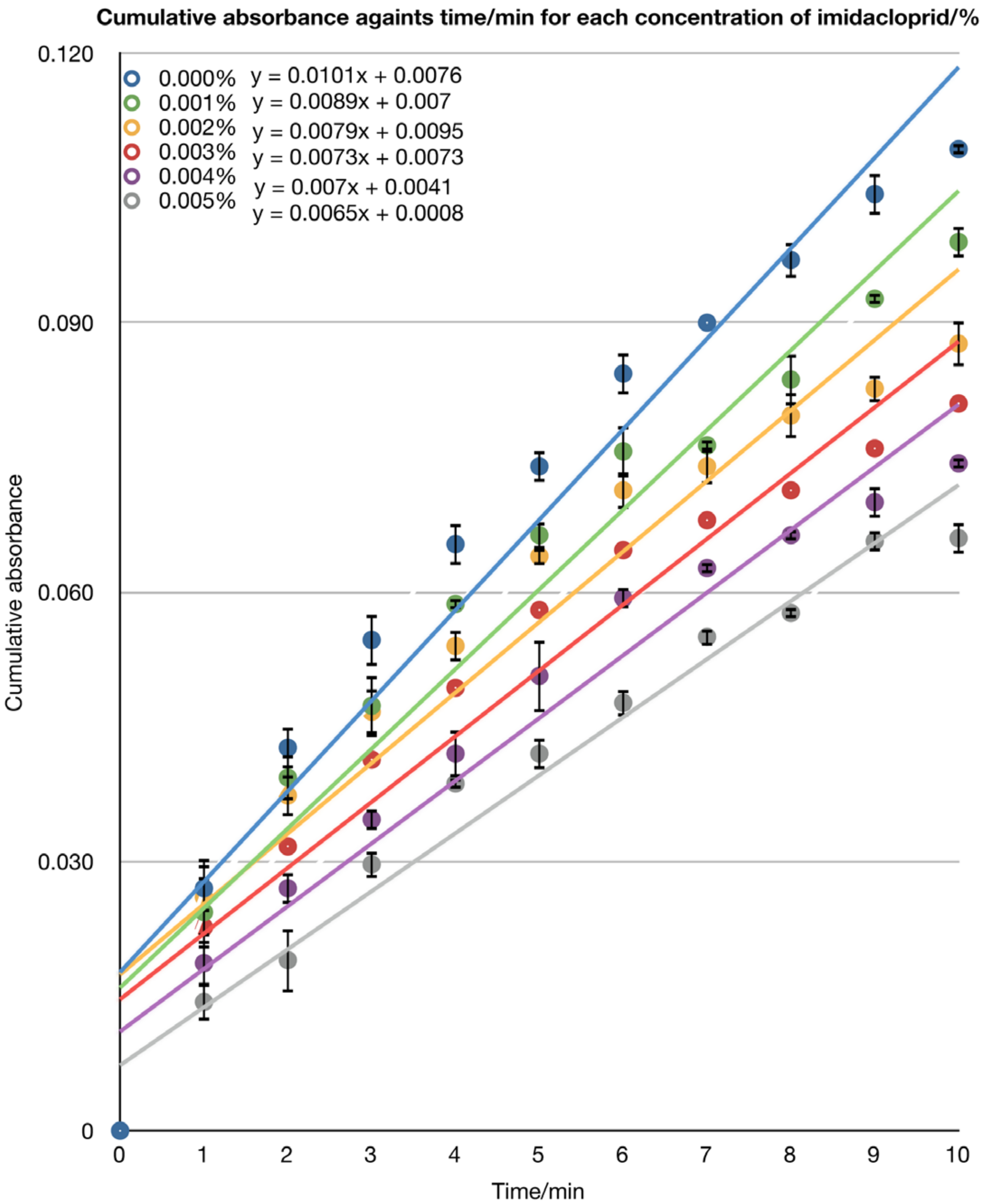
Table 16: Table showing cumulative Absorbance overtime for each concentration of imidacloprid insecticide.

Time taken/ min (± 0.01s)	Standard deviation Concentration of insecticide solution (imidacloprid)%					
	0.000 (control)	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**
0	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000	0.000000000
1	0.002645751	0.004041452	0.004358899	0.000577350	0.002516611	0.002081666
2	0.002309401	0.002516611	0.002309401	0.002516611	0.001732051	0.003605551
3	0.002886751	0.003511885	0.002516611	0.002516611	0.001154701	0.001527525
4	0.002309401	0.000577350	0.001732051	0.002081666	0.002645751	0.000577350
5	0.001732051	0.001527525	0.001000000	0.001000000	0.004041452	0.001732051
6	0.002309401	0.002886751	0.002081666	0.001527525	0.001154701	0.001527525
7	0.000000000	0.000577350	0.002000000	0.002000000	0.000577350	0.001000000
8	0.002000000	0.002886751	0.002516611	0.000577350	0.000577350	0.000577350
9	0.002309401	0.000577350	0.001527525	0.002000000	0.001732051	0.001154701
10	0.000577350	0.001732051	0.002516611	0.001000000	0.000577350	0.001732051

Table 17: Table showing the standard deviation cumulative Absorbance overtime for each concentration of imidacloprid insecticide.



Graph 5: Graph showing Cumulative absorbance against time/min for each concentration of Cypermithrin/%



Graph 6: Graph showing Cumulative absorbance against time/min for each concentration of imidacloprid/%

Concentration of insecticide solution/%	Rate of photosynthetic electron transport/ (micromol/min)		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.00231	0.00253	0.00284
0.001	0.00205	0.00225	0.00250
0.002	0.00174	0.00200	0.00222
0.003	0.00166	0.00172	0.00205
0.004	0.00152	0.00180	0.00197
0.005	0.00138	0.00163	0.00183

Table 17: Table showing the rate of photosynthetic electron transport of each concentration of the insecticides.

Concentration of insecticide/%	Percentage inhibition/% (trial 1)		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.00	0.00	0.00
0.001	12.4	11.2	10.1
0.002	30.3	22.4	22.0
0.003	32.6	35.7	26.6
0.004	36.0	34.7	31.2
0.005	43.8	42.9	38.5

Table 18: Table showing percentage inhibition of each concentration of the insecticides after ten minutes. (trial 1)

Concentration of insecticide/%	Percentage inhibition/% (trial 2)		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.00	0.00	0.00
0.001	18.3	10.0	10.9
0.002	31.2	24.0	20.0
0.003	38.7	37.0	25.5
0.004	39.8	38.0	32.7
0.005	49.5	41.0	39.1

Table 19: Table showing percentage inhibition of each concentration of the insecticides after ten minutes. (trial 2)

Concentration of insecticide/%	Percentage inhibition/% (trial 3)		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.00	0.00	0.00
0.001	20.0	16.5	7.3
0.002	29.5	22.7	17.4
0.003	31.6	32.0	25.7
0.004	42.1	38.1	32.1
0.005	41.1	39.2	41.3

Table 20: Table showing percentage inhibition of each concentration of the insecticides after ten minutes. (trial 3)

Concentration of insecticide/%	Percentage inhibition/% (average)		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.00	0.00	0.00
0.001	20.0	16.5	7.3
0.002	29.5	22.7	17.4
0.003	31.6	32.0	25.7
0.004	42.1	38.1	32.1
0.005	41.1	39.2	41.3

Table 21: Table showing percentage inhibition of each concentration of the insecticides after ten minutes. (average)

Concentration of insecticide/%	Standard deviation		
	Malathion	Cypermithrin	Imidacloprid
0.000	0.0000000000	0.0000000000	0.0000000000
0.001	4.0079707303	3.4510635873	1.8701834511
0.002	0.8504900548	0.8504900548	2.3065125189
0.003	3.8431757701	2.5942243542	0.5859465277
0.004	3.0805843601	1.9347695814	0.7549834435
0.005	4.2883563284	1.8502252115	1.4742229591

Table 22: Table showing standard deviation of each concentration of the insecticides after ten minutes of the average percentage inhibition results

11.2 Photographic appendix

Figure 15- Me using the spectrophotometer

Figure 16- Cuvette under aquarium light

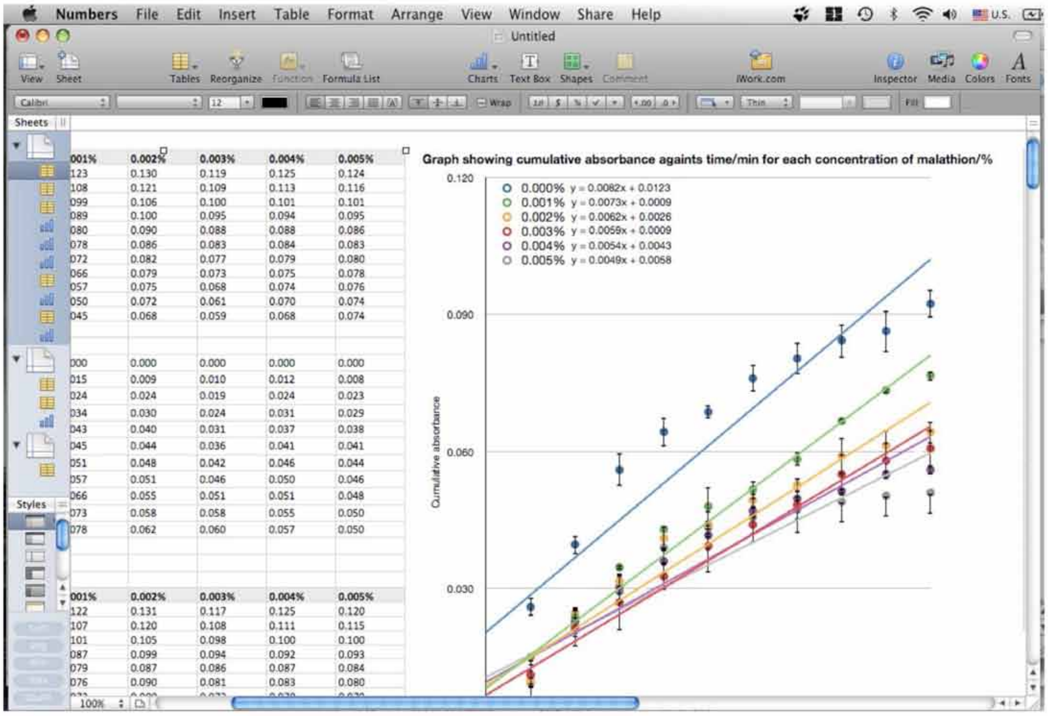


Figure 17- Computer screenshot of using Mac Microsoft Excel to plot graphs